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Effect of heat stress on semen characteristics and genetics in Thai native grandparent roosters

Jiraporn Juiputta,* Thirawat Koedkanmark ,* Vibuntita Chankitisakul,*,† and Wuttigrai Boonkum *,†,1

*Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand; and
†Network Center for Animal Breeding and Omics Research, Khon Kaen University, Khon Kaen 40002, Thailand

ABSTRACT Grandparent roosters are crucial in poultry breeding programs and significantly influence future bird generations' genetic makeup and performance. However, these roosters face considerable challenges from heat stress, which can adversely affect their reproductive performance, semen quality, and overall health and welfare. Our study aimed to investigate the effects of heat stress on the genetics of semen characteristics, identify the appropriate temperature and humidity indices (THI), and determine the threshold point of heat stress to prevent thermal stress. We analyzed data from 3,895 records of 242 Thai native grandparent roosters in conjunction with the THI using 7 THI functions and the regression method. The threshold point of heat stress, genetic parameters, rate of decline of semen characteristics per level of THI, estimated breeding values and selection index values were analyzed using the multivariate test-day model in the AIREML and BLUPF90 programs. Based on the regression coefficient and statistical criteria of the lowest −2logL and AIC values, the results showed that a THI of 78 was considered the threshold point of heat stress. The estimated heritability values ranged from 0.023 to 0.032, 0.066 to 0.069, 0.047 to 0.057, and 0.022 to 0.024 for mass movement, semen volume, sperm concentration, and the semen index, respectively. The reduction rates of mass movement, semen volume, sperm concentration, and semen index at a THI of 78 were −0.009, −0.003, −0.170, and −0.083 per THI, respectively. The genetic correlations among the semen traits were moderately to strongly positive and ranged from 0.562 to 0.797. The genetic correlations between semen traits and heat stress were negative and ranged from −0.437 to −0.749. The permanent environmental correlations among the semen traits (0.648 −0.929) were positive and greater than the genetic correlations. Permanent environmental correlations between semen traits and heat stress were negative and ranged from −0.539 to −0.773. The results of the selection indices showed that the higher the selection intensity was, the greater the degree to which the selection index corresponded to genetic progress. The recommendation for animal genetic selection is that the top 10% is appropriate because it seems most preferred. Therefore, using a multivariate test-day model and selection index for the high genetic potential of semen traits and heat tolerance in Thai native grandparent roosters makes it possible to achieve genetic assessment in a large population.

Key words: heat tolerance, temperature and humidity index, genetic parameter, indigenous chicken

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INTRODUCTION

Thai native chickens, also known as "Kai Baan," possess a myriad of unique characteristics that set them apart from broilers. Their meat flavor, textural uniqueness, and nutritional content are distinct and intriguing, making them fascinating meats for consumers (Jaturasitha et al., 2008; Charoensin et al., 2021). Moreover, these native breeds demonstrate remarkable resilience and adaptability to a wide range of climates, often thriving in harsh weather conditions without the need for extensive climate control (Padhi, 2016; Boonkum et al., 2024). This natural resilience allows them to be raised in a more organic environment, with free access to outdoor spaces, fresh air, and natural sunlight, a practice commonly observed among small-scale farmers and rural households (Asnawi et al., 2023).

Although Thai native chickens raised in tropical areas are more tolerant to heat stress compared to those in temperate regions (Loengbudnark et al., 2023), their reproductive efficiency, notably semen quality in roosters,

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[1]Corresponding author: wuttbo@kku.ac.th

is still affected by heat stress. The temperature-humidity index (**THI**), representing the combined effects of ambient temperature and relative humidity, is widely used to measure the impact of heat stress across various species (Bohmanova et al., 2007; Fragomeni et al., 2016; Ratchamak et al., 2021). High environmental temperatures and high relative humidity (especially during the summer and rainy seasons) contribute to elevated THI values. Our previous study indicated that THI values often exceed the threshold of 78, leading to decreased sperm production in Thai native roosters (Pimprasert et al., 2023). A concerning fact is that Thailand experiences THI values above this threshold for up to eight months of the year. This reveals that Thai native roosters are exposed to unsuitable temperatures and humidity for most of the year, resulting in reduced reproductive potential of sperm production. Similarly, adverse climatic conditions during hot periods significantly impact semen production, as evidenced by reductions in semen volume and sperm concentration in other poultry species (Obidi et al., 2008; Harsha et al., 2021; Prabakar et al., 2022). These reductions could result from abnormalities in spermatogenesis and decreased testosterone production (Chen et al., 2015; Xiong et al., 2020). Heat stress also reduced intracellular calcium ions, which ultimately depressed sperm motility (Karaca et al., 2002), leading to reduced fertility in roosters.

While the impact of heat stress on reproductive efficiency is a significant concern, several promising methods exist to mitigate this issue. Environmental modification techniques and ventilation equipment are generally used in intensive broiler farming systems. However, the high costs associated with these methods could be a limitation for local farmers in Thailand (Haitook, 2006; Tirawattanawanich et al., 2011). Therefore, developing heat-tolerant Thai native chicken breeds presents an interesting solution, offering hope for a more sustainable and efficient poultry farming industry.

Previous genetic studies on improving reproductive systems in poultry have mostly focused solely on factors related to semen quality without incorporating environmental factors into the research (Barbato, 1999; Gebriel et al., 2009). This might be because commercial chickens raised intensively under controlled environments have already had their temperature conditions regulated; hence, it is unnecessary to do research by considering environmental factors, while there are few reported for native species (Hu et al., 2013; Daryatmo et al., 2024). In contrast, Thai native chickens are raised under an open system, where environmental temperature significantly impacts the development and quality of semen (Sonseeda et al., 2013; Pimprasert et al., 2023; Islam et al., 2024).

Similarly, breeding improvements in other tropical livestock, such as cattle, have shown that incorporating THI into the selection equations increases prediction accuracy (Menegassi et al., 2016, Llamas-Luceño et al., 2020). Additionally, similar improvements have been reported in pigs when using such integrated approaches (Zumbach et al., 2008; Fragomeni et al., 2016). Thus, sustainable breeding improvements must also consider environmental factors. Therefore, this research aims to integrate these aspects to further enhance the heat tolerance and reproductive efficiency of Thai native chickens. We identified the most appropriate THI for assessing heat stress effects on semen characteristics and estimating genetic parameters and selection indices for genetically selecting Thai native grandparent roosters. The findings are expected to be applicable to other regions with climates similar to Thailand's.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee approved the experimental procedures following the National Research Council of Thailand's Ethics of Animal Experimentation (Record no. IACUC-KKU-82/66).

Animals and Management

The experiment was conducted using 242 Thai native grandparent roosters (Pradu Hang Dum) obtained from the Network Center for Animal Breeding and Omics Research, Faculty of Agriculture, Khon Kaen University, Thailand. Each grandparent rooster was reared in an open-housing system in a single cage with a size of $45 \times 50 \times 60$ cm^3 and an average of 12 h of natural light daily. Each rooster was fed 130 grams/bird/day of commercial feed (90.07% dry matter, 17.15% crude protein, 3.35% crude fiber, 3.99% ether extract, and 9.75% ash), and clean water was always available.

When the roosters reached 30 wk of age, they began to train in collecting semen, and the data recordings were started when the roosters reached 32 wk of age. Roosters whose semen could not be collected within 30 to 32 wk of age were excluded from the study. Semen was routinely collected for testing semen parameters in terms of semen volume, mass movement, and sperm concentration once per week for a year. Before using the farm data for statistical analysis, we had to verify its accuracy. Some data could have been identified as outliers. Consequently, the dataset used in the analysis contained 3,895 records (test day records). The data used for analysis are summarized in Table 1.

Semen Collection and Semen Evaluation

Semen was collected between 2 and 4 pm (every Saturday) using the dorsal-abdominal massage method

Table 1. Data structure for analysis.

Item	Number	Mean	SD	Min	Max	CV (%)
Animal with records (n)	242	—	—	—	—	—
Animal with pedigrees (n)	638	—	—	—	—	—
Number of records (n)	3,895	—	—	—	—	—
Mass movement (score)	—	3.45	1.22	1.00	5.00	35.36
Semen volume (mL)	—	0.39	0.20	0.10	2.50	51.28
Sperm concentration (10^9/mL)	—	3.44	1.58	0.20	9.48	45.93
Air temperature (°C)	—	28.33	4.78	13.00	43.50	16.87
Relative humidity (%)	—	78.00	10.68	39.50	96.00	13.69

(Burrows and Quinn, 1937) in a 1.5 mL Eppendorf tube containing 0.1 mL of IGGKPh diluent (Chankitisakul et al., 2022). The semen samples were protected from light and kept at 22 to 25 °C during transport to the laboratory within 20 min after collection for macroscopic and microscopic evaluation. Semen collection was always carried out by the same person with more than 7 y of experience to ensure the best quality and quantity of semen collected, and the semen was handled carefully to prevent cross-contamination during semen collection.

The quality of fresh semen, in terms of mass movement, semen volume, and sperm concentration, was evaluated as described by Authaida et al. (2023) and Chalah and Brillard (1998). The waves of sperm mass movement were scored on a scale of 1 to 5 (1 = no sperm movement or very slow, less than 10%; 5 = very rapid waves and whirlpools visible, with more than 90% of the sperm showing forward movement). The semen volume was measured using a syringe (1 mL). The sperm concentration was determined using a hemocytometer counting chamber and is expressed as billion (10^9) sperm cells/mL. Moreover, all three semen traits were calculated as the semen index by standardizing the data. The quality of the raw data from the experimental farm, including the data distribution, anomalies, and descriptive statistics, was checked using the PROC UNIVARIATE package in SAS version 9.0 software.

Weather Data

Weather data, including daily air temperature (**T**) and relative humidity (**RH**), were collected using the ELITECH GSP-6 Temperature Data Logger. To investigate the impact of heat stress on semen, T and RH data were calculated as the temperature and humidity index (**THI**) and linked with semen data. The summary of weather data is shown in Table 1.

It is essential to first test whether THI values are suitable for further evaluation by determining the period during which THI values have the highest correlation with sperm parameters. The THI values from 1 d to 7 d before semen collection were tested for correlation with sperm parameters. These correlations were computed using PROC CORR in the SAS program. The results found that the average THI values from 3 to 1 d before semen collection had the highest correlation with semen quality, suggesting that this period is crucial for assessing and mitigating heat stress to maintain optimal semen quality in roosters. Consequently, we used the THI values from this period to study changes in semen quantity and quality and to estimate genetic parameters.

Determination of Appropriate THI Function and the THI Threshold

Due to the lack of previous studies on THI and semen parameter characteristics, it is essential to establish the appropriateness of any THI functions for this study. This can be achieved by evaluating the highest R-square and the lowest MSE values from 7 THI functions previously reported in studies involving broiler and layer chickens. The following is a list of temperature and humidity indices compared in this study. Simple regression analysis using statistical criteria such as the highest R-square (**R^2**) and lowest mean squared error (**MSE**) was used to determine the most appropriate THI functions for the dataset and to determine the THI threshold that affects changes in semen characteristics. The forms of the THI functions are shown as follows.

$$\mathrm{THI1} = \left(0.8 \times \mathrm{T_{avg}}\right) + \left[\left(\frac{\mathrm{RH_{Avg}}}{100}\right) \times \left(\mathrm{T_{avg}} - 14.4\right)\right] + 46.4$$ (Mader et al., 2006)

$$\mathrm{THI2} = 0.8 \times \mathrm{T_{avg}} + \mathrm{RH}\left(\mathrm{T_{avg}} - 14.3\right)/100 + 46.3$$ (de Moraes et al., 2008)

$$\mathrm{THI3} = \left(1.8 \times \mathrm{T_{avg}} + 32\right) - \left(0.55 - 0.0055 \times \mathrm{RH_{Avg}}\right) \times \left(1.8 \times \mathrm{T_{avg}} - 26\right)$$ (NOAA, 1976)

$$\mathrm{THI4} = \mathrm{T_{avg}} + 0.36 \times \mathrm{T_{avg}} + 41.2$$ (Yousef, 1985)

$$\mathrm{THI5} = 0.60(\mathrm{T_{max}}) + 0.40(\mathrm{T_{min}})$$ (Zulovich and DeShazer, 1990)

$$\mathrm{THI6} = 0.85(\mathrm{T_{max}}) + 0.15(\mathrm{T_{min}})$$ (Tao and Xin, 2003)

$$\mathrm{THI7} = \mathrm{T_{avg}} - \left(0.31 - 0.31\left(\frac{\mathrm{RH_{Avg}}}{100}\right)\right) \times \left(\mathrm{T_{avg}} - 14.4\right)$$ (Marai et al., 2001)

where $\mathrm{T_{min}}, \mathrm{T_{max}}, \mathrm{T_{avg}}$ are the minimum, maximum, and average temperatures in degrees Celsius, respectively, and $\mathrm{RH_{Avg}}$ is the average relative humidity as a percentage.

Statistical Analysis and Genetic Model

A multivariate test-day model was used to analyze the effects of heat stress on genetics, the rate of decline of semen characteristics per THI, estimated variance components, and genetic parameters (heritability, genetic correlation, and permanent environmental correlation). The model is shown as follows:

$$\mathrm{y_{ijkl}} = \mathrm{RHS_i} + \mathrm{RW_j} + \mathrm{RA_k} + \alpha(\mathrm{THI}) + \mathrm{a_{0l}} + \mathrm{a_{1l}}[\mathrm{f(THI)}] + \mathrm{p_{0l}} + \mathrm{p_{1l}}[\mathrm{f(THI)}] + \mathrm{e_{ijkl}}$$

where y_{ijkl} is the observed value of test-day semen data for rooster hatch set (**RHS**) class i, rooster body weight (**RW**) class j, rooster age (**RA**) class k, and animal l; α(**THI**) is the rate of decline of semen characteristics per temperature and humidity index (**THI**); a_{0l} is random additive genetic effects without consideration of heat stress in animal l, assumed to be $a \sim N(0, A\sigma_a^2)$, where A is an additive relationship matrix and σ_a^2 is the additive genetic variance; p_{0l} is random permanent environmental effects without consideration of heat stress in animal l, assumed to be $pe \sim N(0, I\sigma_{pe}^2)$, where I is the identity matrix and σ_{pe}^2 is the permanent environmental variance; $a_{1l}[f(THI)]$ and $p_{1l}[f(THI)]$ are the random additive genetic and permanent environmental effects of animal l under heat stress conditions; and e_{ijkl} is the random residual effect, assumed to be $e \sim N(0, I\sigma_e^2)$, where σ_e^2 is the residual variance.

The heat stress function is shown as follows:

$$f(THI) = \begin{cases} 0 & ; THI \leq THI_{threshold} \text{ (no heat stress)} \\ THI - THI_{threshold} & ; THI > THI_{threshold} \text{ (heat stress)} \end{cases}$$

The THI included in the multivariate test-day model was set at various critical values or threshold points. Different thresholds, at 74 (THI74), 76 (THI76), 78 (THI78), and 80 (THI80) of the THI, were tested in the model. The best model was considered to come from the lowest minus twice the logarithm of the likelihood (**−2logL**) and the lowest Akaike information criterion (**AIC**).

Heritability ($\mathbf{h^2}$) was calculated as:

$$h^2 = \frac{\sigma_{a0}^2 + \sigma_{a1}^2 + 2\sigma_{a01}}{\sigma_{a0}^2 + \sigma_{a1}^2 + 2\sigma_{a01} + \sigma_{p0}^2 + \sigma_{p1}^2 + 2\sigma_{p01} + \sigma_e^2}$$

Genetic correlations ($\mathbf{r_g}$) with and without consideration of heat stress effects of the additive genetic effects and correlations between permanent environment and permanent environment heat tolerance effects ($\mathbf{r_p}$) were calculated as follows:

$$r_g = \frac{COV\sigma_{a0,a1}}{\sqrt{\sigma_{a0}^2 * \sigma_{a1}^2}}; r_p = \frac{COV\sigma_{p0,p1}}{\sqrt{\sigma_{p0}^2 * \sigma_{p1}^2}}$$

The variance components and genetic parameters, such as heritability, genetic correlations, and correlations between permanent environment and permanent environment heat tolerance effects, were estimated using the average information-restricted maximum likelihood (**AI-REML**); moreover, the breeding values were estimated using the BLUPF90 family programs (Misztal et al., 2019).

Selection Indices

The selection indices (**I**) were calculated based on the estimated breeding value (**EBV**) between semen traits and heat tolerance. The relative economic value (**v**) of each semen trait was calculated as a proportion of the standardized economic value to the total economic importance of all traits undergoing genetic evaluation, with values ranging from 0 to 1. The determination of relative economic values in the selection indices was based on the following conditions: 1) traits that significantly affect profit margins and help improve the quality of the final product should be prioritized and 2) traits that reduce negative environmental impacts or enhance sustainability. In this case, semen characteristics are more important in profit margins than heat tolerance characteristics. For this reason, this study assigned the economic value of each semen trait as 0.7 or 0.3 for heat tolerance. The selection index equations are shown as follows:

$$I = (v_{mass\ movement} \times EBV_{mass\ movement}) + (v_{heat\ tolerance} \times EBV_{heat\ tolerance})$$

$$I = (v_{semen\ volume} \times EBV_{semen\ volume}) + (v_{heat\ tolerance} \times EBV_{heat\ tolerance})$$

$$I = (v_{sperm\ concentration} \times EBV_{sperm\ concentration}) + (v_{heat\ t\ tolerance} \times EBV_{heat\ tolerance})$$

$$I = (v_{semen\ index} \times EBV_{semen\ index}) + (v_{heat\ tolerance} \times EBV_{heat\ tolerance})$$

where I is the selection index; v_{trait} is the relative economic value for mass movement, semen volume, sperm concentration, and semen index traits; and EBV_{trait} is the estimated breeding value of the semen traits, which correspond to the economic values.

The genetic progress (ΔG) was calculated using the following formula:

$$\Delta G = h^2 \times \overline{SD}$$

where ΔG is genetic progress, h^2 is heritability and $\overline{SD}$ is the selection differential between before and after selection.

RESULTS

Determination of Appropriate THI Function and the THI Threshold

The impacts of THI on mass movement, semen volume, sperm concentration, and the semen index are presented in Table 2. The results showed that the THI1 function was the most appropriate for all semen traits from the seven THI functions based on the highest R-square and the lowest MSE values. The R-square and MSE values of the THI1 function were 0.064 and 1.390 for mass movement, 0.015 and 0.036 for semen volume, 0.544 and 2.490 for sperm concentration, and 0.270 and 3.849 for semen index, respectively. Furthermore, the THI threshold value of the THI1 function was 78. This value means that animals will begin to show the heat

Table 2. Regression analysis of statistical parameters (R^2 and MSE) and heat stress thresholds (threshold) between the temperature and humidity index (THI) and mass movement, semen volume, sperm concentration, and the semen index in Thai native grandparent roosters.

	Mass movement			Semen volume			Sperm concentration			Semen Index		
THI functions	R^2	MSE	Threshold	R^2	MSE	Threshold	R^2	MSE	Threshold	R^2	MSE	Threshold
THI1	0.064	1.390	78	0.015	0.036	78	0.544	2.490	78	0.270	3.849	78
THI2	0.062	1.392	78	0.014	0.037	78	0.542	2.491	78	0.268	3.850	78
THI3	0.061	1.393	78	0.013	0.038	78	0.542	2.492	78	0.267	3.852	78
THI4	0.059	1.396	78	0.013	0.038	78	0.477	2.509	78	0.251	3.858	78
THI5	0.056	1.395	76	0.013	0.038	76	0.469	2.511	76	0.249	3.857	76
THI6	0.054	1.394	76	0.011	0.038	76	0.434	2.521	76	0.242	3.860	76
THI7	0.054	1.398	78	0.011	0.038	78	0.436	2.499	78	0.253	3.862	78

R^2 = coefficient of determination; MSE = mean squared error.

stress phenotype when the environment has a THI value of 78.

Monthly differences in semen traits caused by environmental changes throughout the year according to the THI1 function are presented in Figure 1. The results showed that January, February, November, and December (corresponding to the winter season in Thailand) were the months with the highest semen production, corresponding to a temperature and humidity index in the range of 73 to 79. However, from March to October

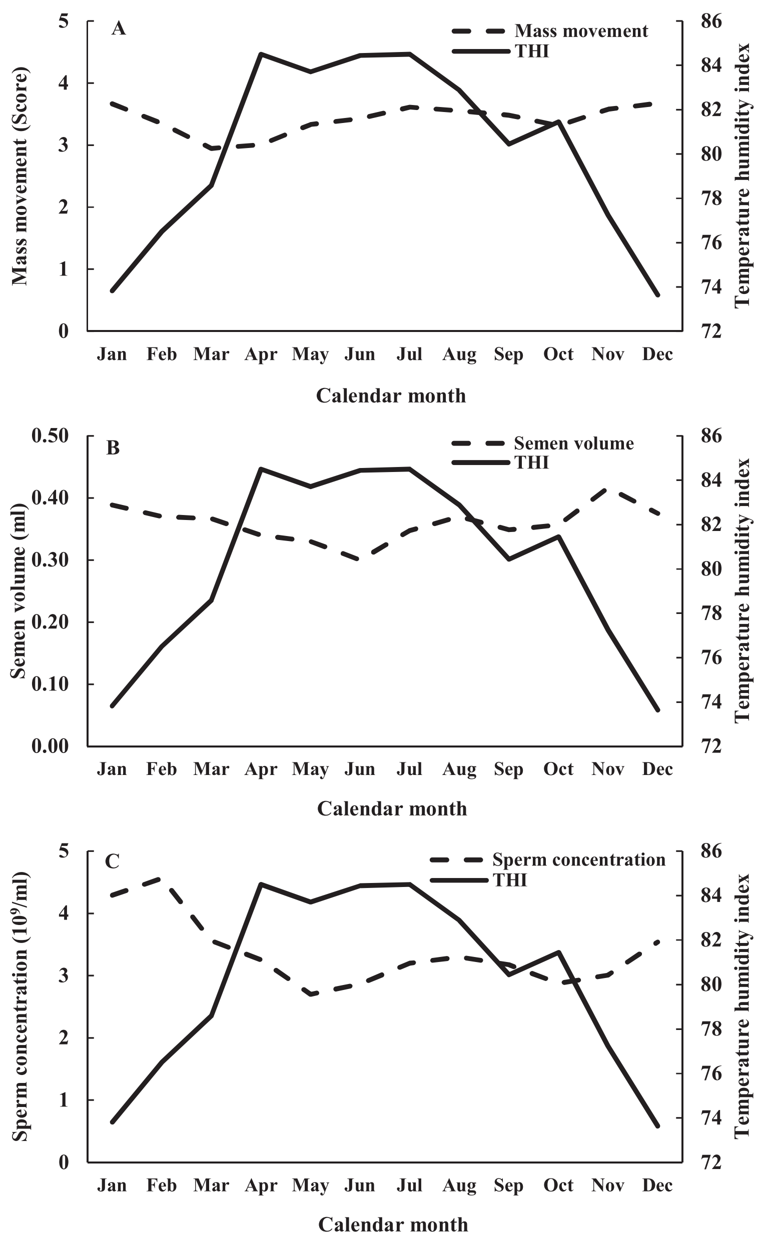

Figure 1. Monthly average temperature–humidity index and semen characteristics in Thai native grandparent roosters.

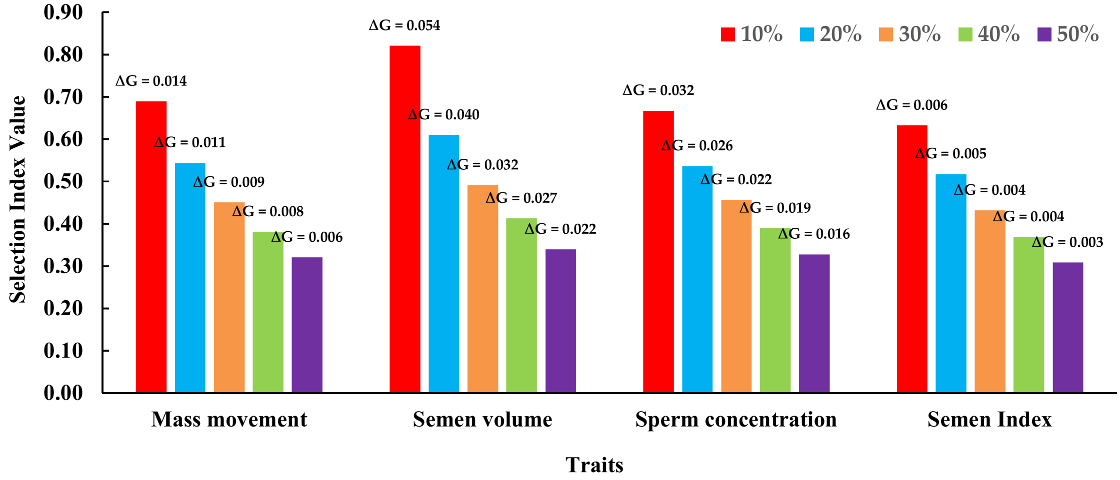

Figure 2. Top 10, 20, 30, 40, and 50% of selection index values and genetic progress (separated and combined semen characteristics) in Thai native grandparent roosters.

(corresponding to the summer and rainy seasons in Thailand), semen production was low, corresponding to a temperature and humidity index in the range of 80 to 85. In particular, the semen concentration (Figure 2C) fluctuated more than the mass movement (Figure 1A) and semen volume (Figure 1B).

Effects of Heat Stress on Variance Components and Heritability

The variance components, heritability, and statistical criteria used to determine the threshold point of heat stress on genetics and the rate of decline of semen traits in Thai native grandparent roosters at various THIs are presented in Table 3. Based on the −2logL and AIC values, the threshold point for heat stress manifestation in this study was found to be a THI of 78. For variance components, the additive variances of animals decreased when the THI increased; meanwhile, the additive variances of the heat stress effect increased when the THI increased for all semen traits. Permanent environmental variances in animal and heat stress effects were greater than those in additive effects. The results were the same for all semen traits. The estimated heritabilities for all semen traits and semen indices were low (<0.1). The estimated heritabilities ranged from 0.023 to 0.032, 0.066 to 0.069, 0.047 to 0.057, and 0.022 to 0.024 for mass movement, semen volume, sperm concentration, and the semen index, respectively. The reduction in semen trait values is related to the increase in THI values; at higher THI levels, there is a greater decline in all semen traits. The reduction rates of mass movement, semen volume, sperm concentration, and the semen index at a THI of 78 were −0.009, −0.003, −0.170, and −0.083 per THI, respectively.

Effects of Heat Stress on Genetic and Permanent Environmental Correlations

The genetic and permanent environmental correlations between mass movement, semen volume, sperm concentration, the semen index, and heat stress at a THI of 78 are presented in Table 4. The genetic correlations among the semen traits (mass movement, semen volume, and sperm concentration) were moderate to high and ranged from 0.562 to 0.797. However, the genetic correlations between semen traits and heat stress were negative and ranged from −0.437 to −0.749. The permanent environmental correlations among the semen traits (0.648−0.929) were positive and greater than the genetic correlations. Permanent environmental correlations between semen traits and heat stress were negative and ranged from −0.539 to −0.773. The genetic and permanent environmental correlations between the semen index and heat stress were −0.548 and −0.729, respectively.

Selection indices

The top 10, 20, 30, 40, and 50% of the selection index values are presented in Figure 2. The results showed that the greater the selection intensity was, the greater the degree to which the selection index corresponded to genetic progress. The selection index values (genetic progress; Δ) for the top 10, 20, 30, 40, and 50% for mass movement were 0.689 (0.014), 0.544 (0.011), 0.450 (0.009), 0.381 (0.008), and 0.320 (0.006); the values for semen volume were 0.821 (0.054), 0.610 (0.040), 0.491 (0.032), 0.413 (0.027), and 0.339 (0.022); and the values for sperm concentration were 0.667 (0.032), 0.536 (0.026), 0.456 (0.022), 0.389 (0.019), and 0.328 (0.016), respectively. The selection index values for combined semen traits, such as the semen index, were 0.633 (0.006), 0.517 (0.005), 0.432 (0.004), 0.369 (0.004), and 0.309 (0.003) and were slightly lower than those for the separated semen traits.

DISCUSSION

Although studies on the effects of heat stress on the genetic parameters of growth and production characteristics in poultry are continuously published, data on the genetics of reproductive characteristics are limited, and

Table 3. Variance components, heritability, model statistical criteria, and rate of decline of the semen traits in Thai native grandparent roosters (Pradu Hang Dum) at various temperature and humidity indices (**THIs**) using a multivariate test-day model with the THI1 function.

	Mass movement				Semen volume				Sperm concentration				Semen index			
Parameters	THI74	THI76	THI78	THI80	THI74	THI76	THI78	THI80	THI74	THI76	THI78	THI80	THI74	THI76	THI78	THI80
σ^2_{a0}	0.053	0.050	0.046	0.041	0.003	0.003	0.003	0.003	0.186	0.165	0.152	0.153	0.143	0.132	0.125	0.118
σ^2_{a1}	0.001	0.001	0.002	0.005	0.000	0.000	0.000	0.000	0.001	0.002	0.003	0.006	0.003	0.004	0.006	0.013
$\sigma_{a0,a1}$	−0.005	−0.005	−0.006	−0.009	−0.000	−0.000	−0.000	−0.000	−0.013	−0.014	−0.016	−0.023	−0.016	−0.014	−0.015	−0.022
σ^2_{p0}	0.434	0.399	0.386	0.350	0.016	0.015	0.014	0.013	1.251	1.144	1.050	0.877	3.097	2.847	2.673	2.366
σ^2_{p1}	0.004	0.006	0.010	0.019	0.000	0.000	0.000	0.000	0.007	0.010	0.015	0.025	0.020	0.028	0.045	0.080
$\sigma_{p0,p1}$	−0.031	−0.035	−0.045	−0.059	−0.001	−0.001	−0.001	−0.001	−0.076	−0.083	−0.097	−0.102	−0.192	−0.210	−0.253	−0.296
σ^2_{e}	0.952	0.953	0.957	0.960	0.024	0.024	0.023	0.022	1.577	1.578	1.580	1.587	2.001	2.004	2.010	2.016
σ^2_{total}	1.372	1.330	1.299	1.239	0.042	0.040	0.038	0.036	2.844	2.705	2.574	2.398	4.848	4.567	4.323	3.957
h^2	0.032	0.032	0.028	0.023	0.069	0.068	0.069	0.066	0.057	0.051	0.048	0.047	0.024	0.024	0.023	0.022
Model statistic criterion																
-2logL	9947.15	9937.17	9932.89	9935.51	9947.15	9937.17	9932.89	9935.51	9947.15	9937.17	9932.89	9935.51	9947.15	9937.17	9932.89	9935.51
AIC	10099.2	10089.2	10084.9	10087.5	10099.2	10089.2	10084.9	10087.5	10099.2	10089.2	10084.9	10087.5	10099.2	10089.2	10084.9	10087.5
Rate of decline of semen traits (unit by semen trait/level of THI)																
$\propto (THI)$	−0.005	−0.002	−0.009	−0.022	−0.001	−0.001	−0.003	−0.005	−0.114	−0.136	−0.170	−0.219	−0.070	−0.079	−0.083	−0.091

THI, temperature and humidity indices; σ^2_{a0}, additive variance of animal; σ^2_{a1}, additive variance of heat stress; $\sigma_{a0,a1}$, covariance between additive variance of animal and heat stress; σ^2_{p0}, permanent environmental variance of animal; σ^2_{p1}, permanent environmental variance of heat stress; $\sigma_{p0,p1}$, covariance between permanent environmental variance of animal and heat stress; σ^2_{e}, residual variance; h^2, heritability; SE, standard error; −2 logL, minus twice the logarithm of the likelihood; AIC, Akaike's information criterion.

Table 4. Genetic (above the diagonal) and permanent environmental correlations (below the diagonal) between mass movement, semen volume, sperm concentration, semen index, and heat stress in Thai native grandparent roosters at a THI threshold of 78.

Traits	Mass movement	Semen volume	Sperm concentration	Semen index	Heat stress
Mass movement	-	0.562	0.782	-	−0.626
Semen volume	0.648	-	0.797	-	−0.437
Sperm concentration	0.929	0.834	-	-	−0.749
Semen index	-	-	-	-	−0.548
Heat stress	−0.724	−0.539	−0.773	−0.729	-

most studies have focused only on genetic parameters. This study is the first to focus in detail on the effects of heat stress on phenotypes and genetics in terms of genetic selection in individual animals with breeding values using a selection index. The monthly temperature and humidity index (**THI**) in Thailand affects mass movement, semen volume, and sperm concentration (Figure 1); this finding is consistent with several previous studies (Harsha et al., 2021; Prabakar et al., 2022; Pimprasert et al., 2023). As the THI increases, the quantity and quality of semen decreases. A comparison of the changes in semen characteristics between the winter, summer, and rainy seasons revealed that the semen of chicken roosters during the summer and rainy seasons was different from that during the winter season, with a reduction of 6.95, 12.33, and 23.56% in characteristic mass movement, semen volume, and sperm concentration, respectively, compared to that in the winter season. These findings provide a clearer numerical picture of the effects of heat stress on the quality and quantity of native chicken semen. The results also revealed that the sperm concentration trait was most affected by heat stress. The results of this study were in accordance with those of Prabakar et al. (2022), who reported seasonal variations in semen quality in both turkeys and egg-type chicken roosters, with winter showing better values of physical parameters, sperm concentration, and biochemical parameters than summer. Furthermore, Bhatti et al. (2023) reported that the seasonal photoperiod and temperature significantly impact the semen quality of exotic and native roosters; all chicken rooster breeds exhibited better semen quality in the winter in terms of semen volume, sperm mortality, sperm concentration, and live sperm than in the summer. In addition, Wannaratana et al. (2021) and Karaca et al. (2002) reported increased sperm production in pigeons, turkeys, and white-legged birds in the winter. The above results can be explained as follows: during the summer, heat can cause thermal stress, negatively impacting the reproductive physiology of roosters. High temperatures can lead to oxidative stress and reduce the functionality and viability of sperm (Surai et al., 2001; Shanmugam et al., 2012; Xiong et al., 2020; Capela et al., 2022). At the same time, high humidity levels, often present during the rainy season, can also stress chickens. This may indirectly result from reduced heat dissipation, as heat dissipation reduces respiratory efficiency and moisture evaporation. Generally, high humidity decreases moisture evaporation by panting, increasing the susceptibility of roosters to heat stress. Consequently, these roosters are highly affected and their reproductive functions decrease, with reduced semen quality and quantity (Balnave, 1998; Lara and Rostagno, 2013). For this reason, winter typically brings more stable and favorable environmental conditions for maintaining reproductive health.

The THI (Figure 1 and Table 2) represents the combined effects of environmental temperature (°C) and relative humidity (%) and is widely used to measure the impact of heat stress. This study showed that when the THI exceeds 78 (a temperature greater than 32 ± 1 °C with a humidity of 55−65%), a decrease in the quantity and quality of semen begins to occur. By comparing the results obtained in this study with those of previous studies, it was found that the threshold point of heat stress in this study had a THI value similar to or greater than that in earlier studies; for example, Pimprasert et al. (2023) reported that at a THI of approximately 78 to 81, the lowest sperm concentration and average sperm number were found; however, semen volume and mass movement were not significant in Thai native roosters. Prabakar et al. (2022) reported that a THI of 73 to 76% is considered a period of discomfort in terms of the semen quality of turkey and egg-type chicken male breeders in India. Furthermore, a THI threshold value of 70 was previously reported for chickens in both layers and broilers (Tao and Xin, 2003). Interestingly, Thai native males began to experience stress due to the influence of temperature and humidity at a THI of 78, which is greater than that in many previous reports. The genetics of native animals are adaptable, and native animals are therefore more resistant to heat stress than chickens from temperate regions. These chickens have evolved efficient thermoregulatory mechanisms. They can dissipate heat more effectively through behaviors such as panting, wing spreading, and seeking shade. Their bodies are better at managing internal temperatures through vasodilation (widening of blood vessels) and increased blood flow to the skin. In addition, native chickens typically have a lower basal metabolic rate. This means that they produce less internal heat during normal metabolic processes, helping them stay cooler in hot environments (Yalçin et al., 1997; Jiang et al., 2010; Fathi et al., 2013; Kim et al., 2021).

The heritability of mass movement, semen volume, sperm concentration, and semen index ranged from 0.008 to 0.071, showing low estimates for all semen traits (Table 3). The estimated heritabilities were similar to those previously reported by Thepnarong et al. (2019) for the semen characteristics (semen volume, mass movement, sperm concentration, and total sperm) of Betong chickens ($h^2 = 0.04−0.12$) and by Wolc et al. (2019) for the sperm motility and sperm count of White

Leghorn roosters (h^2 = 0.08 and 0.13). However, the results of this study were lower than those of many previous studies, such as in Chinese male chickens, which studied 7 characteristics (semen volume, semen pH, semen color, sperm viability, sperm motility, sperm deformities, and sperm concentration), and showed that the heritability values ranged from 0.03 to 0.85 (Hu et al., 2013). Moreover, the results of a study of seven sperm characteristics (semen color, semen volume, sperm progressive motility, sperm concentration, total sperm per ejaculate, concentration of live spermatozoa, and percentage of abnormal sperm) in Rhode Island red and white breeder cocks in Nigeria showed heritability values ranging from 0.33 to 0.83 (Kabir et al., 2007). The low heritability of the semen traits means that approximately 10% of the variation indicates that genetic factors contribute to the trait variability within the population. In comparison, the remaining 90% of the variation is due to environmental factors.

In addition, the heritability values in this study tended to decrease as the THI increased, which can be explained as the influence of the environment on phenotypic traits becoming stronger relative to genetic influence under heat stress conditions. In other words, environmental factors such as temperature and humidity contribute more to the variability in traits, reducing the proportion of variability attributable to genetics. Furthermore, high THI induces physiological stress responses in chickens, leading to greater phenotypic plasticity. Phenotypic plasticity refers to the ability of an organism to change its phenotype in response to environmental conditions. When chickens face high heat and humidity, their bodies might exhibit a wide range of responses to cope with stress, making it more difficult to distinguish genetic effects from environmental effects. Theoretically, as environmental stress increases, the environmental variance component of the phenotypic variance ($Vp = Vg + Ve$, where Vg is genetic variance and Ve is environmental variance) increases. Since heritability (h^2) is defined as the ratio of genetic variance to total phenotypic variance ($h^2 = Va/Vp$), an increase in Ve leads to decreased heritability; in addition, a high THI can lead to significant genotype-by-environment interactions (G×E). This result suggested that different genotypes may respond differently to heat stress. Such interactions complicate the genetic architecture of traits and can mask heritability under extreme conditions.

The estimated genetic correlations between semen traits were moderate and positive (>0.5) (Table 4). These results indicated that the same genes or sets of genes similarly influence all semen traits. In other words, if one trait is expressed due to certain genetic factors, the other trait will also be expressed. This situation will be beneficial for planning the genetic improvement of poultry because it is easier to improve both traits simultaneously through genetic selection. However, the genetic correlations between semen traits and heat stress were negative. These results indicated that selecting chicken roosters for high semen quality and quantity could increase the susceptibility of roosters to heat stress. Although the genetic correlations were negative, the combined selection of semen quality and quantity with heat tolerance using the breeding value index is possible (Ravagnolo et al., 2000). The permanent environmental correlations among the semen traits were positive (0.648−0.929), and the permanent environmental correlations between the semen traits and heat stress were negative and ranged from −0.539 to −0.773. These results confirmed that environmental factors play a more critical role in the expression of traits than genetic factors. Therefore, genetic selection combined with environmental modification will help reduce the severity of heat stress effects on the expression of semen traits.

The selection indices were calculated using EBVs of the traits (mass movement, semen volume, sperm concentration, semen index, and heat tolerance) and economic values (Figure 2). Values from the selection index also allow for balanced animal genetic selection decisions. Animals are ranked according to the overall genetic value of the traits to be genetically improved (Cole and VanRaden, 2018). Genetic improvement occurs when genetic merit is improved through selection. The improvement in genetic value refers to the overall improvement in a flock brought about by selection for several traits that contribute to the flock's breeding objective. The results revealed that the genetic progress of the selection index combining sperm concentration and heat tolerance was greater than that of the other selection indices studied. Moreover, if all semen characteristics are selected simultaneously, a selection index that considers the semen index and heat tolerance can be used in genetic improvement planning and individual animal selection in this population. In addition, the present study presented the percentage of animals selected for future replacement herds, which revealed that if many animals are selected, the selection index values will also be lower. It is directly related to increased genetic progress (Khazraji et al., 2020). However, selecting animals too intensely (a small number of animals are selected) can result in greater inbreeding and decreased genetic variation (Weigel, 2001). Therefore, an appropriate selection intensity or proportion is important for balancing genetic improvement and inbreeding. It is recommended that the selection of the top 10% from the flock seems to be preferred compared to other proportions since it is not too intense. The time may have come for us to develop a poultry breed that is both heat tolerant and produces well.

In conclusion, heat stress negatively affected the phenotypic and genetic quality and quantity of semen characteristics in Thai native grandparent roosters. A THI of 78 is the starting point at which animals begin to experience heat stress. Genetic selection of Thai native grandparent roosters for good quality and quantity of semen may decrease heat tolerance. However, using a genetic evaluation combined with a selection index can help individuals genetically improve both characteristics.

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DISCLOSURES

The authors declare no conflicts of interest.

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