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Genetic parameter estimates for alternative growth traits and their relationship with the absolute and relative growth rates of Thai black-bone chickens

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Abstract

The objective of this study was to estimate the genetic parameters for alternative growth traits including age at the inflection point (TI), weight at the inflection point (WI), and maximum increment (MI), and their relationship with the absolute growth rate (AGR) and relative growth rate (RGR) of Thai black-bone chickens (KU-Phuparn). Three non-linear models (Gompertz, Logistic, and von Bertalanffy) were fitted to measure the body weight of 2,933 Thai black-bone chicken from hatch to 12 weeks of age. The coefficients of determination (R²), root mean squared error (RMSE), and mean absolute error (MAE) were used to determine the most appropriate model. Alternative growth traits for each bird, including the coordinates for TI, WI, and MI, were calculated using the individual growth curve parameter from the best non-linear model. Genetic parameters for AGR, RGR, TI, WI, and MI traits were estimated by the average information restricted maximum likelihood algorithm. Heritability estimates for AGR and RGR were moderate to high, whereas low heritability values were observed for the alternative growth traits. The genetic correlations among the alternative growth traits were low to high and positive (0.06 to 0.94). A moderate genetic correlation between AGR and RGR was observed. Genetic correlations between the alternative growth traits (TI and WI), AGR, and RGR were low (<0.7). The results of this study reveal that selection for the alternative growth traits TI and WI could be included in the breeding objectives for Thai black-bone chickens when selecting animals for both age and weight simultaneously is desired.

Keywords: Absolute growth rate, Thai black-bone chicken, Correlations, Heritability, Relative growth rate

1. Introduction

The Thai black-bone chicken (KU-Phuparn) is well known in the functional foods industry in Thailand and other Asian countries. The black-bone chicken was developed at Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus [1]. They are generally raised in extensive and semi-extensive conditions due to their ability to adapt to both cold and hot conditions. The selection objective is to improve growth traits (body weight at 12 weeks of age and chest circumference) and the egg production trait (number of eggs from the first 17 weeks of lay). KU-Phuparn chickens positively impact the local economy in poor areas of northeastern Thailand. AS they are slower maturing compared to other commercial broiler breeds or lines, it is important to improve productivity, especially growth traits to reduce day-to-market weight and production costs and thereby improve producer profitability and economic efficiency.

Broiler growth rate is a key performance indicator for livestock production related to profitability. Average body weight at market in the U.S. broiler is 2.9 kg at 47 days of age [2]. In Thailand, the body weight (BW) at market for Thai native chickens is 0.8 to 1.2 kg at 112 days of age [3]. Body weight has been used to select for growth rate over the decades because it has moderate heritability and is easy to select for [4]. Growth traits not

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only include average daily gain (ADG) and BW, but also includes the growth curve for the birds. Growth rate includes the relationship between age and BW (the increase in growth per unit time) [5], in which a typical sigmoid or S-shaped curve is obtained. Growth curve can be divided into two phases: 1) the accelerating phase includes growth from hatching to an inflection point (maximum growth rate), which depends on non-linear function characteristics and 2) a decelerating phase where the growth rate starts decreasing (or growth rate in increasing at a decreasing rate) until to a mature weight or limiting value (asymptote) [6]. Body weight at different ages has been used to describe the growth curve using non-linear mathematical models that have parameters with biological meaning [7-9].

Growth curve parameters are used to estimate the expected weight at a specific age [10] and age and weight at the inflection point. It is possible to growth curves as an alternative to growth traits to identify the appropriate market weight and market age. Therefore, selection based on age and weight at the inflection point as alternative growth traits represent an opportunity to improve growth performance in Thai black-bone chickens in a tropical climate and under extensive farming situations. Studies from the scientific literature have reported genetic parameters from the black-bone chicken [3] that are based on growth performance traits such as BW and ADG. Genetic parameter estimates for age and weight at the inflection point (alternative growth traits) of black-bone chickens could not be found in the scientific literature. Hence, the objective of this study was to estimate the genetic parameter for age and weight at the inflection point and their relationship with the absolute growth rate (AGR) and relative growth rate (RGR) in Thai black-bone chickens.

2. Materials and methods

2.1 Animals, housing, and management

The data from 1,814 male and 1,466 female KU-Phuparn chickens were obtained from the Animal Research Farm of the Kasetsart University Chalermphrakiat Sakon Nakhon Province Campus. The chickens were housed in an open housing system [11]. Wing bands were used to identify individual birds (National Band & Tag Company, KY, USA). Twenty chickens were raised in each open pen. Pens (1.4 m wide x 1.5 m deep x 2.5 m high) were separated by wire mesh from floor to roof and made of concrete blocks (0.5 m height), with each pen containing floor litter (rice husks), one water bowl, and one feeder (plastic hopper) (produced by Mitrkasetphand Co., Ltd., Thailand). The space allowance for each chicken was 0.1 m², which met or exceeded Good Agricultural Practices for chicken farms recommendations [11]. From 0 to 4 weeks of age, the chickens were fed a commercial diet (pelleted) with 21% crude protein (CP) 3,200 Kilocalories (kcal) Metabolisable Energy (ME)/kg, and from 5 to 12 weeks they were fed with 18% CP (3,200 kcal ME/kg). This diet met or exceeded the nutritional requirements for chickens [12]. The chickens were provided ad libitum access to feed and water throughout the experiment. The vaccination program was administered by the farm veterinarian and followed the commonly utilized broiler vaccination protocols of the Thai commercial broiler industry. The management and feeding program were provided and followed the standard operating procedures of the Thai Agricultural Standard. Selection criteria are applied to the replacement birds. Selection for growth and egg production traits is based on phenotypic selection.

The individual records kept for each bird included hatch date, hatch number, and generation (Table 1). Individual bird BW was measured at 2-week intervals from hatch to 12 weeks of age (Tanita KD-200, Tanita Corp., Japan). Chicken records that contained incomplete BW information or missing data were deleted from the dataset. Bird mortalities and associated data were excluded from the study. Outlying data was defined using the Mahalanobis distance method, and the chi-square value was used as the cut-off when identifying outliers. The chickens and associated BW classified as outliers were excluded from the final dataset. After editing, the dataset included 2,933 birds (1,559 males and 1,374 females), as shown in Table 2.

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Items	Total	Generation						
		1	2	3				
Animal with records	2,933	822	872	1,239				
Parent without records	243	195	200	201				
Total number of animals	3,176	1,017	1,072	1,440				

Table 1 Pedigrees information of Thai black bone chickens

ILGC (WOOR)	whate					remaie					
	Ν	Mean	Min	Max	SD	N	Mean	Min	Max	SD	
0 (Hatch)	500	36	25	50	8	322	34	20	50	9	
2	500	195	125	260	38	322	171	90	260	45	
4	500	361	230	480	55	322	317	175	455	62	
6	500	542	370	720	64	322	488	285	670	73	
8	500	757	520	995	83	322	658	445	900	90	
10	500	969	660	1,255	107	322	842	575	1,140	105	
12	500	1,198	825	1,545	119	322	1,020	720	1,365	128	
0 (Hatch)	410	40	25	55	8	462	36	25	50	6	
2	410	211	140	270	29	462	186	125	265	31	
4	410	391	225	540	62	462	350	235	475	45	
6	410	576	410	755	76	462	529	390	700	56	
8	410	779	565	1,015	88	462	722	565	905	63	
10	410	1,006	775	1,305	109	462	939	745	1,165	76	
12	410	1,223	950	1,645	120	462	1,157	920	1,435	86	
0 (Hatch)	649	40	25	55	7	590	38	25	50	7	
2	649	210	150	280	28	590	195	125	265	33	
4	649	421	280	580	64	590	399	280	535	49	
6	649	652	455	925	99	590	602	455	825	71	
8	649	916	645	1,285	144	590	813	645	1,080	88	
10	649	1,150	865	1,680	166	590	1,052	850	1,365	99	
12	649	1,376	1,055	2,015	172	590	1,263	1,050	1,625	96	
	0 (Hatch) 2 4 6 8 10 12 10 12	$\begin{array}{c c} (NOR) & \underline{N} \\ \hline N \\ \hline 0 (Hatch) & 500 \\ 2 & 500 \\ 4 & 500 \\ 6 & 500 \\ 8 & 500 \\ 10 & 500 \\ 10 & 500 \\ 12 & 500 \\ 0 (Hatch) & 410 \\ 2 & 410 \\ 4 & 410 \\ 6 & 410 \\ 4 & 410 \\ 6 & 410 \\ 10 & 410 \\ 12 & 410 \\ 10 & 410 \\ 12 & 410 \\ 0 (Hatch) & 649 \\ 2 & 649 \\ 4 & 649 \\ 6 & 649 \\ 8 & 649 \\ 10 & 649 \\ 12 & 649 \\ \end{array}$	$\begin{array}{c cccc} \underline{\text{NN}} & \underline{\text{Mean}} \\ \hline 0 (\text{Hatch}) & 500 & 36 \\ 2 & 500 & 195 \\ 4 & 500 & 361 \\ 6 & 500 & 542 \\ 8 & 500 & 757 \\ 10 & 500 & 969 \\ 12 & 500 & 1,198 \\ 0 (\text{Hatch}) & 410 & 40 \\ 2 & 410 & 211 \\ 4 & 410 & 391 \\ 6 & 410 & 576 \\ 8 & 410 & 779 \\ 10 & 410 & 1,006 \\ 12 & 410 & 1,223 \\ 0 (\text{Hatch}) & 649 & 40 \\ 2 & 649 & 210 \\ 4 & 649 & 421 \\ 6 & 649 & 652 \\ 8 & 649 & 916 \\ 10 & 649 & 1,150 \\ 12 & 649 & 1,376 \\ \end{array}$	N_{0} MeanMin0 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Table 2 Descriptive statistics of body weight (g) by generation, age, and sex of Thai black-bone chickens.

Gen = generations of chickens, N = number observed, SD = standard deviation.

In the present study, alternative growth traits for each bird—the coordinates for age at the inflection point (TI), weight at the inflection point (WI), and maximum increment (MI), which identifies where the growth rate is at a maximum—were calculated using the individual growth curve parameter from the best non-linear model [13]. Equations for the alternative growth traits are provided in Table 3.

Table 3 Equations for non-linear models and alternative growth traits.

Model	Equation*	TI	WI	MI
Gompertz	$y = A^* exp(-b^x exp(-k^*t))$	ln(b)/k	A/e	k*WI
Logistic	$y = A/(1+b^*exp(-k^*t))$	ln(b)/k	A/2	k*WI/2
von Bertalanffy	$y = A^{*}(1-b^{*}exp(-k^{*}t)^{3})$	ln(3b)/k	A*8/27	3k*WI/2

*y = body weight (g) at age (t), A = asymptotic weight (g), b = integration constant, k = maturity rate, t = age in weeks, TI = age at the inflection point (week), WI = weight at the inflection point (g), MI = maximum increment (g/week), e = 2.71828.

The growth curve parameters were estimated by the nlsLM function from R software [14]. Absolute growth rate from hatch to 12 weeks of age (AGR) and relative growth rate from hatch to 12 weeks of age (RGR) were calculated as follows [15]:

$$AGR = \frac{W_2 - W_1}{t_2 - t_1} \tag{1}$$

where $W_1 = BW$ at hatching (week 0), $W_2 = BW$ at week 12 of age, $t_1 =$ hatching, and $t_2 =$ week 12 of age.

$$RGR = \frac{lnW_2 - lnW_1}{t_2 - t_1}$$
(2)

where $W_1 = BW$ at hatching (week 0), $W_2 = BW$ at week 12 of age, $t_1 =$ hatching, $t_2 =$ week 12 of age, and ln = natural logarithm.

2.2 Growth curve parameter estimation and model selection criteria

Individual growth curve parameters were estimated for each bird using three non-linear models—Gompertz, Logistic and von Bertalanffy—and were fitted to the hatching weight (at week 0) and BW from 2 to 12 weeks of age measurement. Equations for the three non-linear models are provided in Table 3. The growth curve parameters for each model were estimated using the nlsLM function with the Levenberg-Marquardt algorithm in minpack.lm packages [16] from R software [14]. Repeated 10-fold cross validation was used to evaluate the performance for the 3 non-linear models [17]. The 10-fold cross validations were repeated 5 times for each model, the most common model validation practice reported in the scientific literature [17,18]. After building a model, the average prediction errors across all folds and all repeats for each model were calculated. The selection criteria, including coefficients of determination (R²), root mean squared error (RMSE), and mean absolute error (MAE), were used to determine the most appropriate model. The R², RMSE, and MAE were calculated as follow [17]:

$$R^2 = 1 - \frac{SSE}{SST}$$
(3)

where SSE is the sum of squares from the residuals and SST is the total sum of squares.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{n}}$$
(4)

where y_i is the observed BW at age *i*, \hat{y}_i is the estimated BW at age *i*, and n is the number of observations.

$$MAE = \frac{\sum_{i=1}^{n} |y_i - \hat{y}_i|}{n}$$
(5)

where y_i is the observed BW at age *i*, \hat{y}_i is the estimated BW at age *i*, and n is the number of observations. The non-linear model with the lowest RMSE and MAE and the highest R² is preferred and was utilized to identify the "best" model. The growth curve parameters were used to calculate TI, WI, and MI.

2.3 Genetic parameter estimation

After model selection, the alternative growth traits (TI, WI, and MI) were calculated from growth curve parameters from the best non-linear model. In this study, the fixed effects included sex (male and female), hatch (3 levels), and generation (3 levels). Genetic parameters (heritability and genetic correlations) for AGR, RGR, TI, WI, and MI traits were estimated by the average information restricted maximum likelihood (AIREML) algorithm implemented in the AIREMLF90 program [19]. The following multivariate linear animal model was used in the analysis:

$$y = X\beta + Za + e \tag{6}$$

where y is the vector of observations for the five traits (AGR, RGR, TI, WI, and MI); β is the vector for the fixed effects that included sex, hatch, and generation; a is the vector for the additive direct genetic effect; e is the vector for residual effects, and X and Z are incidence matrices. The random effect vectors a and e were assumed to be a~ N (0, A \otimes G₀) and e~ N (0, I \otimes R₀), respectively, where G₀ and R₀ are the additive genetic and residual variance-covariance matrices across the five traits respectively, I is the identity matrix, \otimes is the Kronecker product operator, and A is the pedigree-based relationship matrix.

3. Results

The goodness of fit associated with the three non-linear models from repeated k-fold cross validations are presented in Table 4. The model with the lowest RMSE and MAE values from the present study was observed using the von Bertalanffy model (RMSE = 109.1 ± 4.6 ; MAE = 75.0 ± 2.5). However, R² values from the three

non-linear models were close to 1 (>9.0). Based on the R², RMSE and MAE, the von Bertalanffy growth curve was determined to be the "best" model describing the growth curve for Thai black-bone chicken population.

Hence, growth curve parameters from von Bertalanffy were also used to calculate the alternative growth traits (TI, WI, and MI).

Statistic	Non-inear model			
	Gompertz	Logistic	von Bertalanffy	
RMSE	110.0 (±3.9)	113.1 (±4.2)	109.1 (± 4.6)	
MAE	77.3 (±2.6)	82.1 (±2.4)	$75.0 (\pm 2.5)$	
b	3.26 (±0.01)	13.16 (±0.10)	$0.71 (\pm 0.01)$	
А	1986.5 (±18.6)	1479.1 (±7.0)	2631.4 (± 39.6)	
\mathbb{R}^2	0.93 (±0.00)	0.93 (±0.00)	$0.93 (\pm 0.00)$	
k	0.159 (±0.002)	0.342 (±0.002)	$0.096 (\pm 0.001)$	

Table 4 Growth curve parameter estimates (\pm SE) and model statistics (\pm SD) of the three non-linear models.

 $k = maturity rate, R^2 = coefficients of determination, b = integration constant, MAE = mean absolute error, RMSE = root mean squared error, A = asymptotic weight.$

The results for the estimated alternative growth traits, AGR and RGR are shown in Table 5. The average AGR was 103.3 ± 13.7 g and 94.5 ± 11.5 g for male and female chickens, respectively while average RGR was 29.2 ± 1.9 g and 29.2 ± 1.9 g for male and female chickens, respectively. The age at the inflection point that was predicted using growth curve parameters from the von Bertalanffy model was 7.7 ± 2.0 and 7.7 ± 1.2 weeks for male and female chickens, respectively. In the present study, the weight at the inflection point was 762 ± 200 g and 753 ± 124 g for male and female chickens, respectively. The maximum increment was 121 ± 20 and 130 ± 23 g/week for male and female chickens, respectively. Average TI for male and female chickens was not significantly different, while the average WI and MI for male and female chickens were significantly different (p<0.01).

Table 5 Descriptive statistic for growth traits¹ for the Thai black-bone chicken population.

Trait [*]	Mean $(\pm SD)$			
	Male $(n = 1,559)$	Female $(n = 1,374)$	Total $(n = 2,933)$	
MI	121 ^b (±20)	130 ^a (±23)	132 (±23)	
WI	$762^{a}(\pm 200)$	753 ^b (±124)	927 (±345)	
RGR	29.2 (±1.9)	29.2 (±1.9)	29.2 (±1.9)	
TI	7.7 (±2.0)	7.7 (±1.2)	8.2 (±2.9)	
AGR	$103.3^{a}(\pm 13.7)$	94.5 ^b (±11.5)	99.2 (±13.5)	

TI = age at the inflection point (week), RGR = relative growth rate from hatch to 12 weeks of age (%), AGR = absolute growth rate from hatch to 12 weeks of age (g/week), WI = weight at the inflection point (g), MI = maximum increment (g/week).

The heritability estimates for the alternative growth traits (TI, WI, and MI), AGR, and RGR are presented in Table 6. Heritability estimates for AGR and RGR traits were moderate to high (0.27 and 0.51, respectively), whereas low heritability values were observed for the alternative growth traits (TI = 0.19, WI = 0.14 and MI =0.09). Genetic and phenotypic correlations among the alternative growth traits (TI, WI, and MI), AGR, and RGR are shown in Table 7. The genetic correlations between alternative growth traits and AGR varied and ranged between -0.31 to 0.84 while genetic correlations between alternative growth traits and RGR were low to moderate and positive (0.11 to 0.52). A moderate genetic correlation (0.41) between AGR and RGR was observed. A relatively high genetic correlation (0.84) was observed between MI and AGR. Unsurprisingly, favorable genetic correlations between TI and AGR were observed (-0.31). The genetic correlations among the alternative growth traits were low to high and positive (0.06 to 0.94). Most of the genetic correlation estimates the among alternative growth traits were moderate to high (0.36 to 0.94), except for TI and MI (0.06). It should be noted that TI was highly correlated with WI, both genetically and phenotypically (0.94 and 0.96). Similarly, high genetic and phenotypic correlations between AGR and MI were observed (0.84 and 0.81, respectively). Variations in the phenotypic correlations among the alternative growth traits, AGR, and RGR were observed (0.05 to 0.81), while positive and moderate to high phenotypic correlations were observed among the alternative growth traits (0.21 to 0.96).

Trait [*]	Variance comp	Variance components**				
	σ_a^2	σ_{e}^{2}	$\sigma_{ m p}^2$	h ² (±SE)		
WI	16377	101420	117797	0.14 (±0.03)		
MI	31.83	309.09	340.92	0.09 (±0.03)		
AGR	30.99	84.62	115.60	0.27 (±0.04)		
RGR	1.87	1.82	3.69	0.51 (±0.05)		
TI	1.47	6.47	7.94	0.19 (±0.03)		

Table 6 Variance component and heritability (h^2) estimates for absolute growth rate, relative growth rate and alternative growth traits.

*RGR = relative growth rate, TI = age at the inflection point, AGR = absolute growth rate, MI = maximum increment, WI = weight at the inflection point. ** σ_a^2 = additive genetic variance, σ_e^2 = residual variance, σ_p^2 = phenotypic variance, SE = standard deviation.

Table 7 Genetic (\pm SE; above diagonal) and phenotypic correlation estimates (\pm SE; below diagonal) with their standard deviation in parenthesis between absolute growth rate, relative growth rate and alternative growth traits.

Trait*	AGR	RGR	TI	WI	MI	
AGR	-	0.41 (±0.09)	0.31 (±0.13)	0.01 (±0.15)	0.84 (±0.06)	
RGR	0.47 (±0.02)	-	0.11 (±0.11)	0.19 (±0.12)	0.52 (±0.12)	
ΤI	$0.08 (\pm 0.02)$	0.05 (±0.02)	-	0.94 (±0.02)	0.06 (±0.18)	
WI	0.33 (±0.02)	0.14 (±0.02)	0.96 (±0.00)	-	0.36 (±0.17)	
MI	0.81 (±0.01)	0.39 (±0.02)	$0.20 (\pm 0.02)$	0.42 (±0.02)	-	
*						

AGR = absolute growth rate, RGR = relative growth rate, TI = age at the inflection point, WI = weight at the inflection point, MI = maximum increment

4. Discussion

In the present study, the average AGR between male and female chickens was significantly different (p<0.01) males grew faster compared with females. On the other hand, the average RGR between male and female chickens was not significantly different. These results agree with previous studies, where the average growth rate of males was greater than females, and males grew faster than females [7]. Moharrery and Mirzaei (2014) [20] compared the BW of commercial broiler and Iranian chickens at different weeks of age and reported that the average BW of native chickens differeds from commercial chickens.

Previously published findings have reported relatively high age at the inflection point values from the von Bertalanffy model [7,21] compared with the present study. Similarly, Aggrey (2002) [22] estimated inflection point values using the Richards, Gompertz, logistic, and spline regression models on the Athens-Canadian chicken population and all were greater than those observed in the present study. Zhao et al. (2015) [21] reported that Chinese indigenous chickens had greater values for weight at the inflection point using the von Bertalanffy model (875 to 1064 g). Similarly, Mata-Estrada et al. (2020) [7] reported a greater value for weight at the inflection point using the von Bertalanffy model for male chickens (892 g) and a lower value for female chickens (596 g). In the present study, the age and weight at the inflection point differed when compared to previous studies. These differences could be due to genetic selection for growth rate based on best linear unbiased prediction which was not considered in the selection program for the KU-Phuparn chickens. Moreover, in the present study, the genetic background, management, and feeding program were different to those in the scientific literature [7,21].

N'Dri et al. (2007) [23] reported moderate (0.25 to 0.30) heritability estimates for age at the inflection point in French chickens. Growth curve parameters have been used when performing genetic evaluations for growth traits in chickens [24-27]. However, no findings have been reported in the scientific literature no the genetic parameters for AGR, RGR, WI, and MI for Thai black-bone chickens. Studies have reported moderate to high heritability estimates for BW at different ages [24-27], while Mebratie et al. (2019) [24] and Manjula et al. (2018) [28] reported that heritability estimates for weight gain varied from 0.05 to 0.46.

While no other scientific studies have reported the genetic parameters for alternative growth traits (TI, WI, and MI) of Thai black-bone chicken, some studies have reported genetic and phenotypic relationships among growth curve parameters and BW at difference ages. For example, Manjula et al. (2018) [28] reported that heritability estimates for weight gain at different ages and associated growth curve parameter traits in Korean native chicken varied from -0.97 to 0.99. Niknafs et al. (2012) [25] reported that genetic correlations among BW traits varied from moderate to high (0.36 to 0.91) in Mazandaran native chickens. Mebratie et al. (2019) [24] reported genetic correlations between BW and weight gain that were moderate ranging from 0.16 to 0.50.

In the breeding industry, BW at fixed age has commonly been used as a selection trait to improve growth rate in broiler breeding programs, as BW is easy to measure in a normal farm routine and has a moderate

heritability [29]. However, extensive and semi-extensive producers that utilize relatively small labor and capital inputs, require chickens with the ability to adapt to a wide range of housing and climate conditions. The objective of improved growth rates in Thai black-bone chickens is a balance between age and weight to meet both customer and chicken producer demand. Selecting growth traits that combine age at a market weight such as age and weight at the inflection point can be used to improve growth rates in Thai black-bone chickens, even though heritability estimates for the alternative growth traits are low.

5. Conclusion

Genetic correlations between the alternative growth traits TI and WI, AGR, and RGR were low (<0.7). From a genetic viewpoint, the selection for the alternative growth traits will not be in the same direction as AGR and RGR. Therefore, selection for TI and WI could be included in the breeding objectives for Thai black-bone chickens when selecting animals for both age and weight simultaneously is desired. The findings from the present study could provide another point of view for using growth curve parameter information to modify the growth curve shape to optimize breeding programs for the Thai black-bone chicken population.

6. Ethical approval

The experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of Kasetsart University (Approval number: ACKU64-CSC-003) and the Ethical Review Board of the Office of the National Research Council of Thailand (NRCT license number: U1-04518-2559).

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8. Conflict of Interests

All authors certify that there is no conflict of interest with or involvement in any financial organization regarding the material discussed in this manuscript.

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