

**Studies on the empirical growth curve estimations considering
seasonal compensatory growth in Japanese Thoroughbred horses**

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General Introduction

Thoroughbred horses, well-known horse breeds for horseracing worldwide, are seasonal breeders, and engage in their mating activities during early spring, so that their foals born 340 days later can take advantage of the milder temperatures and abundant forage. Due to the seasonal mating, young Thoroughbred foals experience their first winter season at the almost same ages. Then, the young foals generally show seasonal compensatory growth (CG) patterns in their yearly spring, where their growth rates (Hintz et al., 1979; Staniar et al., 2004; Brown-Douglas et al., 2005; Brown-Douglas and Pagan, 2006) decline in winter and dramatically increase in spring due to the coldness of winter seasons. The seasonal CG is clearly found in the change of body weight or average daily gain of yearling Thoroughbreds especially in northern regions or countries (Brown-Douglas and Pagan, 2006).

There have been many studies investigating the seasonal change of horses' growth rate (Hintz et al., 1979; Staniar et al., 2004, 2005; Brown-Douglas et al., 2005) or determining the appropriate growth curve for the surveyed horses (Santos et al., 1999; Morel et al., 2007). However, continuous univariate empirical growth curve equations considering the CG have not been estimated so far. Accounting for CG in Thoroughbreds, Staniar et al. (2004) assumed that the growth rate consists of baseline and other systematic deviation components, even though they did not propose any continuous single variable growth curve equations handling CG. France et al. (1996) showed a biphasic growth curve equation by combining 2 different mathematical equations with different phases or periods, and this is an alternative approach to handle CG instead of discarding the useful merits of a continuous equation. Yin et al. (2003) proposed a continuous bivariate equation handling 2 different growth phases by using an additional timing parameter. Wan et al. (1998) and Porter et al. (2010) proposed flexible alternative equations for describing growth, but their equations are intended for the

development of main (baseline) equations and seemed to be unsuitable for handling CG.

In Japan, the Equine Research Institute, Japan Racing Association (JRA) published standardized growth curves of body weight for young Japanese Thoroughbreds based on the plots of longitudinal averages of the actual weight-age data up to 25 months covering the first winter season (Equine Research Institute, 2004). The CG patterns were clearly observed in their growth curves, but the curves were not the mathematical equations.

The young Thoroughbred growth data in Japan is mainly collected in the Hidaka region in Hokkaido, the northern island in Japan. The Hidaka region is famous for the intensive production of racehorses where there are about 815 racehorse farms corresponding to 82 % of all Japanese racehorse farms (JBBA, 2012). The JRA Hidaka Training and Research Center is located in the Hidaka region. The annual average temperature in Hidaka is 6.0 °C (°F = 1.8 * °C + 32) and average monthly temperature for January and February are minus 7.9 and 7.2 °C, respectively (JMA, 2013). Everywhere is covered by snow in Hidaka during winter, and green pastures only begin to grow in the middle of May. During winter seasons, the foals are housed alone in stalls without room heating. Due to this coldness in winter, the typical CG phenomenon appears in the growth of Japanese Thoroughbred foals. Concerning developmental orthopaedic disease in horses, Donabedian et al. (2006) investigated the relationship between nutrient intake and growth rate, and Raub (2010) discussed the making of a durable equine. The standard growth curve equation handling CG would be useful for such experiments.

During the periods of CG, careful feeding management is important to secure the optimum development of young Thoroughbred horses based on useful standard growth curves, because the relationship between gain and feeding amount tends to be unbalanced in these periods. In this study, the continuous single variable empirical growth curve estimations

considering seasonal CG in Japanese Thoroughbred horses, are examined. All animal procedures in this study were approved by the Animal Care and Use Committee at the JRA Hidaka Training and Research Center, and procedures for handling horses complied with those specified in the Basic Guidelines for Comprehensively Promoting Measures on the Welfare and Management of Animals (MEJ, 2006).

Chapter 1

**Empirical growth curve estimation using sigmoid sub-functions
adjusting compensatory growth**

Introduction

Goldstein (1979) expressed the general idea of the longitudinal studies concerning the growth curve estimations. In the book, there were many options for constructing the mathematical equations for the growth of organisms. Among the options, we focused on the sigmoid functions because of its simplicity and applicability as noted by Goldstein (1979).

The objective of this chapter was to construct the continuous single variable growth curve equation empirically adjusted for seasonal compensatory growth (CG) for body weight of Thoroughbreds by using sigmoid sub-functions. In this chapter, the male body weight was only analyzed in order to illustrate an example for this approach.

Materials and Methods

A total of 1,633 male body weight (BW) and age measurements, for 232 Thoroughbred colts, were collected by the Japan Bloodhorse Breeders' Association (JBBA) between 2005 and 2009. The data with extreme values over ± 4 residual standard deviations were preliminary analyzed by using Logistic growth curve equation, and removed from the analysis. The scatterplot of the weight-age data is shown with gray dots in Fig. 1-1. In the figure, the tendency of the seasonal CG can be recognized between about 300 and 600 days, which is the period of CG.

Seven sigmoid growth curve equations (Logistic, Gompertz, von Bertalanffy, Brody, Richards, Bridges and Janoscheck; see Kohn et al., 2007) were fit to the weight-age data. The best-fit equation based on Akaike's Information Criterion (AIC) was the Richards equation (Richards, 1959) as follows:

$$BW = \frac{A}{\left(1.0 + B e^{-k t}\right)^{\frac{-1.0}{m}}} \quad (\text{Equation 1-1})$$

In this “general” Richards equation (Eq. 1-1), BW (kg) is described as a function of age (t , day). The biological interpretations of the four parameters (A , B , k and m) have been discussed previously (Stanier et al., 2004). Briefly, A is the asymptotic limit for BW as t approaches infinity (i.e., mature BW), B is a scaling parameter that defines the degree of maturity, k is a rate constant that determines the spread of the curve along the time axis, and m is the point of inflection in the sigmoid curve in relation to age. These unknown parameters were estimated by the SAS NLMIXED procedure (SAS Institute Inc., 2008). The estimated Richards equation using the weight-age data was as follows:

$$BW = \frac{575.0}{\left(1.0 - 0.94513 e^{-0.00213 t}\right)^{\frac{-1.0}{1.2412}}} \quad (\text{Equation 1-2})$$

In Fig. 1-1, the thick gray line indicates the estimated Richards growth curve listed on Eq. 1-2, and the thin black line is a plot of the expected (actual) data averages of the BW, where the averages were computed based on the monthly averages of the BW data and their monthly daily gain. This thin black line is identical to that published in the Japanese Feeding Standard for Horses (Equine Research Institute, 2004). The deviation of the thin black line (expected data averages) from the thick gray line (Richards growth curve) is clear between about 300 and 600 days. The deviation of the expected data averages from the Richards growth curve is shown in Fig. 1-2, where the largest deviations are recognized during the period of CG.

To adjust for the deviation caused by CG shown in Fig. 1-2, a sigmoid sub-function was designed as follows. A general sigmoid function $f(t)$ is expressed as:

$$f(t) = \frac{1.0}{1.0 + e^{-\alpha t}} \quad (\text{Equation 1-3})$$

where e is the base of the natural logarithm, t is time, where $-\infty < t < \infty$, and $0 \leq f(t) \leq 1$. A sigmoid function is a monotonous increase function, and the shapes of the function depend on the α values. We chose α values of 5 and 10. The results around $-1 \leq t \leq 1$ are shown in Fig. 1-3.

The subtraction between two sigmoid functions with different α values leads to a function having only a single wave (down and up wave) at $t = 0$. By using this subtraction and application of several modifications around the t parameter, the following sigmoid sub-function $f'(t)$ can be obtained:

$$f'(t) = \frac{1.0}{1.0 + e^{\frac{-10.0(t-432.0)}{268.49}}} - \frac{1.0}{1.0 + e^{\frac{-5.0(t-432.0)}{268.49}}} \quad (\text{Equation 1-4})$$

and the shape of the sub-function is shown in Fig. 1-4. This sub-function curve crosses the x axis at 432 days with the wave length (i.e., distance between the starting and ending point of the wave) of 268.49×2 . This sub-function has zero value when $t = -\infty$ or ∞ . Only when t is around 432 days, do the non-zero $f'(t)$ values appear. This characteristic of the sub-function is like the shape of the deviation shown in Fig. 1-2 especially for the period of CG. Therefore, it was assumed that the $f'(t)$ sub-function can empirically adjust the deviation caused by CG. In the original Richards equation (Eq. 1-1) the maturity related parameter is B , and the newly developed $f'(t)$ sub-function can be used for the adjustment of the B parameter.

Results and Discussion

In general, the newly developed $f'(t)$ sigmoid sub-function can be added to the B parameter in Eq. 1-1. Specifically, this leads to the replacement of “-0.94513” by “-0.94513 +

$\beta f'(t)$ in Eq. 1-2 where β is a coefficient of $f'(t)$. With this replacement and combination of Eq. 1-2 and Eq. 1-4, the growth curve equation adjusting for the seasonal CG was obtained as:

$$BW = \frac{575.0}{\left(1.0 + (-0.94513 + 0.3582 f'(t)) e^{-0.00213 t}\right)^{\frac{-1.0}{1.2412}}} \quad (\text{Equation 1-5})$$

$$\text{where } f'(t) = \frac{1.0}{1.0 + e^{\frac{-10.0(t-432.0)}{268.49}}} - \frac{1.0}{1.0 + e^{\frac{-5.0(t-432.0)}{268.49}}}.$$

The optimal coefficient value of 0.3582 (i.e., β , the coefficient of $f'(t)$) and also the optimal wave length of 268.49 were estimated again by using the weight-age data and the SAS NLMIXED procedure. Eq. 1-5 looks complicated but is a continuous single variable function of age t in days. The insertion of $f'(t)$ in Equation 1-5 affects the BW only when t is within about 432 ± 268.49 days. The growth curve of this new Richards equation (Eq. 1-5) combined with the sigmoid sub-function is shown with thick gray line in Fig. 1-5, where the black line and dots are the same as in Fig. 1-1. As shown in Fig. 1-5, the shapes of the two lines were almost identical in the period of CG. By using this approach, the AIC value decreased from 13,053 (Eq. 1-2) to 12,794 (Eq. 1-5), indicating the better fit of Eq. 1-5 to the weight-age data than Eq. 1-2.

The deviation of the expected data averages from the new Richards growth curve adjusting for CG is shown in Fig. 1-6. The deviation in the period of CG is clearly reduced when compared to the case of non-adjusted (Fig. 1-2). These results suggest the usefulness of this proposed approach for handling CG typical in Japanese Thoroughbred horses.

As Richards (1959) noted, some researchers may consider that the parameter B is unimportant biologically. We can choose an alternative approach to adjust parameter k by the sub-function. If k was adjusted by the $f'(t)$ sub-function, the combined equation became more

complicated and difficult in numerical computation for parameter estimation because parameter k is the one used in the exponential. We aimed at simplicity for the combined equation, and chose B parameter to be adjusted in the $f'(t)$ sub-function.

The proposed method in this chapter is one of the useful approaches for adjusting seasonal CG in growth curve estimations for Thoroughbreds. Based on this approach, the optimal growth curve equations can be estimated also for female BW data of Thoroughbreds or another growth traits such as body height or entire width of chest that are considered to be also affected by seasonal CG. This approach is easily applicable to the general sigmoidal growth curve equation families such as those having biological parameters (e.g. A , B and k parameters in Richards equation).

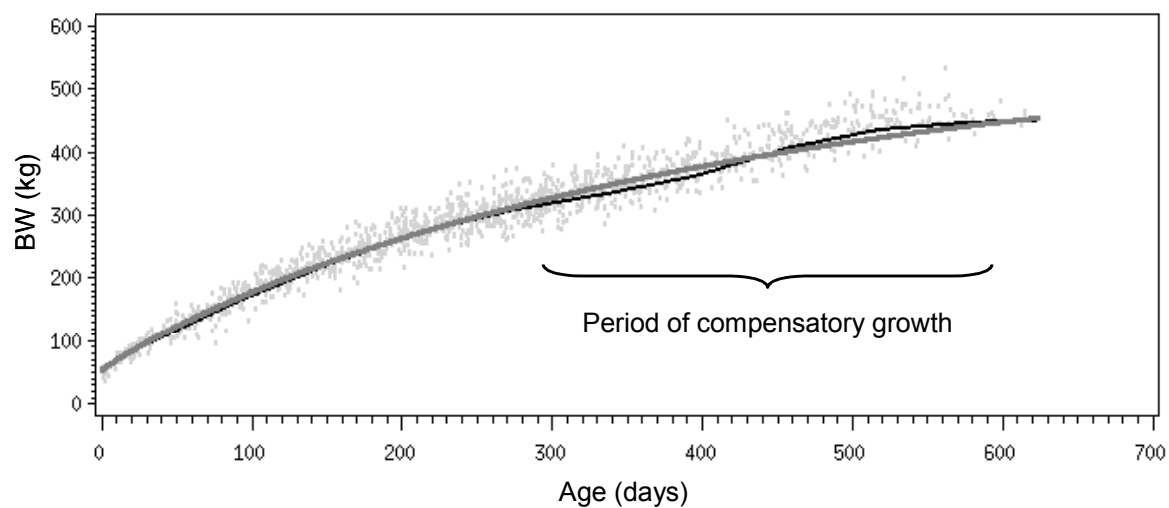


Figure 1-1. Scatterplot of the 1,633 male BW data of Thoroughbred colts (light gray dots). Thin black line indicates expected (actual) data averages of BW, and the thick gray line indicates estimated Richards growth curve (i.e., Eq. 1-2).

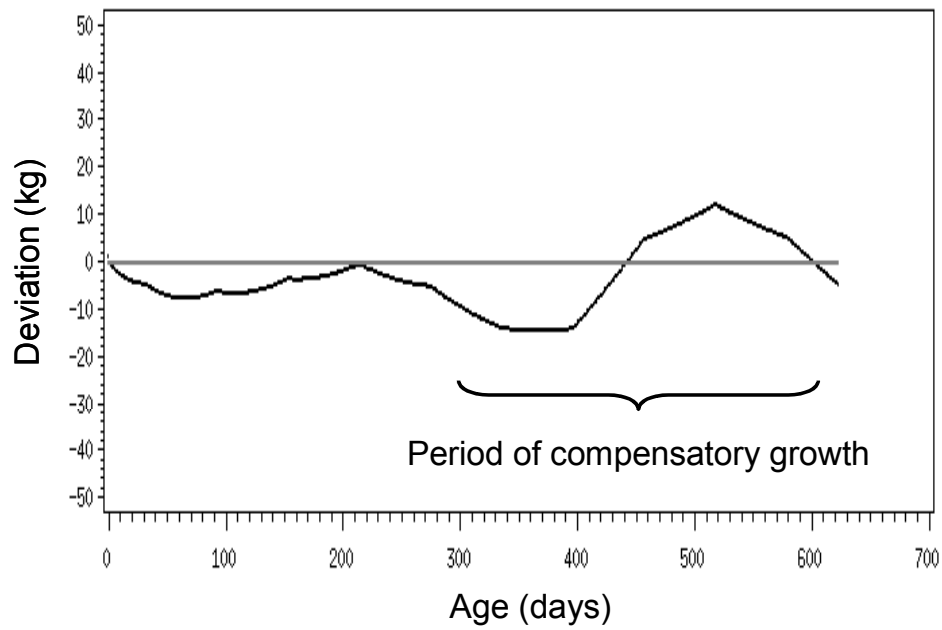


Figure 1-2. Deviation of the expected (actual) data averages of the male BW from the estimated Richards growth curve (i.e., Eq. 1-2).

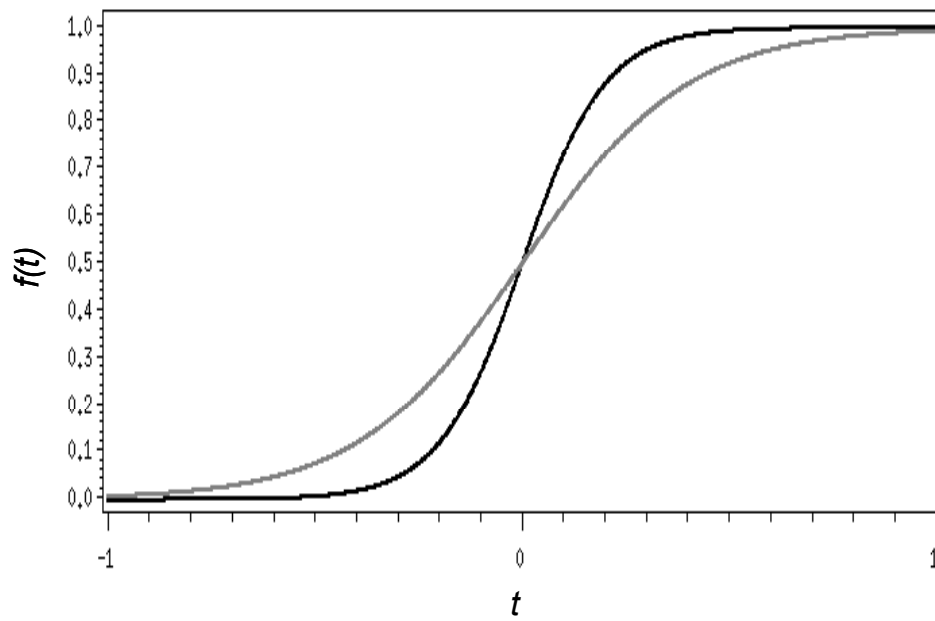


Figure 1-3. Sigmoid curve with two different values of α . Black and gray lines are with $\alpha = 10.0$ and $\alpha = 5.0$, respectively.

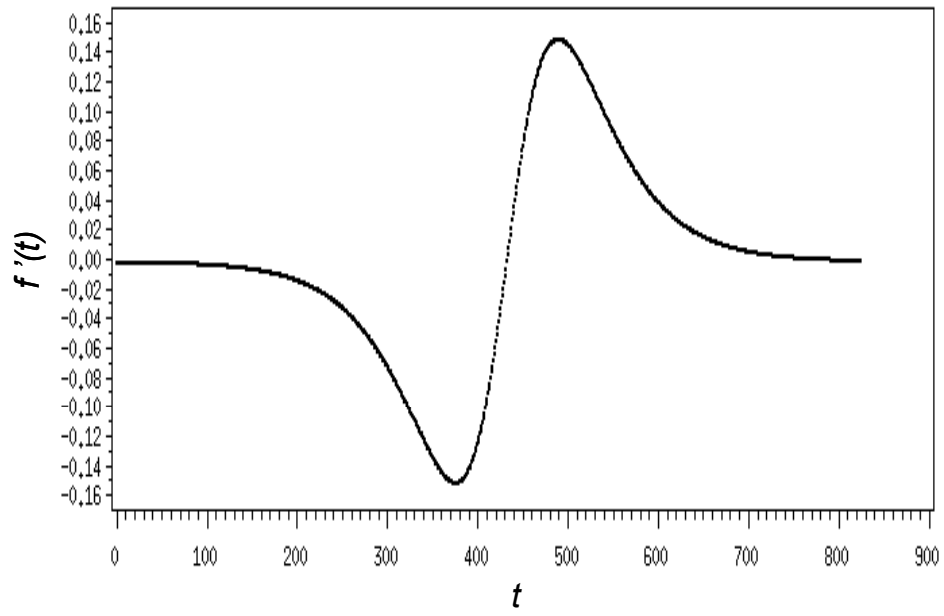


Figure 1-4. Constructed sigmoid sub-function $f'(t)$ for adjustment of seasonal compensatory growth.

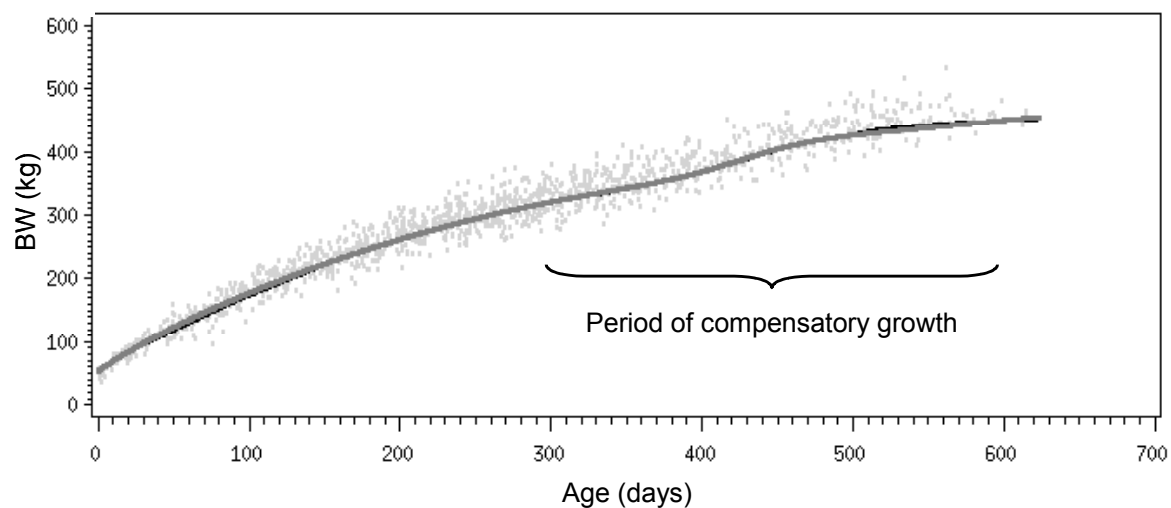


Figure 1-5. Scatterplot of the 1,633 male BW data of Thoroughbred colts (light gray dots). Thin black line indicates expected (actual) data averages of BW, and thick gray line indicates estimated Richards growth curve with the developed sigmoid sub-function (i.e., Eq. 1-5).

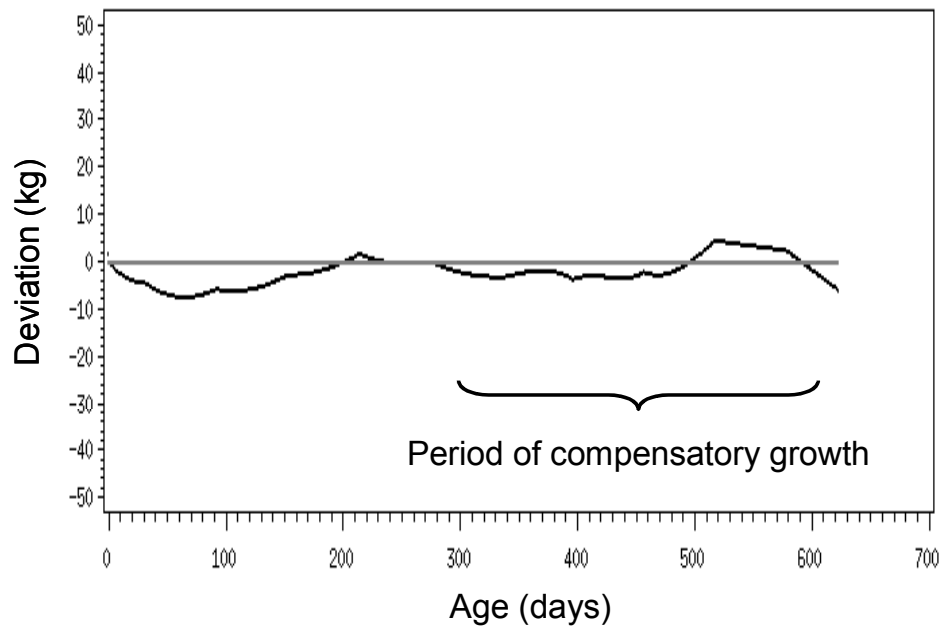


Figure 1-6. Deviation of the expected (actual) data averages of the male BW from the estimated Richards growth curve with the developed sub-function (i.e., Eq. 1-5).

Chapter 2

Empirical growth curve estimation considering multiple seasonal compensatory growths for body weights of Thoroughbred horses

Introduction

In Chapter 1, an empirical adjustment approach has been proposed to adjust a single (i.e., first year only) seasonal CG when growth curve equations are estimated in Japanese Thoroughbreds. For the adjustment of the single CG, a new sigmoid subfunction was developed and combined with the traditional Richards growth curve equation.

Thoroughbred horses generally experience 2 major winter seasons, first and second year seasons, before their debut in horseracing. Multiple applications of the proposed approach would be useful for considering multiple CG in Thoroughbred horses. The objective of this chapter was to estimate the growth curve equations empirically adjusted for multiple (i.e., first and second year) seasonal CG for BW of young Japanese Thoroughbreds based on the approach of the Chapter 1.

Materials and Methods

Data Description

For 39 Thoroughbred colts and 42 fillies, respectively, collected by the Hidaka Training and Research Center, JRA between 1999 and 2008, we analyzed a total of 3,961 and 4,341 BW (kg) and age (day) measurements. The maximum age in the data was about 1,100 days, which covers 2 major winter seasons before the foals' debut in horseracing. The frequency distributions of birth months of the foals are shown in Fig. 2-1. The weaning months of the foals is about 5 to 6 months. Taming training for the foals begins generally in August to October of their yearling year. The rations in winter are hay and grains fed based on the foals'

age in months after consulting the Japanese Feeding Standard for Horses (Equine Research Institute, 2004). Body weights of foals were measured weekly in the morning by using a 1 t load-cell type scale (Kubota Corporation, Osaka, Japan 1990) and two 1.5 ton electric balances (Mettler-Toledo International Inc., Tokyo, Japan, 1996) for pre- and postweaning periods, respectively.

Growth Models

In Chapter 1, seven sigmoid growth curve equations (Logistic, Gompertz, von Bertalanffy, Brody, Richards, Bridges and Janoscheck; see Köhn et al., 2007) were compared using Akaike's information criterion (AIC; Akaike, 1973). The chosen equation was the Richards equation (Richards, 1959) is already shown in Eq.1-1 as follows:

$$BW = \frac{A}{\left(1.0 + B e^{-k t}\right)^{\frac{-1.0}{m}}} . \quad (\text{Equation 1-1})$$

In the traditional Richards equation, BW (kg) is described as a function of age (t , age in days). Concerning the other parameters, A is the asymptotic limit for BW as t approaches infinity; B is a scaling parameter that defines the degree of maturity; k is a rate constant that determines the spread of the curve along the time axis; m is the point of inflection in the sigmoid curve in relation to age.

Due to the lack of mature BW data of the analyzed foals, the mature BW (i.e., parameter A) of Japanese Thoroughbreds was fixed as 575.0 kg for both sexes based on The Japanese Feeding Standard for Horses (Equine Research Institute, 2004). The other parameters (B , k and m) are estimated by the SAS NLMIXED (SAS Inst. Inc., Cary, NC) procedure separately

in each sex. We used the Richards equation as the main growth curve equation following the Chapter 1.

Adjustments of Compensatory Growth

To adjust the changes of growth rate during the first and second CG, the B parameter (i.e., degree of maturity) of the traditional Richards equation was modified by multiple applications of subfunctions [$f'(t)$ and $f''(t)$] as follows:

$$BW = \frac{575.0}{\left(1.0 + (B + B' f'(t) + B'' f''(t)) e^{-kt}\right)^{\frac{-1.0}{m}}},$$

in which $f'(t) = \frac{1.0}{1.0 + e^{\frac{-10.0(t-432.0)}{268.49}}} - \frac{1.0}{1.0 + e^{\frac{-5.0(t-432.0)}{268.49}}}$

and $f''(t) = \frac{1.0}{1.0 + e^{\frac{-10.0(t-797.0)}{268.49}}} - \frac{1.0}{1.0 + e^{\frac{-5.0(t-797.0)}{268.49}}}$.

(Equation 2-1)

Based on Chapter 1, the sigmoid subfunctions $f'(t)$ and $f''(t)$ for the CG adjustment were developed. A general sigmoid function $f(t)$ is shown in Eq.1-3 as follows:

$$f(t) = \frac{1.0}{1.0 + e^{-\alpha t}} \quad (\text{Equation 1-3})$$

A sigmoid function is a monotonous increase function, and the shapes of the function depend on the α values. The subtraction of 2 sigmoid functions with different α values leads to a function having only a single wave (down and up wave) such as Fig. 1-4. We chose arbitrary α values of 5 and 10 (in Chapter 1). By using the subtraction between the 2 sigmoid functions

with several modifications of the t parameter, the sigmoid subfunction $f'(t)$ in Eq. 2-1 above is obtained. The shape of the subfunction $f'(t)$ is shown in Fig. 1-4. The $f'(t)$ value crosses the horizontal axis at 432 days with the wave range of 268.49×2 days. These values of 432 and 268.49 are commonly estimated in both sexes as in Chapter 1. The 432 days is the averaging day of the first CG for all horses and determined by the crossing point between the actual data averages of all data with both sexes and traditional Richards equation, based on Chapter 1. The half of the wave range, 268.49 days, is also determined by the preliminary parameter estimation by the SAS NLMIXED procedure (SAS Inst. Inc., Cary, NC) using the data of both sexes. This subfunction has zero value when $t = -\infty$ or ∞ . Only when t is around the averaging day of CG (i.e., around 432 days) do the typical nonzero $f'(t)$ values appear as in Fig. 1-4. Then, it was assumed that the $f'(t)$ subfunction can empirically explain the changes of growth rate caused by CG (i.e., decreased growth rate in winter and increased growth rate in spring).

The developed $f'(t)$ subfunction was adapted for the B parameter, because B is a major maturity related parameter, as explained above. This subfunction is applied multiply for the B parameter in order to explain multiple CG with different winter seasons with minor modifications. For the construction of the subfunction $f''(t)$ for the second year seasonal CG, the 432 days is simply modified as 797 ($= 432 + 365$) as shown in Eq. 2-1 above, assuming that the second year seasonal CG comes just 1 year after from the first CG. The coefficient B' and B'' in Eq. 2-1 are estimated in the parameter estimation.

Parameter Estimation and Model Comparison

Unknown parameters in the equation were estimated by the SAS NLMIXED procedure

(SAS Inst. Inc., Cary, NC). The fundamental parameters (B , k , and m) were initially estimated using Eq. 1-1, and then B' and B'' coefficients for subfunctions were estimated using Eq. 2-1 by fixing the estimated fundamental parameter values. This 2-step estimation is practical and computationally feasible. It is also useful for getting information about the main (i.e., baseline) growth curve without CG and additional modifications of the baseline due to CG.

After the parameter estimation, indices of goodness-of-fit were calculated and used for the model comparisons among the models with or without subfunctions for CG. The chosen indices were AIC, Bayesian information criterion (BIC), -2 log likelihood and the average of residual sum of squares (RSS).

Results and Discussion

The scatter plot of the weight-age data is shown with gray dots in Fig. 2-2. In the figure, the tendencies of the seasonal CG are recognized around 432 d (and slightly 797 d) corresponding to the first and second CG periods, respectively.

The newly developed $f'(t)$ and $f''(t)$ sigmoid subfunctions were simply added to the B parameter in Eq. 1-1 with the coefficients B' and B'' . Specifically, this leads to the replacement of B by $B + B'f'(t) + B''f''(t)$ as shown in Eq. 2-1. With these replacements and parameter estimation in each sex, the growth curve equations for male and female BW considering the 2 seasonal CG were obtained as

$$BW_{male} = \frac{575.0}{\left(1.0 + (-0.94513 + 0.3582 \times f'(t) + 0.7466 \times f''(t))e^{-0.00213 t}\right)^{\frac{-1.0}{1.2412}}}$$

(Equation 2-2)

and

$$BW_{female} = \frac{575.0}{\left(1.0 + (-0.94880 + 0.3582 \times f'(t) + 0.6705 \times f''(t))e^{-0.00204t}\right)^{\frac{-1.0}{1.2708}}},$$

(Equation 2-3)

respectively. The subfunctions $f'(t)$ and $f''(t)$ were common in both sexes and the same as Eq. 2-1. In this chapter, considering the consistency of the established growth curve equations of the previous studies, the parameter B and B' were based on the Chapter 1.

Equations 2-2 and 2-3 look complicated but are a continuous single variable function of t (age in days). The insertions of $f'(t)$ and $f''(t)$ affect the BW only when t is within about 432 ± 268.49 days or 797 ± 268.49 days, respectively. The growth curves of the new Richards equation combined with these sigmoid subfunctions (i.e., Eq. 2-2 and 2-3) are shown with black lines in Fig. 2-3, and they express the changes of growth rate in the first and second CG periods. Departures of the prematuring BW (i.e., BW around 1000 days) in the analyzed data from the estimated growth curves are recognized due to the fixed usage of the maturing BW of 575.0 kg in this chapter. The body size of the recent colts and fillies are somewhat larger than the Japanese Thoroughbreds population standards on The Japanese Feeding Standard for Horses published earlier (Equine Research Institute, 2004).

For male equations, the AIC value of traditional Richards equation (Eq. 2-2 without all subfunctions) was 33,666 (Table 2-1). The AIC value of the equation adjusting only the first seasonal CG (Eq. 2-2 without $f''(t)$) was 33,107, and showed a better fit than the traditional Richards equation. Based on the complete male equation of Eq. 2-2, the AIC value also decreased to 32,942, indicating the best fit to the actual weight-age data. For females, the

complete equation of Eq. 2-3 showed the best fit, too. Concerning BIC, -2 log likelihood and RSS, the same tendencies for the goodness-of-fit were recognized. In these model comparisons, the parameter values in each model were fixed as in Eq. 2-2 and 2-3. Even if all the parameters were re-estimated by using each model, the same tendencies were also recognized. These results showed the strong evidence of the presence of both first and second CG in the analyzed Japanese Thoroughbred horse population.

Some researchers may consider that the parameter B is biologically unimportant, as Richards (1959) noted. We tested an alternative to adjust parameter k by the subfunctions. If k was adjusted by the $f'(t)$ and $f''(t)$, however, the combined equation became more complex and we met difficulty in numerical computations. For the simplicity and computational feasibility of the combined equations, we adjusted the parameter B .

One useful property of our approach is the introduction of continuous single variable growth curve equations. The developed subfunctions are applicable multiply for any biological parameters in any growth curve equations if necessary or computationally feasible. The combined growth curve equation with these subfunctions is always a continuous single variable mathematical equation of time. With this property, for example, empirical percentile growth curves with Z-values considering seasonal CG are easily generated based on the variations of the analyzed data around the estimated growth curves.

The young Thoroughbreds generally undergo physical training for horseracing when they become about 2 year of age even though they are still growing. As in our data (Fig. 2-2 and 2-3), the larger data variations of BW are recognizable in the second year season probably due to the growth and training. Better understanding of the development of young horse body

compositions during their growing and training seasons are important based on some mathematical standard growth curves considering CG, as suggested by the recent studies of the body compositions of young Thoroughbreds (Tozaki et al., 2011; Fonseca et al., 2013).

The proposed method in this chapter is one of the useful approaches for considering and understanding seasonal CG in growth curve estimations for young Thoroughbreds in northern regions or countries where the seasonal CG are distinctive. Knowledge of the estimated growth curves handling CG would be worthwhile for the horse raising activities that relate to secure development of Thoroughbred horses in winter and spring seasons. Based on this approach, the unique optimal growth curve equations considering multiple seasonal CG could be empirically estimated also for other seasonal breeding animals or other growth traits such as body heights or entire width of chest that would be affected also by seasonal CG.

Table 2-1. Akaike's information criterion (AIC), Bayesian information criterion (BIC), -2 log likelihood (-2 Log L) and average of residual sum of squares (RSS) for Thoroughbreds growth curve models

Models	Sex ¹	AIC	BIC	-2 Log L	RSS ²
Traditional Richards	M	33,666	33,669	33,662	269.5 $\pm$ 6.8 ^a
Including $f'(t)$	M	33,107	33,110	33,103	233.4 $\pm$ 6.4 ^b
Including $f'(t)$ and $f''(t)$	M	32,942	32,947	32,936	223.7 $\pm$ 6.3 ^c
Traditional Richards	F	37,407	37,411	37,403	305.2 $\pm$ 7.5 ^a
Including $f'(t)$	F	36,754	36,757	36,750	262.1 $\pm$ 7.2 ^b
Including $f'(t)$ and $f''(t)$	F	36,594	36,599	36,588	252.4 $\pm$ 7.4 ^c

¹ M = Male; F = Female.

² Average of RSS $\pm$ SE.

^{a,b,c} Averages with different superscripts differ within sex ($P < 0.05$).

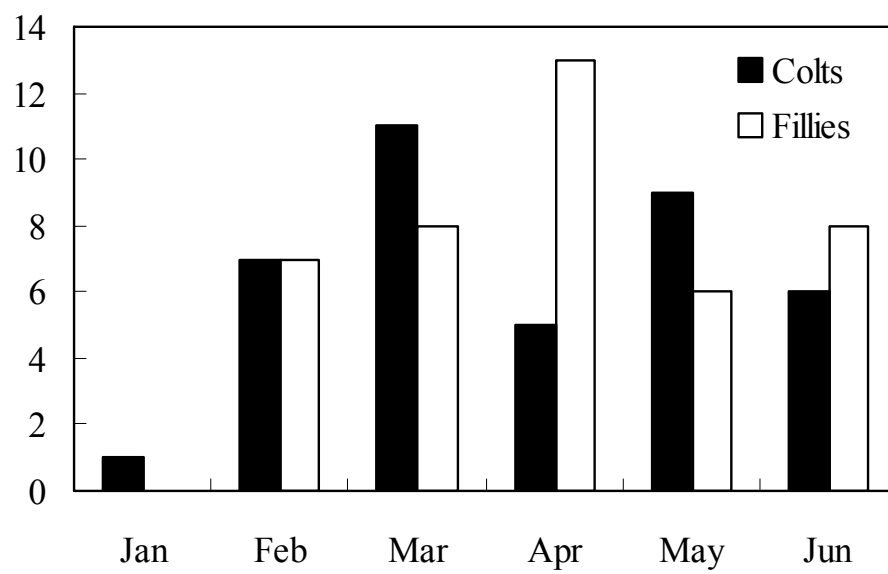


Figure 2-1. Frequency distribution of the analyzed young horses by birth months.

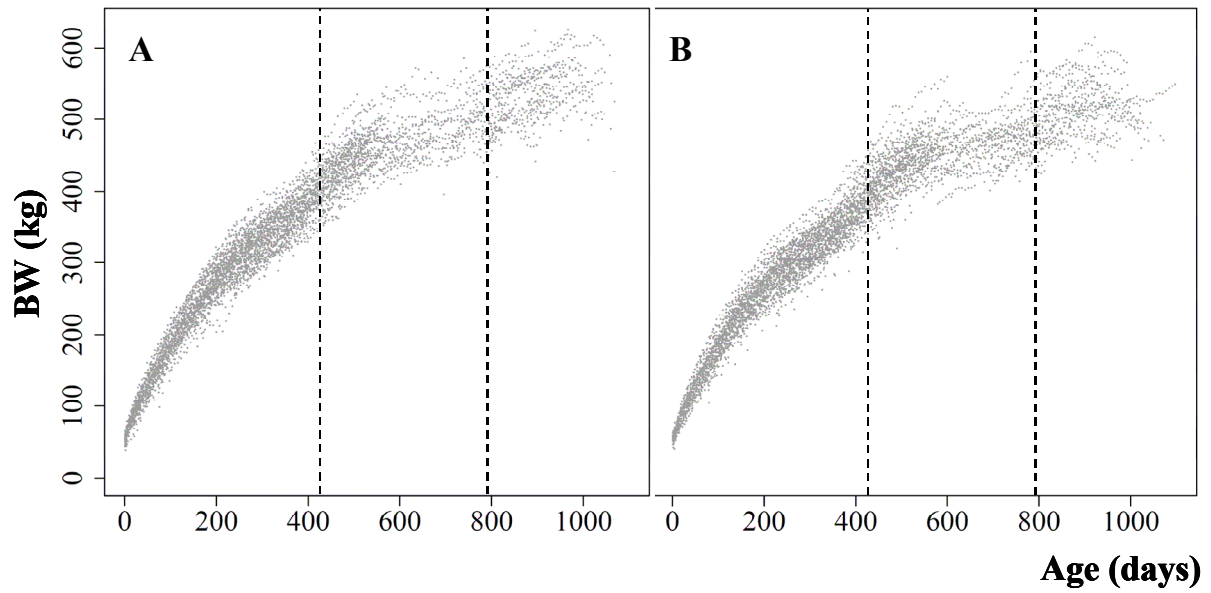


Figure 2-2. Scatter plot of the BW data of 3,961 colts (A) and 4,341 fillies (B) of Japanese Thoroughbreds (gray dots). Dashed lines indicate the averaging days of the periods of compensatory growth: 432 (left) and 797 days (right).

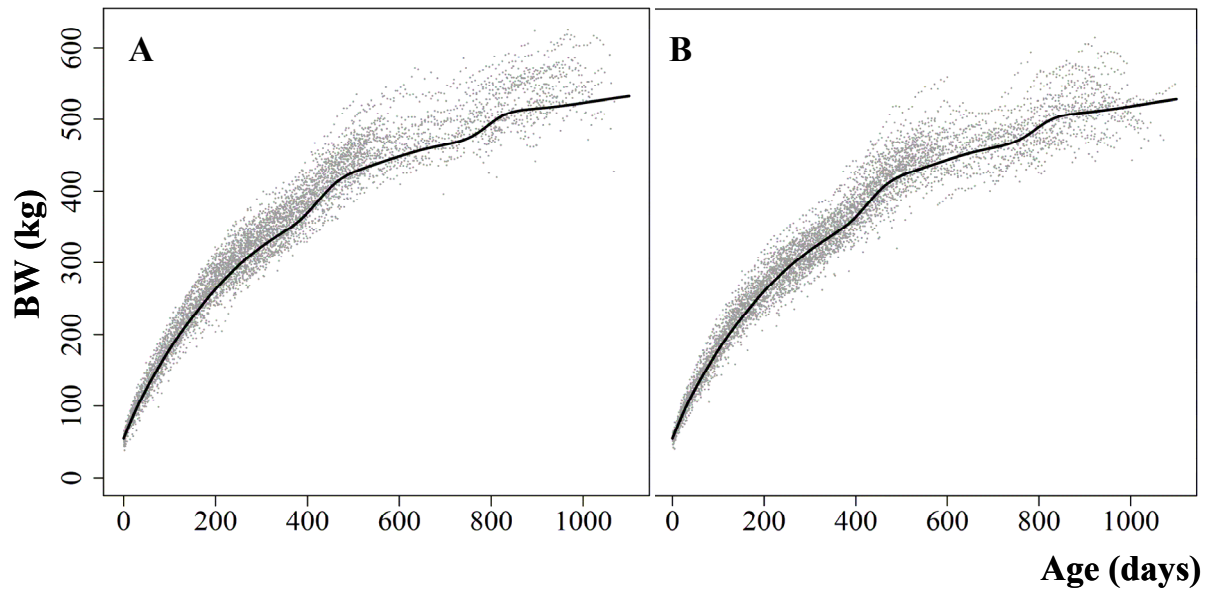


Figure 2-3. Scatter plot of the BW data of 3,961 colts (A) and 4,341 fillies (B) of Japanese Thoroughbreds (gray dots). Black lines indicate estimated growth curves (Eq. 2-2 and 2-3 for colts and fillies, respectively).

Chapter 3

Empirical percentile growth curve estimation for Japanese Thoroughbred horses

Introduction

Percentile growth curves, which track the values of anthropometric indices visually with percentages, are useful as a clinical indicator of monitoring growth and health conditions by evaluating variations of children's growth status, such as under- or over- nutrition conditions. Percentile growth curves have been widely used in many medical and health care areas for humans (Waterlow et al., 1977; Hamill et al., 1979; Kumar and Bhalla, 1988; Neyzi et al., 2006; WHO, 2006; Tatsumi et al., 2012) and animals (Masoud et al., 1986; Corson et al., 2008). For Thoroughbred horses, the construction of percentile growth curves have been investigated (Crepaldi et al., 2005; Kocher and Staniar, 2013), where the percentile BW charts for Thoroughbreds were available. The percentile growth curves directly or empirically considering CG patterns, however, have not been estimated so far.

Mohammed (1990) reported that Thoroughbred yearlings were at a high risk of developmental disorders (e.g. osteochondrosis and orthopaedic diseases). Due to the dramatic change of the growth rate during winter and spring seasons, the seasonal CG may be an additional risk factor for the physical developments of yearling Thoroughbred foals. Concerning BW for young Thoroughbreds, we proposed mathematical equations for empirical growth curves considering seasonal CG in Chapter 2. These growth curve equations would be useful for the establishment of the percentile growth curves considering seasonal CG because the equations are continuous single variable functions of the age of horses, and the variances of the growth data can be used as the empirical percentiles with Z-scores under the assumption of normal distributions of the data.

The establishment of the percentile growth curves would give us useful indicators for feeding managements in order to achieve sound musculoskeletal developments or desirable

body compositions of growing horses. In this chapter, we propose a method for constructing empirical percentile curves for body weight and withers height of Japanese Thoroughbred horses by using Z-scores, considering CG that have typical characteristics in seasonal breeding animals.

Materials and Methods

Data description

A total of 5,594 and 5,680 BW (kg) and age (days) measurements of 271 colts and 237 fillies of Thoroughbreds, respectively, were collected by the Hidaka Training and Research Center, Japan Racing Association (JRA) and the Japan Bloodhorse Breeders' Association (JBBA) between 1999 and 2009. In addition, a total of 3,770 withers height (WH; cm) and age (days) measurements of 422 Thoroughbred colts and fillies was also collected. The maximum age in the BW data was about 1100 days, which covers two winter seasons (CG periods) before the foals' debut in horseracing. The maximum age in the WH data was about 800 days, which covers only the first winter season.

Growth curves for body weight

For BW of male and female Thoroughbreds, the following mixed model equations (Eq. 3-1 and 3-2) were used based on the growth curves derived in Chapter 2, where the traditional Richards' growth curve equation (Richards, 1959) was modified for considering CG effects with the sigmoid sub-functions $f(t)$ and $f'(t)$. The sigmoid sub-functions $f(t)$ and $f'(t)$ adjust the first and second year CG at 432 and 797 (= 432 + 365) days of age, respectively, and were adapted to the biological parameters responsible for maturity in the Richards equation (Richards, 1959). The 432 days of age were determined by the crossing points of the actual

data averages and traditional Richards equation as explained in Chapter 1 and 2.

In the mixed model equations (Eq. 3-1 and 3-2), both individual horses (a_i) and residuals (e_{ij}) for individual i and his/her age ij were included as random effects with their variance components of σ_a^2 and σ_e^2 , where the population averages of the a_i and e_{ij} were both zero and they were independent of each other.

The a_i was added to the maturity size (575.0). Then, the maturity size of each individual became $575.0 + a_i$, indicating that the final maturity size differs according to each individual. Based on Chapter 2, the equations for BW for male and female Thoroughbreds were expressed as:

$$BW_male_{ij} = \frac{575.0 + a_i}{\left(1.0 + (-0.94513 + 0.3582 \times f(t) + 0.7466 \times f'(t)) e^{-0.00213 t_{ij}}\right)^{\frac{-1.0}{1.2412}}} + e_{ij} \quad (\text{Equation 3-1})$$

and

$$BW_female_{ij} = \frac{575.0 + a_i}{\left(1.0 + (-0.94880 + 0.3582 \times f(t) + 0.6705 \times f'(t)) e^{-0.00204 t_{ij}}\right)^{\frac{-1.0}{1.2708}}} + e_{ij}, \quad (\text{Equation 3-2})$$

$$\text{where } f(t) = \frac{1.0}{1.0 + e^{\frac{-10.0(t_{ij} - 432.0)}{268.49}}} - \frac{1.0}{1.0 + e^{\frac{-5.0(t_{ij} - 432.0)}{268.49}}},$$

$$f'(t) = \frac{1.0}{1.0 + e^{\frac{-10.0(t_{ij} - 797.0)}{268.49}}} - \frac{1.0}{1.0 + e^{\frac{-5.0(t_{ij} - 797.0)}{268.49}}},$$

$$a_i \sim N(0, \sigma_a^2), \text{ and}$$

$$e_{ij} \sim N(0, \sigma_e^2).$$

BW_male_{ij} and BW_female_{ij} are the BW for individual i measured at age (time) t_{ij} . The unknown variance components (i.e., σ_a^2 and σ_e^2) were estimated for each sex by the SAS

NLMIXED procedure (SAS Institute Inc., 2008).

Growth curve for withers height

For WH of Thoroughbreds, the growth curve equation was constructed by following the method of the growth curve constructions for BW. We combined both sexes in the WH analysis, because the difference of WH between sexes was small in our preliminary analyses (about 0.5 cm in maximum) which corresponded to the small gender difference of WH with 2 cm in maximum (Equine Research Institute, 2004), and the total number of data available for WH was small. Furthermore, only the WH data of less than about 800 days which corresponds to the first seasonal CG period was available. Thus, the sub-function $f(t)$ handling the first CG was only included in the equation (Eq. 3-3). The $f(t)$ was identical to that used in Eq. 3-1 and 3-2 assuming that the CG affects WH at the same period as in BW. The equation for WH was expressed as:

$$WH_{ij} = \frac{161.0 + a_i}{\left(1.0 + (-0.8452 + 0.4304 \times f(t)) e^{-0.00293 t_{ij}}\right)^{\frac{-1.0}{3.8465}}} + e_{ij} \quad (\text{Equation 3-3})$$

$$\text{where } f(t) = \frac{1.0}{1.0 + e^{\frac{-10.0(t_{ij} - 432.0)}{268.49}}} - \frac{1.0}{1.0 + e^{\frac{-5.0(t_{ij} - 432.0)}{268.49}}},$$

$$a_i \sim N(0, \sigma_a^2), \text{ and}$$

$$e_{ij} \sim N(0, \sigma_e^2).$$

The unknown variance components were also estimated by the SAS NLMIXED procedure (SAS Institute Inc., 2008).

Empirical percentile growth curves using Z-scores

In the definition of ‘percentile’, the value of 50 percentile is the median of the data, and not the average. If the data is not normally distributed, the data value of the 50 percentile is not the mean of the data, and if the data is normally distributed, the data value of the 50 percentile is identical to the mean of the data. To compute the exact 50 percentile curve for BW, for example, numerical medians of the BW data must be computed at all ages, as according to Kocher and Staniar (2013). For the computation of other percentile curves (e.g. 25 percentile curve or so), the same manner of the computations is necessary. Even though this approach to get exact percentile curves is useful for the exact illustrations of the data variations, it is generally difficult to obtain the continuous single variable mathematical growth curve function of age (time).

Using Z-scores is an alternative way to obtain empirical percentile growth curves. Z-score is a standardized score that indicates how many standard deviations a data point is apart from the population mean based on the assumption of normal distributions. Based on the Z-scores of the estimated variance components, the empirical percentile growth curves were easily constructed. Combined with the Eq. 3-1, 3-2 and 3-3 and the information of the estimated variance components and their Z-scores, the equations for empirical percentile growth curves for BW (Eq. 3-4 and 3-5) and WH (Eq. 3-6) were constructed as follows:

$$BW_{male_percentile} = \frac{575.0 + Z\sqrt{\sigma_a^2}}{(1.0 + (-0.94513 + 0.3582 \times f(t) + 0.7466 \times f'(t))e^{-0.00213t})^{\frac{-1.0}{1.2412}}} + Z\sqrt{\sigma_e^2},$$

(Equation 3-4)

$$BW_{female_percentile} = \frac{575.0 + Z\sqrt{\sigma_a^2}}{(1.0 + (-0.94880 + 0.3582 \times f(t) + 0.6705 \times f'(t))e^{-0.00204t})^{\frac{-1.0}{1.2708}}} + Z\sqrt{\sigma_e^2},$$

(Equation 3-5)

and

$$WH_{percentile} = \frac{161.0 + Z\sqrt{\sigma_a^2}}{\left(1.0 + (-0.8452 + 0.4304 \times f(t)) e^{-0.00293 t}\right)^{\frac{-1.0}{3.8465}}} + Z\sqrt{\sigma_e^2} .$$

(Equation 3-6)

These equations are the continuous single variable function of age (t). In these equations, Z is the Z-score that corresponds to an empirical percentile. We assigned the empirical percentile curves for BW and WH of Thoroughbreds as 3%, 10%, 25%, 50%, 75%, 90% and 97%. Each empirical percentile corresponds to Z-scores of -1.881 , -1.282 , -0.675 , 0.000 , 0.675 , 1.282 and 1.881 , respectively. When the Z-score is zero that corresponds to the empirical 50 percentile curve, Eq. 3-4 and 3-5 become identical to the equations proposed in Chapter 2.

Validation of the estimated percentile growth curves

For the validation of the constructed empirical percentile growth curves, the data percentages between these percentile curves were counted. For the validation analyses, the age data periods were assorted from zero to 180 days repeats. The percentages of the data positioned between each percentile curve intervals (i.e., 0% to 3%, 3% to 10%, and so on) were counted from 0 to each age periods (i.e., 0 to 180, 360, 540, 720, 900, and all days).

Results

Based on Eq. 3-1 and 3-2, the estimates of the variance components for male BW Thoroughbred horses were $\sigma_a^2 = 1,431.14$ and $\sigma_e^2 = 191.51$, and for female BW are $\sigma_a^2 = 1,431.01$ and $\sigma_e^2 = 228.95$. For WH of both sexes, based on Eq. 3-3, the variance components were $\sigma_a^2 = 16.14$ and $\sigma_e^2 = 3.52$. All variance components were significantly different from

zero ($P < 0.0001$). The square root of these values of the estimated variances were combined with the Eq. 3-4, 3-5 and 3-6 for the construction of the empirical percentile growth curves.

The scatterplots of the weight- and height-age data and estimated empirical percentile growth curves are shown in Fig. 3-1 and Fig. 3-2. These percentile curves express both the changes of growth rate in the first and second CG periods, and the distribution of the data in the analyzed population. In these figures, the tendency of the seasonal CG can be recognized around 432 and 797 days, corresponding to the first and second CG periods, respectively. Our former studies illustrated the clear evidences for the presence of both first and second CG for the BW based on the model comparison of with and without CG (see in Chapter 2). Concerning the WH, we also confirmed the presence of the CG according to the same manner of the model comparison procedure.

The results of validation analyses for estimated percentile growth curves are shown in Tables 3-1, 3-2 and 3-3 for male and female BW and the WH for both sexes, respectively. In these tables, there was a greater number of data positioned over 50% curves for any age periods and traits.

Discussion

The growth curve equation models used in this chapter were based on the previous chapters but in this chapter the data from both JBBA and Hidaka Training and Research Center were combined in order to estimate the variance components with a large data set. Interestingly, the estimated variances of the individual horses (σ_a^2) for male and female BW were quite similar to each other in this chapter (i.e., 1,431.14 vs. 1,431.01). For the application of this information to other horse populations, however, other studies will be necessary.

Slightly higher deviations of the data variation from the estimated percentile growth curves can be recognized (Fig. 3-1 and 3-2). In Tables 3-1, 3-2 and 3-3, the higher deviations are also clearly recognized. Our growth curves considering CG were based on the former knowledge of the Japanese Thoroughbreds with the assumption of the maturing weight of 575.0 kg and the maturing withers height of 161.0 cm (Equine Research Institute, 2004). The higher deviations of BW and WH in the current Thoroughbred population suggest the possibility that the body size of the recent colts and fillies seemed to be rather larger than the former Japanese Thoroughbreds population on The Japanese Feeding Standard for Horses (Equine Research Institute, 2004).

The latent problems of using the current underestimated percentile curves obtained in this study would relate to the misunderstanding of the absolute superiority of the superior growing foals. In the recent Japanese Thoroughbred population, the 50%-75% percentile intervals seem to be ordinary (see Tables 3-1, 3-2 and 3-3). Racehorse managers in Hidaka region should recognize this information when using these underestimated percentile curves for the practical management of their horses. Even though these percentile curves in this study are currently underestimated, the personal growing profile for each foal can be precisely obtained and it will be used for the secure feeding managements of the foal during CG periods. The more numerous the data of the maturing weights and heights of the recent Japanese Thoroughbred population is available, the more accurately the growth curve models and variances will be estimated.

A simple way for the construction of the exact percentile growth curves is to compute the data summarizing statistics (e.g. ordered data percentages or medians) at all age and to plot them on the graph visually, as noted. This approach gives us percentile graphs that are ‘data dependent’, and the obtained graphs tended to have sharp notches (i.e., not smooth lines). A

variety of empirical methods were used for developing smoothing curves using the actual data plots (Flegal, 1999; Corson et al., 2008). Methods for smoothing include cubic splines, kernel regression, locally weighted regression and lambda-mu-sigma (LMS) method (Cole and Green, 1992). The LMS method is often used for estimation of smoothed percentiles (Corson et al., 2008; Gökçay et al., 2008; Tatsumi et al., 2012). These methods are alternatives to get other empirical percentile growth curve equations for Thoroughbreds. As discussed by Wang and Chen (2012), the use of Z-scores for the development of empirical percentile growth curves is still useful because of its ability to quantify the extreme data values based on the parametric statistical estimations and hypothesis testing. Based on the assumption of normal distribution of the analyzed data, the estimated empirical percentile growth curves using Z-scores give us standardized scales for the analyzed data distributions.

Practical control of WH would be a challenging task compared with the practical control of BW. The estimated empirical percentile curves of WH, however, will be still useful for a reference of the development of young horses when combined with the percentile information of his/her BW. The integrated percentile information from many growth traits gives us valuable references for the sound development of young Thoroughbred horses.

As already noted in Chapter 1 and 2, a useful property of our approach is the introduction of continuous single variable growth curve equations considering CG, which can be easily applicable to obtain empirical percentile curve equations as shown in this chapter. The developed empirical percentile growth curves using Z-scores are computationally feasible and worthwhile for understanding the aspect of BW and WH distributions and for managing young Thoroughbred horses especially during CG periods.

Table 3-1. Data distributions for the male Thoroughbred BW for different periods of age

Age (days)	Percentile intervals (%)							
	0-3	3-10	10-25	25-50	50-75	75-90	90-97	97-100
180	0.06	0.64	6.07	28.58	43.41	17.07	4.16	0.00
360	0.09	0.55	5.41	27.10	40.65	20.43	5.41	0.37
540	0.07	0.40	5.36	25.26	40.38	22.18	5.70	0.67
720	0.06	0.39	4.97	25.16	40.09	22.33	5.91	1.10
900	0.06	0.41	4.85	25.36	39.15	22.75	6.23	1.16
all	0.07	0.39	4.74	25.01	39.01	22.99	6.42	1.29

Table 3-2. Data distributions for the female Thoroughbred BW for different periods of age

Age (days)	Percentile intervals (%)							
	0-3	3-10	10-25	25-50	50-75	75-90	90-97	97-100
180	0.07	0.35	5.10	34.78	42.88	12.92	3.84	0.07
360	0.07	0.77	7.12	34.42	41.04	13.11	3.33	0.10
540	0.07	0.78	6.34	32.62	41.27	14.58	3.85	0.48
720	0.06	0.76	6.39	31.59	41.47	15.15	3.96	0.61
900	0.06	0.76	6.87	31.63	40.91	14.90	4.24	0.65
all	0.05	0.08	7.22	31.37	40.81	14.82	4.26	0.69

Table 3-3. Data distributions for the male and female Thoroughbred WH for different periods of age

Age (days)	Percentile intervals (%)							
	0-3	3-10	10-25	25-50	50-75	75-90	90-97	97-100
180	0.00	0.35	3.49	12.15	23.81	25.91	10.54	2.23
360	0.00	0.50	5.39	19.99	30.60	20.85	6.75	1.10
540	0.00	0.39	5.07	21.73	29.92	18.64	6.00	0.81
all	0.03	0.45	6.00	27.22	35.81	22.33	7.22	0.96

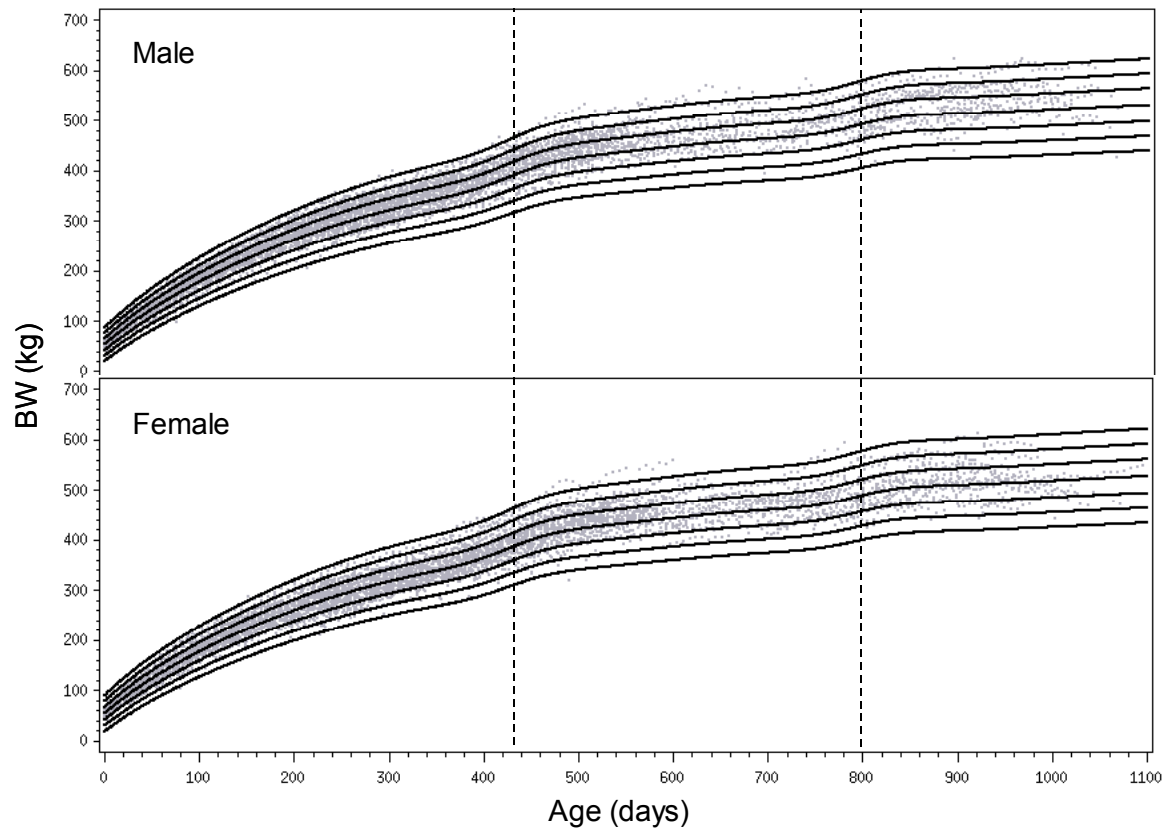


Figure 3-1. The scatterplot of the weight-age data and the empirical percentile growth curves for male and female BW of Japanese Thoroughbred horses. The weight-age data is shown with gray dots and the percentile curves are shown with black lines: from the bottom line, 3, 10, 25, 50, 75, 90, and 97 percentile curves are shown. Dashed lines indicate the centers of the first and second CG periods (432 and 797 days, respectively).

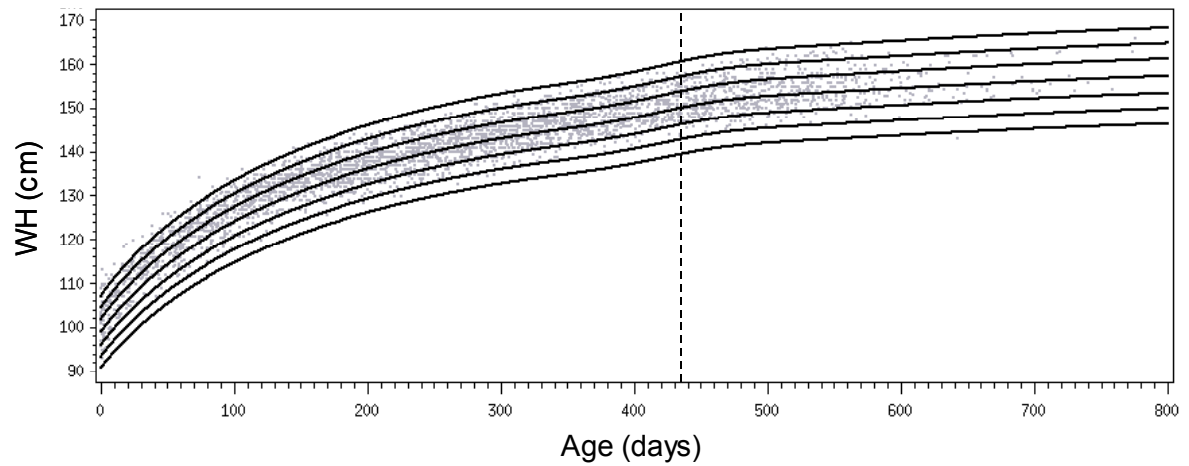


Figure 3-2. The scatterplot of the height-age data and the empirical percentile growth curves for WH of Japanese Thoroughbred horses of both sexes. The height-age data is shown with gray dots and the percentile curves are shown with black lines: from the bottom line, 3, 10, 25, 50, 75, 90, and 97 percentile curves are shown. Dashed line indicates the center of the first CG period (432 days).

General Discussion

Chapter 1:

A new empirical adjustment approach is proposed to adjust for compensatory growth (CG) when growth curve equations are estimated, by using 1,633 male body weights of Thoroughbreds as an illustrating example. Based on general Richards growth curve equation, a new growth curve equation was developed and fit to the weight-age data. The new growth curve equation had a sigmoid sub-function that can adjust the CG, combined with the Richards biological parameter responsible for the maturity of animals. The unknown parameters included in the equations were estimated by the SAS NLMIXED procedure. The goodness of fit was examined by using Akaike's Information Criterion (AIC). The AIC values decreased from 13,053 (general Richards equation) to 12,794 (the newly developed equation), indicating the better fit of the new equation to the weight-age data. The shape of the growth curve was improved during the period of CG.

Chapter 2:

A new empirical approach is proposed considering the multiple CG when growth curve equations are estimated, by using body weights of Japanese Thoroughbred colts and fillies raised in Hidaka, Hokkaido. Based on the traditional Richards growth curve equation, new growth curve equations were developed and fit to the weight-age data. The foals generally experience 2 major winter seasons before their debut in horseracing. The new equations had sigmoid sub-functions that can empirically adjust the first and second year CG, combined with the Richards biological parameter responsible for the maturity of animals. The unknown parameters included in the equations were estimated by the SAS NLMIXED procedure. The goodness-of-fit was examined by using several indices of goodness-of-fit (i.e., Akaike's

information criterion, Bayesian information criterion, -2 log likelihood, and residual sum of squares) for the multiple applications of the sub-functions. A total of 3,961 and 4,341 body weight and age measurements for male and female Thoroughbreds, respectively, were used for the analysis. The indices indicated the best fit of the new equations including both sub-functions for the first and second CG to the weight-age data. The shapes of the growth curves were improved during the periods of CG.

Chapter 3:

Empirical percentile growth curves using Z-scores are developed for Japanese Thoroughbred horses, with considerations of the seasonal CG that is a typical characteristic in seasonal breeding animals. Based on new growth curve equations for Japanese Thoroughbreds adjusting the CG proposed in Chapter 1 and 2, individual horses and residual effects were included as random effects in the growth curve equation model and their variance components were estimated. Based on the Z-scores of the estimated variance components, empirical percentile growth curves were constructed. A total of 5,594 and 5,680 body weight and age measurements for male and female Thoroughbreds, respectively, and 3,770 withers height and age measurements were used for the analyses. The unknown parameters included in the equations were estimated by the SAS NLMIXED procedure.

Discussion:

In this study, a useful approach for empirically considering multiple seasonal CG in growth and percentile curve estimations for Japanese Thoroughbreds is proposed. Based on the combinations of several nonlinear sigmoid functions, the developed empirical growth curves and percentile growth curves using Z-scores are always continuous single variable

mathematical equations of time and computationally feasible, that are useful for monitoring individual growth conditions for body weight and withers height of young Thoroughbred horses especially during CG periods. Based on this approach, the optimal growth curve equations can be estimated also for other growth traits affected by seasonal CG.

The theoretically essential part in the proposed approach is explained in Chapter 1, and Chapter 2 and 3 denote the useful application examples of it.

When constructing complex mathematical models such as considering CG, one possible option is to use linear polynomial models as discussed in Goldstein (1979). By increasing the order of unknown parameters in a polynomial model, you can easily get some accurate approximated mathematical functions within the observed range of the analyzed data. However, these approximation models are merely based on the observed relationship between the data and the included parameters, and do not concern any theoretical considerations about the underlying mechanisms that produce the data. Furthermore, the increase of the size of growth data variation during the aging is even not considered with this polynomial approach, which is inapplicable to construct some empirical percentile curves for the growth data. As Pinheiro and Bates (2000) discussed, the interpretability, parsimony, and validity should be focused on in the selection of mathematical models for growth data and variation.

We chose nonlinear models in order to achieve our own purpose in this study. There have been many nonlinear sigmoid functions for the growth curve estimations as noted in Chapter 1, such as Logistic, Gompertz and Richards functions. These historical nonlinear models were composed based on a model for some mechanisms of producing the growth data. The parameters in these nonlinear models admit some rational interpretations for growth of organisms. These nonlinear models also provide some reliable predictions for the variable outside the range of observed data. These useful properties of the nonlinear models are clearly

recognized in the results of this study, too.

As discussed in Chapter 3, the estimated growth and percentile curves in this study were slightly underestimated compared to the current tendency of growth status in the recent Japanese Thoroughbred population. The possible problems of using the current underestimated curves would relate to the misunderstanding of the absolute superiority of the superior growing foals. Racehorse managers in Hidaka region should recognize this information when using these underestimated curves for the practical management of their horses. Even though these curves in this study are currently underestimated, the personal growing profile for each foal can be obtained and it will be used for the secure feeding managements of each foal during CG periods. The more numerous the data of the maturing weights and heights of the recent Japanese Thoroughbred population is available, the more accurately the growth curve models and variances will be estimated based on the approach of this study.

One useful application of the proposed approach in this study is the construction of the tailor made growth curve equation for the growth status of each individual horse. Each individual has his/her own birthday information. Due to the seasonal mating animals, the expecting day of the meeting midpoint of CG period differs in each individual, and is easily calculated for each individual. Based on birthday information and the population average of the birth-date, the tailor made growth curve equation for each individual can be obtained by sifting the center of the midpoint of CG (i.e., 432 and 797 days: see Chapter 2) with the deviation of his/her birthday from the averaging birth-date of the analyzed Thoroughbred population. Such tailor made growth curve equation for each individual would be helpful for the secure management of horses.

In conclusion, this study proposed a new empirical approach of getting continuous single

variable growth curve equations considering CG, which can be applicable to obtain other equations such as percentile curves or tailor made growth curves. The growth curve equations proposed in this study are worthwhile for understanding the aspect of growth traits of Japanese Thoroughbred horses during CG periods, giving Japanese horse managers some useful diagnostic references for the sound development of young Thoroughbred horses.

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