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The use of allometric and nonlinear functions for longitudinal data analysis of physical and chemical body composition in pigs

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Abstract

Allometric and sigmoidal functions were used to analyse changes in total body composition (soft tissue, bone, viscera; primal carcass cuts and their components; organs; chemical composition) during growth of pigs, obtained from a three generation full-sib design used to identify QTL. Allometric functions showed high goodness of fit ($R^2 = 0.98$) for the mass of chemical body protein, lipid, ash and water in relation to empty body weight with allometric growth coefficients b = 0.99, 1.68, 0.99 and 0.86, respectively. However, increase in lipid deposition at higher weight (120-140 kg) deviated from the allometric pattern. If this growth period is of interest, nonlinear functions were more appropriate. Additionally, we analysed protein and lipid accretion with two-stage nonlinear models, fitting Gompertz functions in relation to age for individual pigs. This resulted in estimates for maximum protein accretion rate at 132 and 135 g/d and for mature protein mass at 27.9 and 28.0 kg for females and castrated males, respectively. Allometric functions of empty body weight and nonlinear logistic functions of age mostly fitted our data well; their few and biologically interpretable parameters can therefore be used to identify genomic regulation of growth with DNA markers.

Introduction

In molecular genetic analysis, longitudinal observations are very often treated as different traits and than analysed separately for QTL in a genome scan. However, then not all data information is used simultaneously and this is expected to result in higher sampling variance compared to a longitudinal data analysis. In addition, environmental effects can be estimated more accurately and thus be separated from genetic effects. Furthermore, the shape of the curve of each trait can be estimated with a longitudinal data analysis. There are different functions used for physical and chemical body composition in the literature. Especially, allometric functions are known to well describe body compositional changes throughout the growth of meat animals (Gu et al., 1992). The allometric functions mainly describe body composition in relation to body weight. In contrast, growth, feed intake, feed conversion ratio are mainly described in relation to time using polynomial functions (Schnyder, 2001; Lorenzo Bermejo et al., 2003ab). However, for growth, numerous studies showed its sigmoidal shaped pattern. Nonlinear functions such as Gompertz, Logistic and Richard function, etc. are expected to fit these sigmoidal shaped curves. Using these nonlinear functions, it is possible to identify QTL for shape parameters as suggested by Roehe et al. (2003). The authors expect that this will give more information about the regulation of growth traits during the growing-finishing period. Therefore, the objective of this study was to show which functions are appropriate to descibe the change of physical and chemical body composition during growth. In future studies, these functions will be used to identify QTL for the shape of body composition curves.

Material and methods

Animals

In a trial, a two generation full sib design was build up to obtain longitudinal growth rate, feed intake, protein deposition rate, fat deposition, carcass characteristics, etc. The final goal of the study using experimental data was to identify quantitative trait loci of the different traits during growth. Data on weekly measured weights were used to obtain the genetic determination of growth over an extended test period from 15 to 140 kg. Physical and chemical body composition was obtained at six target weights (20, 30, 60, 90, 120, and 140). Seven Pietrain sires and 14 dams of a hybrid line from PIC Germany were mated to obtain the F1 animals. These mated parents were used as base animals and were unrelated to each other. Thereafter, F1 animals were mated to each other avoiding the mating of related animals. Data of the F1 generation (116 animals) and the F2 generation (197 first and 189 second parity animals) were used in the present analysis for physical and chemical body composition. Chemical body composition was determined on live animal using deuterium dilution technique. The description of this technique and its association to reference methods such as magnetic resonance imaging is described by Landgraf et al. (2004abc) and Mohrmann et al. (2004ab). In order to measure the response of selection on change in shape of feed intake curves two lines of PIC (2938 of line 3, 2307 of line 4) were used.

Statistical methods

Three different groups of functions were used to fit longitudinal data. The first group consist of allometric functions in which physical and chemical body composition Y is related to a reference component X, mostly body weight, as given in the following function:

$$Y = a\,X^{b},$$

where a is the value of Y when X = 1 and b is the allometric growth coefficient relating the growth of Y to that of X. The allometric equation $Y = aX^{b}$ was fitted by linearizing the function as $\log_{10} Y = \log_{10} a + b \log_{10} X$.

The second group of functions was using polynomials within a random regression model to reflect the pattern of the longitudinal data. The random regression model was as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wp} + \mathbf{e}\,,$$

where **y** is the vector containing traits such as body weight, feed intake, protein deposition at different weeks on test, **b** is a vector of fixed effects and covariates, **a** and **p** represents the coefficient of intercept, week on test (slope) and week on test squared for additive genetic effects and permanent environmental effects, respectively. The vector **e** represents the residual effects and **X**, **Z**, **W** are the corresponding incidence matrices. The vector **b** consists of the fixed effects sex, birth farm, batch, week and week squared as well as the interaction of sex, birth farm and batch with day and day squared. The variance of the observation was $\mathrm{Var}(\mathbf{y}) = \mathbf{ZGZ}' + \mathbf{WPW}' + \mathbf{R}$, where $\mathbf{G} = \mathbf{A} \otimes \mathbf{G_0}$, $\mathbf{P} = \mathbf{I} \otimes \mathbf{P_0}$ and $\mathbf{R} = \mathbf{I} \otimes \mathbf{R_0}$. **A** is the numerator relationship matrix among animals and $\mathbf{G_0}$, $\mathbf{P_0}$ and $\mathbf{R_0}$ represent the additive genetic, permanent environmental and residual variance covariance matrix, respectively. The temporary residual matrix accounts for the correlation among residuals and for the heterogeneity of the residual variance. For the estimation of variance components, the ASREML program (Gilmour et al., 1999) was used.

The third group of functions was using nonlinear function in order to reflect the sigmoidal shape of longitudinal data. Estimation of genetic parameters of nonlinear function was obtained in a two-stage model. The nonlinear function parameters were estimated in the first stage and the genetic variances of these function parameters were obtained in the second stage. The model fitted to the longitudinal data of growth was:

$$y_{ij} = f(x_{ij}, \beta_i) + \varepsilon_{ij} ,$$

where y_{ij} is the weight of the animal i at age x_{ij}. The function $f(x_{ij}, \beta_i)$ characterises the systematic variation of animal i with β_i being the function parameters to be estimated and ε_{ij} represents the random variation of weight during growth.

The expectation and covariance structure of individual data of growth were assumed to be:

$$E(y_i \mid \beta_i) = f_i(\beta_i)$$

$$Cov(y_i \mid \beta_i) = R_i(\beta_i, \xi),$$

where ξ is the vector of covariance parameters to be estimated.

Functions $f_i(\beta_i)$ used in the present study are logistic function

$$y_{ij} = \alpha_i / [1 + \exp(\beta_i - \gamma_i x_{ij})]$$

and Gompertz function

$$y_{ij} = \alpha_i \exp[-\exp(\beta_i - \gamma_i x_{ij})],$$

which described the systematic relationship between body weight, feed intake, body composition, etc. and age of each individual animal. The covariance matrix $\mathbf{R_i}(\beta_i, \xi)$ describes the pattern of the random variability associated with the growth of animal i. The heteroscedasticity and residual correlation can also be taken into account by:

$$\mathbf{R_i}(\beta_i, \xi) = \sigma^2\, \mathbf{S_i}(\beta_i;\theta,\rho,\lambda)$$

where θ is the heterogeneity factor, ρ is the autoregressive and λ the moving average component.

In the second stage, genetic variances of individual function parameters were estimated based on the following model:

$$\beta_i = \mathbf{X_i b_i} + \mathbf{Z_i a_i} + \mathbf{e_i}\, ,$$

where $\mathbf{b_i}$ is the vector of fixed effects containing sex, birth farm and batch effects. The vectors $\mathbf{a_i}$ and $\mathbf{e_i}$ represent the additive genetic effect $\sim N(\mathbf{0}, \mathbf{A} \otimes \mathbf{G_0})$ and the residual effects $\sim N(\mathbf{0}, \mathbf{I} \otimes \mathbf{R_0})$. Genetic parameters were estimated using the program ASREML (Gilmour et al., 1999) and the estimated asymptotic covariance matrices of the function parameters obtained in the first stage were incorporated using the facility OWN of ASREML.

Results and Discussion

Allometric functions

In Figure 1 the allometric function was fitted for lipid mass in relation to empty body weight. Lipid mass versus empty body weight showed an exponentially shaped curve with higher curvature in barrows than in gilts. The rapid accumulation of lipid with increasing empty body weight was also reflected by substantially higher allometric coefficients of 1. The goodness of fit of the allometric function was high with r=0.99. Also, the solution of allometric functions was stable after log transformation. However, higher deviations of measurements of lipid mass from the allometric function were obtained at the end of the growth period, indicating their reduced goodness of fit for this stage of growth. This shows that allometric functions have less flexibility to fit changes at the beginning and end of the growth period.

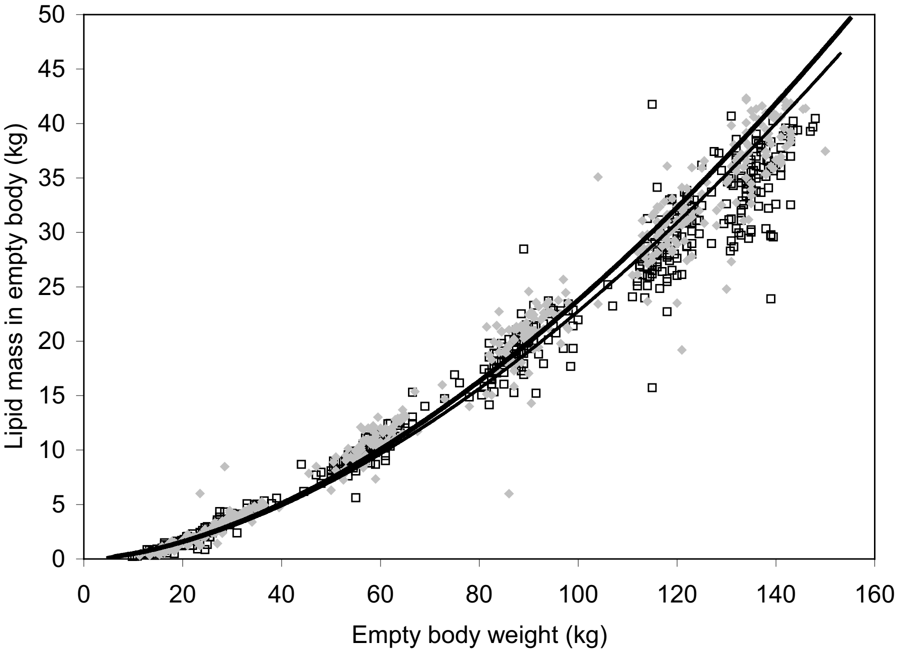

FIGURE 1: Developmental changes in lipid mass in relation to empty body weight (EBWT) for gilts (□; thin line; no. = 96) and barrows (◆; thick line; no. = 78) and using allometric functions. Gilts: $y = 0.0101 \times EBWT^{1.6763}$ (r = 0.99; residual s.e. = 2.5); barrows: $y = 0.0104 \times EBWT^{1.6803}$ (r = 0.99; residual s.e. = 2.5).

In relation to empty body weight gain, the accretion rate of water mass was lower as indicated by *b*-values of the allometric function of less than 1 with b = 0.86 and 0.84 for gilts and barrows, respectively. There was a linear association between protein mass or ash mass and the empty body weight as indicated by an allometric growth coefficient of close to 1, with almost no differences between genders. This information is essential for growth modelling of pigs as described by Knap et al. (2003).

Polynomial functions

For feed intake the second order polynomial was parsimonious compared to those of higher order. The heterogeneous variance was most appropriately accounted for by unstructured diagonal autoregressive moving-average ($ARMA_{diag}$) model (Lorenzo Bermejo et al., 2003b). Estimates of the autocorrelation were 0.80 and 0.81 and of the moving average component 0.15 and 0.11 for line 3 and 4, respectively. The additive genetic and permanent components of the variance were low in comparison with the components of the residual variance. This shows the high variability of daily data of feed intake, where more variation is usually present within than between animals. The additive genetic random coefficients, intercept and linear regression, were positively correlated (0.57 line 3, 0.55 line 4), i.e. selection on initial feed intake will result in a higher linear increment in feed intake per day during the growth period. The estimated correlations between intercept and quadratic regression coefficient were –0.77 for line 3 and –0.89 for line 4, and the estimated correlations between linear and quadratic regression coefficient were –0.48 for line 3 and –0.61 for line 4, i.e. selection on initial feed intake will result in flatter feed intake curves.

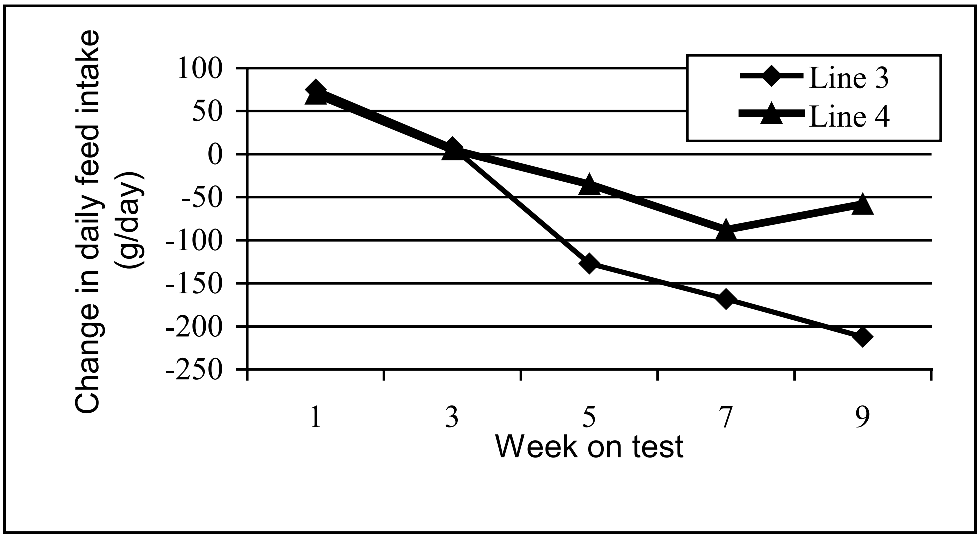

FIGURE 2: Change in daily feed intake of the progeny of the 5% highest ranked boars selected for weighted breeding values, which weighted to result in an increase in feed intake in the early growing period and a decrease in feed intake in the later finishing period.

Several studies have shown that higher feed intake at early stage of growth and a decrease in feed intake at the end of the finishing period will improve feed efficiency (e.g. Eissen et al., 2000; Schulze et al., 2001 and 2002; Mohrmann et al., 2004b). Based on the Eigenfunction of the estimated genetic parameters of feed intake, this will be achieved by the second Eigenfunction. Thus, estimated breeding values for feed intake have to be weighted by this second Eigenfunction to achieve the above-described breeding goal. In order to predict the selection response in the given population, 5% of the boars were ranked according to these weighted breeding values. Then, the mean daily feed intake of the reference population was compared with the mean daily feed intake of the offspring of the 5% highest ranked boars. Selection response is presented in Figure 2. Selection resulted in an increase in daily feed intake at early growing period of 75 and 70g for line 3 and 4, respectively, and resulted in a decrease in daily feed intake in the last finishing period of 212 g and 58 g for line 3 and 4. Thus, selection for change in feed intake pattern using polynomials in a random regression model was very successful to change feed intake pattern during growth. Additionally, polynomial functions showed a better fit to feed intake data than nonlinear functions.

Nonlinear functions

Protein, lipid and ash curves were fitted using Gompertz function in relation to age and their results are shown in Table1.

TABLE 1: Estimates of parameters (± approximate standard errors) of the Gompertz function fitted to protein (P), lipid (L) and ash (A) in relation to age

Gender	P_∞ (kg)	P_{Gomp} (kg/d*kg)	P_{max} (g/d)	L_∞ (kg)	L_{Gomp} (kg/d*kg)	L_{max} (g/d)	A_∞ (kg)	A_{Gomp} (kg/d*kg)	A_{max} (g/d)[‡]
Gilts	27.88	0.0129	132	41.90	0.0166	256	4.94	0.0138	25
(s.e.)	(0.22)	(0.00027)		(0.53)	(0.00057)		(0.04)	(0.00031)	
Barrows	28.03	0.0131	135	45.58	0.0168	282	4.87	0.0145	26
(s.e.)	(0.24)	(0.00030)		(0.61)	(0.00061)		(0.04)	(0.00037)	

[†] Abbreviation: P_∞, L_∞, A_∞ = asymptotic values of mature protein, lipid and ash mass, respectively; P_{Gomp}, L_{Gomp}, A_{Gomp} = Gompertz rate of protein, lipid and ash deposition, respectively; P_{max}, L_{max} and A_{max} = maximum rate of protein, lipid and ash deposition, respectively.

[‡] d = day.

Estimated protein mass at maturity was similar in gilts and barrows at about 28 kg, whereas lipid mass at maturity was 3.7 kg less in gilts than in barrows. Also, the maximum rate of protein deposition was similar for both sexes at about 130 g/day, but maximum rate of lipid deposition was lower in gilts than in barrows with 26 g/day. Mature ash mass and maximum ash deposition showed small differences between genders.

Estimates of function parameters and the goodness of fit of the nonlinear growth functions, logistic and Gompertz are given in Table 2. Both functions resulted in almost equal mean residual standard deviation and coefficient of determination. However, using Gompertz function for 6.5% of the animals the estimation failed to converge, whereas using logistic function this was the case only for 0.9% of the animals. In addition, the estimates of the Gompertz curve for mature weight and age at point of inflection showed much more variation, which may indicate the lower robustness of this function for the given data

TABLE 2: Means ($\bar{x}$), standard deviations (SD), minimum (MIN), maximum (MAX), residual standard deviations (RSD) and coefficients of determination (R^2)

Parameters	Unit	$\bar{x}$	SD	MIN	MAX	RSD	R^2
Logistic function						2.0635	0.997
A	kg	178	29	125	384		
β/γ	days	172	23	134	278		
Γ		0.0203	0.0028	0.0114	0.0269		
Gompertz function						1.999	0.997
A	kg	251	59	151	465		
β/γ	days	174	31	124	285		
Γ		0.0096	0.0019	0.0053	0.0149		

Genetic and residual variances as well as heritabilities of function parameters are presented in Table 3. Highest heritability was found for logistic growth rate. Substantially lower heritability was obtained for age at point of inflection, whereas the parameter of mature weight showed lowest heritability. One reason may be that the mature weight in the given data was estimated outside the observed data. Using Gompertz function, no genetic determination of its parameters, ages at point of inflection and growth rate, was estimated. The absence of genetic determination of the Gompertz parameters γ and β/γ, the convergence problems and the large variation of parameters indicated that the Gompertz function was not the appropriate function for the given data. Also, Lorenzo Bermejo et al. (2003a) found the logistic function to be more appropriate for estimation of feed intake curves. Interestingly, the lowest heritability using random regression model was found at the age of 172 days where the logistic function estimated the point of inflection averaged over all animals of the data. Further information about the sampling variance of the parameters can be obtained by Bayesian analysis as carried out by Blasco et al. (2003).

TABLE 3: Genetic variances (σ^2_a), residual variances, heritabilities of parameters of nonlinear logistic and Gompertz function using a two stage method

Function parameter		Σ^2_a	Σ^2_e	h^2
		Logistic function		
Mature weight	α	16.7	370.6	0.04
Age at point of inflection	β/γ	55.5	295.4	0.16
Logistic growth rate	γ	195.9	419.5	0.32
		Gompertz function		
Mature weight	α	86.4	3187	0.03
Age at point of inflection	β/γ	0.34	813	0.00
Gompertz growth rate	γ	0.33	293	0.00

Conclusion

Allometric functions showed several desirable properties such as stable linear solution after log transformation, parameters with straightforward biological interpretation and good convergence due to stable first derivatives. However, for body composition traits such as lipid deposition, allometric functions were not flexible enough in order to fit changes at the end of the finishing period. In our data, the use of allometric function resulted in overestimation of the lipid deposition. The use of polynomial function was most appropriate for data of high daily variation such as feed intake. But parameters of polynomials of greater than order two have no biological meaning and thus are not useable for direct selection. However, selection on breeding values, weighted by the second Eigenfunction of the additive genetic variance-covariance matrix of the polynomial regression coefficients, resulted in a change in feed intake curve to the desired shape. In contrast, nonlinear logistic functions have parameters with biological meaning per se. Therefore, the parameters can directly be used for selection, e.g. to change the point of inflection. Especially, information of QTL associated with parameters of nonlinear function can be used to get more insight in the genomic regulation of growth.

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