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Multilevel Analysis for Repeated Measures Data in Lambs[1]

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ABSTRACT

The study was conducted to compare the individual growth curves models and to detect individual differences in the growth rate by a performing multilevel analysis. The data set used for this purpose consisted of live weight records of 52 crossbred lambs from birth to 182 days of age. There were 670 observations in level-1 units which were the repeated measurements over time, and there were 52 observations in level-2 units which were lambs. In the study, parameter estimation of time-independent covariate factors, such as gender, birth type and birth weight, was performed by using five different models within the framework of multilevel modeling. LRT, AIC and BIC were used for the selection of the best model. The "*Conditional Quadratic Growth Model-B*" provided the best fit to the data set. The multilevel analysis indicated that linear and quadratic growth in lambs was significant. According to the results of the study, individual growth curves can be investigated using multilevel modeling in animal studies which is an important parameter of the individual growth rate.

Keywords: Repeated data; Multilevel models; Intra-class correlation; Individual growth models

Kuzularda Tekrarlamalı Veriler için Çok Düzeyli Analiz

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ÖZET

Bu çalışma, çok düzeyli analizler kullanılarak bireysel büyüme eğrisi modellerini karşılaştırmak ve büyüme oranındaki bireysel farklılıkları belirlemek amacıyla yapıldı. Bu amaç için kullanılan veri seti, 52 baş melez kuzunun doğumdan 182 günlük yaşa kadar olan canlı ağırlık kayıtlarını içermektedir. Zaman içinde tekrarlanan ölçümlerin olduğu seviye-1'de toplamda 670 gözlem ve kuzuların olduğu seviye 2'de 52 gözlem bulunmaktadır. Bu çalışmada, çok seviyeli modelleme yapısı içinde beş farklı model kullanılarak cinsiyet, doğum tipi ve doğum ağırlığı gibi zamana bağlı olmayan kovaryet etkilere ilişkin parametre tahmini yapıldı. En iyi model seçimi için LRT, AIC ve BIC kullanıldı. Veri setini en iyi açıklayan "*Conditional Quadratic Growth Model-B*" olarak belirlendi. Çok düzeyli analiz, kuzularda doğrusal ve ikinci dereceden büyümenin önemli olduğunu gösterdi. Çalışmanın sonuçlarına göre, bireysel büyüme oranının önemli olduğu hayvancılık çalışmalarında bireysel büyüme eğrileri, çok düzeyli modelleme kullanılarak araştırılabilir.

Anahtar Kelimeler: Tekrarlamalı veri; Çok düzeyli modeller; Sınıf içi korelasyon; Bireysel büyüme modelleri

1 The part of the study was presented as a poster in conference named "Agriculture for Life, Life for Agriculture" in Romania.

1. Introduction

As in various study fields, hierarchical or clustered data structure is commonly encountered in animal studies. Some examples are as follows; milk production of the cows nested within sires (Simsek & Fırat 2011), milk fat ratio of the sheep nested within herds, egg production of the chicken nested within cocks, carcass weight of the sheep nested within sires. All are good examples for a two-level structure. In the example of carcass weight from the sheep nested within sires, sheep are level-1 units and sires are level-2 units. When compared with sheep of different sires, it is inevitable that carcass weights of the sheep from the same sire are more similar. In the last 20 years, multilevel modeling (Goldstein 1999) has become a standard approach in the analysis of such data. Multilevel models are mixed effects models containing both fixed effects and random effects which may take the form of either random intercepts or random coefficients, and are named random coefficient models (Leeuw & Kreft 1986) or hierarchical models (Bryk & Raudenbush 1986; Raudenbush & Bryk 2002) in the literature.

Repeated data, which are naturally dependent, represent all observations of a variable obtained from the same individual sequentially over time. It means that observations on the same individual are related to each other (Singer & Willett 2003; Hedeker 2004; Hedeker & Gibbons 2006). While the assumption of independence of the data is violated, predictions which are made using traditional statistical analyses such as ANOVA and ordinary least-square (OLS) are biased and Type 1 errors increase (Peugh 2010; Shek & Ma 2011). In the recent years, multilevel modelling has been used in the analysis of data in repeated structure (Chen & Cohen 2006; DeLucia & Pitts 2006; Peugh 2010; Shek & Ma 2011). Repeated data are analyzed as a two-level model in multilevel modelling. The measurements obtained repeatedly over time have been classified among individuals; repeated measurements represent level-1 units, while individuals/animals represent level-2 units (Singer 1998; Singer & Willett 2003; Hedeker 2004; Hedeker & Gibbons 2006). The fact that multilevel modelling is used for repeated measures data has some advantages over traditional methods. It does not require a balanced data structure. In other words, the same number of repeated measurements is not necessary for each individual (Green et al 1998). Both the mean change in the population over time and individual changes may be predicted. Parameter estimation is performed for both time-variant and time-invariant independent variables. The best covariance structure for the model may be chosen (Hedeker 2004; Hox 2010; Shek & Ma 2011). Furthermore, multilevel analysis can be easily performed for the models with three and more level (Lancelot et al 2000).

The aim of this study was to compare the individual growth curves models and to detect individual differences in the growth rate by a performing multilevel analysis. For this aim, firstly the appropriate covariance structure was selected, and then the models were compared to find the best model explaining the change in the individual growth curves of lambs.

2. Material and Methods

2.1. Material

The study was carried out at a Research and Application Farm of Yuzuncu Yil University in Van, Turkey. Animal material of the study consisted of (Ile de France x Akkaraman (B_1)) x Karakas crossbred lambs. The live weights of 52 lambs were recorded at two-weekly intervals from birth to 182 days of age. In the data set, 6 lambs had 12 and the remaining 46 lamb had 13 measurements obtained in different time points. Independent variables included in the model were gender, birth type and birth weight. The total number of observations was 670 in level-1 units which were repeated measurements, while level-2 which was the level of lambs which had 52 observations.

Five models were constructed for the data set in the study and were analyzed using SAS, 9.3 (SAS 2014) and MLwiN (2.02) statistics software (MLwiN 2009).

2.2. Method

Model I (Unconditional Mean or Null Model): In this model which is the baseline for 'Unconditional Linear Growth Model', only intercept is present and is known as 'null' model.

Level-1: $Y_{ti} = \pi_{0i} + e_{ti}$

Level-2: $\pi_{0i} = \beta_0 + u_{0i}$

The Equation 1 is obtained when level-2 items are put into their place in level-1.

$$Y_{ti} = \beta_{00} + u_{0i} + e_{ti} \quad (1)$$

Where; Y_{ti}, live weight of the lamb *i* at *t* time; β_{00}, grand-mean of live weight of the lambs; u_{0i}, deviations in a lamb's live weight mean around the grand-mean (β_{00}); e_{ti}, deviation between observed and expected live weights of the lamb. Assumptions made for level-2 (u_{0i}) and level-1(e_{ti}) error terms are $u_{0i} \sim N\left(0, \sigma^2_{u_0}\right)$ and $e_{ti} \sim N\left(0, \sigma^2_e\right)$, respectively. This model (Equation 2) is important to test the variance between level-2 units and calculate intra-class correlation coefficient (ICC) (Goldstein 1999; Raudenbush & Bryk 2002; Singer & Willett 2003).

$$ICC = \frac{\sigma^2_{u_0}}{\sigma^2_{u_0} + \sigma^2_e} \quad (2)$$

Model II (Unconditional Linear Growth Model): It is used to describe the time-dependent change in repeated measurements. Thus, individual variation in growth rate is examined and the model does not include any independent variable, except time (Equation 3).

Level-1: $Y_{ti} = \pi_{0i} + \pi_{1i} T_{ti} + e_{ti}$

Level-2: $\pi_{0i} = \beta_{00} + u_{0i}$; $\pi_{1i} = \beta_{10} + u_{1i}$

$$Y_{ti} = \beta_{00} + \beta_{10} T_{ti} + u_{1i} T_{ti} + u_{0i} + e_{ti} \quad (3)$$

Where; Y_{ti}, described in Model I; T_{ti}, time variable showing *t*. measurement time for the lamb in *i*. order; β_{00}, intercept which is the grand-mean live weight of the lambs at the zero time point; β_{10}, regression coefficient namely slope which is the mean change in live weight over time; u_{0i} and u_{1i}, level-2 error term; e_{ti}, level-1 error term. All error terms have a normal distribution. u_i and e_{ti} errors are independent of each other and error terms in the same level are correlated to each other. So, $e_{ti} \sim N(0, \sigma^2_e)$, $\mathrm{u}_i = [u_{0i}, u_{1i}]^T \sim N(0, \Omega_u)$,

where $\Omega_u = \begin{pmatrix} \sigma^2_{u_0} & \sigma_{u_{01}} \\ \sigma_{u_{01}} & \sigma^2_{u_1} \end{pmatrix}$

Model III (Conditional Linear Growth Model): In addition to Model II, this model also has the variables that do not change over time and these are written in level-2 (Equation 4).

Level-1: $Y_{ti} = \pi_{0i} + \pi_{1i} T_{ti} + e_{ti}$

Level-2: $\pi_{0i} = \beta_{00} + \beta_{01} Z_i + u_{0i}$; $\pi_{1i} = \beta_{10} + \beta_{11} Z_i + u_{1i}$

$$Y_{ti} = \beta_{00} + \beta_{10} T_{ti} + \beta_{01} Z_i + \beta_{11} T_{ti} Z_i + u_{1i} T_{ti} + u_{0i} + e_{ti} \quad (4)$$

Where; fixed and random effects described in Model II; Z_i, time-invariant variables; β_{01}, effect of the time-invariant variables; β_{11}, interaction effect of these effects and the time. In this study the variables are the genders of the lambs, birth type and birth weight.

Model IV (Conditional Quadratic Growth Model-A): This model includes linear end quadratic effects of the time. These effects take place in level-1 equation in Model IV.

Level-1: $Y_{ti} = \pi_{0i} + \pi_{1i} T_{ti} + \pi_{2i} T^2_{ti} + e_{ti}$

Level-2: $\pi_{0i} = \beta_{00} + \beta_{01} Z_i + u_{0i}$; $\pi_{1i} = \beta_{10} + \beta_{11} Z_i + u_{1i}$

Generally for Model IV, a single equation is written using level-2 and level-1 (Equation 5).

$$Y_{ti} = \beta_{00} + \beta_{10}T_{ti} + \beta_{01}Z_i + \beta_{11}T_{ti}Z_i + \beta_{2i}T_{ti}^2 + u_{1i}T_{ti} + u_{0i} + e_{ti} \quad (5)$$

Where; T^2, quadratic effect of the measurement time; β_{2i}, average amount of change caused by the quadratic effect of time in the response variable.

Model V (Conditional Quadratic Growth Model-B): This model pays attention to the similar effects as Model IV. Its only difference is that the quadratic effect is a random effect in the model. This is given in level-2 equation.

Level-1: $Y_{ti} = \pi_{0i} + \pi_{1i}T_{ti} + \pi_{2i}T_{ti}^2 + e_{ti}$

$\pi_{0i} = \beta_{00} + \beta_{01}Z_i + u_{0i}$

Level-2: $\pi_{1i} = \beta_{10} + \beta_{11}Z_i + u_{1i}$

$\pi_{2i} = \beta_{20} + \beta_{21}Z_i + u_{2i}$

A general equation is written for Model V by using the equations of level-1 and level-2 (Equation 6).

$$Y_{ti} = \beta_{00} + \beta_{10}T_{ti} + \beta_{20}T_{ti}^2 + \beta_{01}Z_i + \beta_{11}T_{ti}Z_i + \beta_{21}T_{ti}^2Z_i + u_{1i}T_{ti} + u_{2i}T_{ti}^2 + u_{0i} + e_{ti} \quad (6)$$

Where; T^2, quadratic effect of time; β_{20}, mean change in response variable by quadratic effect of time; $T_{ti}^2Z_i$, interaction effect of quadratic effect of time and the time-invariant variables; β_{21}, mean change in response variable based on this interaction effect; u_{2i}, term of level-2 error recently added to the model. In this case, $\mathrm{u}_i = \left[u_{0i}, u_{1i}, u_{2i}\right]^T \sim N(0, \Omega_u)$ assumption is made for level-2 error types of

$$\mathrm{u}_i = \left[u_{0i}, u_{1i}, u_{2i}\right]^T. \text{ Where } \Omega_u = \begin{pmatrix} \sigma_{u_0}^2 & & \\ \sigma_{u_{01}} & \sigma_{u_1}^2 & \\ \sigma_{u_{02}} & \sigma_{u_{12}} & \sigma_{u_2}^2 \end{pmatrix}$$

To obtain the variance-covariance matrix that is suitable for the study data, Unstructured (UN), Compound Symmetry (CS), first-order autoregressive (AR(1)) and Toeplitz covariance structures were used for each of the models (Littell et al 2000).

2.2.1. Model selection

The likelihood ratio test (LRT) has been used to compare two models. It is computed by the difference in deviance for the two models, which one of them is reduced and the other is current model (Equation 7).

$$LRT = [(-2LL_{Reduced\ Model}) - (-2LL_{Current\ Model})] \quad (7)$$

Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC) are the cohesion criteria used for the model selection. AIC uses log-likelihood and parameter number (Akaike 1974) and BIC uses also sample size together with log-likelihood and parameter (Equation 8) number (Schwarz 1978).

$$AIC = -2LL + 2k$$
$$BIC = -2LL + k\ln(n) \quad (8)$$

Where; *LL*, log-likelihood the model; *k*, number of estimated parameters in the model; *n*, number of observations.

The model having minimum AIC and BIC values is determined as the best model when selecting the model.

3. Results and Discussion

According to the results of Model I with UN and Toeplitz covariance structures having the minimum AIC and BIC values, both level-1 ($\sigma_e^2 = 91.58$) and level-2 variances ($\sigma_{u_0}^2 = 12.26$) were significant (P<0.05, P<0.0001). The ICC value was obtained using the Equation 2 (Goldstein 1999) indicated that the observations were not independent from each other and 12% of the change in the live weight was due to the difference between the lambs.

$$ICC = \frac{\sigma_{u_0}^2}{\sigma_{u_0}^2 + \sigma_e^2} = 0.1181 \cong 0.12$$

In studies with repeated measures data, *ICC* value was as expected (Ip et al 2011), but this criterion alone is not always sufficient. So, effective sample size ($n_{eff} = n/\left[1 + (n_{clus} - 1)\right]ICC$) and design effect ($DE = 1 + (n_{clus} - 1)ICC$) was calculated as 263 and

2.6, respectively. Since the design effect value was more than 2 (Peugh 2010; Simsek & Fırat 2011) and ICC value was different from zero (Gulliford et al 1999; Smeeth & Ng 2002; Ip et al 2011), the performance of multilevel analysis was required.

Based on the covariance structure (UN, CS, AR(1) and Toeplitz) the deviance and AIC and BIC values for Model II, III, IV and V are presented in Table 1. This table revealed that UN covariance structure for each model had the minimum deviance, and AIC and BIC values (Littell et al 2000). Thus, UN was accepted as the best covariance structure for all models. The goodness of fit statistics was sought for to find the best model among the models with UN covariance. The results of likelihood-ratio test for models are shown in Table 2. The difference in the deviance between the two models, $[(-2LL_{Reduced\ Model})-(-2LL_{Current\ Model})]$ proved that Model II was significantly better than Model I (P<0.001), Model III was better than Model II (P<0.01), Model IV was better than Model III (P<0.001) and Model V was better than Model IV ($\chi_3^2 = 324.6$, P<0.001). Additionally, it can be remarked that the Model V was the best fit to the data among other models. This result was also supported by AIC and BIC values given Table 1. Because the best model is the model which has the minimum AIC and BIC values, Model V with UN covariance structure was determined as the best model.

The multilevel analysis results obtained by using UN covariance structure for Model II, III, IV and V are presented in Table 2.

As shown in Table 2, while the intercept for Model V was not significant, random change of each lamb around the intercept was significant ($\sigma_{u_0}^2 = 0.7270$, P<0.001). This finding was supported by previous studies conducted on multilevel models in various areas (Leeden 1998; Lancelot et al 2000; Kristjansson et al 2007; Peugh 2010). It revealed that initial weight (14 days of age) of each lamb was different from each other. The fact that linear effect of time was significant in

Table 1- The models used in the study and the goodness of fit statistics with respect to their covariance structures

Models	*Covariance structure*	*-2LL (Deviance)*	*AIC*	*BIC*
Model II	Unstructured (UN)	2940.1	2952.1	2963.8
	Compound symetry (CS)	3009.3	3017.3	3025.1
	First-order autoregressive AR(1)	3009.3	3017.3	3025.1
	Toeplitz (TOEP)	3009.1	3019.1	3028.9
Model III	Unstructured (UN)	2899.4	2919.4	2939.0
	Compound symetry (CS)	2915.1	2931.1	2946.7
	First-order autoregressive AR(1)	2915.1	2931.1	2946.7
	Toeplitz (TOEP)	2914.4	2932.4	2949.9
Model IV	Unstructured	2370.8	2392.8	2414.2
	Compound symetry (CS)	2430.8	2450.8	2470.3
	First-order autoregressive AR(1)	2430.8	2450.8	2470.3
	Toeplitz (TOEP)	2430.8	2450.8	2470.3
Model V	Unstructured	2046.2	2074.2	2101.6
	Compound symetry, (CS)	2740.6	2758.6	2776.1
	First-order autoregressive AR(1)	2740.6	2758.6	2776.1
	Toeplitz (TOEP)	2321.4	2343.4	2364.8

Table 2- The analysis results of multilevel modeling

	Model II estimate (SE)	*Model III* estimate (SE)	*Model IV* estimate (SE)	*Model V* estimate (SE)
Fixed effect				
Intercept	7.8629 (0.2760)***	-3.0477 (2.0884)	-5.2883 (2.0898)*	0.1258 (1.4181)
Time	2.5473 (0.0539)***	1.5502 (0.2944)***	2.7724 (0.2973)***	2.6227 (0.3047)***
BW		1.9307 (0.2900)***	1.9307 (0.2900)***	1.1918 (0.2015)***
Gender		0.6197 (0.4043)	0.6197 (0.4043)	0.04131 (0.2694)
BT		0.5710 (0.4747)	0.5710 (0.4747)	-0.09565 (0.3163)
Time* BW		0.2129 (0.06197)***	0.2129 (0.06197)***	0.2448 (0.06105)***
$Time^2$			-0.1018 (0.003454)***	-0.1018 (0.00856)***
Random effect				
$\sigma^2_{u_0}$	3.1016 (0.7786)***	1.1102 (0.3910)**	1.6288 (0.3883)***	0.7270 (0.2120)***
$\sigma_{u_{01}}$	0.4005 (0.1152)**	0.1994 (0.0729)**	0.1371 (0.07275)	0.06307 (0.1163)
$\sigma^2_{u_1}$	0.1341 (0.02969)***	0.1061 (0.0242)***	0.1165 (0.02419)***	0.5289 (0.1161)***
$\sigma_{u_{02}}$				0.008435 (0.01012)
$\sigma_{u_{12}}$				-0.03805 (0.008782)***
$\sigma^2_{u_2}$				0.00351 (0.00075)***
σ^2_e	3.1299 (0.1851)***	3.1299 (0.1851)***	1.2420 (0.07344)***	0.6037 (0.03744)***
Deviance	2940.1	2899.4	2370.8	2046.2
Number of parameter	6	10	11	14
χ^2	922.41***	40.7**	528***	324.6***
Degrees of Freedom	3	4	1	3

BW, birth weight; BT, birth type; *, P<0.05; **, P<0.01; ***, P<0.001

Model V meant that the lambs gained an average 2.6227 kg live weight every 14 days (P<0.001). The level-2 error term $\sigma^2_{u_1} = 0.5289$ showing the random change with respect to the linear effect of time was significant (P<0.001). Green et al (1998), Lancelot et al (2000), Dudley et al (2009) and Peugh (2010) proven that linear effect of time and its random change was significant. According to this model, the live weight gained every 14 days was not a stable value and varied from one lamb to another. In this model, quadratic effect of time was also examined and found significant (P<0.001). Supporting the current result, numerous authors (Green et al 1998; Lancelot et al 2000; Dudley et al 2009) also reported a significant quadratic time effect. This significant effect indicated that each lamb had an inflection point in the live weight gain and gradually decrease of weight after the point (-0.1018). The analysis clearly showed that linear growth trajectory (2.6227) was higher than the quadratic growth rate (-0.1018). So, initially, the live weight gain of the lambs increased linearly, and then then this linear increase slowed down. Quadratic effect, such as the linear effect of time, was also included in random part of the model and the variation of this effect between lambs was detected to be significant ($\sigma^2_{u_2} = 0.00351$, P<0.001). The covariance of the linear and quadratic effect of time was significant ($\sigma_{u12} = -0.03805$, P<0.001). These results were supported by the Green et al (1998) and Lancelot et al (2000) who studied tree-level model. This means that quadratic growth started earlier in the lambs which had a faster linear growth. On the other hand, quadratic growth started later in the lambs which had a slower linear growth.

The lamb's birth weight, and the interaction between birth weight and linear effect of time was significant (P<0.001, P<0.001). Accordingly, every 1 kg increase in birth weight led to an average 1.1918 kg increase in the live weight. Each unit increase in time led to an average 2.6227 kg increase in the live weight. Each unit increase in interaction effect led to an average 0.2448 kg increase in the live weight. Since the value of the interaction (time*BW) is positive, significant interaction effect also indicated that high-birth weight lambs would have a higher live weight during the growth period.

Individual growth curves of lambs obtained based on Model V are presented in Figure 1. Figure 1 had three significant random coefficients because of Model V; the random coefficients of intercept, linear and quadratic effects. It means that the lambs had different intercept, linear, and quadratic effects from each other.

Figure 2 represents some details in the individual growth curves. The lamb with the id_13 had the highest initial live weight. Its live weight increased linearly until 182 days of age. Similarly, the lambs with the id_52 and id_6 had a linear increase in live weight up to 182 days of age. The live weight of lamb with the id_21 increased linearly up to 112 days of age (about 8. point). After this point the quadratic increase began for id _21. The lamb with id_32 had the lowest initial live weight and the increase in its live weight was slower than the others. Therefore, the lambs with the id_13 and id_15 may be preferred over the other lambs due to their late start of quadratic growth on the 182 days of age.

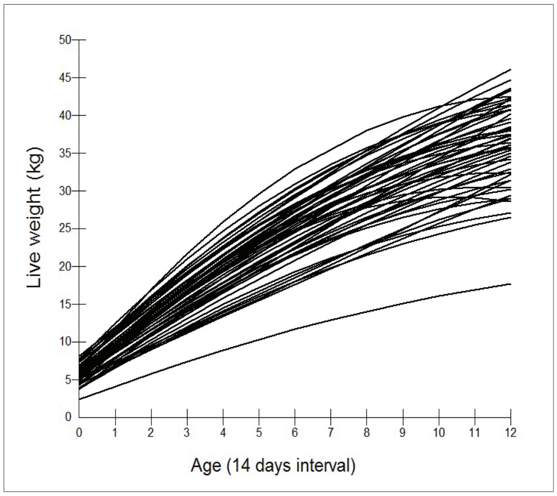

Figure 1- Individual growth curves of the lambs between 14 to 182 days of age

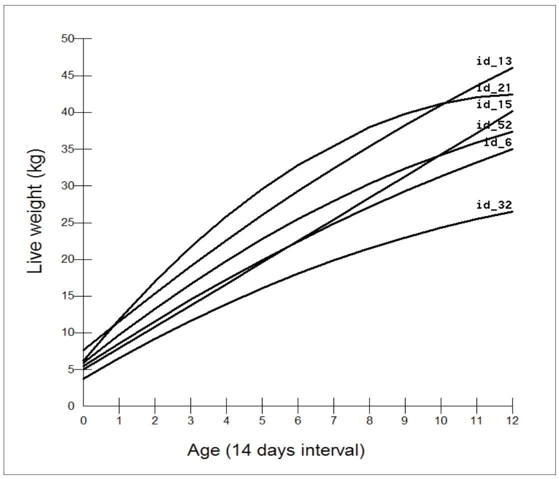

Figure 2- Individual growth curves of randomly selected lambs between 14 to 182 days of age

4. Conclusions

The data sets may have unbalanced repeated data structures, time-variant and time invariant variable, in which case, traditional methods become insufficient. A multilevel analysis is preferred by researchers because it has a powerful and flexible procedure for such data set. The multilevel modeling can be used to investigate the individual growth rate of animals. The growth performance is one of the economically important traits in livestock. There are differences between breeds within a species, and individuals within a breed in respect of the rate of growth. An individual growth curve can be used to determine whether an animal's growth performance is improving, as well as whether the animal is growing faster or slower than other animals. In this study, possible applications of individual growth curves models for repeated data in crossbred lambs were discussed. It was determined that the best model was the Model V. According to the results of the study, the same-age lambs had different growth rates caused by their birth weight. Therefore, the individual growth curves may be indicators for animals which are at high risk of low performance. The multilevel analysis based on Model V indicated that linear and quadratic growth in lambs was significant. As a result, each individual's growth curve can be investigated using multilevel modeling in animal studies which is an important parameter of the individual growth rate.

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