

Comparison of Different Growth Curve Models in Romanov Lambs

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Abstract

This study investigates the modelling of the individual growth curves of Romanov lambs using different equations and the data on the increase in live weight and selects the best model. For this purpose, the live weights of Romanov lambs that were brought to Gürçeşme Village, Niksar, Turkey, from Nikopol, Russia, were recorded from birth to day 180. In the study, the Cubic Spline, Logistic, Gompertz and Richard models were used. For the study, individual growth curves of a total of 278 (178 females, 100 males) lambs were modeled. For the selection of the best model, adjusted determination coefficient (R^2_{adj}), mean square error (MSE), Akaike information criteria (AIC) and Durbin-Watson (DW) values were used. In addition, attention was paid to the parameters and standard errors of the models. The results showed that the mean square error for the male lambs varied from 0.295 to 0.995, while it varied from 0.995 to 2.659 for the female lambs; the R^2_{adj} values were between 0.971 and 0.997 for the male lambs and 0.969 and 0.993 for the female lambs. The AIC values were between -37.12 and 0.094 for the male lambs and -0.196 and 122.12 for the female lambs. The DW values ranged from 1.86 to 2.44 for the female lambs and from 1.02 to 2.79 for the male lambs. Considering the MSE, R^2_{adj} , AIC and DW values of the female lambs (0.295±1.195, 0.997±0.002, -37.12±0.001, 2.23±0.49, respectively) and male lambs (0.995±1.021, 0.993±0.001, -122.12±0.05, 2.31±0.19, respectively), the Cubic Spline model was determined to be the best model, while the Richard model was determined to be the worst fitting model both for the female (0.95±5.143, 0.971±0.002, 0.094±0.31, 2.41±0.01) and male (1.85±2.569, 0.969±0.011, -0.196±0.04, 2.79±0.05) lambs.

Keywords: : Romanov, Lamb, Modelling, Growth curve models

Romanov Kuzularında Farklı Bireysel Büyüme Eğrisi Modellerinin Karşılaştırılması

Öz

Bu çalışmada, Romanov kuzuların canlı ağırlık artışlarına ait veriler ile bireysel büyüme eğrilerinin farklı eşitlikler kullanarak modellenmesi ve en iyi modelin seçimi hedeflenmiştir. Bu amaçla Rusya'nın Nikopol eyaletinden Niksar Gürçeşme köyüne getirilen Romanov koyun ırkı kuzularının canlı ağırlıkları doğumdan 180. günlük yaşa kadar kayıt altına alınmıştır. Çalışmada model olarak, kubik parçalı model (cubic spline model), Lojistik model, Gompertz model ve Richard modelleri kullanılmıştır. Bu çalışma için toplam 278 (178 dişi, 100 erkek) kuzuya ait canlı ağırlık verisi kullanılmıştır. Kullanılan modeller içinde en iyi modelin seçimi için düzeltilmiş belirtme katsayısı (R^2_{adj}), hata kareler ortalaması (HKO), akaike information criteria (AIC) değeri ve Durbin-Watson istatistiklerinden yararlanılmıştır. Ayrıca bu modellere ait parametreler ve standart hatalarında dikkate alınmıştır. Elde edilen araştırma sonuçlarına göre erkeklerde hata kareler ortalaması 0.295 ile 0.995 arasında ve dişilerde 0.995 ile 2.659 aralığında, R^2_{adj} değerleri erkeklerde 0.971 ile 0.997 ve dişilerde 0.969 ile 0.993 aralığında bulunmuştur. AIC değerleri erkeklerde 0.094 ile -37.12 aralığında, dişilerde ise -0.196 ile 122.12 aralığında elde edilmiştir. DW değerleri ise dişilerde 1.86 ile 2.44 aralığında, erkeklerde ise 1.02 ile 2.79 aralığında değişim göstermiştir. Araştırma sonucunda hata kareler ortalaması, düzeltilmiş belirtme katsayısı, AIC değeri ve Durbin-Watson değerleri dikkate alındığında, dişilerde sırasıyla (0.295±1.195, 0.997±0.002, -37.12±0.001, 2.23±0.49) ve erkeklerde sırasıyla (0.995±1.021, 0.993±0.001, -122.12±0.05, 2.31±0.19) bulunmuş olup, bu sonuçlara göre kubik parçalı model en iyi model olarak tespit edilmiştir. En uyumsuz modelin ise dişilerde (0.95±5.143, 0.971±0.002, 0.094±0.31, 2.41±0.01) ve erkeklerde (1.85±2.569, 0.969±0.011, -0.196±0.04, 2.79±0.05) Richard modeli olduğu sonucuna varılmıştır.

Anahtar sözcükler: Romanov, Kuzu, Modelleleme, Büyüme eğrisi modelleri



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INTRODUCTION

Growth is a result of the relationship between an individual's genetic potential for the investigated property and environment. A growth curve shows the changes in yield with time due to this relationship. Yield depends on age and can be the live weight or body properties of an individual. The mathematical models showing the age-growth relationship are used to determine the nutrition program and optimum slaughter age and estimate the effects of the selection methods on farm animals. When modelling the growth, growth rate can be classified as constant growth rate, continuously increasing and decreasing growth rates and varying growth rate. Growth curves depend on the species, environmental conditions and investigated property and thus, selection of the appropriate model requires the use of statistical decision-making. Although growth at a constant rate can occur in certain properties of organisms during certain periods, it has been reported that, in general, the growth rates of organisms are not constant [1-4].

In most cases, linear models fall short in modelling the growth of organisms during an entire life period [2-5]. During periods of varying growth rates, the use of more complex non-linear models is more useful compared to linear models, in fact, it is necessary. Another important advantage of non-linear models is that they can serve as the basis of an objective method that will estimate the growth potential and sustainable production of an organism [6].

The purpose of growth curves is to summarize the information that is hard to interpret and obtained from different points due to age with lower number of parameters that can be interpreted biologically. The structure of the data and purpose of the analysis are the two important criteria used in the selection of the method that will be used in the growth curves. Moreover, the parameters of the model that will be used in the estimation of the growth curves are expected to be biologically interpretable. The biological interpretation of the parameters depends on interpreting the relationship between genetics and environment well [3].

Using the developed asymptotic and monomolecular functions, the age-growth relationship for the investigated property in lambs is estimated. In addition, the parameter values that can be the selection criteria for these models are determined [7]. In animal breeding, growth curves give important information on optimum slaughter age and economic growth threshold. Moreover, a growth model validity of which is controlled and accepted (for live weight and body measurements) can be used for the estimation of the growth at a certain period and, thus, for early selection. Growth curves allow estimating the growth of an individual in the future and, thus, selecting the animals that have good growth for breeding at an early age [2,8].

In sheep breeding in Turkey, the majority of the economic revenue is generated by lamb production. One of the major reasons behind the insufficiency to meet the need for animal products is that sheep breeding is mostly dependent on low-yield local breeds [9]. Romanov lamb is a sheep breed that was obtained in North Russia after regular improvement studies for many years and has a high ability to transfer its properties to its offspring. Compared to other sheep breeds, Romanov lambs have higher breeding capability and viability. Due to their high adaptation and breeding, they are preferred in herd breeding and, thus, data on Romanov lambs were used in the study considering their adaptability to the breeding conditions in Turkey [10].

There are various husbandry studies on the issue investigating different species [11,12]. However, the number of studies on the growth models for Romanov lambs is limited.

The study investigates the fitness of individual growth curves that were modeled using the Richard, Logistic, Gompertz and Cubic Spline models to the data for both female and male Romanov lambs. The MSE, R^2_{adj} , AIC and DW values were compared in both female and male Romanov lambs for the four different growth curve models. In addition, estimations for the parameters of the individual growth curves for four different models are given.

MATERIAL and METHODS

In the study, the increases in the live weights of a total of 278 Romanov lambs (178 female and 100 male) that were brought to Nksar Gürçeşme from Nikopol, Russia, were recorded from birth to day 180 and individual growth curves were modeled.

The lambs were fed with the same ration program from weaning (2.5 months) to the end of the experiment (day 180) and the lambs were not allowed to pasture. Before weaning, each lamb was monitored individually and according to their live weights and suckling, they were daily fed with the 2500 kcal/ME and 12-16% HP-containing initial concentrate feed in an average amount of 200-300 g. After weaning, the lambs were separated from their mothers and divided into special groups according to their birthdays and, again, using the ration programs, they were fed with the 2500 kcal/ME and 18-21% HP-containing concentrate feed and high-quality roughage by paying attention to their live weights and ages and the amount of the feed was homogeneously and carefully calculated to apply the necessary feeding program.

The common feature of the models used in the fitness of growth curves is their use of two main biological parameters: the performance and growth rate of an individual at a certain point, usually at a mature age. However, in some models, another parameter is the increasing or decreasing

changing point of a growth curve in terms of the growth rate. The non-linear models are commonly used in the investigation of the relationship between growth and age.

Four different functions were used for the individual growth curves in Romanov lambs, comprising the Richard, Logistic, Gompertz and Cubic Spline functions. For the sake of clarity, the α , β , k and m parameter representations of the models in Table 1 were replaced by β_0 , β_1 , β_2 and β_3 respectively. For all models included in this study, the parameters are defined as β_0 , the maximum potential of the asymptote or dependent variable; β_1 , biological constant; β_2 , controls the rate at which the dependent variable approaches the potential maximum; β_3 is allometric constant. Knots are determined by considering concave and convex formation points. In Cubic Spline, the number of the knots position is very important [13]. Table 1 shows the mathematical models of the functions [14-17].

The NLIN (non-linear regression) procedure of the SAS 9.0 System for Windows was used in the adaptation of the growth curve models (W and β) to the live weight data and estimation of the parameters [18]. The Marquardt method was used for iteration, which was preferred due to being a representation of a reconciliation between the Gauss-Newton and Steepest descent methods and combining the best aspects of the two methods by eliminating their severe limitations [19]. The Marquardt iteration model requires parameters to be estimated and their initial values, a model with a single dependent variable and partial derivatives of the model for each parameter. The statistical methods that are known and suitable for the linear models are usually not suitable for non-linear models and F-statistics cannot be used to reach a conclusion at any significance level [19]. Thus, the models are compared to each other using unexplained variances [20].

Goodness-of-fit Criteria

Goodness of the fit of the models was evaluated using the adjusted determination coefficient R^2_{adj} , mean square error (MSE), Akaike information criteria (AIC), correlation between observed live weight and residuals (RESC) and Durbin-Watson autocorrelation test (DW) [21].

The goodness-of-fit criteria to compare the functions that will explain the growth of lambs are as follows:

| Models | Expressions |
|--------------|---|
| Richard | $W_t = \beta_0 / (1 + \beta_1 \exp(-\beta_2 t))^{1/\beta_3}$ |
| Logistic | $W_t = \beta_0 / (1 + \beta_1 \exp(-\beta_2 t))$ |
| Gompertz | $W_t = \beta_0 \exp(-\beta_1 \exp(-\beta_2 t))$ |
| Cubic Spline | $W_t = \beta_0 + \beta_1 t + \beta_2 t^2 + \beta_3 t^3 + \beta_4 (t-a)^3$ |

W_t : live weight on the day, β_0 , β_1 , β_2 , β_3 , β_4 : Model constants describing the growth curves of the Richard, Logistic, Gompertz and Cubic Spline models, \exp ; the natural logarithm base, t : Age(days), a : knot

Adjusted Determination Coefficient (R^2_{adj}),

The adjusted R^2 (R^2_{adj}) value, which was developed by Henry Theil to avoid the increase in the R^2 value that is obtained using the explanatory variables, was used.

$$R^2_{adj} = R^2 - \left(\frac{k-1}{(n-k)(1-R^2)} \right) \quad (1)$$

where n is the number of observations and k is the number of parameters.

Different than the R^2 value, adjusted R^2 (R^2_{adj}) only increases when the absolute t value of the added variable is higher than 1. R^2_{adj} is always lower than or equal to R^2 ($R^2_{adj} \leq R^2$) [22].

Mean Square Error (MSE),

$$MSE = SSE / (n - k) \quad (2)$$

where n is the number of observations, SSE is the sum square of errors and k is the number of parameters.

Akaike Information Criteria (AIC),

Akaike information criteria (AIC) are used to select the most compatible model among different models [23]. The model with the lowest AIC value is selected as the most compatible model.

$$AIC = n \times \ln \left(\frac{SSE}{n} \right) + 2k \quad (3)$$

where n is the number of observations, SSE is the sum square of errors and k is the number of parameters [24].

Durbin-Watsons (DW)

There are various tests that were developed to determine autocorrelation. When the number of observations is 13, the most commonly used test is the Durbin-Watson (DW) d statistics.

$$d = \sum_{t=2}^n (e_t - e_{t-1})^2 / \sum_{t=1}^n e_t^2 \quad (4)$$

The value of the d statistics and its comparison to the lower ($d_{LOWER}=d_L$) and upper ($d_{UPPER}=d_U$) limits in the Durbin-Watson (DW) d statistics table ($d_{LOWER}=d_L$) allows deciding whether autocorrelation exists or not. The Durbin-Watson (DW) value is compared with the lower and upper critical values, d_L and d_U . If the calculated DW is lower than d_L , there is a positive autocorrelation between the error terms (DW close to 0) [22]. If the calculated DW is higher than d_U , there is not an autocorrelation (DW close to 2) or there is not a negative autocorrelation between the error terms (DW close to 4). If the calculated DW is between d_L and d_U , the test is inconclusive, i.e. autocorrelation cannot be decided [25].

RESULTS

Table 2 shows the values that were calculated using the live weights and live weight gain values of both female

and male Romanov lambs and the individual growth curves obtained with the Richard, Logistic, Gompertz and Cubic Spline models. The MSE, R^2_{adj} , AIC and DW values of the models were given both for female and male lambs. According to [Table 2](#), the model with the lowest MSE value was the Cubic Spline model for both female and male lambs, while the Richard model and Logistic model had the highest MSE values for the female and male lambs, respectively.

[Table 2](#) shows that the MSE values for the female lambs were 0.95, 0.534, 0.405 and 0.295, in the Richard, Logistic, Gompertz and Cubic Spline models, respectively, while the MSE values for the male lambs were 1.850, 2.659, 1.369 and 0.995, respectively. Moreover, the R^2_{adj} values of the female lambs were between 0.971 and 0.997, while they were between 0.969 and 0.993 for the male lambs.

Furthermore, [Table 2](#) shows the AIC and DW values in different models for both male and female lambs. As seen in the [Table 2](#), the lowest AIC value of the female lambs was -37.12 and obtained with the Cubic Spline method, while it was -122.12 in the male lambs and, again, obtained with the Cubic Spline model. The DW value was in the desired ranges for both male and female lambs and the values indicated no autocorrelation.

[Table 3](#) shows the growth parameters of the female and male lambs estimated by Richard, Logistic, Gompertz, and Cubic Spline models. The highest mean β_0 parameter

values (205.7 for female lambs and 214.1 for male lambs) were estimated by the Richard model. The β_0 parameter calculated with the Richard model was higher than those estimated with other models.

The β_1 parameter was estimated by all models used in the study and represents the ratio of live weight gain after birth to adult live weight. In male lambs, the highest β_1 parameter was obtained when the Gompertz model was used (5.012), followed by the Logistic, Cubic spline and Richard models. In female lambs, the highest value for the β_1 parameter was obtained with the Logistic model (2.223), followed by the Gompertz, Cubic Spline and Richard models.

Furthermore, the β_2 parameter that was commonly estimated by the Richard, Logistic, Gompertz and Cubic Spline growth curve models shows at what rate the live weight at age t approaches the adult live weight. In male lambs, the highest β_2 value giving information about the growth rate was obtained with the Gompertz (0.895) model, followed by the Richard (0.711), Logistic (0.0389) and Cubic Spline (-0.027) models. In female lambs, the highest maturation rate (β_2 parameter) was obtained with the Richard (0.842) model, followed by the Logistic (0.0228), Gompertz (0.018) and Cubic Spline (-0.006) models.

Moreover, [Fig. 1](#) and [Fig. 2](#) show the growth curves of different growth models for both female and male lambs. When the weight measurements of an organism that are taken during its life cycle or a certain period are adjusted

Table 2. MSE, R^2_{adj} , AIC and DW values and their standart error of the models

| Gender | Model | MSE | R^2_{adj} | AIC | DW |
|--------|--------------|-------------|-------------|--------------|-----------|
| Female | Richard | 0.950±5.143 | 0.971±0.002 | 0.094±0.31 | 2.41±0.01 |
| | Logistic | 0.534±2.215 | 0.972±0.004 | -5.41±0.05 | 2.44±0.69 |
| | Gompertz | 0.405±7.152 | 0.990±0.015 | -23.44±0.07 | 1.86±0.33 |
| | Cubic Spline | 0.295±1.915 | 0.997±0.002 | -37.12±0.01 | 2.23±0.49 |
| Male | Richard | 1.850±2.569 | 0.969±0.011 | -0.196±0.04 | 2.79±0.05 |
| | Logistic | 2.659±0.476 | 0.990±0.009 | -12.32±0.08 | 1.02±0.57 |
| | Gompertz | 1.369±8.978 | 0.986±0.002 | -21.44±0.09 | 1.58±0.59 |
| | Cubic Spline | 0.995±1.021 | 0.993±0.001 | -122.12±0.05 | 2.31±0.19 |

MSE: Mean Square Error, R^2_{adj} : Adjusted Determination Coefficient, AIC: Akaike Information Criteria, DW: Durbin-Watson Statistic

Table 3. Estimations for the parameters of the grow h functions

| Gender | Model | β_0 | β_1 | β_2 | β_3 | β_4 | Knot |
|--------|--------------|-------------|------------|-------------|-------------|-------------|------|
| Female | Richard | 205.70±0.02 | 0.005±0.05 | 0.842±1.02 | - | - | - |
| | Logistic | 33.46±2.11 | 2.223±0.15 | 0.0228±0.01 | - | - | - |
| | Gompertz | 47.79±3.09 | 1.008±0.25 | 0.018±0.22 | - | - | - |
| | Cubic Spline | 3.35±0.21 | 0.106±0.01 | -0.006±0.05 | 0.0009±0.03 | -0.002±0.01 | 75 |
| Male | Richard | 214.10±0.01 | 0.045±0.15 | 0.711±1.89 | - | - | - |
| | Logistic | 38.45±3.19 | 4.213±0.05 | 0.0389±0.89 | - | - | - |
| | Gompertz | 41.72±1.09 | 5.012±0.02 | 0.895±0.06 | - | - | - |
| | Cubic Spline | 3.11±0.01 | 0.356±0.18 | -0.027±0.01 | 0.0011±0.06 | -0.027±0.04 | 75 |

to the growth models, the resultant curves usually have an S-shape and, thus, are called sigmoidal curves. Sigmoidal curves are the best models in explaining biological growth. As seen in Fig. 1 and Fig. 2, although the distributions of the models and calculated values were close to each other until a certain period (until about the age of three months), they partially diverged from each other during the period until the age of six months.

This is also revealed by the R^2_{adj} values of the models. The R^2_{adj} values in the Richard model for both male and female lambs were especially lower than other models. As seen in the Fig. 1 and Fig. 2, the curve of the Richard model diverged from the curves of other models.

As revealed by the curves, the curve that was closest to the real value was the curve of the Cubic Spline model, indicating that the best model was the Cubic Spline model.

DISCUSSION

The study was carried out to determine the best model among four different models by using the data on the

increase in the live weights of Romanov lambs until the age of 180 days. For this purpose, MSE, DW, AIC and R^2_{adj} values were used.

In the study, four different values were primarily used to determine the best model. When the model fitness is sorted in accordance with the AIC values, the model with the lowest AIC value is accepted as "the best" model. According to the AIC, the models with an AIC value lower than 2 can be considered to have a good support [23,26].

According to the results, in females, the lowest mean square error was 0.295 ± 1.915 , the highest R^2_{adj} value was 0.997 ± 0.002 , the lowest AIC value was -37.12 ± 0.001 and DW value was 2.23 ± 0.49 and obtained with the Cubic Spline model, while, in males, the lowest MSE was 0.995 ± 1.021 , the highest R^2_{adj} value was 0.993 ± 0.001 , the lowest AIC value was -122.12 ± 0.05 and DW value was 2.31 ± 0.91 and, again, obtained with the Cubic Spline model. The results agree with the results obtained in other studies [27-31].

Sengul and Kiraz [32] reported that high R^2 values for Logistic and Gompertz models in a their study of growth curves of turkeys. Tekel et al. [33] concluded that Gompertz, Logistic

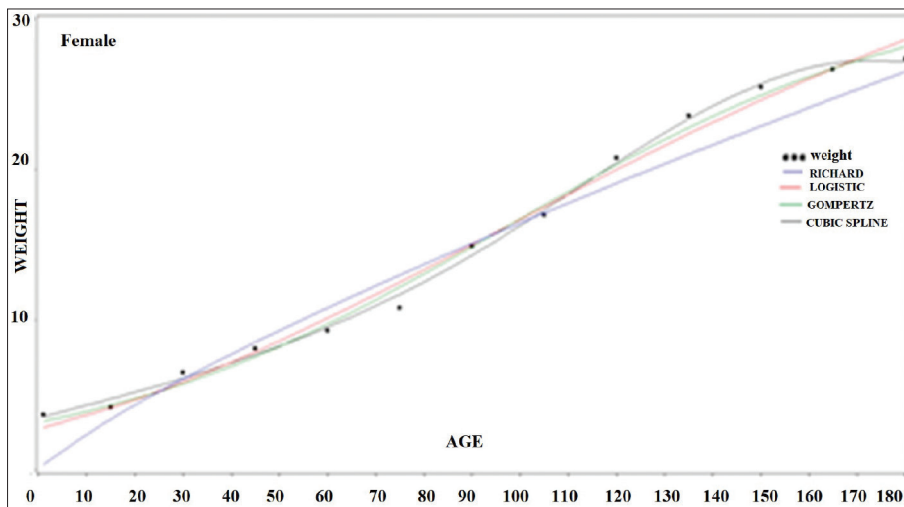
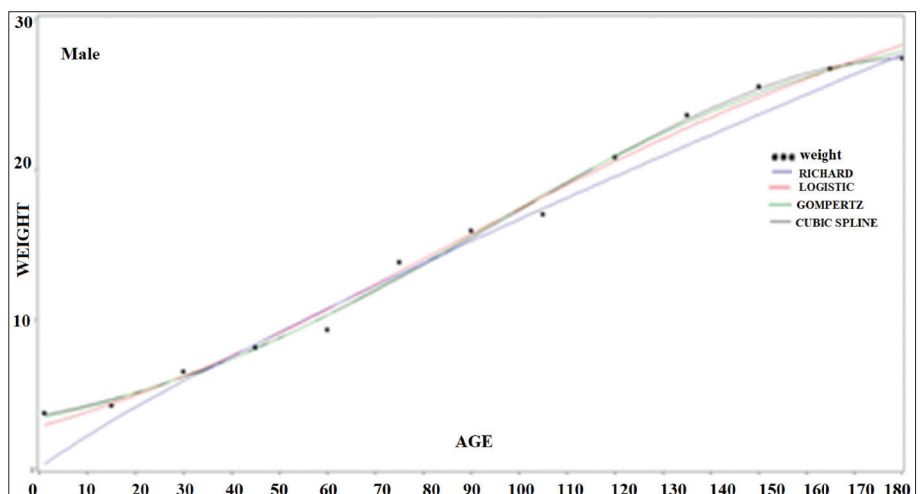


Fig 1. Growth curves of the female lambs by different growth functions

Fig 2. Growth curves of the male lambs by different growth functions



and Bertalanffy models described growth of Awassi lambs better than Brody and Negative exponential models.

Yildiz et al.^[31] used the Gompertz and Logistic models on the determination of the growth curves of Merinos x Kivircik hybrid lambs. The determination coefficient (R^2) in the Gompertz model (R^2) was 0.986 for female lambs and 0.990 for male lambs, while it was 0.982 in the Logistic model both for the male and female lambs. In a similar fashion, in this study, the adjusted determination coefficient (R^2_{adj}) in the Gompertz model was 0.990 for female lambs and 0.986 for male lambs, while the R^2_{adj} value in the logistic model was 0.972 and 0.990 for the female and male lambs, respectively.

In Aggrey's study on the determination of the growth curves of poultry with different models^[26], Aggrey used non-linear models (Gompertz, Logistic, Richard) and the Cubic Spline model. The researcher found that the determination coefficient (R^2) in the Richard and Gompertz models was 0.981 for female lambs and 0.982 for male lamb, while it was 0.978 for female lambs and 0.980 for male lambs in the Logistic model; the R^2 value in the Cubic Spline model was 0.960 and 0.964 for female and male lambs, respectively. Aggrey^[26] found that the Cubic Spline model had the lowest R^2 value, whereas the R^2 value in the Cubic Spline model was 0.997 for the females and 0.993 for the males in this study. This indicates that the growth curve models yield different results for different species, even for different breeds. On the contrary to the study carried out by Aggrey^[26], in this study, the best model for the estimation of the growth curves of Romanov lambs was determined to be the Cubic Spline model.

Aytekin and Zulkadir^[30] used the Gompertz, Logistic and Cubic Spline models to determine growth curves of Malya sheep and found that the determination coefficients were 0.915, 0.912 and 0.921 in the Gompertz, Logistic and Cubic Spline models, respectively. Their results agree with the results obtained in this study. Celikeloğlu et al.^[34] used the Gompertz and Logistic models to determine growth curves of Pirlak sheep and found that the determination coefficients were 0.950 and 0.942, respectively. These results agree with the results obtained in this study. Keskin and Dag^[35] used the Linear and Quadratic models to determine growth curves of Anatolian Merino lambs and found that the determination coefficients were 0.990 and 0.984, respectively. The fattening periods of this study and our study are similar and there is a difference between the models used.

Balan et al.^[36] used the Gompertz, Logistic and Richards models to determine growth curves of Mecheri sheep and found close R^2 values to those obtained in our study.

The study revealed that the best growth model for both male and female Romanov lambs was the Cubic Spline growth function with its lowest MSE, highest R^2_{adj} and

lowest AIC values. In addition, the Cubic Spline model attracted the attention as the model with the highest auxiliary values. DW value indicated that the Cubic Spline model did not have an autocorrelation problem.

The shape of growth curves varies depending on the species, environmental conditions and investigated property. In this case, there are certain factors the researcher should consider when attempting to obtain a model. The first factor is deriving a growth/time equation that will be used as the growth function from a differential equation and second factor is selecting biologically interpretable parameters for this equation^[4,37].

In conclusion, considering the comparison criteria for the live weight values of Romanov lambs, the best model was determined to be the Cubic Spline model and the Richard model was determined to be the least compatible model.

We would also like to point out that the growth differences due to genotype can result in the need to use different models for the fitness of growth data. Model fitness for growth curves can vary depending both on genotype and investigated property. In the fitness of growth curves, when selecting a model, emphasis should be put on the structure of the data, ease of estimation and biological interpretability of the parameters that will be estimated.

Another factor affecting model selection is the fluctuations in live weight due to age. This can stem from the physiological differences between the individuals as well as the differences between the environments. For example, factors that cause sudden changes in live weight such as reaching sexual maturity at different periods, climate, production level, diseases and stress can affect model selection and the shape of growth curves.

Furthermore, Romanov sheep's high reproductive performance, meat yield and adaptability to the geographical conditions of Turkey render it an important breed for the breeders in Turkey. In the study, the live weight values of the Romanov lambs during a six-month period were determined to be close to those of local breeds. Cross-breeding studies can increase the reproductive performance of the local breeds without decreasing their meat yield.

AUTHOR CONTRIBUTIONS

Y. Tahtali conceived the ideas of the study and writing manuscript; M. Sahin performed data collection and analysis; L. Bayyurt performed data analysis and writing manuscript

CONFLICT OF INTEREST

The authors declare no conflict of interest

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