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Choice of non-linear models to determine the growth curve of meat-type quail

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ABSTRACT: The objective of this study was to compare non-linear models fitted to the growth curves of quail to determine which model best describes their growth and check the similarity between models by analyzing parameter estimates. Weight and age data of meat-type European quail (Coturnix coturnix coturnix) of three lines were used, from an experiment in a 2 × 4 factorial arrangement in a completely randomized design, consisting of two metabolizable energy levels, four crude protein levels and six replicates. The non-linear Brody, Von Bertalanffy, Richards, Logistic and Gompertz models were used. To choose the best model, the Adjusted Coefficient of Determination, Convergence Rate, Residual Mean Square, Durbin-Watson Test, Akaike Information Criterion and Bayesian Information Criterion were applied as goodness-of-fit indicators. Cluster analysis was performed to check the similarity between models based on the mean parameter estimates. Among the studied models, Richards' was the most suitable to describe the growth curves. The Logistic and Richards models were considered similar in the analysis with no distinction of lines as well as in the analyses of Lines 1, 2 and 3. Key words: adult weight, age, cluster analysis, Logistic model, Richards model.

Escolha de modelos não-lineares para determinar a curva de crescimento de codornas de corte

RESUMO: Objetivou-se, neste estudo, comparar modelos não lineares ajustados às curvas de crescimento de codornas para determinar qual modelo que melhor descreve o crescimento de codornas e verificar a similaridade dos modelos analisando as estimativas dos parâmetros. Para as análises foram utilizados os dados peso e idade de codornas européias de corte (Coturnix coturnix) proveniente de três linhagens, em um esquema fatorial 2x4, instalado em um delineamento inteiramente casualizado, com dois níveis de energia metabolizável e quatro níveis de proteína bruta, com seis repetições. Os modelos não lineares utilizados foram: Brody, Von Bertalanffy, Richards, Logístico e Gompertz. Para a escolha do melhor modelo utilizou-se o Coeficiente de Determinação Ajustado, o Percentual de Convergência, o Quadrado Médio do Resíduo, o Teste de Durbin-Watson, o Critério de informação Akaike e o Critério de informação Bayesiano como avaliadores da qualidade do ajuste. Utilizou-se a análise de agrupamento para verificar, baseado nas estimativas médias dos parâmetros, a similaridades entre os modelos. Entre os modelos estudados, o Richard foi o mais adequado para descrever as curvas de crescimento. Os modelos Logístico e Richards foram considerados similares nas análises sem distinção de linhagem, bem como nas análises das Linhagem 1, 2 e 3. **Palavras-chave**: análise de agrupamento, idade, modelo Richards, Modelo Logístico, peso adulto.

INTRODUCTION

The quail farming activity has become increasingly popular, as the species is a valuable protein source for humans (KHOSRAVI et al., 2016). However, little is known about the growth pattern of this animal.

In a meat-type quail production system, weight-age variables are measured at pre-defined intervals, with the weight behavior analyzed over time. Non-linear regression models are recommended for this type of analysis, as they possess biologically interpretable parameters.

Non-linear models can be used to describe the growth of animals over time, making it possible to evaluate genetic and environmental factors that influence the growth curve. In this way, the growth curve can be altered through selection, i.e., by identifying animals with a faster growth rate without changing their adult weight rather than selecting increasingly large animals (SARMENTO et al., 2006).

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Much research has been done on the growth curves of sheep (SARMENTO et al., 2006; MALHADO et al., 2008; AFONSO et al., 2009; SILVEIRA et al., 2011), goats (CARNEIRO et al., 2009), cattle (MAZZINI et al., 2003; SILVA et al., 2004) and other animal species such as shrimp, frog, rabbit, chicken, goat, sheep, swine and cattle (FREITAS et al., 2005). However, few studies have looked into the growth of meat quail.

In many studies on growth curves, it is common for two or more models to generate similar estimates, with curves graphically fitted near each other. For this reason, it is necessary to know to what extent these models may or may not be considered statistically similar. An alternative to address this situation is the use of multivariate methods such as cluster analysis to group models with similar results according to their traits (variables), based on some criterion of similarity or dissimilarity (MAIA et al., 2009; SILVEIRA et al., 2011; SOUZA et al., 2013). Cluster analysis, which forms homogeneous groups based on a simultaneous evaluation of several traits of interest, can be used to group models with similar parameter estimates.

This study examined non-linear models to describe the growth curve of meat-type European quail in order to identify the model that best fits the data. Additionally, cluster analysis was employed to classify the non-linear regression models used to describe the growth curve that were considered similar given the obtained parameter estimates of each one, which were analyzed with no line distinction as well as for the three different lines of meat quail.

MATERIALS AND METHODS

The analyzed data originated from an experiment conducted in the experimental shed at the Quail Farming Unit of the Experimental Farm Professor Hélio Barbosa of the Veterinary School of the Federal University of Minas Gerais. 10,368 seven-day-old meat-type European quail (*Coturnix coturnix coturnix*) of three lines, of both sexes, were used for six experimental periods of seven days each (1-7, 8-14, 15-21, 22-28, 29-35 and 36-42 days of age).

The experiment was set up as a completely randomized design with six replicates of 12 quail per experimental unit, in a 2×4 factorial arrangement with two metabolizable energy levels (2900 and 3100 kcal ME/kg of diet) and four crude protein levels (22, 24, 26 and 28% CP), totaling 864 observations. Treatments were represented by the combinations of the factors' levels. Average quail weight was calculated at each time interval for Lines 1, 2 and 3, considering the eight treatments and six replicates.

Five non-linear models were used to estimate the quail's growth curve and the curve parameters (Table 1). To compare the growth models examined in this study, six goodness-of fit indicators were applied, as described next.

Convergence rate (C%), whereby a curve was fitted for the treatments (eight diets) in each line (three lines), for each one of the models, totaling 24 observations (N = 24). In this way, it was possible to determine which model showed the highest convergence number (n). The percentage of converging fits was calculated as follows: $C\% = \frac{n}{N} \times 100$

Adjusted coefficient of determination (R^{2}_{aj}) , which was used to compare the goodness fit of models with different numbers of parameters (p) (SILVEIRA, 2012). Its formula is given below:

$$\mathbf{R}_{aj}^{2} = \mathbf{R}^{2} \cdot \left(\frac{\mathbf{p} \cdot \mathbf{1}}{\mathbf{N} \cdot \mathbf{p}}\right) \left(\mathbf{1} \cdot \mathbf{R}^{2}\right)$$

where:

$$R^{2} = 1 - \frac{SQR}{SQT}$$
$$SQR = \sum_{i=1}^{n} (Y_{i} - \hat{Y}_{i})^{2}$$

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Table 1 - Non-linear regression models for growth curves.

Model	Growth curve model
Richards	$\boldsymbol{y}_{i} = \frac{\boldsymbol{\beta}_{1}}{\left(1 + e^{\left(\boldsymbol{\beta}_{2} - \boldsymbol{\beta}_{3} \boldsymbol{x}_{i}\right)}\right)^{\frac{1}{\beta_{4}}}} + \boldsymbol{e}_{i}$
Gompertz	$\boldsymbol{y}_i = \boldsymbol{\beta}_1 e^{\left(-e^{\left(\boldsymbol{\beta}_2 - \boldsymbol{\beta}_3 \boldsymbol{x}_i\right)\right)}} + \boldsymbol{e}_i$
Logistic	$\boldsymbol{y}_{i}=\frac{\beta_{1}}{\left(1+e^{\left(\beta_{2}-\beta_{2}\boldsymbol{x}_{i}\right)}\right)}+\boldsymbol{e}_{i}$
Brody	$\boldsymbol{y}_{i}=\beta_{1}\left(1\!-\!\beta_{2}e^{-\beta_{3}\boldsymbol{x}_{i}}\right)\!+\boldsymbol{e}_{i}$
Von Bertalanffy	$y_i = \beta_i \left(1 - \beta_2 e^{-\beta_3 x_i}\right)^3 + e_i$

 y_i : body weight at age x_i ; β_1 : weight at maturity; β_2 : integration constant without a defined biological interpretation; β_3 : maturity rate or growth speed; β_4 : parameter that shapes the curve, whose fixation determines the inflection point.

is the residual sum squares,

$$\hat{\mathbf{Y}}_i = f(\mathbf{x}_i, \hat{\mathbf{y}})$$

$$SQT = \sum_{i=1}^{n} (Y_i - \overline{Y})^2$$

is the total sum of squares, N is the number of observations used to fit the curve and

p is the number of parameters in the function, including the intercept.

Residual Mean Square (RMS), calculated by dividing the residual sum of squaresby the number of observations, which is the maximum likelihood estimator of residual variance. The RMS of the different models was compared, given the different numbers of parameters to be estimated in each model (SARMENTO et al., 2006).

The Durbin-Watson Test, which carries the name of its inventors, who created it in 1950, and remains the most important test to check autocorrelation (HILL et al., 2003). Its statistics is given by:

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

where e_t are the deviations of the regression fitted by the ordinary least squares method.

Hill et al. (2003) suggested using computer programs that calculate the P-value for the explanatory variables of the model in question. Rather than comparing the calculated *d* value to some tabulated d_c values, they propose using the computer to calculate the P-value of the test. If this P-value is lower than the specified significance level, $H_0: Q = 0$ is rejected and the existence of autocorrelation is confirmed.

However, to simplify test execution, we tabulated intervals which contain the critical value for different n (sample size) and p (number of parameters) values at the significance levels of 1% and 5% (unilateral). Their expressions, hypotheses and table are presented by HOFFMANN & VIERA (1998).

To test $H_0: \varrho = 0$ against, the *d* value is compared to d_L and d_U . If $d < d_L$, the result is significant and H_0 is rejected in favor H_A . If $d > d_U$, the result is not significant; that is H_0 is not rejected. If $d_L < d < d_U$, the result is inconclusive. To test $H_0: \varrho = 0$ against $H_A: \varrho < 0$, the *d* value is compared to $4 - d_L$ e $4 - d_U$. The result is significant if $d > 4 - d_L$ and not significant if $d < 4 - d_U$. If $4 - d_U < d < 4 - d_L$, the result is inconclusive. The result would obviously be the same if we compared $4 - d \operatorname{com} d_L$ and d_U .

Akaike's Information Criterion (AIC), which allows the use of the parsimony principle in

the choice of the best model; that is, according to this criterion, the model with the most parameters is not always the best, and lower AIC values indicate a better fit (AKAIKE, 1974). Its expression is given by: AIC = -2loglike + 2p

where p is the number of parameters and *loglike* is the logarithmic value of the likelihood function considering the parameter estimates.

Like AIC, the Bayesian Information Criterion (BIC) also takes into consideration the model's degree of parameterization "p", and, likewise, the lower the BIC value (SCHWARZ, 1978), the better the model fit. Its expression is given by: BIC = -2loglike + pln(n)

where n is the number of observations used to adjust the curve; and p is the number of parameters.

Once the convergence of all models was determined, we obtained the mean values corresponding to each goodness-of-fit indicator for each model and defined the best model for the growth curve of quail. For cluster analysis, we used the centroid method and quadratic Euclidean distance between the mean vectors-centroids-of both groups. The use of this algorithm provides a maximum number of groups corresponding to the number of individuals or units, and all these possible groups can be visualized through the construction of a graph, the dendrogram. MOJENA (1977) proposed a criterion to determine the k number of groups that optimizes the goodness of fit of data clustering. The idea is to achieve the greatest amplitude in the joining distances of the formed groups.

The non-linear regression models (Table 1) were adjusted to the quail weight-age data using the PROC MODEL procedure of SAS software (SAS Institute 2002), via the ordinary least squares method with the Gauss-Newton algorithm. After the parameter estimates were estimated for each model, multivariate data sets were formed with the models corresponding to the units and the parameter estimates corresponding to the variables. These were subjected to cluster analysis using the PROC CLUSTER procedure of SAS (SAS Institute 2002), considering the centroid method. Subsequently, the PROC TREE procedure of SAS (SAS Institute 2002) provided the dendrogram to analyze the obtained groups in relation to the parameter estimates.

RESULTS AND DISCUSSION

The average weights of the eight treatments for Lines 1, 2 and 3 (Table 2) were used to obtain

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	СР	22	24	26	28	22	24	26	28
Age (da	ays)	T1	T2	T3	T4	T5	T6	T7	T8
e 1	7	23.83	23.23	24.00	23.90	24.23	23.58	23.75	23.21
	14	63.09	68.45	73.81	73.36	65.18	68.16	68.75	71.49
	21	113.20	124.17	132.40	129.46	120.93	125.56	127.18	129.39
Line	28	165.09	175.73	187.27	182.88	176.33	179.36	178.29	182.68
Π	35	206.52	219.62	227.15	222.99	218.01	223.23	218.71	220.12
	42	257.32	263.14	264.95	261.20	257.13	263.45	257.22	259.63
e 2	7	26.22	28.69	27.30	27.31	27.28	26.51	26.52	28.24
	14	62.73	69.40	70.00	70.60	64.12	64.91	69.12	73.10
	21	115.69	127.50	128.15	131.39	116.30	126.72	127.32	132.87
Line	28	167.18	184.12	180.37	186.16	166.29	179.23	183.70	187.87
Π	35	199.17	211.06	212.41	209.95	202.22	216.85	221.57	231.48
	42	227.37	240.69	238.09	249.99	236.75	246.81	251.43	253.77
Line 3	7	25.50	25.33	25.68	25.14	26.14	25.14	24.90	26.27
	14	62.35	66.00	67.57	71.13	60.33	65.34	67.59	65.35
	21	113.22	123.38	125.74	127.29	115.17	121.42	126.81	124.48
	28	160.92	172.51	174.54	175.35	167.26	173.25	180.24	175.28
	35	207.72	219.12	214.70	214.86	218.95	215.75	223.09	219.98
	42	220.58	232.75	231.11	225.13	228.55	230.14	236.10	232.52

Table 2 - Mean weights¹ obtained per Treatment (T) in the Lines of meat-type quail.

¹Standard error = 2.6827; ME: metabolizable energy (kcal); CP: crude protein (%).

the parameter estimates of the non-linear Logistic, Gompertz, Von Bertalanffy, Brody and Richards models (Table 3). Consequently, the results of the criteria used to define the model that best describes the growth curve (Table 4) were obtained by the means of each criterion, for each model.

Oftentimes, authors choose a model to estimate the growth curve due to simplicity of interpreting and ease of determining its parameters. Other choose a model because of desirable properties of a growth function. However, the use of goodnessof-fit parameters allows for a more adequate choice of the model that best describes the growth curve.

Several authors have used some of the evaluation criteria mentioned in the present study

to define which model to use in their analyses (MAZZINI et al., 2003; SILVA et al., 2004; FREITAS 2005; GUIMARÃES et al., 2006; SARMENTO et al., 2006; MALHADO et al., 2008; MAIA et al., 2009; AFONSO et al., 2009; CARNEIRO et al., 2009; SILVEIRA et al., 2011).

Only the Logistic and Gompertz model obtained 100% convergence (Table 4) and, based on the adjusted coefficient of determination (R^2_{aj}), the Richards (99.41%), Logistic (98.75%) and Gompertz (98.70%) models best fit the data. As for RMS, the Richards model (41.46) obtained the lowest value.

In an experiment examining the growth curve of male Hereford cattle, Mazzini et al. (2003) fitted the Brody, Gompertz, Logistic, Richards and

Model	Estimate					
	$\widehat{\beta_1}$	$\widehat{\beta_2}$	$\widehat{\beta_3}$	$\widehat{eta_4}$		
Logistic	273.28 ± 14.90	13.81 ± 1.53	0.1151 ± 0.01	-		
Gompertz	344.29 ± 58.55	3.26 ± 0.23	0.0561 ± 0.01	-		
Von Bertalanffy	457.34 ± 152.34	0.70 ± 0.04	0.0350 ± 0.01	-		
Brody	2286.29 ± 826.70	0.99 ± 0.02	0.0024 ± 0.01	-		
Richards	252.56 ±21.30	$137.74 \pm 278,\! 15$	$0.1604{\pm}\ 0.01$	-0.6329 ± 0.38		

Table 3 - Mean estimates and standard error of parameters for the non-linear models for the growth of meat-type quail.

 β_1 : weight at maturity; β_2 : integration constant without a defined biological interpretation; β_3 : maturity rate or growth speed; β_4 : parameter that shapes the curve, whose fixation determines the inflection point.

Table 4 - Results of the goodness-of-fit indicators.

Model	С%	\mathbb{R}^2_{aj}	RMS	DW	AIC	BIC
Logistic	100	0.9875	89.34	2.5971	41.66	41.04
Gompertz	100	0.9870	92.58	2.4093	45.30	44.68
Von Bertalanffy	41.66	0.7240	2160.51	1.9950	52.87	52.25
Brody	4.16	0.9563	281.30	2.1260	52.70	52.07
Richards	66.66	0.9941	41.46	3.2897	39.94	39.10

C%: Convergence rate; R²_{aj}: Adjusted Coefficient of Determination; RMS: Residual Mean Square; DW: Durbin-Watson Test; AIC: Akaike's Information Criterion; BIC: Bayesian Information Criterion.

Von Bertalanffy functions and compared them in terms of Convergence Rate, Mean Residual Square, Adjusted Coefficient of Determination, Mean Prediction Error and Student's t test. The researchers concluded that the best-fitting functions were the Von Bertalanffy and Gompertz, followed by the Logistic function. The Brody and Richards functions, in turn, showed low convergence rates (23.16% and 46.25%, respectively), indicating their unsuitability to describe the studied data. The same convergence problem was reported in the present study. Additionally, the Brody function overestimated weight at maturity, corroborating the findings of MAZZINI et al. (2003).

When compared to the tabulated values at the 1% significance model, results of the DW test (Table 4) for the models with three parameters (n = 6, $d_U \approx 1,46$) and for the model with four parameters ($n = 6, d_U \approx 1,70$) revealed that there is no autocorrelation between the regression deviations.

Although, the Von Bertalanffy model obtained the lowest value (1.9950) in the DW test (Table 4), it validated the decision for non-rejection of H_0 , i.e., it confirmed that the errors are independent. However, it did not satisfactorily meet the other measures of goodness of fit, namely, convergence lower than 50% (41.66%); higher RMS (2160.51); and higher AIC (52.87) and BIC (52.25). Results of the DW test and of the Logistic, Gompertz, Brody and Richards models also confirmed the independence of errors.

When the models were compared based on the AIC and BIC criteria, the Logistic (41.66; 41.04) and Richards (39.94; 39.10) models obtained the lowest respective values (Table 4).

The Logistic and Richards models disagreed only for RMS (89.34 and 41.46, respectively) and %C (100% and 66.66%, respectively). The Richards model had the best result for RMS, whereas the Logistic model was superior for %C (Table 4). Although, Richards model was penalized by the parsimony principle due to four parameters, which interfered in the interactions; consequently, leading to a lower convergence rate, it presented the lowest AIC and BIC values.

In a study on growth curves, FREITAS (2005) fitted the Brody, Gompertz, Logistic, Richards and Von Bertalanffy models to weight-age data of eight animal species (shrimp, frog, rabbit, chicken, goat, sheep, swine and cattle) considering convergence, the coefficient of determination and the biological interpretability of parameters as criteria for fit evaluation. The Logistic, followed by the Von Bertalanffy model, was the most versatile to fit the growth data of the studied animal species, whereas the Richards model exhibited computational difficulties and was thus not suitable to represent the growth curve.

MALHADO et al. (2008) analyzed those non-linear models to describe the growth of Santa Inês × Texel crossbred sheep adopting the Residual Mean Square; Converge Rate; and graphic analysis of the models with the observed average weight as the criteria to select the best model. The authors concluded that the Logistic model was the most suitable for modeling animal growth in the studied period, as it precisely estimated their growth according to the graphic analysis. Although the Gompertz and Von Bertalanffy models showed the best fit, they overestimated growth. CARNEIRO et al. (2009) used the same goodness-of-fit indicators as well as the Absolute Mean Residual Deviation and reported that the Logistic model showed the best fit in describing the growth curve.

MAIA et al. (2009) used the Brody, Gompertz, Logistic and Von Bertalanffy models and RMS, %C, BIC and coefficient of determination as goodness-of-fit indicators and reported that the Logistic model showed the best goodness of fit for all indicators, to describe the growth curve of banana tree.

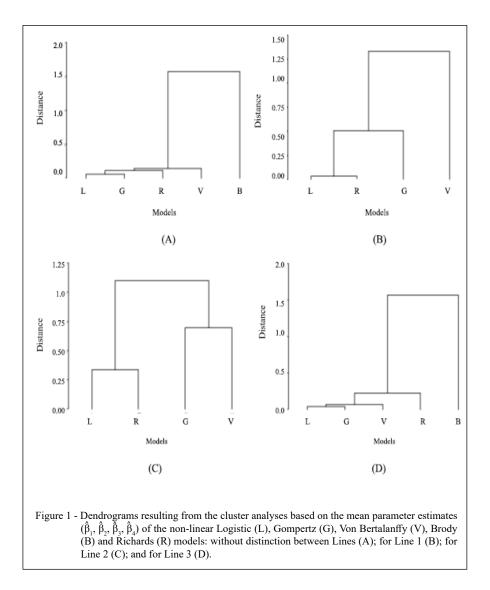
DRUMOND (2013) used the asymptotic index, which takes into consideration the Coefficient of Determination, Asymptotic Standard Deviation and Absolute Mean Residual Deviation as goodness-of-fit indicators, and recommended the Logistic model as the best model to describe the growth curve of quail.

SARMENTO et. al. (2006) studied the growth curve of Santa Inês sheep and observed that the differences between the Von Bertalanffy, Gompertz and Richards models were small, which suggested they satisfactorily fit the growth data.

The higher the number of criteria adopted, the more reliable is the indication of the best models. Conversely, when a high number of indicators was used, model choice may become a complex process (SILVEIRA et al., 2011). The use of cluster techniques based on the means of each indicator facilitates the identification of the best models (SILVEIRA et al., 2012).

Based on the results of cluster analysis, a dendrogram was formed for the mean parameter estimates of the models without distinctions between lines and for Lines 1, 2 and 3 (Figure 1 - A, B, C and D). In these results, the greatest amplitude of distances was noted between the juncture points given in the migration from two groups to one group. Thus, the suggestion was to choose k = 2 groups of non-linear models.

For the models with no distinction between lines (Figure 1A), one group was formed only with the Brody (B) model, and another with other models (Logistic, Gompertz, Richards and Von Bertalanffy).



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In the dendrogram corresponding to Line 1 (Figure 1B), one group was formed by the Von Bertalanffy model only, whereas the second group contained the Logistic, Richards and Gompertz models. In the dendrogram referring to Line 2 (Figure 1C), one group was formed by the Gompertz and Von Bertalanffy models and the second group was composed of the Logistic and Richards models. In the dendrogram of Line 3 (Figure 1D), one group contained only by the Brod model, while the second group was formed by the Logistic, Gompertz, Von Bertalanffy and Richards models. The last dendrogram was similar to the dendrogram resulting from the mean parameter estimates of the non-linear models without distinction of lines (Figure 1A). The Logistic, Gompertz and Richards models were considered similar in the analyses of Lines 1 and 2 and in the analysis without line distinctions. However, in the analysis of Line 2, only the Logistic and Richards models were similar.

Goodness-of-fit indicators are important as an objective criterion for the choice of the model that best fits the data. However, the combination of multivariate analysis (cluster analysis) helps to identify the models which are most similar in describing animal growth, based on the parameter estimates.

In situations in which models are similar in relation to the parameters and the model with the best fit does not show the highest convergence rate, the choice of the most suitable model is up to the researcher, who should prioritize which one best describes growth or represents the growth of the largest number of animals (TEIXEIRA NETO et al., 2016).

In the present study, the Logistic and Richards models were similar in cluster analysis. Although, the Richards model showed lower convergence than the Logistic model, the former exhibited better results for the goodness-of-fit indicators. Nevertheless, considering that adult weight in quail is usually approximately 260 g (MÓRI et al., 2005), and comparing the models considering the intervals by the standard error of the estimates, the Logistic (258.38 $\leq \beta_1 \leq 288.18$) and Richards models (231.26 $\leq \beta_1 \leq 273.86$) were similar to estimate for adult weight and Gompertz (285.84 $\leq \beta_1 \leq 402.84$), Von Bertalanffy (305.00 $\leq \beta_1 \leq 609.68$) and Brody models (1459.59 $\leq \beta_1 \leq 3112.99$) overestimated adult weight (Table 3).

SOUZA et al. (2013) investigated alternative non-linear models for the study of growth in Morada Nova sheep. The authors undertook a multivariate classification based on cluster analysis, using goodness-of-fit indicators, and reported that Meloun I and Meloun IV were the models that best represented animal growth.

TEIXEIRA NETO (2016) used eight linear models in the study of the growth of Santa Inês sheep and concluded, via cluster analysis, that the Mitscherlich, Meloum I and Brody models are suitable and equivalent to describe growth. The Brody model was chosen, as it showed slightly better values for the goodness-of-fit indicators.

SILVEIRA et al. (2011) used cluster analysis to classify non-linear regression models to describe the growth curve of crossbred sheep considering the results of different goodness-of fit indicators. The analysis indicated the Richards model as the most suitable to describe the growth curve of the three evaluated gene pools.

CONCLUSION

The non-linear Logistic and Richards models showed the best results for the adopted evaluation criteria. However, the Richards model is recommended to describe the growth curve of quail, and the use of non-linear models associated with multivariate classification, via cluster analysis, showed to be suitable for use in studies of growth curves of meat quail. The Logistic and Richards models were considered similar in all analyses; therefore, their use is recommended for this species.

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DECLARATION OF CONFLICTS OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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