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Cross-sectional and longitudinal method for describing growth curve of rabbits

[*Método transversal e longitudinal para descrição da curva de crescimento de coelhos*]

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ABSTRACT

Rabbit farming is an activity with high growth potential due to its easy handling, high prolificacy, low polluting impact, and easy adaptability to family farming systems, producing meat of high biological value. Therefore, the aim of this work was to evaluate, using von Bertalanffy's nonlinear model, growth curves of weight as a function of age in ‘Flemish Giant Rabbits’ and ‘New Zealand White’ crossbred rabbits. Two different data collections were used: the longitudinal method and the cross-sectional method. The experiment was carried out at the Federal University of Lavras, located in the municipality of Lavras, Minas Gerais, Brazil, where 10 crossbred rabbits were evaluated, and animals were weighed from 0 to 150 days of age. Both methods proved to be adequate to describe the development of rabbits and the cross-sectional method proved to be an adequate alternative to obtention of growth curves, saving time in data collection and showing consistent estimates.

Keywords: growth curves, rabbit farming, regression, modeling, von Bertalanffy

RESUMO

A cunicultura é uma atividade com alto potencial de crescimento devido à facilidade em seu manejo e à alta prolificidade, por apresentar baixo impacto poluidor, por se enquadrar bem em sistemas próprios de agricultura familiar, além de produzir carne de alto valor biológico. Diante disso, o objetivo deste trabalho foi avaliar, por meio do modelo não linear de von Bertalanffy, curvas de crescimento de coelhos mestiços de Gigante de Flandres e Nova Zelândia Branco, utilizando-se dois métodos distintos da coleta dos dados: o método longitudinal e o método transversal, a fim de estimar o crescimento do peso em função da idade. O experimento foi realizado na Universidade Federal de Lavras, situado no município de Lavras, Minas Gerais, Brasil. Foram avaliados 10 coelhos mestiços, cuja pesagem foi realizada de 0 a 150 dias de idade. Os dois métodos se mostraram adequados para descrever o crescimento de coelhos, e o método transversal se revelou uma boa alternativa, com ganho de tempo na coleta dos dados e apresentando estimativas consistentes.

Palavras-chave: curvas de crescimento, regressão, modelagem, von Bertalanffy

INTRODUCTION

Appropriate rabbit breeding management it is very important, because it is related to the growth. Rabbits have a high growth rate (Marín-García *et al*., 2020), and in the post-weaning phase (from the 28th day of age) is the time when the greatest influence of daily management occurs, which is directly correlated with animal breed, food quality, health and thermal environment in which the animals will be submitted throughout the breeding period (Ferreira *et al*., 2012).

Thus, it is important to evaluate methods that can model the growth traits of these animals in view of the importance of management in rabbit in

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cages. In addition, the use of new technologies that enable increasing the rabbit selection accuracy and reducing intervals between generations are of great value to maximize production, avoiding economic losses (Migdal *et al*., 2018; Petrescu and Petrescu-Mag, 2018).

The von Bertalanffy's nonlinear model is widely used in several areas when a functional relationship of a response variable with one or more explanatory variables is searched (Bertalanffy, 1957; Mischan and Pinho, 2014; Lee *et al*., 2020). Thus, the use of this model for the study of natural phenomena is appropriate, especially when it comes to animal growth, since these events do not always behave in a linear manner and depend on multiple factors, ranging from diet composition to social classification of animals. This model also has the advantage of presenting good adjustment quality with small number of parameters with biological interpretations (Kühleitner *et al*., 2019; Brunner *et al*., 2021). According to Teleken *et al*. (2017), the von Bertalanffy's model was the most suitable among all others to describe the growth of 'Nova Zelândia' rabbits.

The evaluation of the animal growth and development process, measured by the increase in morphometric measurements over time, is fundamental for the proper management of the species, allowing the establishment of strategies to enhance or mitigate certain traits of animals under study (Teleken *et al*., 2017; Fernandes *et al*., 2019).

Commonly, studies of animal growth curves are performed using the longitudinal method, which seeks to monitor its evolution over time. However, some researchers have used the cross-sectional method, which uses similar animals at different times, for example, animals of the same breed raised in the same environment (Ribeiro *et al*. 2018; Souza *et al*., 2019). The von Bertalanffy model is also used for plants growth, and in some cases, the cross-sectional data methodology is used when it is not possible to use longitudinal data, especially in dry matter and drying studies (Macedo *et al.*, 2017).

The use of the cross-sectional method is recommended to solve problems when the researcher does not have the time that the animal species under study takes to reach the end of the growth phase. However, despite having some losses in the analysis such as loss of continuity when not following the same animal over time, which theoretically could result in increase in weight variability over the ages, this methodology brings the gain of rapid data collection. However, studies comparing methodologies using rabbit's data are still scarce.

Some researchers have already used the cross-sectional method as a substitute for the longitudinal method, as can be seen in the works by Ribeiro *et al*. (2018) and Souza *et al*. (2019) for 'Mangalarga Marchador' horses. Thus, for this type of growth curve analysis, comparing results using different methods is plausible, since the cross-sectional method may be a possible alternative for this type of study.

To validate the analysis, Popper (1972) proposed the application of the hypothetical deductive method, which states that in the construction and / or validation of a theory, one must seek a scientific hypothesis using tests to verify whether this hypothesis may or may not be validated. This non-exclusive theory is accepted in the scientific community and is known as conjectural science.

Given the above, the aim of this study was to evaluate the results of longitudinal and cross-sectional methodology using the von Bertalanffy's model in the description of the growth curve of crossbred rabbits.

MATERIAL ANS METHODS

Rabbits used in this experiment came from the didactic collection of the Rabbit Farming Sector of the Department of Zootechnics - Federal University of Lavras (DZO / UFLA), located in the municipality of Lavras, Minas Gerais, Brazil. Ten 'Gigante de Flandres' (GF) x 'Nova Zelândia Branco' (NZB) crossbred rabbits were evaluated, where GF is the paternal line and NZB the maternal line. Animals were weighted from birth to 150 days of age, and weights were collected on random days. The experiment was conducted between October 2017 and March 2018. The research project was previously submitted and authorized by the Ethics Committee in the use of animals of the Federal University of Lavras, under protocol number 022/17.

Rabbits were weaned at 30 days of age and subsequently placed in groups of 5 animals per cage until 90 days of age, after which, they were rehoused in individual cages until the end of the experimental period. Cages are built with galvanized wire with dimensions of 80 cm in length x 60cm in width x 40cm in height. During the experimental period, rabbits received pelleted feed with 16% crude protein and free water distributed by nipple-type drinkers. Weighing of animals always took place in the morning before feeding, so that the weight was not influenced by food intake and was always measured by two people throughout the experiment.

The longitudinal method considered the mean value, in each age, of the weight of 10 crossbred rabbits divided into classes, for this method, the standard deviation of the weight of rabbits in each age class was also calculated. For the cross-sectional method, a random draw was carried out in which the individual weight of one rabbit in a given time *t* was used, simulating a situation in which the researcher arrives at the breeding site and measures the weights of several animals of different ages at the same time. Despite considering the weight of only one rabbit there is no loss of generalities since a random draw was carried out, so this unit value could also come from an average. Data can be seen in Table 1.

Table 1. Data on the weight of crossbred rabbits over time, with weight (average) in grams used in the longitudinal method and weight used in the cross-sectional method, identifying the number of the drawn rabbit

Class (Days)	Weight (average)	Standard deviation	Days (*t*)	Weight	Rabbit
0	57.33	4.8442	0	52	8
1-7	88.70	25.6540	4	92	10
8-14	178.10	40.5435	9	172	1
15-21	245.65	44.1018	17	170	5
22-28	309.55	64.1137	24	382	9
29-35	487.48	124.6204	30	478	2
36-42	719.10	155.3525	37	610	4
43-49	1019.80	181.3851	45	702	5
50-56	1335.90	188.6078	52	1180	7
57-63	1577.77	191.4398	59	1744	9
64-70	1906.35	171.4321	67	2104	10
71-77	2170.93	179.8482	75	2268	2
78-84	2400.08	198.4010	82	2423	1
85-91	2699.07	192.4267	89	2804	3
92-98	2826.07	208.3235	96	3116	4
99-105	2988.25	233.8523	103	3228	1
106-112	3128.45	226.5597	111	2848	6
113-119	3195.60	213.1326	119	2917	8
120-126	3261.90	205.5989	123	3230	10
127-133	3376.85	230.8545	129	3367	9
134-140	3423.15	194.5489	135	3494	1
141-147	3544.50	217.5053	145	3760	3
148-154	3594.50	215.9965	150	3650	7

Animal growth can be considered a result expressed by equation 1:

$$\frac{dP}{dt} = \alpha P^m + \beta P \quad (1)$$

where P represents the weight of the animal as a function of time t; α and β are anabolism and catabolism constants, respectively, and m is an exponent that indicates the last values proportional to some body weight power P (Bertalanffy, 1957; Fernandes *et al*., 2020).

The allometric coefficient m is directly linked to the pattern of animal development and the correct use of this value allows better estimates for the study of growth curves; therefore, $m = 3/4$ was used, which was the most suitable for this type of study (Shi *et al*., 2014; Fernandes *et al*., 2019).

The parameterization used will be as follows:

$$y_i = a\left(1 - \frac{exp(k \times (b - x_i))}{4}\right)^4 + \varepsilon_i \quad (2)$$

for $i = 1, 2, \ldots, n$, in which: y_i is the i-th observation of the dependent variable , x_i is the i-th observation of the dependent variable; a is the asymptotic value, that is, the expected value for the maximum growth of the object under study, when $x_i \rightarrow \infty$; b is associated with the abscissa of the inflection point; k is a maturity or precocity index associated with growth, and the higher its value, the less time it will take for the object under study to reach the asymptotic value a; ε_i is the random error associated with the i-th observation which is assumed to be independent and identically distributed following a normal distribution of zero mean and constant variance, that is, $\varepsilon_i \sim N(0, I\sigma^2)$.

In the von Bertalanffy's model, the inflection or maximum point occurs at $y_i = 8a/27 \approx 0{,}30a$, indicating that it occurs at approximately 30% of the maximum horizontal asymptote value. To find the maximum point, the first derivative of the model was performed, and the 95% confidence interval (CI) was evaluated to verify whether there are significant differences between points found in each method.

The von Bertalanffy model was initially adjusted for each longitudinal and cross-sectional methodology. The model parameters were estimated to describe the growth curves of crossbred rabbits. The estimates of these parameters were obtained by the Gauss-Newton iterative method implemented in the nls () function of the R software (R Core Team, 2020). The significance of parameters ($\beta_i \neq 0$) was verified using the t test at 5% level (P<0.05). Initially, it was considered that all assumptions about errors (ε) were met.

From the error vector of this adjustment, residual analysis was performed based on statistical tests using the Shapiro-Wilk (SW), Durbin-Watson (DW) and Breusch-Pagan (BP) statistical tests to verify normality, independence, and residual homoscedasticity, respectively. If any of the assumptions is not met, the deviation must be corrected or incorporated into the parameter estimation process.

If the DW test is significant (P <0.05) for the longitudinal method, a new adjustment will be made using the generalized least squares method, incorporating a first-order autoregressive parameter (ϕ) - AR (1) into the model. In this case, the error of model expression is given by: $\varepsilon_i = \phi_1 \varepsilon_{i-1} + u_i$. The verification of the independence assumption after the inclusion of the AR (1) will be done through graphical analysis.

The 95% confidence interval for the model parameter βi is calculated as follows:

$$IC(\beta i) : bi \pm t(v; 025) \times S(bi)$$

Where: bi is the estimate for the parameter (βi); $S(bi)$ is the standard error of the estimate and $t(v; 025)$ is the upper quantile of the student's t distribution, considering $\alpha = 5\%$ and the degree of freedom $v = n - p$.

The quality evaluators used were:

Determination coefficient:

$$R^2 = \left(1 - \frac{SQR}{SQT}\right) \quad (3)$$

Standard error of the mean:

$$SEM = \left(\frac{\sigma}{\sqrt{n}}\right) \quad (4)$$

Residual standard error:

$$RSE = \sqrt{\frac{\sum_{i=1}^{n}(Y-\hat{Y})^2}{n-p}} \qquad (5)$$

where: SQR is the square sum of residuals; SQT is the total square sum; n is the sample size; σ is sample standard deviation; $\hat{Y}$ is the regressor variable estimated by the model; Y is the observed variable; p is the number of parameters. These evaluators were obtained using the following functions *Rsq ( )* in the R software. Thus, the most suitable model is the one that presents the highest R^2 value and the lowest SEM value.

RESULTS

When analyzing the results obtained by the Shapiro-Wilk (SW) and Breusch-Pagan (BP) tests considering 5% significance level, it was found that results were not significant (P>0.05), that is, the estimated model did not violate normality and homogeneity assumptions.

For the longitudinal methodology, residual dependence was assessed by the Durbin-Watson test (DW) at 5% significance level. For the cross-sectional method, this assumption of residual dependence was not evaluated, since the observations are obtained from different animals and the weight of the rabbit at a given time t will not depend on the weight of the rabbit at the previous time t-1.

The DW test proved to be significant (P < 0.05) for the longitudinal method, indicating that residues showed dependence; therefore, a new adjustment was made using the generalized least squares method, incorporating a first-order autoregressive parameter (ϕ) into the model - AR (1).

After the inclusion of AR (1), a new analysis of residues was performed to verify if the independence assumption was met. The graph of the partial autocorrelation function of adjustment residuals (Figure 1) demonstrated that only the first-order autoregressive parameter (ϕ) was sufficient to model the residual dependence, thus ensuring greater precision of estimates (Jane *et al*., 2020).

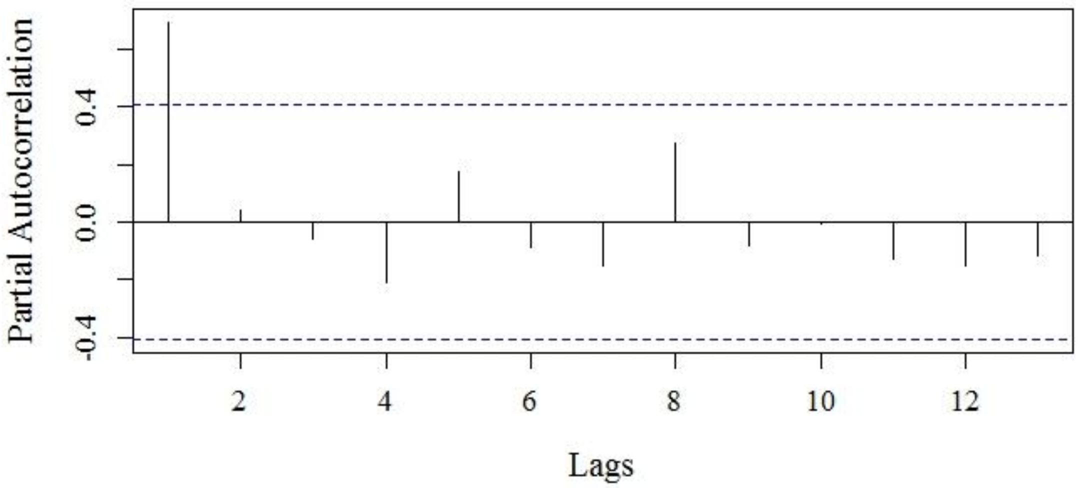

Figure 1. Graph of the partial autocorrelation function of residuals for the adjustment of the von Bertalanffy's model adjusted to the growth of crossbred rabbits using the longitudinal method.

The adjustments of methodologies under study using the von Bertalanffy's model all have significant parameters, by the test *t*, at 5% significance for weight as a function of age and can be seen in Table 2.

Table 2. Estimates of parameters with respective 95% confidence intervals (CI) for the adjustment of the von Bertalanffy's model to the growth data of crossbred rabbits and the adjustment quality evaluators for each method under study

Method	Parameters	Estimate	CI	R^2	SEM	RSE
Longitudinal	*a*	3809.4950	[3522.65; 4096.34]	0.9951	181.6349	109.96
	b	52.7677	[48.71; 56.82]			
	k	0.0274	[0.02; 0.03]			
	ϕ	0.9217	[0.4333; 0.9916]			
Cross-sectional	*a*	3770.0000	[3481.30; 4198.42]	0.9852	212.0352	179.90
	b	50.1800	[46.32; 54.41]			
	k	0.0296	[0.02; 0.04]			

Determination coefficient values R^2 were greater than 0.98, indicating satisfactory adjustments (Table 2). However, it was possible to observe greater residual standard error (RSE) in data when using the cross-sectional methodology in comparison with the longitudinal methodology, with values of 179.90 and 109.96 respectively (Table 2). Thus, it could be concluded that there is greater distance (variability) from values observed in relation to curve estimated using the longitudinal method, this is corroborated when analyzing *SEM*.

In this study, it was observed that for both methodologies, the von Bertalanffy's model underestimated the weight of rabbits in the first 30 days of life, and values estimated by the longitudinal method showed slight superiority in relation to values estimated by the cross-sectional method (Figure 2).

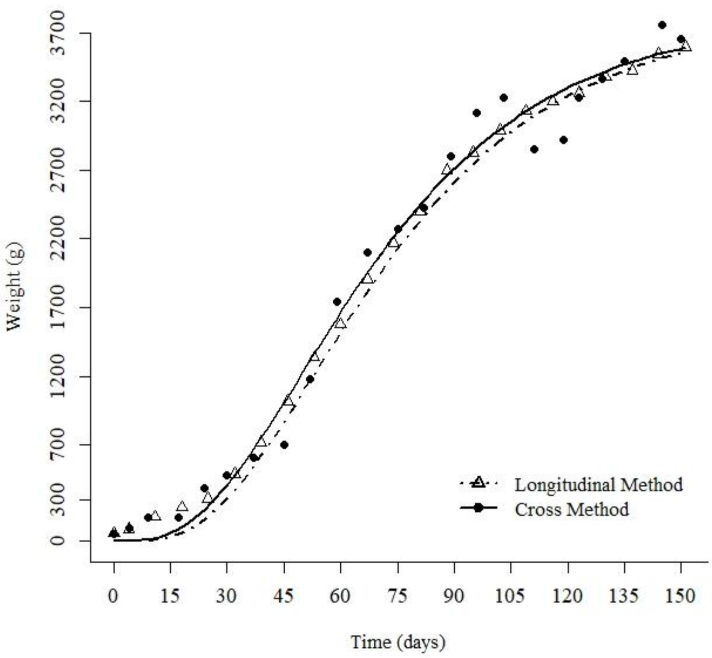

Figure 2. Growth curves observed using the von Bertalanffy's model using longitudinal and cross-sectional methods in crossbred rabbits.

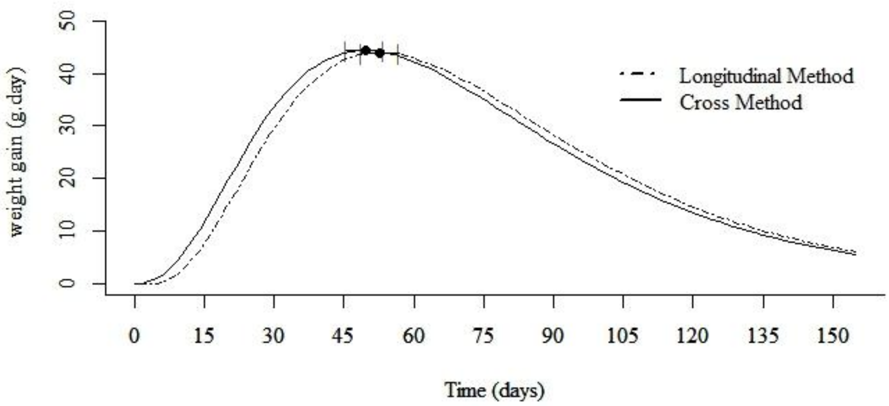

Figure 3. Graph of the daily growth rate in grams per day and the 95% CI for the maximum growth point using the cross-sectional and longitudinal method with von Bertalanffy's model for crossbred rabbits.

Figure 3 shows the point of maximum growth (inflection point), through the first derivative of the von Bertalanffy model, with the respective CI for both methods: longitudinal and cross-sectional.

DISCUSSION

Adding a first-order autoregressive parameter (ϕ) to model residuals was necessary to model this autocorrelation and incorporate it into the model, so that data adjustments produce more accurate predictions and allow analyses of series measured in a few temporal observations, thus ensuring greater precision of estimates and better adjustment quality (Fernandes *et al*., 2019). Silva *et al*. (2021b) mention in his paper that incorporating residual dependence does not necessarily improve the quality of fit, but it is the most coherent, since the assumption of residual independence was not met.

According to Table 2, through parameter a, the estimated value for the maximum weight of crossbred rabbits did not change significantly when the data collection methodology was changed, as there is an intersection in the confidence interval. Point estimates were 3,809.49 grams for the longitudinal methodology and 3770.00 grams for the cross-sectional methodology, in addition, to estimate values near in according with of rabbit's weights at 150 days age.

Weight gain control for this type of production is of paramount importance, as this performance index allows measuring the efficiency of the handling method used in rabbit breeding, consequently leading to improvement in carcass quality and yield (Sampaio *et al*., 2003; Petrescu and Petrescu-Mag, 2018).

Rabbit has meat with excellent nutritional and dietary properties, with high nutritional value and easy digestibility, being recommended even for children and older adults (Migdal *et al*., 2018; Hernández and Zotte, 2020). In rabbit farming, from the rearing of cubs, producers can increase profit margin according to the purpose and destination of production to be marketed. But, for this to occur adequately and for maximum return to be achieved, correct management of animals is required (Silva *et al*., 2021a).

Several authors like Fradinho *et al*. (2016), Brunner *et al*. (2021) and Teixeira *et al*. (2021) showed in their studies the great utility of the parameters of nonlinear models possess practical or biological interpretation in animal growth analyses. Particularly, in the study by Teixeira *et al*. (2021) the cross-sectional data collection method was also used to describe the growth of Campolina horses. These authors claim that cross-sectional data collection together with the use of non-linear models proved to be efficient for the description of the animal growth curve.

Thus, a great advantage of the parameterization used in the von Bertalanffy's model is related to parameter *b*, since its estimates are directly interpreted as the abscissa of the inflection point, that is, the aforementioned parameter represents the maximum growth point, and after this moment, the animal continues to grow, but at a lesser pace, until it reaches adult weight (Fernandes *et al*., 2019).

Therefore, for rabbits aged 45-60 days, their growth is more accentuated, and, after this period, it will reduce until reaching stabilization, it is observed that there is an intersection between the CIs of the inflection points (parameter b), indicating the suitability of the transversal method, which can also be seen in Figure 3. Possibly, the accentuated growth observed between the ages of 45 and 60 days is because animals already produce all the digestive enzymes necessary for the digestion and absorption of nutrients from diet and reduction of diarrhea and stress of starting a new life without the mother (Jaruche, 2013).

However, after 70 days of age, the growth of rabbits tends to reduce, since from that age, rabbits enter the puberty phase and their growth is slowed down (Ferreira *et al*., 2012).

Jacob *et al*. (2015) evaluated 'Chinchila Soviética' and 'Gigante Branco' breeds and concluded that the von Bertalanffy's model described the average weekly growth of rabbits of both breeds, being effective for regulating regimes and feeding management to improve productivity and the overall profitability of rabbits.

The maximum growth point was evaluated for both methodologies using the first derivative of the model under study (Figure 3) and when evaluating CI, it was found that there were no significant differences (P<0.05) between points found in each method, since intervals have intersection. This indicates that on the 50^{th} day of life (approximately), the greatest weight gain occurs and the data collection method did not influence this estimate. As in the study by Jacob *et al*. (2015), weight gain percentage decreased after the maximum growth point with increasing age.

Figure 3 shows that rabbits have greater weight gain when they are between 35 and 80 days old, with average daily weight gain (GMD) of approximately 40 g day^{-1}. This result corroborates the studies available in the literature, being established that occurs slaughter of these animals between 75 and 80 days old. (Machado and Ferreira, 2011; Ferreira *et al*., 2012; Jaruche, 2013).

According to results, the cross-sectional method, despite not being commonly used and presenting greater data variability in relation to the adjusted curve, proved to be a good alternative for describing the growth of crossbred rabbits. This method can be recommended when there is need for rapid assessment of animal growth to assist in nutrition and management.

Thus, using efficient techniques to describe the growth curve throughout the productive life of rabbits is fundamental in establishing the productive potential of a herd that can directly influence the final production results (Rebollar *et al*., 2009; Szendro *et al*., 2012). Aiming at commercial production, modern rabbit production seeks earlier, more prolific, productive, and resistant animals, so that they can be slaughtered earlier, with higher carcass yield, optimizing feeding costs (which corresponds to 70% of the total production cost), without violating environmental and animal welfare precepts (Ferreira *et al*., 2012).

CONCLUSION

The von Bertalanffy's model proved to be suitable for evaluating the growth of crossbred rabbits using both the longitudinal and cross-sectional methods. The longitudinal method presents better adjustment quality evaluators, since data show less variability of values observed with the adjusted curve. The cross-sectional method proved to be a good alternative for evaluating the growth of crossbred rabbits, and this type of analysis, in addition to saving time in data collection, provides model estimates and the maximum growth point statistically equal to the longitudinal method, with 95% confidence.

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