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Sample size and power calculations for body weight in beef cattle

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Abstract

Estimates of minimum sample sizes are calculated in order to test differences in rates of changes over time for longitudinal designs. In this study, body weight of crossbred beef cattle, considering 14 measurements on individuals, taken at birth, weaning (7 months of age) and monthly from 8 to 19 months of age, were analyzed by an usual mixed model for repeated measures. The number of individuals n required to detect significant differences (δ) between any two consecutive measurements on the individual, was obtained by a SAS program considering a t -variate normal distribution ($t = 14$), sample variance-covariance matrix among the repeated measures, F -distribution with noncentrality parameter, type I error (α), power test ($1-\beta$) and minimum correlation between repeated measures. Figures showing the n estimated as function of number of measurements on the individual, α (0.01 and 0.05); power of test (0.80 and 0.90); minimum correlation (0, 0.2, 0.4 and 0.6) and δ (1.0 standard deviation, 1.5 standard deviation and 2.0 standard deviation) are presented. Keywords: Longitudinal data, power calculation, repeated measures, sample size estimate.

Introduction

Animal production has been experimented considerable genetic improvement advancement in some performance traits. In order to obtain significant improvement of the animal breeding in future programs, may require molecular marker-assisted selection, which require a identification of candidate genes or anonymous genetic markers associated with the traits of economic interest. The use of candidate genes has been proposed with the objective of directly searching for QTL (Quantitative Trait Loci).

The polymorphism for growth hormone (GH) gene has been associated with growth traits (Rocha et al., 1992; Unanian et al., 2000), carcass composition and meat quality (Taylor et al., 1998). Polymorphisms of kappa-casein (κ -Cas) and beta-lactoglobulin (β -Lac) have been associated with growth traits (Moody et al., 1996). The influence of candidate genes on performance traits of beef cattle generally has been analyzed considering each trait individually, for example body weight at weaning or body weight at doze months of age, which is a difficult approach for detecting significant effects. The mainly reason for non detection of significance among treatments in studies of candidate genes or molecular markers associate with QTL, it is due to high cost of laboratory analyses, which may be imply in reduced sample size. Another reason is that the influence of candidate genes on performance traits of cattle may manifest later in life of individual. Paz et al. (2003a; 2003b) analyzed the effects of polymorphism of κ -Cas, GH and β -Lac on growth curve of three beef cattle crosses: $\frac{1}{2}$ Canchim-Nellore, $\frac{1}{2}$ Angus-Nellore and $\frac{1}{2}$ Simmental-Nellore and concluded that major differences started at 12-13 months of age. An more efficient alternative for analyzing body weight data, when several measurements are taken in the same individual, is to consider repeated measurements (Little et al. 1996, 1998; Reiezigel, 1999). The power of any repeated measurements (RM) analysis can be enhanced by estimating sample size that considering simultaneously the type I error (α), power test ($1-\beta$) and minimum correlation between repeated measures (Vonesh, 1983; Vonesh and Schork, 1986; Guo and Johnson, 1996; Arndt et al., 2000; Foster 2001). An important contribution of sample size in RM was given by Freitas et al. (1999) for scrotal circumference of Nellore cattle.

The estimate of sample size in repeated measurement studies can help to researchers to solve a important question (Arndt et al., 2000): "Do I gain more statistical precision by adding individuals or by adding additional follow-up measurements?"

The purpose of this study was to estimate minimum sample size required for evaluate the influence of candidate genes (GH, κ -Cas and β -Lac) on body weight in crossbred beef cattle, considering 14 measurements by individual, taken from birth to 19 months of age, analyzed as repeated measurements.

Material and methods

This study used data collected on 213 individuals (75 ½Canchim-Nellore, 74 ½Angus-Nellore and 64 ½Simmental-Nellore) born in 1998 and 1999 in Southeast Brazil. The data considered as RM were 14 measurements of weight collected at birth, weaning (7 months of age) and monthly from 8 to 19 months of age.

The model used to describe the data in order to determine the sample size n , was the standard model for repeated measure (Little et al., 1998): $y_{ijk} = \mu + \tau_i + d_{ij} + t_k + (\tau t)_{ik} + \varepsilon_{ijk}$, that include the overall effect (μ), genotype group (τ_i), random effect of individual within genotype group (d_{ij}), repeated measurements (t_k), interaction of τ_i with t_k ($(\tau t)_{ik}$) and random error (ε_{ijk}). For a proposal of sample sizes studies, was considered the reduced model:

$$y_i = \mu + \varepsilon_i \quad (i = 1, \dots, n), \quad \varepsilon_i \sim \text{IID } N_t(0, \Sigma),$$

where $y_i = (y_{i1}, \dots, y_{it})$ is the response vector of the i^{th} individual across t repeated measurements; $\mu = (\mu_1, \dots, \mu_t)$ is the mean response vector in time t , which include all effects considered in the standard model for repeated measures; ε_i is the experimental error, independently and identically distributed as t -variate normal distribution with mean vector 0 and covariance matrix Σ .

The test to reject or accept the null hypothesis of equal measurements effects $H_0: \mu_1 = \dots = \mu_t$, is based on the statistic:

$$T^2 = n\bar{Y}'C(C'SC)^{-1}C'\bar{Y},$$

where $\bar{Y} = n^{-1} \sum_{i=1}^n Y_i$; $S = (n-1)^{-1} \sum_{i=1}^n (Y_i - \bar{Y})(Y_i - \bar{Y})'$ is the sample

covariance matrix among repeated measurements (RM) positive defined, which estimate Σ ; and C' = any $(t-1) \times t$ orthogonal contrast matrix.

The T^2 statistic is distributed according to the Hotelling T^2 with $(t-1)$ and $(n-1)$ degrees of freedom (df) and noncentrality parameter $\delta^2 = n\mu'C(C'\Sigma C)^{-1}C'\mu$. Under true H_0 , obtain $F = (n-t+1)[(n-1)(t-1)]^{-1}T^2$, which has distribution F with $(t-1)$ and $(n-t+1)$ df and noncentrality parameter δ^2 . For a particular type I error (α), then it rejects H_0 if $F > F(t-1, n-t+1; \delta^2)$; the type I error is the probability of incorrectly rejecting the null hypothesis; the maximum risk of committing α that one is willing to accept, is traditionally set to 0.05.

The minimum sample size n is determined by power considerations associated with Hotelling's T^2 , F -test, values of μ and Σ , in which H_0 is rejected. It was specified for any pair of RM a minimum difference (Δ), subject to the restriction $|\mu_j - \mu_k| = \Delta$ for any $j \neq k$, whose significance should be detected, considering a level of probability α and power of test $(1-\beta)$. The minimum value

of δ^2 subject to the restriction that any two consecutive measurements of a total of 14 repeated observations per individual, $|\mu_j - \mu_k| = \Delta$, defined by δ^2_{Δ} , is equal to $n\Delta^2/\max_{j<k}\{\sigma_j^2+\sigma_k^2-2\sigma_{jk}\}$, where σ_j^2 and σ_k^2 ($j<k$) are the variances and σ_{jk} is the covariance associated to measures j and k , respectively. Considering Σ , any variance-covariance matrix, defined positive satisfying $\rho_{jk}\geq 0$, for all $j < k$, it can be demonstrated that $n\Delta^2/[2\sigma_{\max}^2(1-\rho_{\min})] \leq \delta^2_{\Delta}$ is appropriated for estimating sample size n (Vonesh and Schork, 1986). In this expression, ρ_{\min} is the lower correlation coefficient between repeated measures, $\sigma_{\max}^2 = \text{maximum}(\sigma_j^2)$ and Δ is measured in units of σ_{\max} . Using this expression, the n estimated for $t \geq 2$ repeated measures, in functions of distribution F with $(t-1)$ and $(n-t+1)$ df, α and power of test $(1-\beta)$, were obtained by SAS program that considered an integral and a noncentral F -distribution (Hardison et al., 1983).

Results

The sample variance-covariance matrix, positive defined, is showed by Table 1. The maximum standard deviation σ_{\max} was 85.4235, obtained of $\sigma_{\max}^2 = \sigma_i^2 + \sigma_j^2 - 2\sigma_{ij}$, where i and j are body weight measures ($j < k$). Using the values of σ_{\max} and $\rho_{\min} = 0.05$, has the expression $0.0000685n\Delta^2/(1-\rho_{\min})$ (Vonesh and Schork, 1986). For including ρ_{\min} , this expression takes on account the fact that the correlation between repeated measures decreases as the repeated measures become far apart; for considering σ_{\max}^2 , it takes on account a common fact in growth studies, that is, the variance is linearly proportional to the increment in the response function. By considering the extremes values (σ_{\max}^2 and ρ_{\min}) of an sample variance-covariance matrix, positive defined, these properties assures reliability of the sample size estimate (Brownie et al., 1990; Cullis and McGilchrist, 1990).

The Figures 1 and 2 shows the n estimates obtained by evaluating the expression $0.0000685n\Delta^2/(1-\rho_{\min})$ and the integral of a central and noncentral F -distribution in functions of a range of values: power of test $(1-\beta)=0.80$ and 0.90 ; minimum correlation (ρ_{\min}) = 0, 0.2, 0.4 and 0.6, detectable difference $\Delta = 1.0\sigma$, 1.5σ and 2.0σ , and $\alpha = 0.01$ and $\alpha=0.05$.

Discussion

The minimum sample size or minimum number of individuals, necessary for detecting significant difference between repeated measures, increases in the following order (Figures 1 and 2): ($\Delta=2.0\sigma$; Power=0.80); ($\Delta=2.0\sigma$; Power=0.90); ($\Delta=1.5\sigma$; Power=0.80); ($\Delta=1.5\sigma$; Power=0.90); ($\Delta=1.0\sigma$; Power=0.80) and ($\Delta=1.0\sigma$; Power=0.90).

For example, if someone desires to detect a significant difference between any two of 14 measurements, considering a minimum difference of 1.0σ , power

of test equal 0.90, minimum correlation equal to 0.4, it is necessary a minimum of 50 and 40 individuals for $\alpha=0.01$ and $\alpha=0.05$, respectively. At the same conditions, a minimum of 31 and 26 individuals are needed to detect a significant between repeated measures when Δ changes from 1.0σ to 1.5σ , for $\alpha=0.01$ and $\alpha=0.05$, respectively. Vonesh and Schork (1986), studied the sample size varying from three to six measurements, seven values of Δ (1.0σ to 3.0σ), power of test of 0.80 and 0.90, and minimum correlation varying from 0.1 to 0.9. They observed greater reduction in the estimates of n when Δ changed from 1.0σ to 1.25σ .

In all Figures, can be seen that the stronger the autocorrelation is, the smaller the sample size that is required. Similar results were obtained by Kirby et al. (1996), when examined the effect of autocorrelation of repeated measures on the assessment of sample sizes methods. They concluded that taking account of the autocorrelation structure of longitudinal data, may lead to more efficient designs.

Procedures for determining minimum sample sizes that are required for power in testing differences in rates of change in multivariate repeated measures experiments, based on power considerations associated with Hotelling's T^2 and the F -test also has increased in the latest years. Guo and Johnson (1996), estimated minimum sample sizes required for several hypotheses from multivariate analysis, and several tables were presented. Arndt et al. (2000), analyzing a data set on post-stroke patients containing six follow-up assessments of six standard rating scales, studied a method for evaluating the relative benefit of adding individuals versus adding measurement times. The data suggested that collect five or six repeated measurements were sufficient for accurately assessing changes and that attempts to further precision should be accomplished by increasing the sample size. King and Dobson (2000), described a method that generalizes the effect size for paired differences to more than two repeated observations per individual, for a range of sample sizes, varying both the number of individuals (n) and the number of observations per individual (t).

The determination of sample sizes as implemented in this study, plays an important role in the planning of the number of individuals requested in an longitudinal data experiment. Suppose a similar study is planned in order to evaluate the influence of candidate genes on body weight in cattle, from birth to two years of age. In this case, it is reasonable to admit that the sample correlation matrix among the repeated measures will follow the same pattern of variation in relation to the matrix obtained in this study. So, it is reasonable to the researcher to do a balance of number of individuals and repeated measures considering the results presented in Figures 1 and 2. Adequate experimental planning reduces the risks of conducting a study that will not produce useful results, and provide a desired power for detecting an effect of scientific interest.

The number of individuals necessary to detect significant differences between any two consecutive measurements of body weight of cattle, from birth to approximately 19 months of age is influenced by a minimum difference significant (Δ), correlation among the repeated measures, type I error (α) and power of test ($1-\beta$). For a particular Δ value, it is necessary a bigger sample size (n) to prove significant difference between repeated measures response, when α moves from 0.05 to 0.01 and the power goes from 0.80 to 0.90. Independently of the power of the test, ρ_{\min} and Δ , significant difference between means of any two measurements at $\alpha=0.01$ requests a sample size about 30% greater as compared with $\alpha=0.05$.

The main idea of this study was based on growth curves approach, i.e., considering that the 14 measurements of body weight collected from 8 to 19 months of age, when group of polymorphisms were considered both separately or as a group, were adequately described by Logistic model, a sigmoid growth curve, as shown by Paz et al.(2003a, 2003b). It should be considered also that the minimum sample sizes n estimated in this study, using the same data set, increases the power to reject the null hypothesis of no difference between any two consecutive measurements per individual. Considering this minimum, as size increases the narrower the confidence interval of a fitted growth curve becomes, and the fitted growth curve gets closer to the real population growth curve. In these conditions, the researcher has the opportunity to balance the number of individuals and the number of measurements per individual, in order to obtain the desired precision in future experiments of longitudinal studies for considering several purposes: to estimate a global response function, i.e., considering all measurements per individual (for example, growth curve studies); to analyze parallel treatment groups in repeated measures, and to compare variation across-individuals in a fixed age.

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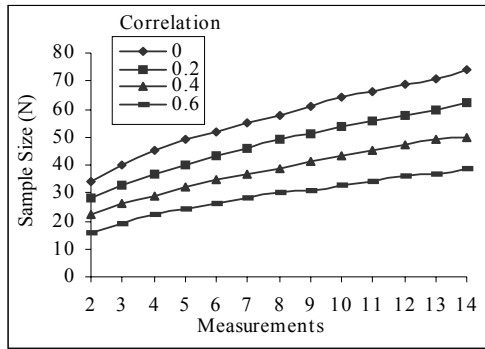
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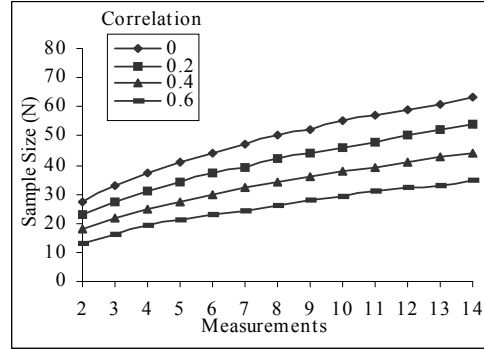
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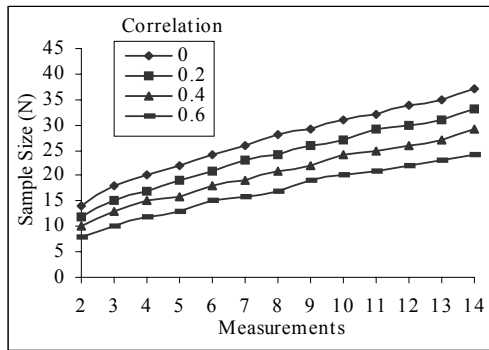




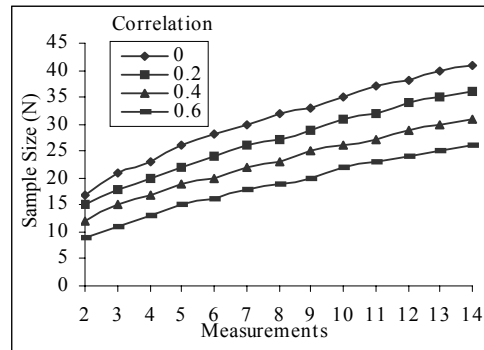
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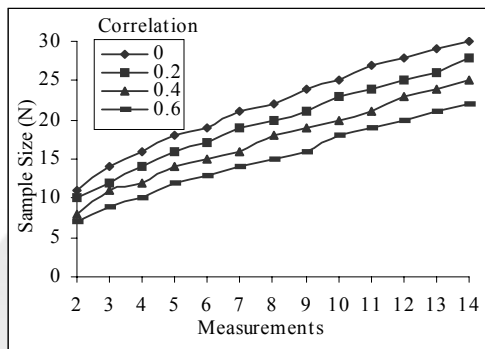
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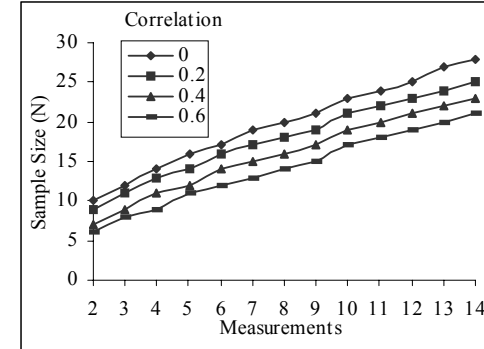
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$\Delta=1.5\sigma$ and Power=0.80

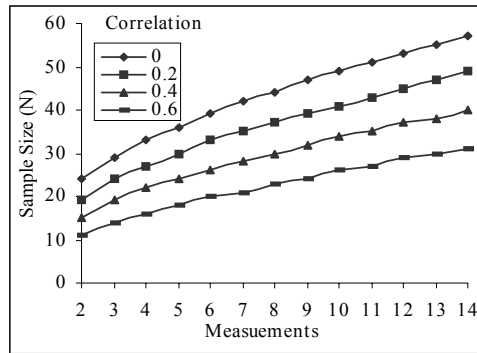


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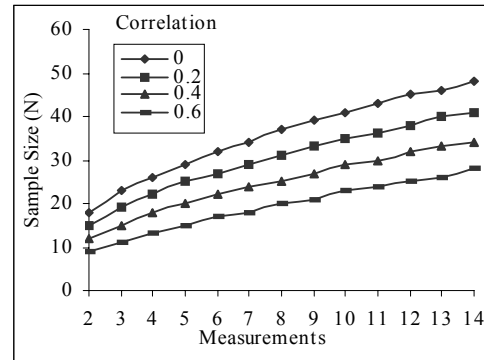


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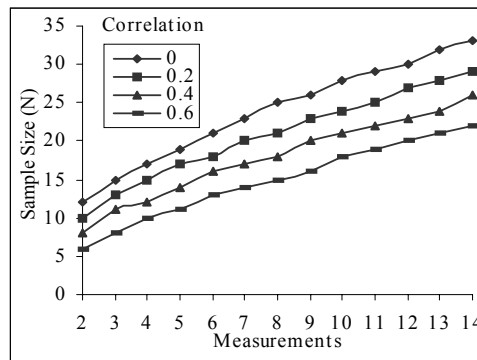
Figure 1. Estimates of sample size (n) in repeated measurements of body weight (BW) in beef cattle for $\alpha=0.01$; power of test ($1-\beta$) =0.80 and 0.90; minimum correlation (ρ_{\min})=0, 0.2, 0.4 and 0.6 and detectable difference (Δ)= 1.0σ , 1.5σ and 2.0σ .



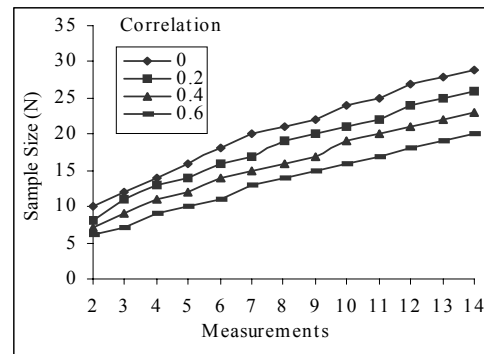
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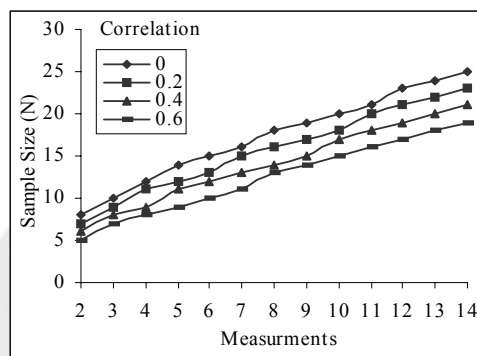
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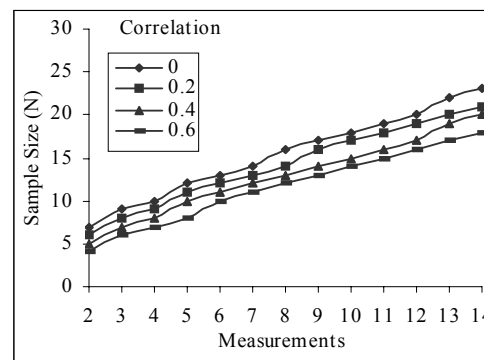
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$\Delta = 1.5\sigma$ and Power=0.80



$\Delta = 2.0\sigma$ and Power=0.90



$\Delta = 2.0\sigma$ and Power=0.80

Figure 2. Estimates of sample size (n) in repeated measurements of body weight (BW) in beef cattle for $\alpha=0.05$; power of test ($1-\beta$) =0.80 and 0.90; minimum correlation (ρ_{\min})=0, 0.2, 0.4 and 0.6 and detectable difference (Δ) = 1.0σ , 1.5σ and 2.0σ .

Table 1. Sample size covariance matrix considering 14 measurements of body weight by individual from beef cattle. At birth (BW), weaning (WW, 7 months of age) and monthly from 8 to 19 months of age (W8 to W19).

	BW	WW	W8	W9	W10	W11	W12	W13	W14	W15	W16	W17	W18	W19
BW	15.74	30.03	24.73	24.66	14.80	9.51	15.27	29.83	37.36	38.25	51.39	47.32	57.82	54.00
WW		895.17	731.74	666.2	646.50	657.10	664.07	775.82	845.96	838.46	603.12	518.06	484.57	513.16
W8			897.79	871.81	941.62	1008.17	1075.96	1230.33	1344.93	1388.75	1032.71	743.73	703.41	690.68
W9				1001.42	1205.30	1338.05	1488.38	1628.57	1760.24	1802.27	1272.66	780.84	808.81	858.32
W10					1687.74	1947.17	2184.26	2338.83	2547.15	2671.20	1804.78	865.97	854.59	940.13
W11						2332.44	2636.18	2842.04	3108.08	3304.52	2293.61	1118.50	1075.25	1043.49
W12							3126.02	3397.73	3702.03	3954.01	2861.16	1374.13	1336.85	1273.06
W13								4054.05	4499.38	5049.70	4068.05	2157.98	2006.29	1434.57
W14									5128.68	5873.77	4866.17	2477.24	2291.06	1546.32
W15										7357.94	6391.95	3165.24	2909.19	1726.48
W16											7133.51	3810.11	3507.61	1959.42
W17												3956.20	3691.66	2031.52
W18													3843.44	2222.13
W19														2384.02

