

Growth curves for buffaloes (*Bubalus bubalis*) using random regression mixed models with different structures of residual variances

D. M. Bolívar^{1,2}, M. F. Cerón-Muñoz², M. A. Elzo^{*3}, E. J. Ramírez², D. A. Agudelo⁴

National University of Colombia, Medellín, Colombia¹, University of Antioquia, Medellín, Colombia², University of Florida, Florida, USA³, Lasallian University Corporation, Caldas, Colombia⁴



SUMMARY

The objective of this study was to analyze buffalo growth based on body weight (BW), *Longissimus dorsi* muscle area (AOL), and fat deposition over the hip (FOH) using random regression mixed models of first (FORRM) and second order (SORRM), each with nine different variance structures. Ten measurements for each trait were taken on 26 animals during the first performance test (93 d test plus 23 d adaptation period) developed for buffaloes in Colombia. Computations were performed using the nlme procedure of the nlme library of program R. Preliminary analyses determined that an SORRM was appropriate for BW and FOH and an FORRM was suitable for AOL. The maximum likelihood ratio (MLR), the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) were used to compare models. The best models were an SORRM with homogeneous residual variances for BW, an FORRM with heterogeneous animal residual variances for AOL, and an SORRM with heterogeneous residual variances among farms times an exponential function of age for FOH. Heterogeneity of residual variances was likely due to environmental differences among farms, and to genetic differences among buffaloes not accounted for by FORRM and SORRM. Fixed intercepts with the best models for each trait were 227 ± 7.90 kg for BW, 34.82 ± 0.99 cm² for AOL, and 4.19 ± 0.229 mm for FOH. Fixed linear regression coefficients were 1.289 ± 0.073 g/d for BW, 0.0584 ± 0.0042 cm²/d for AOL, and 0.0035 ± 0.0032 mm/d for FOH. The fixed quadratic regression coefficient indicated that BW rate decreased after one year of age whereas FOH rate continued to increase until the end of the test. Random regression coefficients suggested that there was considerable variability among trait curves for individual buffaloes, particularly for FOH.

Keywords: buffalo, growth curves, performance test

INTRODUCTION

Experiments with repeated measures are common in livestock research (Wang and Goonewardene, 2004). In most cases, multiple observations per experimental unit are taken over time. The usual assumptions of independence and homogeneity of variances are usually not valid for data analysis in such experiments because the measurements made in the same animal are often correlated with each other and the variances between measurements may be different. Mixed model methodology allows for correct and efficient data analysis of experiments with repeated measures through modeling of the covariance structure, considering the correlations between repeated measures and the presence of heterogeneous variances (Littell et al., 1998).

Growth is one of the factors of greatest economic importance in beef production systems. Mixed models can be used to describe growth of farm animal species. Incorporating random effects in the models permits accounting for variability among growth curves from individual animals within a population (France et al., 1996). This is useful in breeding programs because it helps to choose the fastest growing animals without altering their adult weight.

The objective of this study was to analyze buffalo growth based on body weights, *Longissimus dorsi* muscle areas, and fat thicknesses over the hip using random regression mixed models with different residual variance structures, and data from the first performance test developed in Colombia for this species.

MATERIALS AND METHODS

Location. The performance test was conducted at the Experimental Center of the University of Antioquia, located in the municipality of Barbosa, Antioquia, Colombia. This area is classified as subtropical moist forest.

Animals and Diet. A total of 26 buffaloes were used in the performance test. Animals came from four breeding farms where milking was not practiced. The performance test was conducted for a period of 116 days. There was an adjustment period of 23 d, and the evaluation period lasted 93 d. Animals entered the test at an average age of 285 ± 25.72 d with an average weight of 261 ± 29 kg, and they reached an average weight of 342 ± 39.08 kg at the end of the test. The animals were confined and fed fresh Maralfalfa grass (*Pennisetum sp.*) *ad libitum*, plus two kilograms of a feed supplement per day.



Traits. A total of 10 measurements per trait were taken over the 116 d of the performance test, 3 measurements during the adjustment period and 7 measurements during the test period. Animals were weighed (BW) after 12 hr of fasting. Area of the *Longissimus dorsi* (AOL) and fat thickness over the hip (FOH) were measured by ultrasound, using an Akila-Pro equipment (Esaote Europe B. V., Holland) with a 3.5-MHz, 18cm transducer. Images were measured with the Eview program (Pie Medical 1996).

Statistical Analysis. Data were first analyzed using additive random regression mixed models of first order (FORRM; Model 1) and of second order (SORRM; Model 2), both with homogeneous residual variance structures. These 2 base models were as follows:

$$y_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})X_j + \epsilon_{ij} \quad \text{Model 1}$$

$$y_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})X_j + \beta_2 X_j^2 + \epsilon_{ij} \quad \text{Model 2}$$

Where, y_{ij} is a BW, AOL and FOH record measured in the i -th animal at the j -th age; β_0, β_1 and β_2 fixed intercept, linear regression coefficient, and quadratic regression coefficient averaged over all animals; X_j is the j -th age; ϵ_{ij} is the random residual due to effects not considered in the model, where $\epsilon_{ij} \sim N(0, \sigma^2)$, and b_{0i} and b_{1i} random intercept and linear regression coefficient for the i -th animal, representing a random deviation from β_0 and β_1 the fixed regression coefficients, respectively.

9 heterogeneous residual variance structures (Table 1) were evaluated within models 1 and 2. Computations were carried out with the nlme procedure of the nlme library of the R program (R Development Core Team, 2008). The nlme library variance functions were used to model the 9 heterogeneous residual variance structures (Pinheiro and Bates, 2000). Structures 3 and 4 took into account the variance among individual animals and among farms, respectively (Zuur et al. 2009). For structure 5, the residual variance was modeled as times the absolute value of age of animal raised to the power of 2δ where δ is unknown and must be estimated. For structure 6, residual variances were calculated as multiplied by an exponential function of 2δ times the age of animal. Residual variance structure 7 is a constant plus a variance covariate power function. Structures 8, 9, 10 and 11 were combination of previous residual covariance structures. Variances were estimated using restricted maximum likelihood procedures and computed by the nlme procedure of the R program.

To evaluate the importance of the adjustment period, the 9 models were run first with all 10 measurements for the 3 traits, and then with only the 7 measurements of the evaluation period (i.e., without the first 3 measurements of the adaptation period).

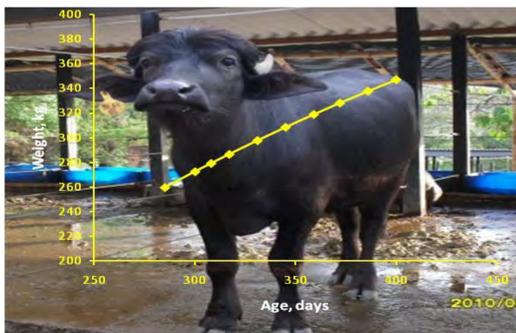


Table 1. Residual variance-covariance structures evaluated in 2 random regression mixed models to describe the growth of buffaloes in a performance test

Function name in R program	Variance structure	Structure
varIdent: Different variances per animal	$\epsilon_{ij} \sim N(0, \sigma_{n1}^2)$	3
varIdent: Different variances per farm of origin of animal	$\epsilon_{ij} \sim N(0, \sigma_{n2}^2)$	4
varPower: power of the variance covariate age of animal	$\epsilon_{ij} \sim N(0, \sigma^2 * age_{ij} ^{2\delta})$	5
varExp: Exponential of the variance covariate age of animal	$\epsilon_{ij} \sim N(0, \sigma^2 * e^{2\delta * age_{ij}})$	6
varConstPower: Constant plus power of the variance covariate age of animal	$\epsilon_{ij} \sim N(0, \sigma^2 * (\delta_1 + age_{ij} ^{\delta_2})^2)$	7
varComb: Combination of functions	$\epsilon_{ij} \sim N(0, \sigma_{n1}^2 + e^{2\delta * age_{ij}})$	8
varComb: Combination of functions	$\epsilon_{ij} \sim N(0, \sigma_{n1}^2 * age_{ij} ^{2\delta})$	9
varComb: Combination of functions	$\epsilon_{ij} \sim N(0, \sigma_{n2}^2 + e^{2\delta * age_{ij}})$	10
varComb: Combination of functions	$\epsilon_{ij} \sim N(0, \sigma_{n2}^2 * age_{ij} ^{2\delta})$	11

ϵ_{ij} = residual for the i -th animal of the j -th age; age_{ij} = explanatory variable used in the variance of the residuals (i.e., variance covariate); $n1 = 26$ (number of animals); $n2 = 4$ (number of farms); δ, δ_1 and δ_2 = coefficients of variance functions (are unknown and must be estimated).

Model Comparison. The FORRM and SORRM with homogeneous residual variance structures (models 1 and 2) were nested within models that assumed heterogeneous structures (models 3 to 11). The Likelihood Ratio Test (LRT) was used to compare these nine models to models 1 and 2 (Zuur et al., 2009). Models that differed significantly from models 1 and 2 using the LRT were compared with each other using the AIC and the BIC.

RESULTS AND DISCUSSION

Body Weight. After measurements obtained during the adaptation period were eliminated, there was a significant decrease in the size of the normalized residuals and residuals by age, by farm, by measurement and by animal. Furthermore, when the 9 heterogeneous residual variance structures were analyzed using all measurements, the LRT found significant differences between models with heterogeneous residual variance structure 5 ($P < 0.0413$), 6 ($P < 0.0001$), 8 ($P < 0.0017$), 9 ($P < 0.0026$), 10 ($P < 0.0002$) and 11 ($P < 0.0043$) and model 2 that assumed homogeneity of variances. In contrast, when the adjustment period was excluded, no significant differences were found between models with heterogeneous variance structures and model 2 (Table 2). These results confirm the importance of considering an adjustment period to allow animals from different production systems to get adjusted to the new environment at the performance test station, leading to a similar residual variation among animals of all farms. Exclusion of measurements from the adjustment period was enough to obtain greater homogeneity of variances for residuals for BW. Thus, model 2 was the best model to evaluate buffaloes for BW during the performance test (Table 2).

Although residuals per animal decreased significantly, there were still heterogeneous variances, possibly a consequence of carry over environmental effects from their farms of origin not eliminated by the adjustment period, and perhaps genetic differences among animals not accounted for by the additive random regression models used here. Buffaloes entered the test with a maximum age difference of 73 d and an initial weight difference of 91 kg (215 to 306 kg). This maximum weight difference increased to 168 kg at the end of the test (244 to 412 kg). In addition, individual differences were observed in growth curves. These differences in growth patterns may help explain the higher residual variances obtained from these individuals.

Table 2. Parameters for body weight estimated by a second-order random regression mixed model with different residual variance structures, in buffaloes under a performance test

Variance structure	Fixed effects			Random effects					Criteria		
	β_0	β_1	β_2	σ_{b_0}	σ_{b_1}	σ_e	$\rho_{b_{b_0}}$	AIC	BIC	LRT [†]	
$\epsilon_{ij} \sim N(0, \sigma^2)$	2	227±7.90	1.289±0.073	-0.0031±0.0003	37.60	0.198	3.44	-0.56	1232	1254	
$\epsilon_{ij} \sim N(0, \sigma_{n1}^2)$	3	226±7.87	1.329±0.066	-0.0033±0.0002	38.22	0.209	2.32	-0.58	1253	1355	28.64 (0.2793)
$\epsilon_{ij} \sim N(0, \sigma_{n2}^2)$	4	226±7.98	1.324±0.073	-0.0033±0.0003	38.13	0.206	3.56	-0.58	1235	1267	2.87 (0.4118)
$\epsilon_{ij} \sim N(0, \sigma^2 * age_{ij} ^{2\delta})$	5	227±7.88	1.289±0.073	-0.0031±0.0003	37.59	0.198	0.63	-0.56	1234	1259	0.25 (0.6191)
$\epsilon_{ij} \sim N(0, \sigma^2 * e^{2\delta * age_{ij}})$	6	228±7.90	1.279±0.073	-0.0030±0.0003	37.50	0.195	4.07	-0.56	1233	1258	0.82 (0.3664)
$\epsilon_{ij} \sim N(0, \sigma^2 * (\delta_1 + age_{ij} ^{\delta_2})^2)$	7	227±7.88	1.289±0.073	-0.0031±0.0003	37.59	0.198	0.63	-0.56	1236	1264	0.25 (0.8837)
$\epsilon_{ij} \sim N(0, \sigma_{n1}^2 + e^{2\delta * age_{ij}})$	8	227±7.86	1.297±0.066	-0.0031±0.0002	37.92	0.201	3.43	-0.57	1253	1358	31.17 (0.2218)
$\epsilon_{ij} \sim N(0, \sigma_{n1}^2 * age_{ij} ^{2\delta})$	9	226±7.85	1.315±0.066	-0.0033±0.0002	37.98	0.205	3.96	-0.57	1253	1358	30.64 (0.2419)
$\epsilon_{ij} \sim N(0, \sigma_{n2}^2 + e^{2\delta * age_{ij}})$	10	226±7.97	1.314±0.073	-0.0032±0.0003	38.00	0.203	3.96	-0.57	1237	1272	3.21 (0.5228)
$\epsilon_{ij} \sim N(0, \sigma_{n2}^2 * age_{ij} ^{2\delta})$	11	225±7.86	1.331±0.066	-0.0033±0.0003	38.22	0.208	1.00	-0.58	1237	1272	2.95 (0.5663)

ϵ_{ij} = residual for the i -th animal of the j -th age; age_{ij} = explanatory variable used in the variance of the residuals (i.e., variance covariate); $k = 1, \dots, 26$ (number of animals) for residual structure 3, and $k = 1, \dots, 4$ (number of farms) for residual structure 4; δ, δ_1 and δ_2 = function coefficients of variance are unknown and must be estimated. β_0 = intercept, corresponding to the initial animal value; β_1 and β_2 = regression coefficients associated with the predictor (age), explaining changes during the performance test. σ_{b_0} : standard deviation of intercept; σ_{b_1} : standard deviation of growth rate; $\rho_{b_{b_0}}$: correlation between the intercept and growth rate; σ_e : standard deviation of error. AIC = Akaike Information Criterion, BIC = Bayesian Information Criterion; LRT = Likelihood Ratio Test. [†]Test conducted between models with heterogeneous variance structures (3-11) and the model that assumes homogeneity of variance (2). Values in parentheses correspond to the level of significance.

Longissimus Dorsi Muscle Area. Residual values increased with age of the animal. Was observed heterogeneity of residual variability among farms, measurements, and animals. Thus, unlike BW, eliminating measurements from the adjustment period was insufficient to reduce residuals and improve the homogeneity of variances. When comparing models with heterogeneous residual variance structure, significant differences (Table 3) were found with the LRT between models with structures 3 ($P < 0.0288$), 8 ($P < 0.0312$) and 9 ($P < 0.0309$) and model 1 (homogeneous variances). Model 3, which assumed a different residual variance for each animal showed the lowest AIC and BIC values (Table 3), thus it was considered to be the best model to analyze AOL. However, although there was a reduction of the size of the residuals by farm, measurements, and animal, heterogeneity of residual variances still remained.

As previously indicated, animals were exposed to different climatic, feeding, and management conditions in their farms of origin. Buffaloes in each of the 4 farms were also likely to be genetically different subpopulations. Thus, although animals entered at a similar age, initial AOL ranged from 25.10 to 45.20 cm², a difference that was maintained until the end of the test, with values between 35.65 and 53.95 cm². Similarly, there were differences among animals in tissue deposition, which may help explain the heterogeneous residual variances.

Table 3. Parameters for *Longissimus dorsi* muscle area estimated by a first-order random regression mixed model with different residual variance structures, in buffaloes under a performance test

Variance structure	Fixed effects			Random effects					Criteria		
	β_0	β_1	β_2	σ_{b_0}	σ_{b_1}	σ_e	$\rho_{b_{b_0}}$	AIC	BIC	LRT [†]	
$\epsilon_{ij} \sim N(0, \sigma^2)$	1	34.58±1.02	0.0611±0.0043	5.033	0.0167	1.063	-0.052	629	647		
$\epsilon_{ij} \sim N(0, \sigma_{n1}^2)$	3	34.82±0.99	0.0584±0.0042	4.914	0.0172	2.291	0.002	639	733	40.05 (0.0288)	
$\epsilon_{ij} \sim N(0, \sigma_{n2}^2)$	4	34.66±1.02	0.0602±0.0043	5.035	0.0171	1.155	-0.063	630	657	4.91 (0.1788)	
$\epsilon_{ij} \sim N(0, \sigma^2 * age_{ij} ^{2\delta})$	5	34.57±1.02	0.0613±0.0043	5.004	0.0165	4.796	-0.030	630	652	0.76 (0.3838)	
$\epsilon_{ij} \sim N(0, \sigma^2 * e^{2\delta * age_{ij}})$	6	34.51±1.01	0.0620±0.0042	4.969	0.0159	1.299	0.013	630	651	0.96 (0.3274)	
$\epsilon_{ij} \sim N(0, \sigma_{n1}^2 + e^{2\delta * age_{ij}})$	8	34.91±0.99	0.0572±0.0041	4.933	0.0168	1.852	-0.008	640	737	40.98 (0.0312)	
$\epsilon_{ij} \sim N(0, \sigma_{n1}^2 * age_{ij} ^{2\delta})$	9	34.90±0.99	0.0572±0.0040	4.936	0.0167	0.001	-0.005	640	737	41.02 (0.0309)	
$\epsilon_{ij} \sim N(0, \sigma_{n2}^2 + e^{2\delta * age_{ij}})$	10	34.61±1.02	0.0608±0.0043	4.996	0.0165	1.321	-0.023	632	662	5.42 (0.2464)	
$\epsilon_{ij} \sim N(0, \sigma_{n2}^2 * age_{ij} ^{2\delta})$	11	34.66±1.02	0.0602±0.0043	5.024	0.0170	2.268	-0.056	632	662	5.01 (0.2867)	

Fat Thickness Over the Hip. Residuals estimated for FOH with model 2 assuming homogeneity of variances suggested that there was a violation of the assumption of homogeneity of residual variances. Residual variation increased with age. The largest variability occurred in the last three measurements. Heterogeneity of residual variance also existed among farms and animals. Table 4 shows the estimates of regression parameters obtained by the models with different variance structures and comparison criteria. All models were significantly different from model 2 (homogeneous variances). The best model was model 10. This model took into account the variance for each farm and an exponential variance for age. Heterogeneity of residual variance was substantially corrected, however, a clear heterogeneity of variance due to age was still observed, and this heterogeneity increased with age and measurement. This increase in residual variance for FOH can be explained by an increase in fat deposition as the animal grows (Putrino et al., 2006). Heterogeneity of variance due to farm and animal was only partially corrected by model 10. In agreement with BW and AOL, animals diverged widely in FOH at the beginning of the test, with values ranging from 2.53 to 6.40 mm.

Table 3. Parameters for *Longissimus dorsi* muscle area estimated by a first-order random regression mixed model with different residual variance structures, in buffaloes under a performance test

Variance structure	Fixed effects			Random effects					Criteria		
	β_0	β_1	β_2	σ_{b_0}	σ_{b_1}	σ_e	$\rho_{b_{b_0}}$	AIC	BIC	LRT [†]	
$\epsilon_{ij} \sim N(0, \sigma^2)$	2	4.48±0.28	-0.0042±0.0045	0.00009±0.00002	1.061	0.0098	0.221	-0.58	196	217	
$\epsilon_{ij} \sim N(0, \sigma_{n1}^2)$	3	4.33±0.23	-0.00008±0.029	0.00007±0.00001	1.038	0.0092	0.044	-0.54	178	275	68.14 (<0.0001)
$\epsilon_{ij} \sim N(0, \sigma_{n2}^2)$	4	4.42±0.27	-0.0024±0.0042	0.00008±0.00002	1.049	0.0095	0.183	-0.56	191	221	11.58 (0.009)
$\epsilon_{ij} \sim N(0, \sigma^2 * age_{ij} ^{2\delta})$	5	4.37±0.25	-0.0010±0.0037	0.00007±0.00002	1.009	0.0083	0.007	-0.53	176	200	22.58 (<0.0001)
$\epsilon_{ij} \sim N(0, \sigma^2 * e^{2\delta * age_{ij}})$	6	4.25±0.24	0.0015±0.0036	0.00006±0.00002	1.005	0.0084	0.080	-0.49	174	199	23.90 (<0.0001)
$\epsilon_{ij} \sim N(0, \sigma^2 * (\delta_1 + age_{ij} ^{\delta_2})^2)$	7	4.37±0.25	-0.0010±0.0037	0.00007±0.00002	1.009	0.0083	0.007	-0.53	178	205	22.58 (<0.0001)
$\epsilon_{ij} \sim N(0, \sigma_{n1}^2 + e^{2\delta * age_{ij}})$	8	4.41±0.21	-0.0021±0.0025	0.00008±0.00001	0.987	0.0086	0.028	-0.48	167	267	80.96 (<0.0001)
$\epsilon_{ij} \sim N(0, \sigma_{n1}^2 * age_{ij} ^{2\delta})$	9	4.36±0.21	-0.0005±0.0026	0.00007±0.00001	0.981	0.0082	0.000	-0.47	169	259	88.88 (<0.0001)
$\epsilon_{ij} \sim N(0, \sigma_{n2}^2 + e^{2\delta * age_{ij}})$	10	4.19±0.23	0.0035±0.0033	0.00005±0.00002	0.985	0.0079	0.073	-0.45	165	198	39.22 (<0.0001)
$\epsilon_{ij} \sim N(0, \sigma_{n2}^2 * age_{ij} ^{2\delta})$	11	4.33±0.24	0.0004±0.0034	0.00006±0.00002	1.004	0.0077	0.003	-0.50	174	207	30.31 (<0.0001)

Growth curves. Individual animal growth curves for BW, AOL, and FOH were computed using parameters from the best models found for each trait. Estimates of were 227 ± 7.90 kg for BW, 34.82 ± 0.99 cm² for AOL and 4.19 ± 0.229 mm for FOH. Estimates of were 1.289 ± 0.073 g/d for BW, 0.0584 ± 0.0042 cm²/d for AOL, and 0.0035 ± 0.0032 mm/d for FOH. These estimated indicate that BW, AOL, and FOH increase throughout the performance test, whereas the values indicated that BW rate decreased after one year of age, and the rate of FOH deposition continued to increase until the end of the test. This pattern of growth was in agreement with the dynamics of cattle growth where muscle and fat deposition occur at a later age (Berg and Butterfield, 1976).

CONCLUSION

The evaluated residual variance structures did not eliminate completely the heterogeneity of variances. Heterogeneity was likely due to environmental differences among farms where animals originated from, and to genetic differences among buffaloes not accounted for by the random regression additive genetic models used here. When BW was analyzed without the 3 weights taken during the adjustment period, residuals were substantially reduced and weight gain increased, showing the importance of allowing buffaloes sufficient time to adjust to the environment at the performance test station. There was no reduction of residuals for AOL and FOH when the adjustment period was eliminated. The best models to describe buffalo growth in this performance test were model 2 with homogeneous residual variances for BW, model 3 with different residual variances for each animal for AOL, and model 10 with heterogeneous residual variances among farms times an exponential function of age for FOH. Estimates of genetic fixed and random regression coefficients were similar across models, however residual variances varied considerably.

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