

## ANALYSIS OF THE LACTATION CURVE OF MURRAH BUFFALOES WITH MIXED NON-LINEAR MODELS

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### ABSTRACT

This study aimed to evaluate the lactation curve of female Murrah buffaloes, using mixed non-linear models (NLM), across three lactation periods (180 d, 210 d, and 240 d). A total of 5334 data on daily milk production (kg) were analyzed. The data were collected every seven days in the interval of one to 250 days of lactation, corresponding to 221 lactations and 145 females, with calvings from 2017 to 2019. The data came from a herd located in the Centro municipality, Tabasco, Mexico. Five NLM were evaluated: Wood (WOD), Wiltmink (WIL), Cobby (COB), Brody (BRO), Sikka (SIK). The best fit model was selected based on the mean prediction error, mean absolute percentage error (MAPE), prediction error variance, coefficient of determination ( $R^2$ ), concordance correlation coefficient (CCC), Akaike (AIC) and Bayesian (BIC) information criteria. A regression analysis was performed between the observed and predicted values. All the NLM had a  $R^2$  above 0.91. They tend to underestimate the predictions, without residual autocorrelation. The MAPE showed an average value of 23.5%. The best fit model was WOD, followed by SIK and BRO. For WIL and COB, the mixed model did not improve the fitting. The shortest lactation period showed the best fit, followed by the 210 d and 240 d periods. The relationship between observed:predicted values fluctuated from 0.65 to 1.00, with an average value of 0.94. The use of NLM transcended in the AIC and BIC. The evaluated models showed goodness of fit, with good predictability, but low values in accuracy and precision of prediction.

**Key words:** Bubalinoculture, lactation period, milk production, model selection, tropical cattle.

## INTRODUCTION

The precise and proper prediction of milk production and performance of offspring is an important pre-requisite in selecting genetically superior animals for the dairy industry. Lactation curves (LCs) describe milk production based on four components: initial production, ascending phase, or increased production, maximum or peak production, and descending rate or reduced production, called persistency. Non-linear models (NLM) are used to characterize and analyze LCs. The regression coefficients are associated to the components and allow to derive other descriptive LC indicators (Papajcsik and Boderó, 1988; Landete-Castillejos and Gallego, 2000; Macciotta et al., 2011), which can be useful in handling, feeding, and breeding programs.

The fitting of NLM has been performed using a non-linear regression analysis with iterative methods (Bates and Watts, 1988). The milk production variable represents a particular case of repeated measures over time, with variance and covariance structures. The data structure is unbalanced, with possible implications in the statistical assumptions of residuals and the certainty of the regression coefficients associated to the model. The non-linear regression does not consider possible sources of variation associated with the animal (Wang and Zuidhof, 2004; Domínguez-Viveros et al., 2017; Vélez et al., 2019). As an alternative to evaluate an LC, the fitting of mixed NLM has been proposed, including sources of variation associated with the animal (Hosseini-Zadeh, 2016; Palacios-Espinosa et al., 2016; Piccardi et al., 2017). The variability in milk production occurs due to genetic and environmental effects, which define and characterize the LC. However, those genetic and environmental effects can change during the lactation period, or, in specific cases, depend on the lactation length (LL) (Madalena, 1988).

The buffalo Murrah breed is a milk type animal, native to India, and the most widespread breed worldwide. Murrah buffaloes are jet black. Male adults can weigh 800 kg, while females can weigh 600 kg. Regarding milk production, lactation can extend from 180 to 305 days, with an average daily production of 5 kg and a fat percentage of 7.3 % (Borghese, 2005; FAO, 2010). In Mexico, the production of buffaloes occurs in the tropical and subtropical regions, and breeders are organized in the Asociación Mexicana de Criadores de Búfalos (AMEXBU, 2019). This study aimed to characterize the lactation period and curve type of female buffaloes as well as determine the production levels and possible sources of variation associated with the animal.

## MATERIALS AND METHODS

A total of 5,334 observations on daily milk production (kg) were analyzed. Data were collected every seven days in the interval of one to 250 days of lactation, corresponding to 221 lactations and 145 female buffaloes, with calvings from 2017 to 2019. These data were obtained from a herd located (La Carolina Ranch) in the Centro municipality, Tabasco, Mexico, grazing native pasture on floodplains and lagoon banks, in a warm-humid climate with summer rains (mean annual precipitation of 2332 mm), and a mean annual temperature of 26.1 °C.

To analyze and characterize the LC, five NLM were evaluated (Ramírez-Valverde et al., 1998; Landete-Castillejos and Gallego, 2000; García-Muñiz et al., 2008): Wood, (WOD;  $y_t = \beta_1 * (t^{\beta_2}) * (\exp(-\beta_3 * t))$ ); Wiltmink, (WIL;  $y_t = \beta_1 + \beta_2 * t + \beta_3 * (\exp(-0.05 * t))$ ); Cobby, (COB;  $y_t = \beta_1 - \beta_2 * t - \beta_1 * (\exp(-\beta_3 * t))$ ); Brody, (BRO;  $y_t = \beta_1 * (\exp(-\beta_2 * t)) - \beta_1 * (\exp(-\beta_3 * t))$ ); and Sikka, (SIK;  $y_t = \beta_1 * (\exp((\beta_2 * t) - (\beta_3 * t^2)))$ ). Where:  $y_t$  = corresponds to the milk production (kg) at day  $t$ ;  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  = are the regression coefficients that comprise each model. Three lactation periods or LL, 180, 210 and 240 days (180 d, 210 d, and 240 d) were evaluated, based on two analyses (ANA1, ANA2), using the NLMIXED procedure in Statistical Analysis System v9.0 (Wolfinger, 1999; Ching-Fan et al., 2005). ANA1 does not include the random effects related to the regression coefficients, only the random effect (e) of residuals. ANA2 included the random effect associated with the female buffalo, which corresponds to the  $\beta_1$  of each model, in addition to the residuals. The random effects (e and  $\beta_1$ ) adhere to the non-correlation assumptions, with a normal distribution, mean equal to zero, and variances equal to  $\sigma_e^2$  and  $\sigma_{\beta_1}^2$ .

The selection of the model with the best fit was made with the coefficient of determination [ $R^2 = (1 - (rss/tss))$ ], the Akaike information criterion [ $AIC = -2 * \text{Log lik} + 2k$ ], and the Bayesian information criterion [ $BIC = -2 * \text{Log lik} + \log(n) * k$ ]. The residuals were evaluated with the mean prediction error [ $MPE = (\sum_{i=1}^n \left( \frac{odp - edp}{odp} \right) * 100) / n$ ], the mean absolute percentage error [MAPE =  $(\sum_{i=1}^n \left( \left| \frac{odp - edp}{odp} \right| \right) * 100) / n$ ], the prediction error variance [PEV =  $\sum_{i=1}^n (odp - edp)^2 / n$ ], and the Durbin-Watson statistic [ $DW = 2(1 - \rho)$ ;  $\rho = \frac{\sum_{t=2}^n (e_t - e_{t-1})^2}{\sum_{t=1}^n e_t^2}$ ] to analyze the error autocorrelations (Motulsky and Christopoulos, 2004; Palacios-Espinosa et al., 2016; Piccardi et al., 2017).

Where: odp = observed daily production; edp

= estimated daily production; n = total number of observations; rss = residual sum of squares; tss = total sum of squares; Log lik = logarithm of the likelihood function; k = number of parameters in the model. For the MPE, PEV, AIC, and BIC, the model with the lowest values was considered as the best fit. The prediction capacity of the NLM was validated by analyzing the linear relationship between the edp and the odp based on the correlation between the two (Y) and the regression analysis based on the model:  $odp = \beta_0 + \beta_i \text{edp}$ ; where  $\beta_0$  is the intercept, and  $\beta_i$  is the slope or change rate of the odp for each change unit in the edp. Additionally, the concordance correlation coefficient was calculated ( $CCC = 2 * \sigma_{odp,edp} / (\sigma^2_{odp} + \sigma^2_{edp} + (\bar{y}_{odp} - \bar{y}_{edp})^2)$ ); which

takes values from 0 to 1 and jointly measures the accuracy and precision of a model (Lin, 1989; Khan et al., 2012). With the results from ANA2, the production variables related to the LC were generated: initial production; accumulated total production (PTOTAL); maximum production at peak lactation; days until maximum production; mean daily production.

## RESULTS AND DISCUSSION

Tables 1, 2, and 3 show the results of statistical criteria used to select the best fit model for each LL. As goodness of fit, all the models explained 91% or more of the variability in the analyzed data, since their R<sup>2</sup> was equal to or greater than

**Table 1. Fit of non-linear models in the analysis of the lactation curve at 180 days of female Murrah buffaloes.**

Item	WOD	WIL	COB	BRO	SIK
Analysis one, non-linear models not including random effects					
$\beta_1$	4.6677	6.5267	6.0960	6.1185	5.7245
$\beta_2$	0.09473	-0.01179	0.008379	0.001538	0.000600
$\beta_3$	0.003033	-1.3824	1.1093	1.0741	0.000012
AIC	15909	15912	15963	15970	15939
BIC	15934	15938	15989	15996	15964
R <sup>2</sup>	0.93	0.93	0.92	0.92	0.91
MEP	-7.6	-7.5	-7.7	-7.6	2.7
MAPE	23.9	23.8	24.1	24.3	22.5
PEV	2.17	2.18	2.20	2.21	2.49
DW	0.78	0.78	0.79	0.77	0.94
Y	0.29	0.29	0.27	0.26	0.27
$\beta_i$	0.99	0.99	0.98	0.99	0.65
CCC	0.15	0.15	0.14	0.13	0.18
Analysis two, non-linear models including the $\beta_1$ random effect					
$\beta_1$	4.6677	6.5267	6.0960	6.1726	5.7245
$\beta_2$	0.09915	-0.01179	0.008379	0.001639	0.000613
$\beta_3$	0.003103	-1.3824	1.1093	0.9621	0.000012
AIC	15838	15914	15965	15908	15869
BIC	15870	15948	15997	15940	15901
R <sup>2</sup>	0.93	0.93	0.92	0.92	0.91
MEP	-9.0	-7.6	-7.7	-7.7	2.8
MAPE	24.3	23.9	24.1	24.1	22.6
PEV	2.18	2.17	2.20	2.20	2.50
DW	0.78	0.78	0.79	0.79	0.94
Y	0.29	0.29	0.27	0.26	0.27
$\beta_i$	0.98	0.99	0.98	0.93	0.65
CCC	0.16	0.15	0.14	0.14	0.18

$\beta_1, \beta_2,$  and  $\beta_3,$  regression coefficients that comprise the evaluated non-linear models. AIC, Akaike information criterion. BIC, Bayesian information criterion. R<sup>2</sup>, coefficient of determination. MPE, mean prediction error. MAPE, mean absolute percentage error. PEV, prediction error variance. DW, Durbin-Watson statistic. Y, correlation coefficient between the observed and estimated values.  $\beta_i$ , slope or change rate of the observed value for each change unit in the estimated value, product of the linear regression analysis. CCC, concordance correlation coefficient. Models: WOD, Wood; WIL, Wiltmink; COB, Cobby; BRO, Brody; SIK, Sikka.

**Table 2. Fit of non-linear models in the analysis of the lactation curve at 210 days of female Murrah buffaloes.**

Item	WOD	WIL	COB	BRO	SIK
Analysis one, non-linear models not including random effects					
$\beta_1$	4.7005	6.4911	6.1440	6.1656	5.7903
$\beta_2$	0.09171	-0.01140	0.008984	0.001664	0.000134
$\beta_3$	0.00296	-1.3204	0.7535	0.9849	0.000008985
AIC	17395	17399	17456	17466	17433
BIC	17421	17425	17482	17492	17459
R <sup>2</sup>	0.93	0.93	0.93	0.93	0.93
MEP	-7.4	-7.5	-7.3	-7.6	-4.7
MAPE	23.4	23.4	23.5	23.6	22.8
PEV	2.05	2.01	2.10	2.10	2.11
DW	0.75	0.75	0.76	0.77	0.77
Y	0.34	0.34	0.33	0.32	0.33
$\beta$	0.99	1.00	0.96	0.99	0.89
CCC	0.21	0.20	0.19	0.19	0.21
Analysis two, non-linear models including the $\beta_1$ random effect					
$\beta_1$	4.6154	6.4911	6.1260	6.2882	5.8059
$\beta_2$	0.09956	-0.001140	0.008837	0.001855	0.000156
$\beta_3$	0.003118	-1.3204	1.0572	0.5938	0.000009345
AIC	17233	17401	17457	17319	17272
BIC	17265	17434	17489	17352	17304
R <sup>2</sup>	0.93	0.93	0.93	0.93	0.92
MEP	-7.4	-7.3	-7.5	-7.4	-4.2
MAPE	23.4	23.4	23.5	23.6	22.7
PEV	2.05	2.06	2.08	2.09	2.11
DW	0.75	0.75	0.76	0.77	0.78
Y	0.34	0.34	0.33	0.32	0.33
$\beta$	0.96	1.00	0.98	0.89	0.86
CCC	0.21	0.20	0.19	0.20	0.21

$\beta_1$ ,  $\beta_2$ , and  $\beta_3$ , regression coefficients that comprise the evaluated non-linear models. AIC, Akaike information criterion. BIC, Bayesian information criterion. R<sup>2</sup>, coefficient of determination. MPE, mean prediction error. MAPE, mean absolute percentage error. PEV, prediction error variance. DW, Durbin-Watson statistic. Y, correlation coefficient between the observed and estimated values.  $\beta$ , slope or change rate of the observed value for each change unit in the estimated value, product of the linear regression analysis. CCC, concordance correlation coefficient. Models: WOD, Wood; WIL, Wiltmink; COB, Cobby; BRO, Brody; SIK, Sikka.

0.91. The DW results showed no autocorrelation in the residuals, with values in the range of 0.71 to 0.94; the MAPE fluctuated from 22.5 to 24.3, with a mean value of 23.5%. For the MPE, the models tend to underestimate the predictions since they presented negative sign results (MPE of -0.42 to -0.90). However, SIK for 180d showed different results that overestimated the prediction (MPE of 2.7). For LL, the AIC- and BIC-based order selection of NLM was best fit for 180 d, followed by 210d and 240d; the order selection of models within LL differs across the analyses. The AIC and BIC values were lower in the WIL and COB models with ANA1; the WOD, BRO, and SIK models showed a reduction with ANA2. The model with the best fit was WOD, followed by

SIK and BRO.

As an indicator of accuracy in the predictions, the Y presented estimates of medium to low magnitude, with a mean value of 0.32. However, a positive trend was observed as LL increased. The  $\beta_1$  obtained from the linear regression analysis indicates the precision of the predictions. Values close to one indicate good precision; values distant to one can be associated with biases in the predictions. The results for  $\beta_1$  ranged from 0.65 (SIK in 180d) to 1.00, with a mean value of 0.94. Table 4 shows the production variables associated with the LC across periods and NLM, while Figs. 1 and 2 show the LC for the three LL through the WOD and SIK models.

LL to which the milk production system

**Table 3. Fit of non-linear models in the analysis of the lactation curve at 240 days of female Murrah buffaloes.**

Item	WOD	WIL	COB	BRO	SIK
Analysis one, non-linear models not including random effects					
$\beta_1$	4.8222	6.4001	6.1050	6.1586	5.8833
$\beta_2$	0.08089	-0.01048	0.008563	0.001649	-0.00045
$\beta_3$	0.002712	-1.1585	1.0944	0.9993	0.000005448
AIC	18349	18357	18403	18412	18398
BIC	18376	18383	18429	18438	18424
R <sup>2</sup>	0.93	0.93	0.93	0.93	0.92
MEP	-7.3	-8.9	-7.3	-7.4	-7.4
MAPE	23.2	23.9	23.3	23.4	23.3
PEV	2.01	2.05	2.03	2.04	2.02
DW	0.71	0.74	0.73	0.75	0.77
Y	0.37	0.36	0.36	0.35	0.35
$\beta$	0.99	0.83	0.99	0.99	0.99
CCC	0.23	0.25	0.22	0.22	0.22
Analysis two, non-linear models including the $\beta_1$ random effect					
$\beta_1$	4.7989	6.4001	6.1050	6.2527	5.9611
$\beta_2$	0.08312	-0.01048	0.008563	0.001788	-0.00072
$\beta_3$	0.002756	-1.1584	1.0943	0.6989	0.000004456
AIC	18168	18359	18405	18233	18222
BIC	18200	18392	18437	18266	18255
R <sup>2</sup>	93.2	93.0	93.1	93.1	92.9
MEP	-7.3	-8.9	-7.3	-7.4	-7.5
MAPE	23.2	23.9	23.3	23.4	23.3
PEV	2.01	2.05	2.03	2.03	2.06
DW	0.71	0.72	0.71	0.73	0.78
Y	0.37	0.36	0.36	0.35	0.35
$\beta$	0.98	0.83	0.99	0.92	0.97
CCC	0.24	0.25	0.22	0.23	0.22

$\beta_1$ ,  $\beta_2$ , and  $\beta_3$ , regression coefficients that comprise the evaluated non-linear models. AIC, Akaike information criterion. BIC, Bayesian information criterion. R<sup>2</sup>, coefficient of determination. MPE, mean prediction error. MAPE, mean absolute percentage error. PEV, prediction error variance. DW, Durbin-Watson statistic. Y, correlation coefficient between the observed and estimated values.  $\beta$ , slope or change rate of the observed value for each change unit in the estimated value, product of the linear regression analysis. CCC, concordance correlation coefficient. Models: WOD, Wood; WIL, Wiltmink; COB, Cobby; BRO, Brody; SIK, Sikka.

extends has different effects. In this sense, LL to which the milk production adjusts, is the basis of adjustment factors and the definition of productive variables in genetic evaluations, with possible implications in the estimation of variance components and genetic parameters (da Silva et al., 2000; Gutiérrez-Valencia et al., 2006; Baldi et al., 2011; Santos et al., 2017). Furthermore, LL is directly related to total milk production and herd income (Suárez and Ramos, 2011; Méndez and Fraga, 2012; Safari et al., 2018), and it can affect the subsequent productive and reproductive life of females (Khan and Chaudhry, 2000; Tonhati et al., 2000; Mendes et al., 2013). Studies of other populations and different production systems have reported various results regarding

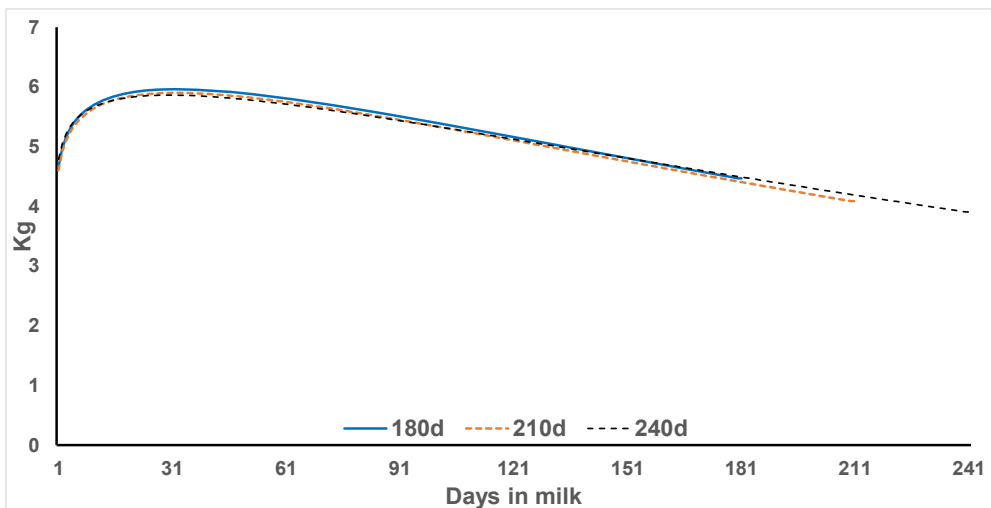
production levels, such as (PTOTAL - LL): Tonhati et al. (2000), 1496 kg in 271 d; Rosati and Van Vleck (2002), 2286.8 kg in 270 d; Gutiérrez-Valencia et al. (2006), 1009.3 kg in 240 d and 1069.6 kg in 270 d; Ramos et al. (2006), 1650 kg in 256 d; Suárez and Ramos (2011), 730 kg in 200 d and 869.9 in 244 d; García et al. (2012), 717 kg in 200 d and 860 kg in 244 d; Méndez and Fraga (2012), 658 kg in 170 d; Shokrollahi and Hasanpur (2014), for two populations 2184.0 kg and 1540 kg at 240 d; Fundora (2015), 867.1 kg in 220 d.

Regarding the LC analysis with NLM, the published results are diverse for the ranking and selection of models to describe the evaluated LC. Quintero-Vélez et al. (2007) reported SIK as the best fit model, followed by BRO and WOD.

**Table 4. Production levels across lactation periods and non-linear models evaluated.**

	WOD	WIL	COB	BRO	SIK
180-day lactation					
PI	4.65	5.20	4.08	4.02	5.72
PTOTAL	955.3	955.8	957.8	958.1	865.4
PMAX	5.88	5.87	6.04	6.05	5.72
DPMAX	27	31	10	10	1
MDP	5.31	5.30	5.32	5.32	4.81
210-day lactation					
PI	4.69	5.22	3.24	4.70	5.79
PTOTAL	1084.3	1084.8	1085.8	1089.9	1057.8
PMAX	5.88	5.86	6.06	6.11	5.79
DPMAX	30	31	11	8	1
MDP	5.16	5.17	5.18	5.19	5.04
240-day lactation					
PI	4.81	6.24	4.05	3.88	5.88
PTOTAL	1210.0	1229.8	1214.5	1216.0	1212.9
PMAX	5.85	6.24	6.05	6.08	5.88
DPMAX	26	1	6	8	1
MDP	5.04	5.12	5.06	5.07	5.05

Models: WOD, Wood; WIL, Wiltmink; COB, Cobby; BRO, Brody; SIK, Sikka. PI, initial production; PTOTAL, accumulated total production; PMAX, maximum production at peak lactation; DPMAX, days until maximum production; MDP, mean daily production.



**Fig. 1. Lactation curves for female Murrah buffaloes based on the Wood model at 180 (180d), 210 (210d), and 240 days (240d).**

Another study conducted by Hossein-Zadeh (2016) reported Nelder and SIK as the best fit models for the first and second lactation, while the ranking of models changed for the third lactation, with WOD being the best fit, followed by Dhanoa and SIK. Additionally, El-Bramony et al. (2016) and Soysal et al. (2016) reported that WOD, followed by COB and WIL, were fit to describe

the production of milk and its components, evaluating from one to seven lactations. Abdel-Salam et al. (2011), Shokrollahi and Hasanpur (2014), and Dezfuli and Babaci (2018) showed similar results, and the WOD model was selected and applied to evaluate and characterize the LC in female buffalo populations. Other studies evaluating linear models have reported the fit

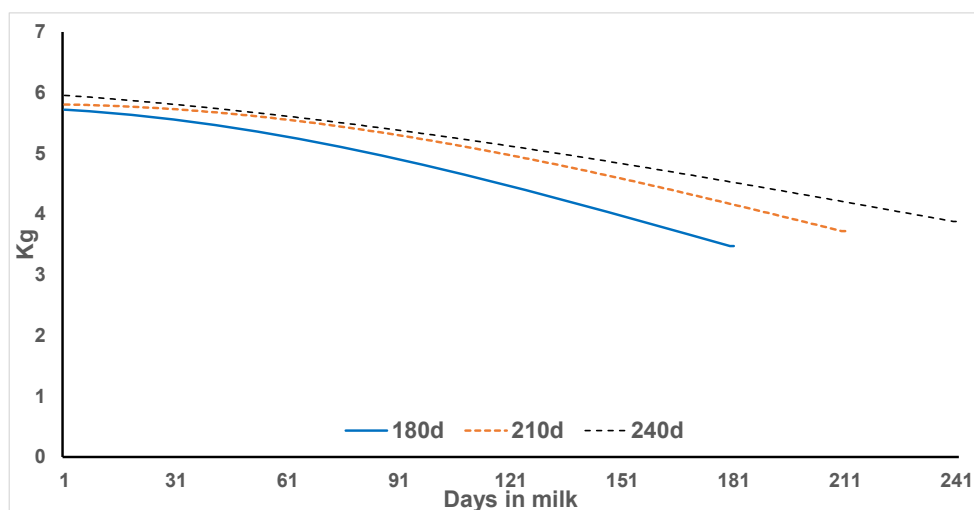


Fig. 2. Lactation curves for female Murrah buffaloes based on the Sikka model at 180 (180d), 210 (210d), and 240 days (240d).

of LC based on quadratic polynomial models and logarithmic components (Fraga et al., 2003; Muñoz et al., 2008; García et al., 2013).

The regression coefficients of the NLM associated with the production, as well as the directly and indirectly derived production variables, can be considered as selection criteria and objectives in breeding programs due to the heritability and genetic correlations estimated for those variables (Rosati and Van Vleck, 2002; Mendes et al., 2013; Safari et al., 2018). In this study, the random effect of the female buffaloes for a regression coefficient improved the fit of WOD, BRO, and SIK. However, future studies should explore the nature of these random effects and their association with genetic or environmental effects. In the ranking of models with LL, the shortest time showed the best fit, based on the AIC and BIC criteria. However, the fit of LL in the estimation of variance components and genetic parameters, as well as in the prediction of genetic values and the ordering of the sires, should be evaluated to develop breeding schemes based on selection.

The production systems of dairy buffaloes under tropical conditions depend on animal, environmental, feeding, and technological factors. In the animal context, the variability in the production levels is attributed to the genotype and additive and non-additive genetic effects as well as to the environmental effects associated with the animal when there are repeated measures over time, as is the case of LC (Tonhati et al., 2000; Ramos et al., 2006; Fooda et al., 2011). The combination of environment,

feeding, and technology defines the production systems. For dairy buffaloes, the production systems transcend the humid and sub-humid tropics of America (Gutiérrez-Valencia et al., 2006; Quintero-Vélez et al., 2007; Suárez and Ramos, 2011; Mendes et al., 2013), Asia (Khan, 1997; Shokrollahi and Hasanpur, 2014; Soysal et al., 2016), and Africa (Abdel-Salam, 2011; Fooda et al., 2011; El-Bramony et al., 2016). The Member States of the South Asian Association for Regional Cooperation make up 73.8% of the world's buffalo population and the populations of the main dairy breeds, Murrah and Nili Ravi, are derived from this region (Hamid et al., 2016).

## CONCLUSIONS

Including the variance associated with the animal transcended in the Akaike and Bayesian information criteria, the evaluated models showed goodness of fit, with good predictability, but low values in accuracy and precision of prediction. The lactation curve of female Murrah buffaloes was fitted to the WOD model, followed by SIK, for the periods of 180, 210, and 240 days.

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