Scientific Paper

Utilization of the response surface methodology to model changes in the productivity of *Cynodon nlemfuensis* Vanderyst

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Abstract

Objective: To use the response surface methodology to model the productivity changes of *Cynodon nlemfuensis* Vanderyst, subject to different fertilization doses and regrowth ages, under cutting conditions, in both seasons of the year.

Materials and Methods: The data were taken from experiments conducted at the Institute of Animal Science in a split-plot design. The main plot was the nitrogen application dose (0,200 and 400 kg N/ha/year). Meanwhile, in the subplots, the 12 regrowth ages (from 1 to 12 weeks) were placed. The total dry matter yield, leaf dry matter yield and stem dry matter yield, were analyzed as response variables. The data were adjusted to multiple regression and response surface models for each variable. They were statistically processed through the packages STATGRAPHICS® Centurion XVI and GeoGebra.

Results: The variables total dry mater yield, leaf dry matter yield and stem dry matter yield, for the dry and rainy seasons, normally decreased for the magnitude of the standardized biases and kurtosis. The second-order model was of good fit, with determination coefficient (R^2) higher than 94 % for all the variables, whose expressions allowed to analyze integrally the management factors in the productive performance of pasture, which facilitates decision-making for its management.

Conclusions: It was proven that the procedures associated to the response surface methodology constitute an efficient tool for pasture growth analysis.

Keywords: *Cynodon nlemfuensis*, experimentation, mathematical models, productivity

Introduction

In agricultural sciences, the conduction of experiments that guarantee appropriate answers to different studied problems or processes is frequent, for which adequate and efficient techniques are required in research processes.

In Cuba, since the 70's and until the present, many studies have been conducted which allowed to create a scientific methodological basis for the study of the growth dynamics and quality of pasturelands under controlled and field conditions through studies that comprise optimum indicators (Rodríguez, 2015; López, 2016; Fernández-Chuairey *et al*., 2017). In these works several factors and their interactions were simultaneously studied. Nevertheless, the statistical analyses were focused on the utilization of simple regressions, although multiple management factors are involved in growth. The application of statistical-mathematical criteria, linked to the response surface methodology (RSM), can contribute to the solution of this problem.

In the international context, this methodology has been used as tool in studies applied to diverse sectors of society, such as the food and chemical industry (Ponce *et al*., 2018; Pillajo *et al*., 2019; Wang *et al*., 2019; De Lima *et al*., 2020) and agricultural sciences (Aredo *et al*., 2014; Del-Ángel-Sánchez et *al*., 2015; Yaguas, 2017). In Cuba, the consulted literature proves that the studies are scarce, particularly, in agricultural sciences, although the works conducted by Guerra-Bustillo (1980), Miranda *et al*. (2013) and Guerra-Bustillo *et al*. (2018) should be mentioned.

Although in scientific literature the research that support the applications of RSM for the characterization of pastureland dynamics and determination of optimum values in different management levels and factors, are not abundant, Guerra-Bustillo *et al.* (2018) that the RSM constitutes a powerful tool of mathematical-statistical modeling in the scientific-research development, by incorporating in its procedures dot-matrix algebra, differential calculus, analytical geometrics, operational research, treatment design an model selection,

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among other aspects, which can be highly useful to study the dynamics of pasture ecosystems.

The objective of this study was to use the response surface methodology to model the productivity changes of *C. nlemfuensis*, subject to different fertilization doses and regrowth ages under cutting conditions, in both seasons.

Materials and Methods

Location. The research was conducted at the Institute of Animal Science (ICA, for its initials in Spanish), located in the San José de las Lajas municipality, Mayabeque province, Cuba, between 22º 53´ North latitude and 82º 02´ West longitude, at 92 m.a.s.l.

Edaphoclimatic characteristics. The experimental works were developed on a compacted ferralitic red soil (Hernández *et al*., 2015), uniform throughout its profile and of fast desiccation. During the experimental period (May, 1992-April, 1993), the mean annual temperature was 24,1 ºC, with maximum values between 31,9 and 32,5 ºC. The rainfall regime in the year is 1 328,27 mm and the average relative humidity, of 79,6 %.

Design and experimental treatments. A split-plot design with three replicas, was used. The main plot was the nitrogen application dose (0, 200 and 400 kg N/ha/year) as ammonium nitrate. Meanwhile, in the subplots the 12 regrowth ages (from 1 to 12 weeks) were placed. A total of 36 treatments were made up, corresponding to the above-described combinations (nitrogen dose x regrowth age).

Experimental procedure. A previously established area of *C. nlemfuensis* (star grass), with more than five years (more than 95 % purity), was used. The remaining area was covered by *Dichanthium annulatum* (Forssk.) Stapf., *Digitaria ciliaris* (Retz.) Koeler and *Cynodon dactylon* (L.) Pers., divided into experimental plots, with harvestable area of 10 m^2. The plots were fertilized with a basal application of 100 kg of P_2O_5/ha/year (triple superphosphate) and 200 kg of K_2O/ha/year (sodium chloride). In the dry season, irrigation by aspersion was applied, at a rate of 500 m^3/ha.

Previous to each experimental cycle (two in the dry season and two in the rainy one), the plots were cut with a harvester to make the cut uniform. Afterwards, the cut was carried out before the six weeks of resting in each treatment between growth cycles.

The harvested green material was weighed in each cut and 200-g parallel samples were taken per each treatment and replica. They were fractioned into their structural components (leaves and stems) and were introduced in an air-circulation stove at 60 ºC until reaching constant weight, in order to determine the dry matter content and the total, leaf and stem dry matter yield.

Measured variables. The study was conducted in the dry and rainy seasons. The total dry matter yield (TDMY), dry matter yield of the leaves (LY) and dry matter yield of the stems (SY), expressed in t DM/ha, were observed as response variables. The age (weeks) and nitrogen doses (kg N/ha/year) were established as independent variables.

Statistical analysis. A data matrix was made with the information of the two seasonal periods: dry season (DS) and rainy season (RS). To characterize all the dependent variables descriptive statistics was used, with central trend and dispersion measures.

The methodological procedure used for obtaining the response surface included for each case:

- Multiple regression analysis.
- Goodness of fit.
- Graphic representation of the surface.
- Obtainment of optimums (analytically).
- Interpretation of the results.

The multiple regression analysis was performed on the three above-mentioned dependent variables. The age and nitrogen dose were considered as dependent variables.

In all the cases first- and second-order models were applied, given by the equations:

$f(x, y) = b_0 + b_1x + b_2y$ First order

$f(x,y) = bo + b_1x + b_2y + b_3x^2 + b_4y^2 + b_5xy$ Second order

Where:

$f(x, y)$: is the dependent variable (response)

x: independent variable (factor 1): regrowth age

y: independent variable (factor 2): fertilizer doses

b_0, b_1, b_2, b_3, b_4 and b_5: are parameters of the models

As fit procedure the method of ordinary least squares was used, according to the statistical criteria published by Burguillo (2005) and Bingham and Fry (2010), who expressed that this is one of the most used methods in the estimation of parameters in linear regression.

For the goodness of fit the statistical criteria referred by Guerra-Bustillo *et al.* (2003) and Torres *et al.* (2012) were used, which include:

- Determination coefficient (R^2).
- Significance of the model.
- Mean absolute error (AE).
- Durbin-Watson statistic.

- Graphic analysis of the residues.
- Selection of the best model.
- Correlation analysis between the observed values and those predicted by the best model.

For the confection of the response surface graph, once the equation is obtained, taking into consideration the goodness of fit and fulfillment of the indicators, the adequate function was defined and the region corresponding to the analyzed data was shown on a graph. For the studied intervals, the optimum responses and combinations, or both, were determined in each case.

For obtaining the optimums through the analytical method, the mathematical criteria, necessary and sufficient conditions for the location of the maximum, minimum or saddle points of all the obtained responses, were applied.

For the doubly differentiable functions, the necessary and sufficient conditions comprised the analysis of the determinant sign of the Hessian matrix H and the annulment of the partial derivatives to find the critical point P. These criteria are shown below:

$H_f(P) > 0$ and $f\_xx\ (P) > 0 \Rightarrow$ P is a relative minimum
$H_f(P) > 0$ and $f\_xx\ (P) < 0 \Rightarrow$ P is a relative maximum
$H_f(P) < 0 \Rightarrow$ P is a saddle point

From the optimums, the presence or absence of these points in the experimental region, was determined. In the case of not being in such region, the values of the independent variables in which the best responses were reached, were estimated.

The data were processed through the statistical package STATGRAPHICS® Centurion XVI for the multiple regression analysis. In addition, the online dynamic mathematics program GeoGebra (2020) was used to determine the optimum values through the described necessary and sufficient conditions.

Results and Discussion

The descriptive analysis of the variables TDMY, LY and SY, for the dry and rainy seasons, is shown in table 1. The data were normally distributed, according to the magnitude of the standardized biases and kurtoses, whose values were between -2 and +2, except the case of the SY in the dry season, with a standardized bias of 2,15. The maximum and minimum values for each variable are within the normal ranges for the species, according to the studied management conditions (Pozo, 1998).

When considering the TDMY, LY and SY values as dependent variables, and the age and nitrogen dose, as independent, the mathematical expressions that allow to describe the performance of the response surface for each studied variable and season, were obtained.

According to Rodríguez-González *et al*. (2018), in the face of the large quantity of developed models, a deep evaluation and validation is necessary, in order to adopt the best model. Table 2 shows the results of the goodness of fit, which contribute high determination coefficients (R^2), with values higher than 84 % in the first-order models, and over 94 % for the second-order ones. The values of the adjusted determination coefficient, which were higher in the quadratic models, were also considered. It should be stated that the variability shown by the yield variables (DMY, LY and SY) is explained by the changes in the values of fertilization and regrowth age (> 97 % in DS and 94 % in RS).

Table 1. Descriptive statistical summary for the total dry matter yield and its morphological components in the rainy and dry seasons.

Statistic	TDMY		LY		SY	
	DS	RS	DS	RS	DS	RS
Number of observations	36	36	36	36	36	36
Mean, t DM/ha	1,52	2,58	0,69	1,25	0,83	1,34
Standard deviation	1,13	1,97	0,43	0,88	0,71	1,09
Minimum, t DM/ha	0,15	0,04	0,09	0,03	0,05	0,01
Maximum, t DM/ha	3,86	5,86	1,46	2,68	2,41	3,22
Range	3,71	5,82	1,36	2,65	2,36	3,21
Standardized bias	1,71	0,63	1,08	0,38	2,15	0,86
Standardized kurtosis	-0,77	-1,74	-1,26	-1,75	-0,30	-1,66

TDMY: total dry matter yield, LY: leaf dry matter yield, SY: stem dry matter yield
RS: rainy season, DS: dry season

Table 2. Results of the goodness of fit of the evaluated models

Variable	First order	Second order	First order	Second order
	Dry season		Rainy season	
Determination coefficient R^2				
(TDMY), %	87,28	98,44	85,78	94,95
(LY), %	88,37	97,06	86,62	95,21
(SY), %	84,85	97,95	84,22	94,44
Adjusted determination coefficient R^2A				
(TDMY), %	86,51	98,19	84,92	94,11
(LY), %	87,67	96,57	85,81	94,41
(SY), %	83,93	97,60	83,27	93,52
Model significance (P - value*)*				
TDMY	0,0000	0,0000	0,0000	0,0000
LY	0,0000	0,0000	0,0000	0,0000
SY	0,0000	0,0000	0,0000	0,0000
Absolute mean of the error				
TDMY	0,29	0,10	0,57	0,35
LY	0,11	0,05	0,25	0,15
SY	0,20	0,07	0,34	0,19
Durbin Watson				
TDMY	2,63 (P=0,96)	1,45 (P=0,04)	2,58 (P=0,95)	2,40 (P=0,84)
LY	2,59 (P=0,95)	2,41 (P=0,85)	2,26 (P=0,75)	2,24 (P=0,71)
SY	2,54 (P=0,94)	0,93 (P=0,0003)	2,74 (P=0,98)	2,53 (P=0,92)
Correlation coefficient r_{op}				
TDMY	0,92	0,99	0,93	0,97
LY	0,93	0,98	0,94	0,97
SY	0,91	0,98	0,92	0,97

TDMY: total dry matter yield, LY: leaf dry matter yield, SY: stem dry matter yield
r_{op} .-correlation coefficient between the observed values and the ones predicted by the model

It was proven that the absolute error means were relatively lower in the second-order models with regards to the first-order ones. The values by Durbin Watson test (DW) showed that there were no problems of autocorrelation of errors, in the first- and second-order models. Nevertheless, in the second-order one, for he variable SY, there was lack of fulfillment in the dry season, with values of the DW statistic < 1,4. This indicates the existence of autocorrelation in the residues.

In addition, there was strong correlation between the predicted values and the ones observed in all the models. It was higher in the second-order expressions, with correlation coefficient r >= 0,97, which suggests the good goodness of fit of the models.

Both models showed good fits. However, the selection criteria showed that the second-order model is the most indicated one to represent and predict the dynamics of the factors nitrogen fertilization and regrowth age in the changes of star grass productivity, in the dry and rainy seasons, respectively.

Thornley and France (1984) stated that the mathematical models, which include more than one variable, represent better the plant growth dynamics, valid criteria for the performance shown by the variables studied in this work. Villegas *et al.* (2019) report that obtaining non-linear models can be very helpful to explain and predict the performance of pastures and forages before certain factors such as age. The studies conducted in Cuba in this same species, with both independent factors, indicate that the performance of the studied variables responds to second-order mathematical expressions.

Table 3 shows the best-fit models for the response variables and graphic expressions, for the

Table 3. Best-fit mathematical models (where: x = regrowth age, y= fertilizer doses).

Response variable	Model
TDMY dry season	*f* (*x*,*y*)="-0.19 + 0.19x + 0.0004y - 0.006" x^2 " - 0.000002" "y" ^2 "+ 0.0006x.y"
TDMY rainy season	f(x,y)="-1.39 + 0.53x + 0.009y - 0.02" x^2 " - 0.00001" "y" ^2 "+ 0.0007x.y"
LY dry season	f(x,y)="-0.09 + 0.10x + 0.0008y - 0.004" x^2 " - 0.000001" "y" ^2 "+ 0.0002x.y"
LY rainy season	f(x,y)="-0.67 + 0.29x + 0.004y - 0.01" x^2 " - 0.000007" "y" ^2 "+ 0.0002x.y"
SY dry season	f(x,y)="-0.10 + 0.08x -0.0003y - 0.001" x^2 " - 6.45" "y" ^2 "+ 0.0004x.y"
SY rainy season	f(x,y)="-0.71 + 0.24x "+" 0.005y - 0.0" 7x^2 " - 0.00001" "y" ^2 "+ 0.0004x.y"

TDMY total dry matter yield, LY: leaf dry matter yield, SY: stem dry matter yield

regrowth ages and nitrogen application doses in the dry and rainy seasons (from left to right, respectively).

For any age and season, the total dry matter yield was higher when nitrogen was supplied, and its differences were more evident from the third and fifth weeks, in the dry and rainy season, respectively. Similar results were reached by Méndez *et al*. (2019), when studying the effect of nitrogen fertilization (0, 40,5 and 81 kg N/ha) on the star grass yield, with responses of up to 40 % as the dose increased (figure 1).

In the graphic representation of the models (figure 1) it was proven that, in both seasons, all the response variables obtained higher values, as the regrowth age and fertilizer dose increased simultaneously, although with particular performances in each season. Similar results were reported by López (2016), Martínez-Martínez *et al.* (2016), Reyes-Pérez *et al.* (2018) and Viteri-Velazco *et al.* (2018). In addition, there was proportional increase of total yield (TDMY) and its structural components (LY and SY) with age and fertilizer. This effect could be given, probably, by the higher metabolic capacity shown by this species in the processes of mobilization and synthesis of organic substances for the formation and functioning of its structures (Pozo *et al.,* 2002).

Similar responses were obtained by Ramos *et al.* (1987) in a study of *C. nlemfuensis* growth with three nitrogen doses (0, 200 and 400 kg N/ha/year) and three cutting ages. These authors recommended to cut between the fifth and sixth week, with the application of 200 kg N/ha/year, dose with which the maximum values of density and growth rate were reached.

Herrera and Ramos (2006) argued that the longest cutting frequencies provided higher yields. Nevertheless, they indicated the progressive decrease of its quality under this management.

When evaluating the effect of regrowth age (35, 42, 49, 56, 63 and 70 days), season and species on the nutritional composition of *Leucaena leucocephala* (Lam.) de Wit and *C. nlemfuensis*, associated in an intensive silvopastoral system, Martínez-Martínez *et al.* (2016) stated that the age and season interaction significantly affected ($p < 0,05$) the dry matter percentage and optimum harvest period of *L. leucocephala* associated with star grass, between 42 and 49 days after pruning or grazing in the rainy and dry seasons, respectively.

The necessary and sufficient conditions for the location of the extreme points in the selected response surface models, showed the maximum TDMY, LY and SY values, in both seasons, out of the ranges of the studied factors (age and nitrogen).

In spite of the above-explained facts, in the studied intervals, the maximum values for the three variables were obtained at the age of 12 weeks and with 400 kg N/ha/year, in both seasons, reaching TDMY of 4,20 t DM/ha in the dry season and 6,38 t DM/ha in the rainy season (table 4).

Maldonado-Peralta *et al*. (2019) stated that the total yield of pastures and its structural components (leaves and stems) was linearly and increasingly related to the increase in the plant age; while the leaf:stem ratio decreased as age increased, aspect that constitutes a regularity in the forage grasses used as pastures and forages.

Conclusions

The utilization of the response surface methodology, which includes the design, modeling and optimization of processes, constitutes a useful tool to represent and analyze the growth of *C. nlemfuensis* and of other species with similar behavior, regarding vegetative growth and similar nitrogen doses and regrowth age, in both seasons.

The application of this methodology allowed the determination of the extremes, which in each indicator were variable in all the expressions for *C. nlemfuensis*, and were reached out of the studied intervals. Yet, they allowed to obtain optimum values in the study region and appraise other alternatives for values foreseen according to the obtained values.

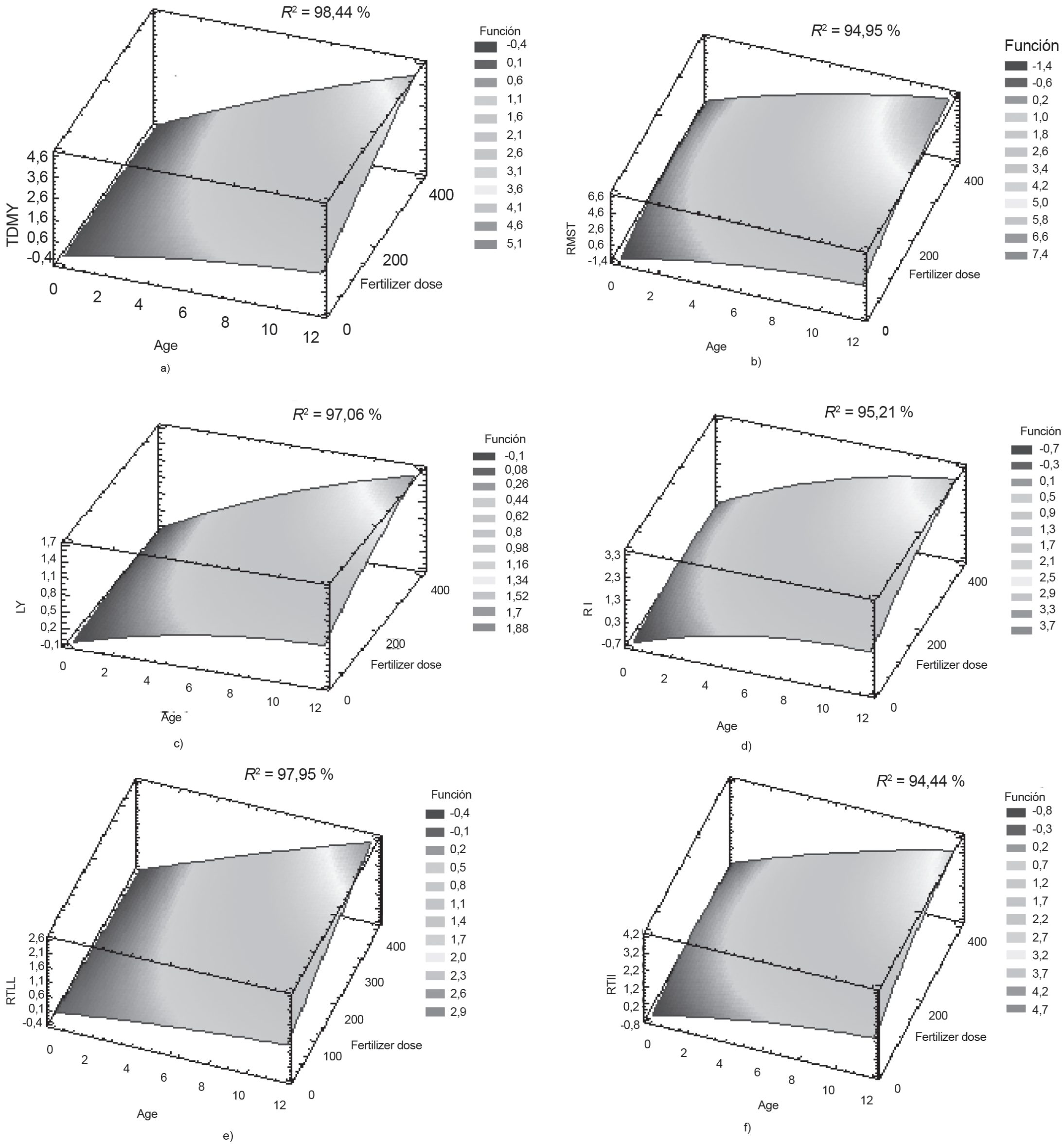

Figure 1. Graphic representation of the quadratic models of response surface for each of the studied variables, in the dry and rainy seasons (from left to right, respectively).Edad de rebrote (semanas), dosis de fertilizante (kg N/ha/año).
Regrowth age (weeks), fertilizer doses (kg N/ha/year).
Where a)- b) represent TDMY; c) - d) LY and e) - f) SY
TDMY: total dry matter yield, LY: leaf dry matter yield, SY: stem dry matter yield.

The second-degree polynomial showed the best fit for the variables related to the yield and a high determination coefficient.

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Authors' contribution

- Liansy Fernández-Domínguez. Conduction of the research, data processing and manuscript writing.
- Pedro Pablo Del Pozo-Rodríguez. Conception of the main research idea, advisory during research development and manuscript writing.
- Lucia Fernández-Chuairey. Statistical analysis and design, research and manuscript advisory.

Table 4. Predicted local maximums in the optimization of the analyzed yield variables.

Variable	Optimum value	
	DS	RS
TDMY	4,20 t DM/ha	6,38 t DM/ha
LY	1,62 t DM/ha	2,86 t DM/ha
SY	2,96 t DM/ha	3,50 t DM/ha

Age of 12 weeks and with a dose of 400 kg N/ha/year
TDMY: total dry matter yield, LY: leaf dry matter yield,
SY: stem dry matter yield

- Rafael Herrera-García. Design and setting up of the experiments, as well as research advisory.

Conflict of interests

The authors declare that there are no conflicts of interests among them.

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