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Milk yield prediction at late lactation in reproductive rabbit does

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Sin vuestro apoyo no habría podido llegar hasta aquí.

LIST OF ABBREVIATIONS

C	commercial diet	MY4	milk yield per day during the 4 th wk of lactation
CP	crude protein	n	number
CV	coefficient of variation	OL	overlap between current lactation and next gestation
d	day	PC	principal component
DE	digestible energy	PCA	principal component analysis
DM	dry matter	PFT	perirenal fat thickness
Δ PFTd	variation of perirenal fat thickness per day between the two points recorded for each data set	RMSE	root mean square error
dpp	days post-partum	SD	standard error
Eq1	equation 1	SQ(LSW)	square of LSW
Eq2	equation 2	SQ(TEI)	square of TEI
Eq3	equation 3	TEI	total energy intake
F	fibrous diet	V16	genetic line constituted from four specialized maternal lines into a composite synthetic line and then selected by litter size at weaning for 16 generations
LP	genetic line founded by reproductive longevity criteria and selected by litter size at weaning for 6 generations	V36	genetic line constituted from four specialized maternal lines into a composite synthetic line and then selected by litter size at weaning for 36 generations
LSW	litter size at weaning	wk	week
max	maximum		
min	minimum		
MLR	multiple linear regression		
MY3	milk yield per day during the 3 rd wk of lactation		

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RESUMEN

Para evitar las consecuencias negativas en el aprendizaje y desarrollo de los gazapos, que podrían aparecer al medir la producción de leche en la cuarta semana, se propuso un modelo, en el que se incluyeron un total de 324 lactaciones, procedentes de dos sets de datos. El ritmo reproductivo fue semi-intensivo en ambos ensayos [inseminación a los 11 d postparto (dpp) y destete a 28dpp], pero las dietas, tipos genéticos, el número de parto y el día de los controles fueron ligeramente diferentes en ambos casos. El modelo incluyó como variables independientes el tamaño de camada al destete (LSW, tanto el efecto lineal como el cuadrático), el consumo total de energía (coneja + camada) (TEI, tanto el efecto lineal como el cuadrático), cambio de condición corporal (Δ PFTd) y producción de leche en la tercera semana (MY3) como rasgos cuantitativos; la existencia de solape entre la lactación actual y la siguiente gestación (variable dummy) y sus interacciones con las variables cuantitativas. Para ajustar este modelo, se propusieron tres ecuaciones que variaban en el uso (Eq2 y Eq3) o no (Eq1) de un suavizado de la distribución de la variable dependiente (Eq3), en el uso de muestras no redundantes y en la eliminación de colinearidades entre variables (Eq3). TEI tuvo una relación cuadrática y MY3 una relación lineal positiva con MY4 en las 3 ecuaciones evaluadas. Los resultados mostraron que, tras la inclusión de TEI y MY3, LSW tuvo un peso relativamente bajo respecto a las otras variables en Eq3 y no se incluyó en Eq1 y Eq2. Δ PFTd sólo se incluyó en el Eq2 y Eq3, denotando un efecto negativo relevante sobre MY4 durante la gestación. La predicción de la producción de leche en la cuarta semana es posible con las variables utilizadas en este estudio, aunque se deben tomar ciertas precauciones. Los principales factores que afectaron a la producción de leche en la cuarta semana fueron la ingesta de energía en la cuarta semana y la producción de leche en la tercera semana. Sin embargo, el uso de un pre-tratamiento de los datos, para suavizar la distribución de la variable dependiente, parece mejorar la predicción, especialmente para valores extremos.

Palabras clave: Ingestión de energía, Tamaño de camada, Condición corporal, Solape.

ABSTRACT

To avoid negative consequences in young rabbit's training and development, that could appear when milk yield measurement at 4th wk is needed, a model was proposed and fitted. A total of 324 lactations coming from two data sets were included in the regressions. The reproductive rhythm was semi-intensive in both trials [insemination at 11 d post-partum (dpp) and weaning at 28dpp], but diets, genetic types, parity order and day of controls were slightly different in some cases. The model included as independent variables litter size at weaning (LSW; both linear and quadratic), total energy intake (doe+litter) (TEI; both linear and quadratic), body condition change (Δ PF_{Td}) and milk yield at 3rd wk (MY₃) as quantitative traits, overlapping degree between current lactation and next pregnancy as dummy variable and their interactions with quantitative traits. To fit this model, three equations differing on the use (Eq2 and Eq3) or not (Eq1) of smoothing sample distribution, non-redundant samples (Eq3) and non-colinearities among variables (Eq3), were tested in order to obtain an accurate equation with biological meaning. TEI had a quadratic relation and MY₃ a positive linear relation with MY₄ in the 3 equations evaluated. The results showed that, after including TEI and MY₃, LSW had a lower relative weight respect to the other variables in the Eq3 and was not included in Eq1 and Eq2. Δ PF_{Td} was only included in the Eq2 and Eq3, denoting a relevant negative effect on MY₄ when pregnant, while slightly positive when non-pregnant. Predicting milk yield at 4th wk is possible with the variables used in this study, although certain precautions must be taken. Main factors affecting milk yield at 4th wk were joined energy intake at 4th wk and milk yield at 3rd wk. However, population pre-treatment of data, to smooth the dependent variable distribution, seems to improve prediction, especially for extreme values.

Key words: Energy intake, Litter size, Body condition, Overlapping.

INTRODUCTION

In the same way than the rest of mammals, milk yield is a strategy of the rabbit female to provide nutrition and ensure survival of the young offspring. Therefore, lactation is a crucial period, not only for survival, but also for a suitable performance of the new-born rabbits. In fact, it has been reported a negative correlation between weaning weight and post-weaning mortality (Lebas, 1993). In addition, lactation is an exigent period for the rabbit females, with a large production of milk characterised for its high nutrient concentration (Maertens *et al.*, 2006).

On the other hand, not only the amount of milk is important, but also the moment, for both does and kits. When rabbits are strictly dependent of milk (17-20 d) high production is interesting. However, high production at late lactation, could reduce litter feed intake (Blas *et al.*, 1990) and cause a more sudden weaning (less progressive transition from milk to solid feeding), which would increase health risk index during growing period (Quevedo *et al.*, 2006). There are several factors affecting milk yield at a concrete lactation stage such as genetic type or selection (Saviotto *et al.*, 2002), diet (Pascual, 2003) or overlapping degree between current lactation and next pregnancy (Lebas, 1972).

Regarding to the feeding behaviour during lactation, which was reviewed by Gidenne and Lebas (2006), new-born rabbits stay into the nest box till 17-20 d of life and rabbit does usually go into the nest only once a day. From this moment, kits are able to move easily and also to go away from the nest and solid feed intake arise progressively. When kits are always into the nest box, milk yield measurement is quite easy, weighting females before and after the milking. However, from this moment on, kits need to be allowed to go away from the nest. Consequently, to be able to measure milk yield, litter and doe separation is required. Nevertheless, this procedure could have consequences in the development of the study

(increases cost and management) and in both female and litter behaviour. According to Gidenne and Lebas (2006) in this moment young rabbits have a transition from a single milk meal per day to a large number, combining liquid and solid meals, and also begin caecotrophy behaviour. In fact, Faraldo *et al.* (2013) showed how separate litter from the doe could worsen the learning of the young rabbits which would reduce their solid feed intake, especially at the end of lactation and the beginning of the growing period.

Therefore, development of a model which could allow milk yield prediction at late lactation would be interesting. When models are proposed, data population is set up promoting variability of the dependent variable (wide range, independent variables, combination of them, etc.), but sometimes the distribution of dependent variable is not frequently considered. For instance, a normal distribution has a high number of values around the mean, whereas very few in the extremes. In these circumstances, coefficients of the regression could sometimes be more influenced by the higher number of values around the mean, and it could lead to a poorer prediction for extreme values. On the contrary, with a uniform distribution in the whole range, it would be expected to predict extreme and around mean values with similar accuracy.

Therefore, the aim of the present work was to develop a model to predict milk yield at the end of lactation which could avoid problems related to the separation of kits from their mothers. On the other hand, not only fitting characteristics, but also proper biological meaning and ability to reproduce differences between treatments were considered to choose the model.

MATERIALS AND METHODS

Databases

In order to increase the robustness of the model, variability of data was promoted including 2 data sets in the regressions. The reproductive rhythm was semi-intensive in both trials [insemination at 11 d post-partum (dpp) and weaning at 28dpp], but diets, genetic types, parity order and day of controls were slightly different in same cases.

Set 1: Consisting on 49 rabbit does (crossbred from the maternal lines A and V, Universidad Politécnica de Valencia) during 5 consecutive cycles (until 6th parturition) with a total of 184 lactations. Does were fed with a commercial diet (10.9 MJ digestible energy (DE)/ kg of dry matter (DM), 170 g crude protein (CP)/ kg DM) and controlled at 18 and 28 dpp for energy intake and perirenal fat thickness (PFT) as described Pascual *et al.* (2004). Litters were standardized at birth to 8-9 kits in primiparous or 10-12 kits in multiparous rabbit does. Litters were into the nest boxes until 21dpp, which were closed, except for the weekends. Once a day in the morning, rabbit does were allowed to nurse and milk yield at 3rd wk was measure weighting females before and after the milking. At 4th wk, litters and does were allocated in different cages. Once a day in the morning each doe was taken to the litter cage to nurse and milk yield at 4th wk was measure weighting females before and after the milking

Set 2: Consisting on 140 primiparous rabbit does from different genetic types from Universidad Politécnica de Valencia: 53 from LP line, founded by reproductive longevity criteria and selected by litter size at weaning for 6 generations (Sánchez *et al.*, 2008); 42 from V16, constituted from four specialized maternal lines into a composite synthetic line and then selected by litter size at weaning for 16 generations (Estany *et al.*, 1989); and 45 from V36, generation 36 of line V. One half was fed with a commercial diet (C diet; 11.6 MJ DE/ kg of

DM, 175 g CP / kg DM) and the other half was fed with low energy fibrous diet (F diet; 9.1 MJ DE/ kg DM, 162 g CP / kg DM). Does were controlled at 14 and 28 dpp to measure PFT, and from 21 to 28 dpp for feed intake. Litters were standardised at birth to 9 kits and milk yield was measured as described in *set I*.

Model traits

Daily milk yield during the 4th wk of lactation (MY4) was used as dependent variable. As independent variables were included traits frequently related to milk yield (Maertens *et al.*, 2006) and frequently available: a) Litter size at weaning (Lebas, 1987), both linear (LSW) and quadratic (SQ(LSW)) described as the main factor affecting milk yield. b) Daily energy intake recorded as total energy intake (doe+litter) at late lactation, both linear (TEI) and quadratic (SQ(TEI)). Although correlated to LSW, this variable was included as higher energy intake of female usually linearly increase milk yield (Xiccato, 1996) while higher feed energy intake of the litter reduced it. c) Daily milk yield during 3rd wk of lactation (MY3) as a measure of the productive level of the doe which would take into account genetic and environmental conditions (e.g. herd-year-season or diet). d) PFT change at late lactation (Pascual *et al.*, 2002) as a measure of the possible antagonism between gain body reserves and milk yield (Δ PFTd). Their main descriptive statistics are presented in Table 1.

Overlapping degree between current lactation and next pregnancy (OL, 1 for pregnant and 0 for non-pregnant does), diet, parity order and genetic type were also recorded as qualitative traits, when two or more levels for the qualitative variable existed in the data set, to evaluate their effect on the obtained models.

PREDICTION OF MILK YIELD AT 4TH WK

Table 1. Main descriptive statistics for the traits included in the models

Variable	Description	mean	min	max	SD	CV×100
<i>All rabbits (n=325)</i>						
MY4	Milk yield at 4 th wk (g/d)	222.1	83.8	373.8	49.4	22.3
LSW	Litter size at weaning	8.88	4.00	12.00	1.39	15.62
SQ(LSW)	Square of LSW	80.83	16.00	144.00	24.34	30.11
TEI ¹	Total energy intake (MJ/d)	3.94	0.63	5.77	0.81	20.48
SQ(TEI) ¹	Square of TEI (MJ ² /d ²)	16.14	0.39	33.30	6.27	38.83
MY3	Milk yield at 3 rd wk (g/d)	254.5	65.0	405.0	62.0	24.3
ΔPFTd ¹	PFT change (mm/d)	0.0003	-0.1250	0.1179	0.0430	14250
<i>Set 1 (n=184)</i>						
MY4	Milk yield at 4 th wk (g/d)	238.3	83.8	373.8	52.1	21.8
LSW	Litter size at weaning	9.50	6.00	12.00	1.29	13.57
SQ(LSW)	Square of LSW	91.90	36.00	144.00	23.74	25.83
TEI ¹	Total energy intake (MJ/d)	3.99	0.63	5.77	0.93	23.36
SQ(TEI) ¹	Square of TEI (MJ ² /d ²)	16.75	0.39	33.30	7.15	42.71
MY3	Milk yield at 3 rd wk (g/d)	286.7	116.7	405.0	54.4	19.0
ΔPFTd ¹	PFT change (mm/d)	0.0074	-0.1176	0.1147	0.0372	504
<i>Set 2 (n=140)</i>						
MY4	Milk yield at 4 th wk (g/d)	200.8	98.8	292.5	36.1	18.0
LSW	Litter size at weaning	8.08	4.00	10.00	1.06	13.16
SQ(LSW)	Square of LSW	66.38	16.00	100.00	16.20	24.41
TEI ¹	Total energy intake (MJ/d)	3.87	2.26	5.39	0.60	15.58
SQ(TEI) ¹	Square of TEI (MJ ² /d ²)	15.35	5.12	29.10	4.79	31.19
MY3	Milk yield at 3 rd wk (g/d)	212.4	65.0	293.3	43.3	20.4
ΔPFTd ¹	PFT change (mm/d)	-0.0089	-0.1250	0.1179	0.0482	539

¹Calculated between the two control points recorded for each dataset (*set 1*: 18-28 dpp; *set 2*: 14-28 dpp for PFT and 21-28 dpp for energy intake). SD: standard deviation. CV: coefficient of variation. PFT: Perirenal fat thickness.

Regression model

The general model used for the regressions was:

$$MY4 = Intercept + \sum_{i=1}^6 b_i \cdot X_i + b_7 \cdot OL + \sum_{i=1}^6 b_{i+7} \cdot X_i \cdot OL + e$$

Where X_i are independent variables mentioned above. It was also used OL as a dummy variable (fixed effect) and its interactions with independent variables, which allows to parameterise equations for pregnant and non-pregnant rabbit does during lactation.

To fit this model three equations were tested, one using a normal distribution for the dependent variable, and the other two trying to use a uniform distribution by two different methods. The three equations were based on multiple linear regression (MLR) with a stepwise procedure ($P < 0.05$). During the regression computing, it was used a threshold of 3 for studentized residuals to find outliers with a high residual value after predicting (e.g. bad fitting to the model) and of 5 times for the leverage points to find outliers that could influence too much in the regression (this statistic measure the influence of each value in determining coefficients of the regression; STATGRAPHICS Centurion XVI, 2010). The three methods developed were:

Equation 1 (Eq1). After outliers depletion for both dependent and independent variables, MLR was computed as previously mentioned. This equation was characterised by a normal distribution.

Equation 2 (Eq2). Firstly, as in Eq1 an outliers search for the independent variables was done. For the dependent variable, it was applied a threshold of [sample mean \pm 2 · sample standard deviation] to eliminate extreme values which are usually not enough represented and could influence highly in the regression. Afterwards, MLR was computed as in Eq1 but adding a

weight variable in order to smooth sample distribution throughout the full range. The histogram of the dependent variable (with 20 classes) was used to obtain as weight variable the ratio between the minimum number of values within a class and the number of values within its class. This equation was characterised by use of a smoothed distribution of the dependent variable without extreme values (e.g. it was expected to smooth sample distribution, but not with underrepresented values).

Equation 3 (Eq3). For this equation it was performed a previous sample selection method to try to improve the smoothing described in Eq2. Firstly, it was also used a threshold [mean \pm 2·standard deviation] for the dependent variable extreme values search. Afterwards, it was computed a principal component analysis (PCA) without deleting any principal component (PC) (STATGRAPHICS Centurion XVI, 2010). Each score from each sample was divided by the corresponding eigenvalue to obtain a dimensionless space with variance equal to one for each dimension. These transformations resulted in a Euclidean space where the distances are easily measured. It was randomly selected 10 samples and the distance from each of these samples to the rest of the population was measured. All the neighbour samples with a distance lower than one were deleted. The process (selection, measuring and deletion) was repeated two times more. Finally, other PCA was computed, where the score from each sample was divided by the new corresponding eigenvalue. The objective was to search for multivariate outliers by measuring distance of each sample to centre of the population (e.g. the centre of the population matches with the mean of the population and the origin of coordinates from the Euclidean Space). The threshold was calculated according to Cuadras (2011) to compare means in multivariate inference, obtaining a value of 15.50 ($P < 0.01$). Samples remaining in the set were used in the regression. The weight variable and MLR were computed as in Eq2, but using as independent variables those obtained in the second PCA (variables were PC's

and values were scores). This equation was characterised by a smoothed distribution, non-redundant samples, non-extreme values for the dependent variable (e.g. as redundant samples were removed from the set, the number of values per class in the histogram was lower and the smoothing of sample distribution lower) and no correlation among independent variables.

To evaluate the different fitting ability of the equations, root mean square error (RMSE) and R^2 were determined, as it has been indicated by Romero and Zúnica (2005). Means and standard deviation of observed and predicted values for each equation were also obtained. To study the capability of the equations to provide useful predicted values, a PROC GLM procedure (SAS Institute, 2002) was computed for the observed values and for the predicted values of each equation. The model included as fixed effects diet, parity order, genetic line and OL. The ability of the predicted values to reproduce the differences obtained with the observed values was evaluated.

RESULTS

The main statistics for the fitted equations are showed in Table 2. It can be observed that RMSE for Eq2 and Eq3 were lower than for Eq1 (-9.4%, -5.7% respectively). Observed and predicted means were similar between them and for the three equations evaluated (maximum difference 0.8% for Eq3). Eq1 had the highest value for the observed standard deviation (+16.7% and +9.5% respect to Eq2 and Eq3), but the lowest for the predicted standard deviation. Predicted range for Eq2 and Eq3 were close and with similar wideness to observed (105.4% and 97.5% of observed range, respectively), whereas in Eq1 predicted range was tighter than observed (74.7% of observed range).

PREDICTION OF MILK YIELD AT 4TH WK

Table2. Main statistics for the fitted equations to predict milk yield during the 4th wk of lactation

Equation	Eq1	Eq2	Eq3
<i>n</i>	321	300	188
RMSE	30.5	27.6	28.7
R ²	0.620	0.578	0.605
Mean			
Observed	222.9	220.9	219.7
Predicted	222.9	221.4	217.8
Range			
Observed	83.8 - 373.8	130.0 - 317.5	130.0 - 315.0
Predicted	101.2 - 317.9	123.1 - 320.7	133.0 - 313.6
Standard deviation			
Observed	49.10	42.07	44.82
Predicted	38.67	39.40	41.66

Eq1: Equation developed using multiple linear regression. Eq2: Equation based on Eq1 but not using extreme values of the dependent variable and weighting these values according with their frequency. Eq3: Equation based on Eq2 but using non-redundant samples and avoiding colinearities among independent variables. RMSE: Root mean square error.

Table 3 shows the regression coefficients obtained for the three equations. Standardised to mean intercept value in Eq3 (216.6) was close to the observed mean (219.7 g/d). LSW was only included in the Eq3, with a low relative weight respect to the other variables. TEI had a quadratic relation and MY3 a positive linear relation with MY4 in the 3 equations evaluated. ΔPFTd was only included in the Eq2 and Eq3, denoting a relevant negative effect on MY4 when pregnant, while slightly positive when non-pregnant. In order to simulate how estimations obtained from proposed equations could be used to evaluate the effect of different treatments on milk yield, Table 4 shows the effect of genetic line, diet and parity order in the observed and predicted MY4. The effect of genetic line on observed MY4 was significant ($P < 0.05$) MY4, but not when predicted values from any predicted equation were used. Observed contrast between LP and V36 line was +18.3 g/d ($P < 0.05$), whereas predicted differences for this contrast were always below +5 g/d. The effect of the diet was significant ($P < 0.001$) for both observed and predicted MY4. Observed contrast between C and F diet was +27.1 g/d ($P < 0.05$), whereas this difference was slightly lower for predicted MY4 (on av.

+18.23 g/d; $P < 0.05$). The effect of parity order was significant ($P < 0.001$) for both observed and predicted MY4, obtaining similar least square means at each parity. However, although differences observed between the parity 2 with the parities 3 and 5 were not significant (+14.0, +11.8 g/d respectively; $P > 0.05$), significant differences were found with the predicted values from all the equations (on av. +20.3, +22.6 g/d; $P < 0.05$).

Table 3. Significant ($P < 0.05$) regression coefficients for the developed equations to predict milk yield during the 4th wk of lactation, parameterised for pregnant and non-pregnant rabbit does

Description	Equation		
	Eq1	Eq2	Eq3 ³
Intercept	-31.47	-101.7	216.6
<i>b value:</i>			
Non-pregnant rabbit does			
LSW			0.315
TEI ¹	53.41	81.07	22.28
SQ(TEI) ¹	-3.893	-7.232	-1.213
MY3	0.407	0.463	27.72
Δ PFTd ¹		21.09	1.323
Pregnant rabbit does			
LSW			3.64
TEI ¹	53.41	81.07	87.73
SQ(TEI) ¹	-3.505	-7.232	-65.10
MY3	0.407	0.491	31.593
Δ PFTd ¹		-140.4	-11.65

Eq1: Equation developed using multiple linear regression. Eq2: Equation based on Eq1 but not using extreme values of the dependent variable and weighting these values according with their frequency. Eq3: Equation based on Eq2 but using non-redundant samples and avoiding colinearities among independent variables. ¹ between the two points recorded for each data set (*set 1*: 18-28dpp; *set 2*: 14-28dpp for PFT and 21-28dpp for energy intake). ³ Coefficients obtained with standardized independent variables.

PREDICTION OF MILK YIELD AT 4TH WK

Table 4. Effect of the genetic line, the diet and the parity order in the milk yield at 4th wk of lactation (lsmeans, standard error in brackets) using the observed values or those obtained from the prediction equations of the set

	Observed	Eq1	Eq2	Eq3
Genetic line (<i>Set 2, n=140</i>)				
LP	209.3(4.6) ^b	208.6(3.3) ^a	209.2(3.9) ^a	205.9(3.8) ^a
V16	201.0(5.2) ^{ab}	198.5(3.8) ^a	197.7(4.3) ^a	195.1(4.3) ^a
V36	191.0(5.0) ^a	206.6(3.6) ^a	205.8(4.2) ^a	201.8(4.3) ^a
P-value	0.0271	0.1144	0.1354	0.1668
Diet (<i>Set 2, n=140</i>)				
C	214.1(4.0) ^b	213.9(2.9) ^b	213.1(3.3) ^b	210.1(3.3) ^b
F	186.8(4.2) ^a	195.3(3.0) ^a	195.3(3.5) ^a	191.8(3.5) ^a
P-value	<.0001	<.0001	0.0004	0.0003
Parity order (<i>Set 1, n=184</i>)				
1	200.7(7.0) ^a	195.6(4.5) ^a	195.7(4.4) ^a	192.9(4.3) ^a
2	235.8(7.5) ^b	230.1(4.9) ^b	231.4(4.8) ^b	227.7(4.6) ^b
3	249.8(8.1) ^{bc}	252.5(5.3) ^c	251.3(5.5) ^c	246.3(5.2) ^c
4	261.5(9.1) ^c	258.4(5.9) ^c	265.2(6.4) ^c	262.2(6.2) ^d
5	247.6(8.2) ^{bc}	254.0(5.5) ^c	254.3(5.5) ^c	248.6(5.3) ^{cd}
P-value	<.0001	<.0001	<.0001	<.0001

Eq1: Equation developed using multiple linear regression (MLR). Eq2: Equation based on Eq1 but not using extreme values of the dependent variable and weighting these values according with their frequency. Eq3: Equation based on Eq2 but using non-redundant samples and avoiding colinearities among independent variables. ^{a,b,c,d} Means at a same effect and column not sharing superscript differ significantly at $P<0.05$.

DISCUSSION

The fact that observed and predicted means were similar, but predicted standard deviations were lower and predicted range tighter to those obtained with the observed values, denotes that values under the mean were overestimate and those over the mean underestimate in general. As it can be seen in the Figure 1a, the slope between observed and predicted values with the Eq1 was lower than one, which was the expected (0.620). However, this deviation was slightly improved when using Eq2 or Eq3 (slopes: 0.727 and 0.736, respectively). As a consequence, there was a relevant higher correlation between residuals after predicted and observed values (Figure 2) for Eq1 ($R^2=0.380$) than for Eq2 and Eq3 ($R^2=0.176$ and 0.178

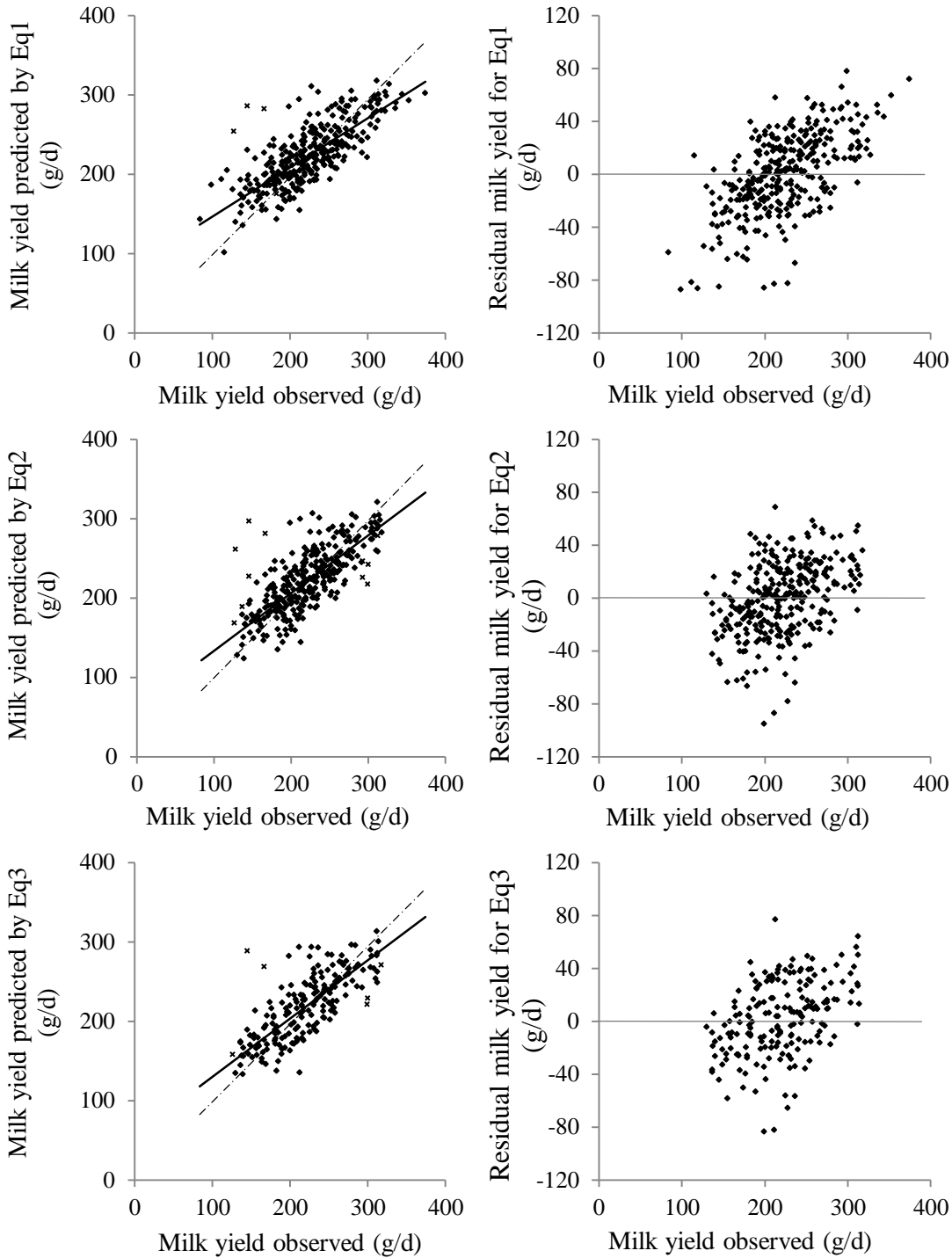


Figure 1. Relationship between predicted and observed milk yield for the developed equations. \blacklozenge Samples used in the regression; \times Regression outliers; $---$ Observed = Predicted relationship; $—$ Least squares regression line for Predicted Vs. Observed.

Figure 2. Relationship between residuals after predicted values for the developed equations and the observed values.

respectively). This higher correlation for Eq1 indicates that prediction error depends on the observed value and therefore it is not in agreement with the independence of the errors assumption on linear regression (Romero and Zúnica, 2005). In this sense, another assumption on linear regression is that residuals should have a normal distribution. In order to evaluate normality of the residuals, kurtosis and skewness were evaluated being kurtosis 0.707, 0.783, 0.139 and skewness -2.585 , -2.489 and -1.317 for Eq1, Eq2 and Eq3, respectively. These results suggest that residuals for Eq1 and Eq2 did not distribute as well as for Eq3, as absolute value for skewness were higher than two. On the other hand, the higher R^2 for Eq1 indicates more variability explained of the dependent variable, but not a better model, as it can be seen in RMSE. This higher R^2 could be explained by the higher range and consequently variability of the Eq1.

Interpreting the regression coefficients can give a biological meaning to the equations. For instance, negative values for Eq1 and Eq2 intercept indicate the average milk yield when all the independent variables are equal to zero, which is impossible as females require, at least, some energy intake to survey. On the contrary, as Eq3 was developed with standardized independent variables, the meaning of this intercept was the average milk yield when all the independent variables had the mean value. So, it would be expected that this intercept value would be very close to the observed and predicted means. Therefore, *b values* for Eq1 and Eq2 indicate the change of MY4 from the intercept per unit of independent variable increased, while *b values* for Eq3 indicate the change from the mean (intercept) of MY4 per unit of standard deviation of independent variable increased. For these reasons comparing coefficients values among equations has no sense and only the sign of the effect was compared.

Positive *b values* for TEI indicate that the more energy intake the more milk yields. These results are in agreement with Xiccato (1996), where only a lineal effect was tested and observed. In the present study, energy intake was measure as the sum of doe and litter intakes, so negative *b values* for SQ(TEI) could denote that higher values of TEI also include a higher contribution of the litter to the joined DE intake. Therefore, this negative quadratic effect would allow to reduce the effect of litter intake. Nevertheless, another explanation could be that females with higher energy intake could allocate part of this extra-energy to other functions. So, if the quadratic effect would be related to the litter, *b values* for SQ(TEI) in pregnant and non-pregnant females should be similar, as occurs in Eq1 and Eq2. However, when colinearities among independent variables were avoided (Eq3), *b values* for SQ(TEI) were completely different for pregnant and non-pregnant females and consequently the hypothesis of allocation to other functions would be more plausible.

According to Lebas (1976), 58.3% of the variability among lactation curves could be explained by the first factor of a PCA which would be related to the total daily amount of milk yield. In this sense, it would expected that high productive does at 3rd wk to have high milk yield at 4th. Consequently, *b values* for MY3 were positives. With regard to litter size at weaning, it was unexpected that these effects were so little relevant as Maertens *et al.* (2006) described it as the main factor affecting to rabbit milk supported by many authors (Lebas, 1969; Torres *et al.*, 1979; Partidge and Allen, 1982; Pascual *et al.*, 1999). However, as litter size also increase TEI and MY3, its effect could be confused or shared among them. In fact, MY3 should take into account genetic and environmental factors (diet, litter size, temperature, etc.) as it could be considered as a measure of the total daily amount of milk yield factor suggested by Lebas (1976) or *k value* proposed by Casado *et al.* (2006).

On the other hand, overlapping between current lactation and next gestation had an interesting effect on predicting equations obtained. When the female was non-pregnant only feed intake and MY3 had relevance on MY4 prediction, whereas when pregnant SQ(TEI) was included at Eq3 and Δ PFTd at Eq2 and Eq3 for a better fitting. When non-pregnant doesn't appear an antagonism between body reserves restore and milk yield. However when pregnant, there is an increased priority for the next offspring (body reserves) at the expenses of the actual (milk yield). The higher are the resources addressed to body reserves the lower are those available for milk yield (Savietto, 2012). In this sense, *b value* for Δ PFTd could be interpreted as priority choice between actual and future litter in pregnant females with a similar energy intake and milk production at 3rd wk. In this sense negative *b value* for SQ(TEI) in pregnant does and close to zero in non-pregnant at Eq3 could denote that pregnant females with higher energy intake could drive more energy to other functions. As it has been described previously, Eq2 and Eq3 presented better slope between observed and predicted values, as well as closer range and standard deviation between predicted and observed values than Eq1, where no significant *b value* for Δ PFTd was established. So, it would be expected that the prediction of extreme values would be better at Eq2 and Eq3. Therefore, it could be hypothesized that high body condition changes could help to fit better extreme values for MY4 in pregnant does during lactation.

Regarding to the simulation of obtained predicted values to evaluate the effect of different treatments on milk yield, observed differences between treatments were in general higher than those obtained with the predicted values, as observed standard deviations were higher than those predicted. It was expected that equations with closer predicted standard deviation to observed would lead to a closer predicted Vs. observed difference between treatments, however, no great differences on mean estimation were observed among equations. In the

three equations, mean observed differences over 25 g/d were always detected as significant, differences between 18 and 25 g/d were detected sometimes and differences lower than 18 g/d were not detected. It could be hypothesized that a higher number of samples would be required to detect differences when these prediction models are used. However, standard errors for means estimation were lower in predicted values than in observed, as predicted standard deviation was lower than observed. Thus, lower differences would be detected with these equations. Another possibility would be considering predicted error as not absolutely random. For instance, there could be systematic errors related to genetic line or parity order, although these systematic errors must always be lower than RMSE. Therefore, it should be avoided interpreting results when observed differences are lower than RMSE.

CONCLUSIONS

Predicting milk yield at 4th wk is possible with the variables used in this study, although certain precautions must be taken and further research would be required to reduce the prediction error. Main factors affecting milk yield at 4th were joined energy intake at 4th wk and milk yield at 3rd wk. However, population pre-treatment of data, to smooth the dependent variable distribution, seems to improve prediction, especially for extreme values. The result showed that pregnant females could address resources in a different way than non-pregnant which also highlighted the importance of reserves mobilization in pregnant does milk yield. The three models proposed showed similar ability to quantify and qualify effects. Nevertheless, Eq3 seemed to be the most statistically correct with a high biological interpretation.

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