

RELATIONSHIP BETWEEN BODY CONDITION AND ENERGY MOBILIZATION IN RABBIT DOES

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Abstract: The present work was performed to examine the relationships between measures of body condition and energy mobilization in rabbit does. The variables studied were body weight (BW), perirenal fat thickness (PFT), basal non-esterified fatty acid concentration (NEFA_b) and non-esterified fatty acids after lipolysis stimulation by isoproterenol (NEFA_i). The effect of time of measuring (at mating, delivery and 10 d after delivery) was estimated in 157 primiparous does. Correlations between body condition components were estimated and a principal component analysis performed. The does decreased BW (6%) and PFT (3%), and increased NEFA_b (25%) and NEFA_i (16%) from mating to delivery. Later, NEFA_b and NEFA_i decreased around 20% from delivery to 10 d after delivery without changing perirenal fat thickness. All BW and PFT lay in the first principal component, and all NEFA traits lay in the second component, showing low correlations with body condition measurements. Both NEFA traits showed high positive correlations when measured at the same time (0.65, 0.72 and 0.69), but low correlations when measured at different times (0.09, to 0.20). We conclude that although body weight and perirenal fat thickness are good predictors of body condition, NEFA should be used when an accurate measurement of energetic mobilization is needed, due to their low correlation.

Key Words: body condition, NEFA, perirenal fat thickness, rabbit.

INTRODUCTION

Body condition is a common tool for assessing the energy status of dams in animal production. Body condition refers to the state of the body energy reserves, i.e. fat deposits, that are used when the does have an energy demand. Different *in vivo* techniques for the estimation of body condition in rabbits have been proposed. Total body electrical conductivity (Fortun-Lamothe *et al.*, 2002), body condition score (Cardinali *et al.*, 2008), computer tomography (Romvári *et al.*, 1998), bioelectrical impedance analysis (Nicodemus *et al.*, 2009) and ultrasound (Pascual *et al.*, 2000) have all been used to assess body condition. Ultrasound scanning is a simple, low-cost and accurate method to estimate fatty deposits. Perirenal fat is the main adipose tissue and is highly correlated with the other adipose tissues (Silva *et al.*, 2012). Due to this, perirenal fat thickness (PFT) has been proposed for the estimation of changes in body condition (Pascual *et al.*, 2000).

Negative energy balance is associated with mobilization of body reserves, predominantly localized in fat and muscle tissues (Gross *et al.*, 2011). Fortun-Lamothe (2006) indicated that an increase of non-esterified fatty acids (NEFA) concentration in blood generally indicates mobilizations of adipose tissue (Gross *et al.*, 2013). An increase in NEFA concentration is interpreted as short-time mobilization, and PFT changes are used to estimate energy changes in the mid-long term. Consequently, body condition and NEFA are both used, as both provide information that helps properly interpret the energy balance of females (Fortun-Lamothe, 2006).

There are 3 key moments when the does need to manage their body condition and energy mobilization; mating (Brechia *et al.*, 2006; Castellini *et al.*, 2006), delivery (Rebollar *et al.*, 2011; Savietto *et al.*, 2016) and early lactation

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(Quevedo *et al.*, 2006). Our objective was to assess the relationships between body condition and energy mobilization measurements at these 3 points in the reproductive cycle of the doe.

MATERIAL AND METHODS

All experimental procedures involving animals were approved by the Miguel Hernández University of Elche Research Ethics Committee (Reference number DTA-MJA-001-11), according to Council Directives 98/58/EC and 2010/63/EU.

Animals

One hundred and fifty-seven primiparous dams were used in this study. All animals were reared at the Miguel Hernández University of Elche (Spain). Rabbits were allowed *ad libitum* access to a standard pelleted diet (218 g acid detergent fibre, 174 g crude protein and 11.0 MJ digestible energy, Cunilactal, Nutreco). The does were kept in individual cages in a farm with a constant photoperiod of 16 h continuous light: 8 h continuous darkness and controlled ventilation. They were first mated at 18 wk of age and at 10 d after parturition thereafter. If the dams were not receptive, they were mated again 1 wk later. Kits were weaned at 28 d of age. Two synthetic maternal lines were used in the analysis.

Traits

All traits were measured at effective mating, delivery and 10 d after delivery, at the second parity. Dam body weight (BW) was recorded. Does' PFT was measured by ultrasound imaging as described by Pascual *et al.* (2000), using Justvision 200 SSA-320A Toshiba ultrasound equipment.

NEFA concentration was determined in basal state (NEFA_b) and in response to the adrenergic agent isoproterenol (NEFA_i), which increases the lipolysis. Blood was sampled before and 7.5 min after isoproterenol injection (50 µg/kg BW, Sigma 15627). This time interval and the isoproterenol concentration were found appropriate by Theilgaard *et al.* (2005) for assessing the lipolytic potential in rabbits. Blood samples were obtained from the central ear artery early in the morning, before feed was distributed, to prevent the effect of feeding, as proposed by Theilgaard *et al.*, (2005). The samples were centrifuged immediately after sampling (4000×g, 4°C, 15 min) and plasma was stored at -20°C for further analysis. Plasma NEFA concentrations were determined using the *in vitro* enzymatic colorimetric methodology prepared by the NEFA test Wako C (Wako Pure Chemical Industries, Ltd, Osaka, Japan). Samples were analysed with a UV spectrophotometer (Model Hewlett Packard 8453).

Statistical analyses

Differences in body condition and energy mobilization indicators were estimated with a model including the effects of time of measurement, line, lactation status (lactating or non-lactating at mating), season and dam permanent effect. All analyses were performed using Bayesian methodology. The posterior median of the difference between time of measurement (D), the highest posterior density region at 95% (HPD_{95%}), and probability of the difference being positive when D>0 or negative when D<0 (P) were calculated. Bounded uniform priors were used for all effects with the exception of the dam permanent effect, considered normally distributed with mean 0 and variance

Table 1: General mean, standard deviation (SD), coefficient of variation (CV) for measures of body condition and energy mobilization at mating, delivery and 10 d after delivery.

	Mating			Delivery			10 d after delivery		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
BW (g)	3637	368	0.10	3411	413	0.12	3556	457	0.13
PFT (mm)	9.30	0.80	0.09	9.1	0.90	0.09	9.20	1.10	0.10
NEFA _b (mmol/L)	0.53	0.25	0.47	0.66	0.31	0.47	0.53	0.21	0.40
NEFA _i (mmol/L)	0.88	0.39	0.44	1.02	0.36	0.38	0.81	0.32	0.40

BW: body weight. PFT: perirenal fat thickness. NEFA_b: basal non-esterified fatty acids concentration. NEFA_i: non-esterified fatty acids after lipolysis stimulation.

σ_p^2 . Residuals were *a priori* normally distributed with mean 0 and variance σ_g^2 . The priors for the variances were also bounded uniform. Features of the marginal posterior distributions for all unknowns were estimated using Gibbs sampling. Convergence was tested using the Z criterion of Geweke (Sorensen and Gianola, 2002) and Monte Carlo sampling errors were computed using time-series procedures described in Geyer (1992). The Rabbit program, developed by the Institute for Animal Science and Technology (Valencia, Spain), was used for all procedures.

Correlations between residuals of a model that included line, lactation status and season effects were estimated. A principal component analysis was performed. All these analyses were performed using the SAS statistical package.

RESULTS AND DISCUSSION

Descriptive results of the traits are presented in Table 1. Body weight of the females was lower than those reported by Quevedo *et al.* (2006) and Theilgaard *et al.* (2009), but perirenal fat thickness was similar (Quevedo *et al.*, 2006) or higher (Theilgaard *et al.*, 2009). This may be due to the different feed composition (Quevedo *et al.*, 2006) or reproductive rhythm applied (Theilgaard *et al.*, 2009). NEFA_b and NEFA_f showed similar results to those of Theilgaard *et al.* (2009), and the NEFA levels were also similar to those obtained by Brecchia *et al.* (2006) after 24 h of fasting. Both NEFA traits showed high variability, with coefficients of variation from 0.40 to 0.47.

Table 2 shows the evolution of body condition indicators for the 3 times at which they were measured. Both NEFA_b and NEFA_f were higher at delivery than at mating (25% and 16%, respectively), as expected due to higher energetic demand (Rebollar *et al.*, 2011) and lower food ingestion of the doe (Pascual *et al.*, 2003) at that moment. In dairy

Table 2: Features of the marginal posterior distribution of the difference (D) between body condition and energy mobilization measurements at different times.

	Mating Delivery	Delivery, 10 d after delivery	Mating, 10 d after delivery
BW			
D	227	-135	92
HPD _{95%}	174, 284	-192, -77	36, 149
P	1.00	1.00	1.00
PFT			
D	0.24	-0.05	0.19
HPD _{95%}	0.08, 0.41	-0.23, 0.12	0.03, 0.36
P	1.00	1.00	0.98
NEFA_b			
D	-0.13	0.13	0.00
HPD _{95%}	-0.20, -0.06	0.05, 0.20	-0.07, 0.07
P	1.00	1.00	0.50
NEFA_f			
D	-0.14	0.21	0.07
HPD _{95%}	-0.24, -0.05	0.10, 0.30	-0.04, 0.15
P	1.00	1.00	0.91

HPD_{95%}: highest posterior density region at 95%. P: probability of the difference being positive when D>0 or negative when D<0. BW: body weight (g). PFT: perirenal fat thickness (mm). NEFA_b: basal non-esterified fatty acids concentration (mmol/L). NEFA_f: non-esterified fatty acids after lipolysis stimulation (mmol/L).

Table 3: Coefficients of correlation between body condition and energy mobilization measurements.

		Mating			Delivery			10 d after delivery				
		PFT	NEFA _b	NEFA _f	BW	PFT	NEFA _b	NEFA _f	BW	PFT	NEFA _b	NEFA _f
Mating	BW	0.53*	0.10	0.02	0.64*	0.41*	0.32*	0.23*	0.57*	0.45*	0.05	0.14
	PFT		0.18	0.25*	0.35*	0.31*	0.24*	0.12	0.34*	0.29*	0.24*	0.26*
	NEFA _b			0.65*	-0.09	-0.09	0.16	0.09	-0.03	-0.03	0.09	0.12
	NEFA _f				-0.07	-0.09	0.18	0.14	-0.08	-0.06	0.18	0.13
Delivery	BW					0.64*	0.10	0.07	0.83*	0.62*	-0.01	0.11
	PFT						0.02	0.00	0.55*	0.51*	0.03	0.04
	NEFA _b							0.72*	0.08	0.04	0.14	0.11
	NEFA _f								-0.01	0.04	0.14	0.20*
10 d after delivery	BW									0.67*	-0.13	-0.06
	PFT										0.09	0.20*
	NEFA _b											0.69*

*P-value<0.05. Body weight (BW), perirenal fat thickness (PFT), basal non-esterified fatty acids concentration (NEFA_b), and non-esterified fatty acids after lipolysis stimulation (NEFA_f).

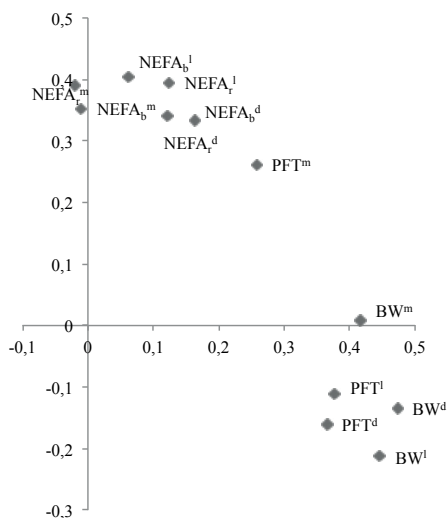


Figure 1: Projection of the traits in the plane defined by the two first principal components. BW: Body weight, PFT: perirenal fat thickness, NEFA_b: basal non-esterified fatty acids concentration, NEFA_t: non-esterified fatty acids after lipolysis stimulation. Superscripts m, d and l mean variable measured at mating, delivery and 10 d after delivery, respectively.

traits are related to energy content, which is highly influenced by the size of the animal. Although high correlations between measurements are expected, some of these correlations are not so high, therefore all of them give useful information about the energy balance in the mid-long term.

All NEFA measurements were located in the 2nd principal component, showing low correlations with the PFT measurements and also with BW. As NEFA measurements do not depend on body weight or perirenal fat, they show the energy balance in the short term. NEFA should be more related to the energy balance due to differences in feed intake (Brecchia *et al.*, 2006), milk yield (Fortun-Lamothe and Prunier, 1999) or heat stress (Savietto *et al.*, 2014). NEFA_b and NEFA_t measurements showed high positive correlations when measured at the same time (0.65 to 0.72), indicating that even in a state of high fat mobilization reserves, the does have at any time an important additional capacity for fat reserves mobilization. NEFA traits showed low correlations between them when measured at different times (0.09 to 0.20), showing that the actual state of fat reserves mobilization should be measured at each time, as when both NEFA traits are measured at the same time they are poor predictors of the capacity for mobilization of reserves at other times. Xiccato *et al.* (2005) also indicated that NEFA levels in blood did not closely reflect the changes in energy balance caused by reproductive rhythm and weaning management.

We conclude that although BW and PFT are good predictors of body condition, NEFA measurements should be used when an accurate measurement of energy mobilization is needed. We also conclude that measuring NEFA after stimulating lipolysis with an adrenergic agent is going to give similar results as basal NEFA, and thus does not seem to be required for a prediction of does' fat reserves mobilization, unless the experiment requires a more accurate prediction of the additional capacity of the does for energy mobilization.

cows, a considerable amount of adipose tissue is known to be mobilized during the transition from late gestation to early lactation, resulting in elevated plasma NEFA (Gross *et al.*, 2013). Perirenal fat thickness and body weight are in agreement with the NEFA measurements, being 3 and 6% lower at delivery. Subsequently, both NEFA traits were around 20% higher at delivery than at 10 d after delivery, but no differences were found for perirenal fat thickness ($D = -0.05$, $HPD_{95\%} = [-0.23, 0.12]$). These variations in NEFA concentrations could be due to variation in the flow of NEFA concentration with respect to its oxidation capability and storage (Gross *et al.*, 2013), so this variation is not necessarily attributable to changes in energy balance. From mating to 10 d after delivery, the balance was negative for BW (92 g) and PFT (0.19 mm). Within a reproductive cycle, the highest value of NEFA was at delivery, which is in agreement with Rebollar *et al.* (2011).

Table 3 shows the coefficients of correlation between traits. To facilitate interpretation of the correlations, we performed a principal components analysis. The first 2 components explained nearly 50% of total variation (30 and 19% respectively). Figure 1 shows the 1st and 2nd principal components. All BW and PFT were located in the 1st principal component, with the exception of PFT at mating. We found substantial positive correlations between them, both at the same time and at different times (0.51 to 0.83). BW and PFT were proposed as predictors of body reserves by Pascual *et al.* (2000). Both

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