World Rabbit Sci. 2004, 12: 7 - 21

ULTRASOUND MEASUREMENTS OF PERIRENAL FAT THICKNESS TO ESTIMATE THE BODY CONDITION OF REPRODUCING RABBIT DOES IN DIFFERENT PHYSIOLOGICAL STATES.

Pascual J.J., Blanco J., Piquer O., Quevedo F., Cervera C.

Unidad de Alimentación Animal, Departamento de Ciencia Animal,
Universidad Politécnica de Valencia, P.O. Box 22012, Valencia 46071, Spain.

Abstract: Sixty New-Zealand×Californian multiparous rabbit does were used to evaluate the capability of a computerized ultrasound system to estimate changes in body condition at different physiological states: parturition, 21st day of lactation, weaning and 28th day of gestation. Perirenal fat weight and carcass energy concentration were significantly correlated with live weight (r = 0.58 and 0.49, respectively; *P*<0.001) and ultrasound measurement of perirenal fat thickness (r = 0.51 and 0.45; *P*<0.001). The inclusion of quadratic and cubic terms of the perirenal fat thickness as independent variables significantly increased the coefficient of determination and lowered the residual standard deviation of the models, based on the live weight of the animals, to estimate the perirenal fat weight and the carcass energy concentration (*P*<0.01). However, these general models tended to overestimate the perirenal fat weight and the body energy concentration of lean animals (<50 g of perirenal fat weight or 8.0 MJ kg^{-1} of carcass energy concentration). When specific multiple regression equations for predicting perirenal fat weight and body energy of multiparous does were developed in function of their physiological state, prediction accuracy of equations improved for rabbits at parturition (R^2 = 0.72 and 0.67), 21 days of lactation (R^2 = 0.84 and 0.67) and weaning (R^2 = 0.76 and 0.65). No model was able to predict either perirenal fat weight or carcass energy concentration of the multiparous rabbit does at 28th day of gestation. In conclusion, excluding animals in late gestation, ultrasound measurement of perirenal fat thickness seems to be a useful technique for *in vivo* estimation of the body condition of reproducing multiparous rabbit does.

Key words: body condition, perirenal fat, ultrasound, reproducing does.

INTRODUCTION

For a complete knowledge of the adequate feeding of reproducing rabbit does, both its effect on the performance traits and its possible effect on the doe's body

Correspondence: J.J. Pascual
E-mail: jupascu@dca.upv.es

condition must be considered, the latter affecting the future reproductive performance. Variables to evaluate the productivity of reproducing rabbit does are clearly defined and harmonized. However, the body condition of the animals is usually controlled by unsuitable records (e.g. following their live weight change) or by destructive techniques (e.g. comparative slaughter). These methods are insufficient to properly evaluate body condition evolution and study the body condition effect on the future performance of the animals.

In recent years, some authors have developed several *in vivo* methods of determining the whole body composition of rabbits: X-ray computer tomography (Szendrõ *et al.*, 1992), magnetic resonance imaging tomography (Köver *et al.*, 1996) and ultrasound measurements of the perirenal fat thickness (Pascual *et al.*, 2000). These non-invasive and non-destructive methods allow study of the *in vivo* body composition changes of particular animals over time. However, some of these techniques still have a low accuracy or are based on the use of medical equipment for humans, which is usually very expensive, located in health centres and turns out to be not very practical in spite of its high precision.

In rabbits, the best predictor of mobilization of body reserves is the change in fat deposit weight, especially perirenal fat (Ouhayoun, 1978; Brun and Ouhayoun, 1988; Masoero *et al.*, 1992). Pascual *et al.* (2000) developed an accurate and precise *in vivo* routine to estimate the body condition of the young reproducing does based on their perirenal fat thickness (PFT) measured by a portable ultrasound equipment. PFT measurements had been confirmed as a very useful technique to evaluate the body condition changes in primiparous rabbit does (Pascual *et al.*, 2002). However, the information about the practical use of this technique in multiparous rabbits is scarce and there is no information about the possible effect of the physiological state of the females on the precision and accuracy of the method, which are crucial for evolution studies.

Consequently, the aim of the present work was to evaluate the ability of computerized ultrasound techniques to estimate the body condition of multiparous rabbit does in different physiological states (parturition, lactation, weaning and late gestation) using currently available portable ultrasound equipment.

MATERIALS AND METHODS

Animals

Sixty New-Zealand × Californian multiparous rabbit does from the experimental farm of the Universidad Politécnica de Valencia were used to evaluate the use of a computerized ultrasound system for estimating changes in body condition in different physiological states. Until control day, animals were fed *ad libitum* a standard commercial diet for rabbit does, in accordance with recent recommendations (De Blas and Mateos, 1998), and were inseminated 11 days after parturition. Animals were classified in four groups in function of their physiological state: Parturition group (PG), constituted by 15 females at the 1st day after parturition (mean live weight ± standard deviation: 4312 ± 444 g); Lactation group (LG), constituted by 15 females at the 21st day of lactation (4390 ± 163 g); Weaning group (WG), constituted by 15 females at 28th day of lactation (4673 ± 512 g); Gestation group (GG), constituted by 15 females at 28th day of gestation (4912 ± 335 g). Seven does of the WG were pregnant and 8 were non-pregnant at weaning date. Eight does of gestation group were inseminated during lactation (11 day) and 7 were after weaning.

Ultrasound measurements

Images were obtained with an ultrasound unit (Justvision 200 “SSA-320A” real-time ultrasound machine; Toshiba), equipped with an electronic micro-convex transducer of multi-frequency (5.0, 6.0 and 7.0 –MHz; PVG-681S). PFT measures were directly obtained using the software of the ultrasound unit.

Thirty minutes before slaughter, fur was removed around the 8th and 9th thoracic vertebrae by shearing, to improve image retrieval and as recommended by Pascual *et al.* (2000). Animals were situated in a box (150 × 370 mm.) during ultrasound measurement to improve image quality. Scanning sites for PFT measurements were located by physical palpation at 8th to 9th thoracic vertebrae, and measurements were made on both sides of the back 3 cm from the vertebral column: right (RPFT) and left (LPFT). Ultrasound gel (Eko Gel) was applied to the scanning sites. The transducer was always placed in the same position to obtain a transversal section of perirenal fat. The same technician did all scanning and interpretation of scans.

Carcass measurements and analytical procedures

After ultrasound measurement, all 60 rabbit does were weighed (live weight; LW) and slaughtered by sodium pentobarbital injection (250 mg per animal). Carcass measurements were made according to the criteria of Blasco and Ouhayoun (1996) and to a comparative slaughter technique (Parigi Bini *et al.,* 1992). The gut and bladder were emptied and returned to empty bodies. After 24 h of cooling, perirenal fat weight (PFW) was recorded. The whole empty body was then minced through a cutter-mincer to produce a homogeneous sample suitable for chemical analysis.

The carcass protein and fat concentrations (CPC and CFC, respectively) of the samples from the empty bodies of the does were determined using the method of the Association Of Official Analytical Chemists (1991), while the carcass energy concentration (CEC) was determined by adiabatic bomb calorimeter.

Statistical analysis

Data were initially evaluated using the Pearson correlation coefficient (ρ; procedure PROC CORR). A regression method was used to fit the least square estimates to the multiple polynomial regression model to obtain general and specific regression equations (procedure PROC STEPWISE) of the SAS (1990). Statistics from regression analysis included the coefficient of determination (R^2) and the residual standard deviation (RSD). Independent variables were measured from the carcass ultrasound images with the exception of LW at slaughter. Carcass measurements were used as dependent variables.

RESULTS AND DISCUSSION

Means, standard deviation, coefficient of variation (CV), and minimum and maximum values of live weight, carcass and ultrasound measurements are presented in Table 1. There was a large variation on PFW, CFC and CEC (CV= 55, 35 and 15%, respectively) of does used in the present work, indicating the suitability of the population for the study of the ultrasound technique accuracy. Ultrasound PFT variables also showed a wide range (CV = 15 to 17%), as a result of perirenal fat weight variations. These results were similar to those obtained by Pascual *et al.* (2000) for LW and estimated CEC (CV = 10.7 and 15.6%; respectively), but variability was higher in this previous work for PFW and PFT (CV = 63.2 and 21.5%, respectively) because of the animals were subjected to different food restriction programs to increase variability.

Table 1: Means, standard deviations and coefficients of variation for carcass and ultrasound measurements.

Variable	Description	Mean	Minimum	Maximum	SD	CV×100
	All rabbits (n=60)					
LW	Live weight at slaughter (g)	4569	3510	5555	437	9.6
PFW	Perirenal fat weight (g)	68.37	2.60	155.00	37.76	55.2
CPC	Carcass protein content (g/kg)	196.7	144.4	233.2	19.8	10.1
CFC	Carcass fat content (g/kg)	110.0	41.5	218.6	38.2	34.8
CEC	Carcass energy content (MJ/kg)	9.09	6.12	12.55	1.37	15.1
RPFT	Ultrasound right perirenal fat thickness (mm)	8.29	5.20	11.50	1.39	16.8
LPFT	Ultrasound left perirenal fat thickness (mm)	8.09	4.60	11.30	1.37	16.9
PFT[1]	Ultrasound mean perirenal fat thickness (mm)	8.31	4.90	11.50	1.37	16.5

(Continue from Table 1)

Variable	Description	Mean	Minimum	Maximum	SD	CV×100
	Rabbits at parturition (n=15)					
LW	Live weight at slaughter (g)	4296	3510	4905	432	10.0
PFW	Perirenal fat weight (g)	66.32	10.00	132.50	35.00	52.8
CPC	Carcass protein content (g/kg)	191.6	164.0	228.6	18.5	9.7
CFC	Carcass fat content (g/kg)	124.8	45.5	218.6	48.1	38.5
CEC	Carcass energy content (MJ/kg)	8.92	6.82	10.50	1.14	12.8
RPFT	Ultrasound right perirenal fat thickness (mm)	7.33	5.20	9.00	1.15	15.7
LPFT	Ultrasound left perirenal fat thickness (mm)	7.21	4.60	9.80	1.36	18.9
PFT[1]	Ultrasound mean perirenal fat thickness (mm)	7.53	4.90	10.00	1.43	18.9
	Rabbits at 21 days of lactation (n=15)					
LW	Live weight at slaughter (g)	4399	4145	4650	161	3.65
PFW	Perirenal fat weight (g)	53.37	16.40	134.00	30.92	57.9
CPC	Carcass protein content (g/kg)	199.7	169.0	233.2	20.9	10.5
CFC	Carcass fat content (g/kg)	94.1	41.5	144.5	28.8	30.6
CEC	Carcass energy content (MJ/kg)	8.84	6.12	11.33	1.27	14.3
RPFT	Ultrasound right perirenal fat thickness (mm)	8.14	6.90	9.20	0.70	8.57
LPFT	Ultrasound left perirenal fat thickness (mm)	8.03	6.70	9.50	0.85	10.6
PFT[1]	Ultrasound mean perirenal fat thickness (mm)	8.36	6.90	10.60	0.94	11.2

(Contiunue from Table 1)

Variable	Description	Mean	Minimum	Maximum	SD	CV×100
	Rabbits at weaning (n=15)					
LW	Live weight at slaughter (g)	4673	3865	5555	493	10.6
PFW	Perirenal fat weight (g)	74.33	2.60	143.20	39.88	53.7
CPC	Carcass protein content (g/kg)	198.6	164.5	228.6	18.80	9.5
CFC	Carcass fat content (g/kg)	100.5	41.5	153.8	32.7	32.5
CEC	Carcass energy content (MJ/kg)	9.28	6.45	11.54	1.49	16.0
RPFT	Ultrasound right perirenal fat thickness (mm)	9.07	7.00	11.50	1.56	17.2
LPFT	Ultrasound left perirenal fat thickness (mm)	8.65	6.30	11.30	1.52	17.6
PFT[1]	Ultrasound mean perirenal fat thickness (mm)	8.74	6.85	11.50	1.50	17.2
	Rabbits at 28 days of gestation (n=15)					
LW	Live weight at slaughter (g)	4908	3975	5295	323	6.59
PFW	Perirenal fat weight (g)	79.45	25.70	155.0	42.70	53.7
CPC	Carcass protein content (g/kg)	197.0	144.4	224.7	21.8	11.1
CFC	Carcass fat content (g/kg)	120.5	58.6	195.7	35.1	29.1
CEC	Carcass energy content (MJ/kg)	9.31	7.28	12.55	1.64	17.6
RPFT	Ultrasound right perirenal fat thickness (mm)	8.63	5.40	11.20	1.45	16.8
LPFT	Ultrasound left perirenal fat thickness (mm)	8.48	4.60	9.90	1.30	15.3
PFT[1]	Ultrasound mean perirenal fat thickness (mm)	8.59	5.00	10.40	1.34	15.6

[1] Mean of right and left measurements. SD: standard deviation, CV: coefficient of variation.

Examining the relationships between pairs of variables is often useful in establishing the basis for a multiple regression model. Therefore, simple correlation coefficients between LW, carcass and ultrasound measurements are given in Table 2. As might be expected, LW was significantly correlated with PFW and CEC (r = 0.580 and 0.490, respectively; $P<0.001$). However, models based only on LW change have been frequently reported as poor in accuracy and precision (PARTRIDGE *et al.*, 1983; PASCUAL *et al.,* 1999).

On the other hand, ultrasound PFT measurements were also significantly correlated with PFW and CEC (r = 0.508 and 0.454; $P<0.001$). Correlation coefficients obtained were lower to those reported by PASCUAL *et al.* (2000) for young rabbit does (around 0.93 and 0.86 for PFW and estimated CEC, respectively), but it was mainly due to the higher PFW range (11 to 229 g) and the absence of differences in physiological state. When the scans were taken on only one side of the body (RPFT and LPFT), the correlations between ultrasound values and PFW and CEC were moderate or even low, suggesting the need to obtain mean measurements of both sides due to the irregular distribution of perirenal fat deposits.

Multiple linear, quadratic and cubic regressions for estimating PFW and CEC of multiparous rabbit does in different physiological states as functions of the LW and the ultrasound PFT measurement are given in Table 3.

Table 2: Simple correlation coefficients between carcass and ultrasound measurements.

Variable	LW	RPFT	LPFT	PFT	PFT^2	PFT^3
LW		0.380**	0.360**	0.389**	0.372**	0.366**
PFW	0.580***	0.391**	0.351**	0.508***	0.509***	0505***
CFC	0.340**	0.173	0.145	0.214*	0.206	0.197
CEC	0.490***	0.324*	0.289*	0.454***	0.459***	0459***

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 3: Multiple regression equations based on live weight (LW, g) and ultrasound measurements (PFT, mm) for predicting perirenal fat weight (PFW, g) and carcass energy concentration (CEC, MJ/kg) in reproducing rabbit does (n=60).

Dependent variable	Eq.	Intercept	*b* value			RSD	R^2
			LW	PFT^2	PFT^3		
PFW	1	-161***	0.0498***			31.0	0.34
	2	-151***	0.0392***	0.567**		28.8	0.44
CEC	3	2.04***	0.0015***			1.21	0.24
	4	2.08***	0.0012**		0.0015**	1.15	0.33

RSD: residual standard deviation
** $P<0.01$; *** $P<0.001$.

The inclusion of quadratic and cubic PFT terms as independent variables significantly improved the R^2 and the RSD of the models based on LW to estimate PFW and CEC respectively ($P<0.01$). Perirenal fat deposit is a tissue characterized by its diffuse and invasive nature, and consequently it does not present a clearly fixed location. Thus, the nature of fat deposits could be the reason for the quadratic and cubic relationship between PFT with PFW and CEC, suggesting that a small increase in PFT (linear measurement) indicates a higher increase in the weight of the perirenal fat deposits (cubic relation with thickness). However, the models obtained in the present work showed a clearly lower accuracy than those obtained by PASCUAL *et al.* (2000) for young rabbit does as for PFW (RSD = 28.83 *vs* 10.22 g) as for CEC estimation (RSD = 1.15 *vs* 0.48 MJ/kg). In fact, when residuals for PFW and CEC prediction based on these general equations are drawn (Figure 1), it can be observed how these models tended to overestimate the PFW and CEC of animals with less than 50 g of PFW or 8.0 MJ/kg of CEC. In addition, general models also tended to underestimate PFW and CEC from these levels, revealing the low predictive accuracy in the body condition estimation of reproducing does based on general ultrasound models.

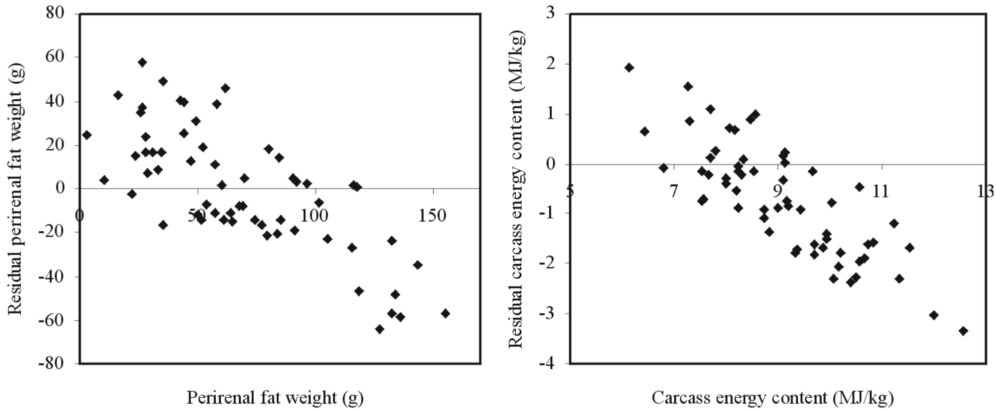

Figure 1: Relationship between residuals after predicting value from general equations (Eq. 2 and 4) based on perirenal fat thickness and actual perirenal fat weight or carcass energy concentration of the reproducing rabbit does (n = 60).

From these results, specific multiple regression equations for predicting PFW and CEC of multiparous does were developed according to their physiological state (Table 4). The prediction of PFW and CEC of animals were clearly improved when specific equations for parturition (R^2 = 0.72 and 0.67 for Eq. 6 and 8 respectively), 21 days of lactation (R^2 = 0.84 and 0.67 for Eq. 10 and 12) and weaning time (R^2 = 0.76 and 0.65 for Eq. 14 and 16) were developed. However, RSD values obtained for PFW prediction remained in some cases very high (20 g), although in other cases they were similar to those obtained by Pascual *et al.* (2000) for PFW (10.2 to 16.5 g) and estimated CEC prediction (0.48 to 0.63 MJ/kg). No model was able to predict PFW or CEC of the does at 28th day of gestation.

As shown in Figure 2, PFW values of the animals at parturition, at 21st day of lactation and at weaning had more or less a linear distribution according to their ultrasound PFT. On the other hand, PFW values from the animals controlled at 28th day of gestation were not correlated with PFT values, showing a random distribution. This result is difficult to explain from the literature, but a hypothesis could be the less homogeneous distribution of the perirenal fat of reproducing does during late gestation as a consequence of the abdominal occupation by the foetuses. This fact

Table 4: Specific multiple regression equations based on live weight (LW, g) and ultrasound measurements (PFT, mm) for predicting perirenal fat weight (PFW, g) and carcass energy content (MJ/kg) in reproducing rabbit does in relation to their physiological state (n=15).

Physiological state[1]	Eq.	Independen variable	Intercept	*b* value				RSD	R^2
				LW	PFT^3	LnPFT	ePFT		
Parturition	5	PFW	-171**	0.0550**				26.5	0.47
	6		-320***	0.0484**		89.2**		20.07	0.72
	7	CEC	0.293	0.0020**				0.768	0.60
	8		-2.58	0.0018**		1.83*		0.733	0.67
21 d lactation	9	PFW	-162	0.0491				31.0	0.07
	10		-19.7		0.1264***			12.5	0.84
	11	CEC	2.02	0.0016				1.07	0.057
	12		6.76***		0.0038***			0.668	0.67
Weaning	13	PFW	-209**	0.0609**				27.3	0.57
	14		-174**	0.0510**			5.79·10-4**	21.3	0.76
	15	CEC	1.25	0.0017*				1.27	0.33
	16		-6.24*			7.20***		0.912	0.65
General (n 45)	17	PFW	-132**	0.0367*	0.0556***			17.8	0.68
	18	CEC	2.51	0.0012*	0.0018***			0.810	0.59

[1] Physiological state of the reproducing does at slaughter. Any model was adjusted to 28 days of gestation data. RSD: residual standard deviation.
*P<0.05; ** P<0.01; *** P<0.001.

could reduce the correlation between the PFT measured at 8-9th thoracic vertebrae and the total size of the perirenal fat deposits (main principle of this technique). This effect was not observed for animals at 17th day of gestation (gestating does in weaning group), showing a similar fit to the equation than non-pregnant does.

When general equations were developed without data concerning the animals at 28th day of gestation, practice models were obtained with relatively high accuracy both for PFW (RSD = 17.8 g and $R^2 = 0.68$ for Eq. 17) and for CEC prediction (RSD = 0.76 MJ/kg and $R^2 = 0.59$ for Eq. 18), independently of the physiological state of the does.

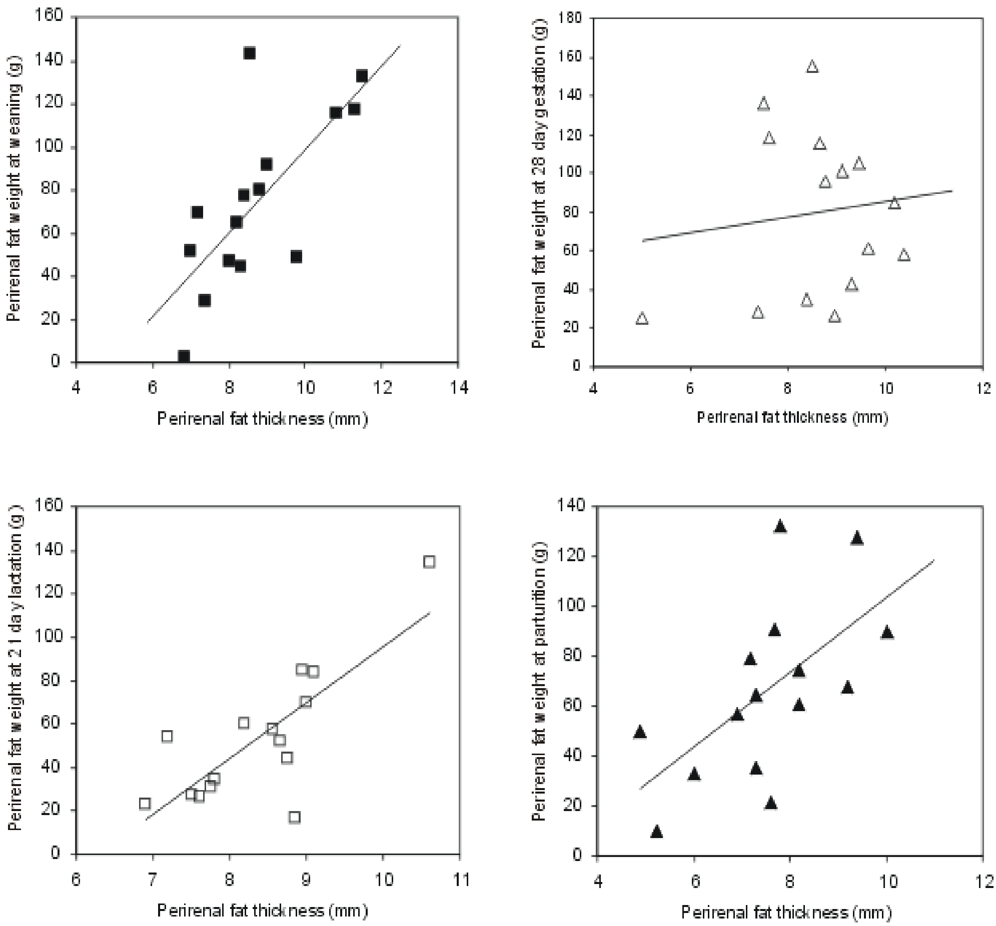

Figure 2: Relationship (linear regression) between the perirenal fat thickness and the perirenal fat weight of reproducing does at different physiological states (n =15).

Avoiding late gestation, ultrasound measurement of PFT seemed to give a better estimate of the body condition of multiparous rabbit does. In fact, the residuals for PFW and CEC of does were homogeneously distributed when specific models in function of the physiological state were used (Figure 3). However, a slight tendency to overestimate the PFW and the CEC of the animals with less than 50 g of PFW or 8 MJ/kg of CEC was maintained. KÖVER *et al.* (1998) and PASCUAL *et al*. (2000) also observed a lower precision in the prediction of low fat weight measurements attributed to their greater relative error. On the other hand, a slight underestimation of the PFW was still observed for the animals with more than 120 g of PFW, perhaps related to the diffuse nature of the perirenal fat depots, especially on high fat animals.

In conclusion, ultrasound measurement of the perirenal fat thickness of multiparous rabbit does seems to be an useful technique for the routine *in vivo* evaluation of their body condition. The precision of the prediction clearly improved when specific models in function of the physiological state of the animals were used, but the body condition cannot be estimated by this technique on rabbit does in late gestation.

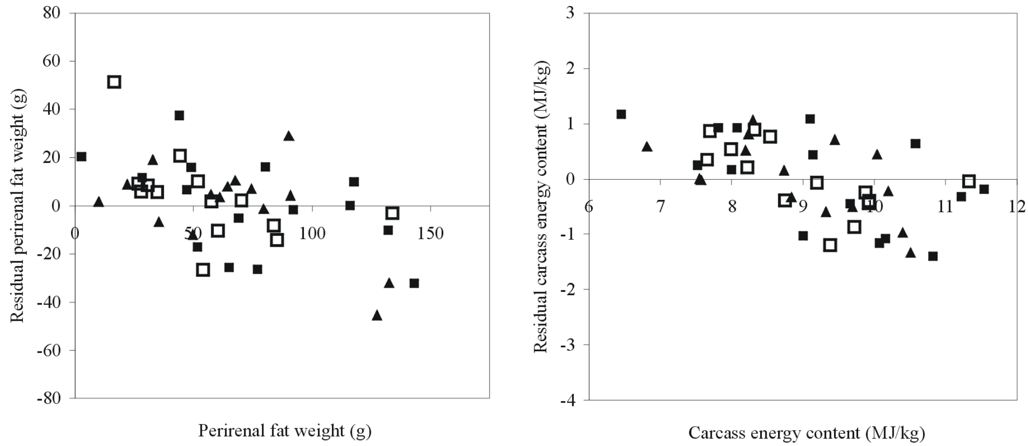

Figure 3: Relationship between residuals after predicting value from the specific equations based on perirenal fat thickness (■ weaning, □ 21 day of lactation and ▲ parturition) and actual perirenal fat weight (Eq. 6, 10 and 14) or carcass energy concentration (Eq. 8, 12 and 16) of the reproducing rabbit does (n= 15).

Acknowledgements: The present work was supported by a grant from the Ministry of Science and Technology (AGL2000-0595-C03-03).

REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1991. *Official methods of analysis of the Association of Official Analytical Chemists, 16th edition*. Association of Official Analytical Chemists, Washington, DC.

BLASCO A., OUHAYOUN J., 1996. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. *World Rabbit Sci., 4, 93-98.*

BRUN, J.M., OUHAYOUN, J. 1988. Growth performance and carcass traits in three strains of rabbits and their two-way crosses. *Proceedings 4th World Rabbit Congress, Budapest, 2: 168-176.*

DE BLAS, C., MATEOS, G.G. 1998. Feed formulation. *In: The nutrition of the rabbit. Ed. C. de Blas and J. Wiseman, CABI Publishing, 241-254.*

KÖVER, GY., SØRENSEN, P., SZENDRÕ, ZS., MILISITS, G. 1996. *In vivo* measurement of perirenal fat by magnetic resonance tomography. *Proceedings 6th World Rabbit Congress, Toulouse. Vol. 3: 191-194.*

KÖVER, GY., SZENDRÕ, ZS. ROMVÁRI, R., JENSEN, J.F., SØRENSEN, P., MILISITS, G. 1998. *In vivo* measurement of body parts and fat deposition in rabbits by MRI. *World Rabbit Science, 6: 191-194.*

MASOERO, G., BERGOGLIO, G., RICCIONI, L., DESTEFANIS G., BARGE, M.T. 1992. Near Infrared Spectroscopy applied to living rabbits to estimate body composition and carcass and meat traits. A calibration study. *Journal of Applied Rabbit Research, 15: 810-818.*

OUHAYOUN, J., 1978. [Comparative analysis of rabbit strains differing in adult weight]. *Doctoral Thesis, Academie de Montpellier.*

PARIGI BINI, R., XICCATO, G., CINETTO, M., DALLE-ZOTTE A. 1992. Energy and protein utilization and partition in rabbit does concurrently pregnant and lactation. *Animal Production, 55:153-162.*

PARTRIDGE, G.C., FULLER, M.F., PULLAR, J.D. 1983. Energy and nitrogen metabolism of lactating rabbits. *British Journal of Nutrition, 49: 507-516.*

PASCUAL J.J., CERVERA C., FERNÁNDEZ-CARMONA J. 1999. The effect of dietary fat on the performance and body composition of rabbit in the second lactation. *Animal Feed Science and Technology, 86:191-203.*

PASCUAL J.J., CASTELLA F., CERVERA C., BLAS E., FERNÁNDEZ-CARMONA J. 2000. The use of ultrasound measurement of perirenal fat thickness to estimate changes in body condition of young female rabbits. *Animal Science, 70:435-442.*

PASCUAL J.J., MOTTA W., CERVERA C., QUEVEDO F., BLAS E., FERNÁNDEZ-CARMONA J. 2002. Effect of dietary energy source on the performance and perirenal fat thickness evolution of primiparous rabbit does. *Animal Science, 75: 267-273.*

SAS. 1990. *User's guide: statistics*. Statistical Analysis Systems Institute Inc., Cary, NC.

Szendrõ Zs., Horn P., Kövér Gy., Berényi E., Radnai I., Bíró-Németh E. 1992. *In vivo* measurement of the carcass traits of meat type rabbits by X-ray computerised tomography. *Journal of Applied Rabbit Research, 15: 799-809.*