World Rabbit Sci. 2014, 22: 187-194
doi:10.4995/wrs.2014.1200

URINE FEATURES USED TO SURVEY NITROGEN EXCRETION IN RABBITS

GASCO L.*, ROTOLO L.*, MASOERO G.*†, MINISCALCO B.‡, ZOCCARATO I.*

*Department of Agricultural, Forest and Food Sciences, University of Torino. Largo Paolo Braccini, 2. I-10095 Grugliasco, Torino, Italy.
†Accademia di Agricoltura di Torino. Via Andrea Doria, 10 – I-10123 Torino, Italy.
‡Department of Veterinary Sciences, University of Torino. Via L. da Vinci 44. I-10095 Grugliasco, Torino. Italy.

Abstract: The aim of this work was to estimate liquid and faecal nitrogen (N) excretion from rabbit herds using 2 clinical analyses of urine samples (urinary urea, UU and creatininury, CU) combined with the daily nitrogen intake (DNI) and metabolic weight of growing and lactating rabbit does. In the framework of 6 experiments, 81 growing rabbits, divided into 17 groups, weighing from 1.8 to 2.8 kg, and 18 multiparous lactating does, divided into 2 groups, were reared in metabolic cages. Five experimental groups of growing rabbits and one of lactating does received diets with lower crude protein content (from –8 to –19% less). The urine was collected (4-d and 1-d collection period for the growing rabbits and lactating does, respectively) and the daily weight (DUW: on av. 188±66 g/d), urinary urea (UU: 1012±463 mg/dL) and creatininury concentrations (CU: 46±25 mg/dL) were recorded. Lactating does showed higher DNI (+127%; $P<0.001$), which was excreted more in the faeces (DFN: +141%; $P<0.001$) than in the urine (DUN: +35%; $P=0.36$), compared to the growing rabbits on a daily *per-capita* basis. Consequently, the faecal-N to urine N ratio was higher for the does compared to growing rabbits (F/U: +93%; $P<0.001$). The percentage of retained N (PRN) for the lactating does and growing rabbits was not different (50.8 *vs.* 56.6%; $P=0.31$). Forward regression models were used to predict the daily nitrogen excretion. Successful r-square fit results were obtained ($P<0.005$) for the *per-capita* daily quantity of urinary N (DUN: $R^2=0.79$) and faecal N (DFN: 0.93, mainly depending on DNI). The individual DNI was accurately fitted ($R^2=0.994$; standard error=0.03), considering the 2 model estimates of the DUN and of the DFN, the metabolic weight and the type of animal. Relativising the N excreta as a percentage of the DNI, or as a ratio of the faecal –to urinary–N, led to less stable results of the regression models. The daily N intake, combined with the collection of urine samples and the measurement of urea and creatinine, led to a reliable estimate of the liquid N excretion.

Key Words: nitrogen intake, nitrogen excretion, urine, urea, creatininury, rabbits.

INTRODUCTION

The nutritional strategies that are adopted to reduce nitrogen (N) and phosphorus excretions from pigs include phase feeding, diet supplementation with 4 synthetic limiting amino acids and the addition of phytase for phosphorus (Aarnink and Verstegen, 2007). A three-phase feeding programme, together with a reduction in volume, can reduce N excretion by 16%, compared to a mono-phase system for growing-finishing pigs, as an improved N catabolism can reduce thirst in pigs to a great extent (Rademacher, 2000). Similar results were obtained by Nahm (2007), who reduced the N excretion and ammonia emission from poultry manure. The use of phase feeding in rabbits improves feed efficiency by reducing N excretion (Maertens and Luzi, 1996). The excretion of ingested N by growing rabbits can be partitioned into faeces, which reaches 25-27%, and urine, which accounts for 17-25%, according to Calvet *et al.* (2008), with an average faecal-to-urine N ratio of 1.24. The use of low protein (LP) diets reduces the urea level in the blood as well as in the urine of rabbits (Masoero *et al.,* 2008). Moreover, a reduction in crude protein (CP), from 18.4 to 16.1%, did not modify the performance of rabbit does (Palomares *et al.,* 2006), while a reduction from

Correspondence: G. Masoero, giorgio_masoero@alice.it. Received *February 2012* - Accepted *April 2014.*
http://dx.doi.org/10.4995/wrs.2014.1200

18.7% to 16.9% improved the milk efficiency of the N intake by 17%, with a 22% reduction in daily urinary urea N excretion (Masoero *et al.*, 2011a).

The aim of this work was to model N excretion from rabbit herds using urine samples analysed for urea (UU) and creatininury (CU), combined with the dietary crude protein level (CP) and daily N intake (DNI), considering the type of animal and their metabolic weight as the covariate variables. The results of this short bottom-up method are limited, and not at all values are comparable with the standard tabulated values, obtained from a global balance between N intake and the N fixed in the carcass during the lifecycle, i.e. the up-down criteria established by the European Commission (ERM/AB-DLO, 1999). Nevertheless, this technical measurement proposal appears quite interesting for the real monitoring of nutritional management evolution.

MATERIALS AND METHODS

In 6 experiments, 81 growing rabbits (from 56 to 77 d of age) and 18 lactating multiparous does were reared in metabolic cages according to the standard harmonised methodology (Perez *et al.*, 1995) and studied for the total collection of faeces and urine. Nineteen experimental groups were created (Table 1), 6 of which received diets with lower CP content (from –8 to –19% less), compared to the control groups in each experiment. The daily urine weight (DUW) of heavy growing rabbits was obtained from a 4-d collection in a single jar, previously acidified with 3 mL of H_2SO_4 concentrate to prevent urease activity and N losses, while the analysis sample was collected on day 5. A single 24 h sample was collected from the does on day 19 of lactation. A clinical examination of the fresh urine, centrifuged for 5 min at 2000 rpm, was performed within 2 h of collection. The UU and CU concentrations were determined on diluted distilled water (1/10) samples using ILAB300 (Instrumentation Laboratory) methods, that is, Urease/GLDH (Cat. No. 00018255440) for urea and Jaffè (Cat. No. 00018257240) for creatinine. The N contents of dried faeces and diets were analysed by means of NIRS equations provided by ratio-performance deviation of 3.2 and 3.0 respectively (Xiccato *et al.*, 2003; Meineri *et al.*, 2009; Núñez-Sánchez *et al.*, 2012) calibrated on an LSP 350-2500P LabSpec-Pro portable spectrophotometer (ASD, Analytical Spectral Devices Inc., Boulder, CO).

The basic variables considered in the study are listed in Table 1, which reports the experimental design of the 19 groups, the CP level of the diet (g/kg), growth performance (g/d), daily feed intake (g/d), DNI (g/d), daily faecal N (DFN, g/d), daily urine weight (DUW, g/d), CU (mg/dL), UU (mg/dL), daily urinary urea and creatinine nitrogen (DUN, g/d). The other variables, featuring nitrogen efficiency and excretion, were formulated as shown in Tables 2 and 3.

The first step of the statistical analysis concerned the significance of the type of animal on N excretion (Table 2) by using a univariate GLM model (SAS/STAT® 9.2. SAS Inst. Inc., Cary, NC). The second step concerned the N excretion model (Tables 3 and 4). The correlation matrix, reported in Table 4, was analysed by means of Ward's Hierarchical Clustering Analysis (HCA), performed via StatBox software *vs.* 6.5 (Grimmer Logiciel, Paris) in order to compare the relative average similarity patterns. To predict the N excretion, a forward regression model (with significance level entry at $P<0.20$) was used that considered seven predictor variables: type of animal (growing rabbit and doe), dietary CP level, UU, CU, UU–to–UC N ratio, as suggested by Moen and Del Giudice (1997), the metabolic weight of the animals in the test period and the DNI. The statistics considered for the prediction features using individual analysis were the r-square of the model, and the mean square error. Since the aim of the work was to assess the reliability of the mean values on the basis of the results of herd examinations from a few samples of urine from growing and doe rabbits, the effective group averages were regressed on the predicted group averages to obtain the r-square coefficient of the group-mean (R^2G) with their standard error as the group averages (SE_G).

The 3rd step concerned the goodness of fit of the bottom-up proposed method; thus, another complete model was built by regressing the DNI on the estimates obtained from the 2 sub-models for DUN and DFN, and considering the metabolic weight of the animals (Table 5).

In the final step, 10 groups of growing rabbits taken from other publications were considered together with the 19 groups of the present work for comparison purposes, and some general trends for growing rabbits together with the does were developed using a quadratic regression.

Table 1: Summary of the basic variables recorded for the 19 groups from 6 experiments.

Experiment[1]	No.	LP[2]	CP (g/kg)	PR (%)[3]	Animal type[4]	Live Weight (g)	ADG (g/d)	ADFI (g/d)	DNI (g/d)	DFN (g/d)	DUW (g/d)	CU (mg/dL)	UU (mg/dL)	DUN (g/d)
0	4	1	170	-	G	2165	29.5	135	3.66	1.10	195	43.1	1118	0.89
0	4	1	167	-	G	2170	13.3	121	3.25	0.90	175	32.4	783	0.6
0	4	1	167	-	G	2081	29.5	136	3.63	1.00	219	22.3	635	0.53
0	4	1	167	-	G	2311	7.3	119	3.18	0.92	139	49.2	773	0.42
1	8	2	149	–11%	G	2271	49.8	191	4.56	1.64	261	23.8	661	0.80
1	9	1	167	-	G	2381	48.0	186	4.96	1.52	259	24.1	816	0.93
2	3	1	165	-	G	1786	32.3	142	3.74	1.18	162	28.2	715	0.54
2	4	1	164	-	G	1790	34.7	116	3.05	0.99	172	26.5	743	0.49
2	4	2	154	–8%	G	1813	40.8	136	3.35	1.21	125	32.9	733	0.37
2	4	2	152	–6%	G	1793	37.3	145	3.53	1.27	140	35.5	868	0.53
3	5	2	141	–19%	G	2675	36.4	181	5.02	1.57	181	62.3	1214	1.00
3	4	1	173	-	G	2826	39.8	209	5.80	1.65	224	52.7	1640	1.61
3	4	2	141	–19%	G	2753	38.0	163	4.53	1.28	202	67.2	1413	1.16
3	4	1	173	-	G	2779	41.0	195	5.41	1.50	217	55.3	1525	1.42
4	6	1	144	-	G	2632	47.7	160	3.68	0.91	125	64.9	1247	0.69
4	5	1	147	-	G	2442	59.8	164	3.85	1.03	147	41.3	904	0.63
4	5	1	151	-	G	2506	35.5	168	4.04	1.05	117	65.8	1170	0.64
5	9	1	165	-	D	4379	14.4	370	9.80	2.97	201	67.6	1257	1.13
5	9	2	150	–9%	D	4528	13.9	373	8.96	3.05	198	65.8	1047	0.90
Total variation coefficient (%)						33	61	44	44	49	35	54	46	49

[1]0: Zunino *et al.*, 2010; Peiretti *et al.*, 2011; 1: Masoero *et al.*, 2011b; 2: Meineri *et al.*, unpublished; 3: Zoccarato *et al.*, 2008; 4: Gasco *et al.,* unpublished; 5: Masoero *et al.*, 2011a.
[2]Low Protein: 1, normal diet; 2, low protein diet supplemented with 4 amino acids (Met, Lys, Trp, Thr).
[3] PR: protein reduction with respect to the protein level of the normal diet (for each experiment % of the control).
[4] G: growing; D: lactating does.
CP: crude protein; ADG: average daily gain; ADFI: average daily feed intake; DNI: daily N intake; DFN: daily faecal N; DUW: daily urine weight; CU: creatininury; UU: urinary urea concentration; DUN: daily urinary N.

Table 2: Differences between growing rabbits and lactating does in predictor and target variables, featuring nitrogen efficiency and excretion, derived from the basic variables (Table 1).

	Growing		Lactating does			
Variables	Means	SEM[1]	Means	SEM	*P*-value	D/G[2]
Predictor variables						
Daily N intake (DNI) (g/d)	4.16	0.11	9.35	0.25	<0.0001	125%
Urinary urea concentration (UU) (mg/dL)	985	51	1146	112	0.19	16%
Creatininury (CU) (mg/dL)	42	2.6	67	5.7	0.001	58%
Urinary urea N -to- CU N ratio (g/g)	4.37	0.15	2.91	0.32	<0.0001	–34%
Faecal CP (g/kg DM)	140	2.6	157	5.7	0.010	12%
Target variables						
Daily urine weight (DUW) (g/d)	185.5	7.3	199.4	16.0	0.43	7%
Daily urinary N (DUN) (g/d)[3]	0.81	0.05	1.05	0.10	0.030	29%
Percentage of the N intake in urine (PUN) (%)[4]	19.2	0.71	11.2	1.57	<0.0001	–42%
Daily faecal N (DFN) (g/d)	1.25	0.04	3.02	0.09	<0.0001	141%
Faecal-to-Urine N ratio (F/U) (g/g)[5]	1.80	0.13	3.54	0.28	<0.0001	96%
Daily retained N (DRN) (g/d) [6]	2.10	0.07	5.29	0.14	<0.0001	152%
Percentage of retained N (PRN)[7]	50.9	0.8	56.3	1.7	0.001	11%
Efficiency of digestible N (%)[8]	72.7	1.0	83.5	2.2	<0.0001	15%
Efficiency of urine N (g/g)[9]	3.15	0.24	6.16	0.52	<0.0001	96%
Percentage of faecal N (PFN) (%)[10]	30.0	0.5	32.5	1.0	0.030	8%
Crude protein apparent digestibility (%)[11]	70.0	0.5	67.5	1.0	0.030	–4%

[1]SEM: standard error of means. [2] D/G: deviation % of the doe mean from the growing rabbit mean. [3] DUN=DUW×UU×0.46×10^{-5}. [4]PUN=DUN/DNI×100. [5] F/U=DFN/DUN. [6] DRN: calculated as residual not-excreted N, i.e. by subtracting the urinary urea and creatinine N and the faecal N from the ingested N; this residual N was used for maintenance as well as for production (retention in body mass and milk). [7]PRN=DRN/DNI×100. [8]DRN/(DNI–DFN)×100. [9]DRN/DUN. [10]PFN=DFN/DNI×100. [11]DFN/DNI×100.

RESULTS AND DISCUSSION

All the variables showed a high variability (coefficient of variation ≥33%. Table 1), which reflected the short term testing and the great differences in the weight of the rabbits and the dietary characteristics. The most stable trait appeared to be the percentage of retained N (PRN: 13%, data not shown), which was calculated by subtracting from DNI the daily excretion of N (in urinary urea, creatinine and in the faeces).

The relationships between the 3 urine parameters (daily weight, UU, CU) reflect the range of the between-group variation. These data, which are shown in Figure 1, are useful to check whether the urine had been contaminated by feed residues or faeces.

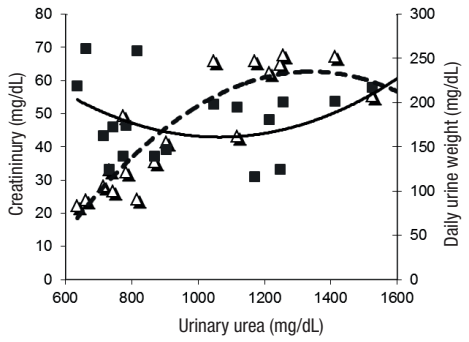

Figure 1: Relationship between the urinary urea concentrations (UU, mg/dL), with the creatininury (CU: Δ, mg/dL, R^2: 0.80; *P*<0.001) and the daily urine weight (DUW: ■ ; R^2: 0.21; *P*=0.16) for 19 groups of growing rabbits and rabbit does.

The lactating rabbit does showed a higher DNI than the growing rabbits (+125%; *P*<0.001) (Table 2), and retained a superior N amount in milk and body (DRN: +152%; *P*<0.001). As regards solid excretion, the does recovered a higher protein concentration in the faeces (CPF: +12%; *P*<0.01), due to lower N digestibility (CPAD: –4%; *P*=0.03) and an increased proportion of faecal N excretion (PFN: +8%; *P*=0.03). In contrast, the liquid N pathway was favourable to rabbit does and reduced the percentage of N in urine by 42% (PUN: *P*<0.001), with an improved utilisation because the does used 6.16 g of N per g of N excreted in urine, while the growing rabbits only utilised 3.15 (+96%; *P*<0.001). As a net result of the 2 processes, in the does the N excretion was prevalent in the solid phase, thus the ratio of the solid-to-liquid N was 3.54 *vs.* 1.80 in the growing rabbits (F/U: +96%; *P*<0.001).

Table 3: Models of the nitrogen efficiency parameters considering the animal type, protein content of the diet, urine parameters, metabolic weight and nitrogen intake, assessed by means of a forward regression using 99 single records or the 19 group-means.

Intercept and Predictor variables	DUN (g/d)	DFN (g/d)	F/U (g/g)	PUN (%)	PFN (%)	DUW (g/d)
Intercept growing rabbits	–1.78	0.61	7.03	–19.1	47.5	–205
Intercept lactating does	–2.26	0.87	8.29	–22.3	47.5	–308
Crude Protein feed (CP) (g/kg)	0.0059	–0.0027[a]	–0.0083[a]	0.15	–0.046[a]	1.19
Urinary Urea, (UU) (mg/dL)	0.0007	–0.0001[a]	–0.0007[a]	0.015	–0.0015[a]	–0.052
Creatininury (CU) (mg/dL)	–0.0052	–0.0020[a]	–0.020	–0.16	–0.030[a]	–0.77[a]
Urea/Creatinine N, (UN/CN) (mg/mg)	0.041[a]	0 [b]	–0.39	0 [b]	0 [b]	14.6
Metabolic Weight (kg $BW^{0.75}$)	0.38	0 [b]	–1.13	6.43	–4.66	88.8
Daily N Intake (DNI) (g/d)	0.069	0.30	0.34	–1.72	0.86[a]	12.5
R^2	0.79	0.93	0.58	0.63	0.18	0.56
SEP	0.81	0.20	0.70	4.10	3.90	44
r^2_G	0.97	0.96	0.81	0.91	0.45	0.86
SE_G	0.07	0.12	0.35	1.63	2.53	19

All intercepts and coefficients were significant at $P<0.05$, except [a]($P<0.20$) and [b]($P>0.20$; coefficients set to 0).

R^2: R-square of multivariate regression from 99 single records.

SEP: Standard error of prediction.

r^2_G: Simple bivariate r-square of the 19 group-means from the 99 estimated single values *vs.* measured group-means. *P*-values of R^2 and r^2_G <0.001.

SE_G: Standard error for group-means.

It must be noted that rabbit does and growing rabbits were not fed the same diets.

Parigi-Bini *et al.* (1992) observed that the efficiency of utilisation of digestible protein for milk protein production was 25% greater than for body protein (76 and 61%, respectively). However, these authors used the comparative slaughter technique, and the different partition of the N excretion between urine and faeces was not investigated. In the present paper, the efficiency of retention of digestible N (Table 2) was confirmed significantly higher in rabbit does (72.7% *vs.* 83.5%; +15%; $P<0.0001$). The urea-to-creatinine N ratio (UN/CN) was 34% lower in the lactating does than in the growing rabbits ($P<0.001$), and this result also indicated a better N efficiency in the rabbit does. In wild and domestic ruminants, when the body conditions decline due to a severe sub-nutrition period, the urinary UN/CN ratio increases because the catabolism of body protein increases the concentration of urea nitrogen in the blood (Moen and Dal Giudice, 1997). In general, lactating does at 19 d after parturition can be considered to be in a re-nutrition phase (body weight increase) in almost 2/3 of cases. Pascual *et al.* (1999), when studying pluriparous does, found that, in the first 21 d of lactation, the live weight increased around 80-100 g, but with high variability.

Successful in r-square fit results ($P<0.001$) were obtained from the regression models (Table 3) developed to estimate DFN and DUN (0.93 and 0.79, respectively). When the N excreta was expressed as a percentage of the DNI, or as a ratio of the faecal-to-urinary N (F/U), less stable solutions of the regression models were obtained. In fact, the models for urinary N excretion (PUN: 0.63), faecal N (PFN: 0.18) and the for the F/U ratio (0.58) and also the individual urine weight (0.56) were less accurate. The predictor variables were not always significant at $P<0.05$. In particular, the

Table 4: Correlation between the absolute daily *per-capita* and the relative fractions of the N ingested balance. Pearson correlation values (n= 99).

	DNI	DUN	DFN	F/U	PUN	PFN	CPAD
Daily N Intake (DNI)	1	0.47[a]	0.96[a]	0.39[a]	–0.18	0.18	–0.19
Daily Urinary N (DUN)		1	0.36[a]	–0.44[a]	0.67[a]	–0.13	0.12
Daily Faecal N (DFN)			1	0.52[a]	–0.24[a]	0.44[a]	–0.44[a]
Faecal N – to urinary N ratio (F/U)				1	–0.55[a]	0.50[a]	–0.49[a]
Percentage urinary N (PUN)					1	–0.25[a]	0.24[a]
Percentage faecal N (PFN)						1	–0.98[a]
Crude protein apparent digestibility (CPAD)							1

[a]*P*-value<0.05.

Table 5: Modelling of daily N intake based on the real predictor measurements of urinary (DUN) and faecal (DFN) excretion or on the estimated predictor urinary N (Est_DUN) and estimated faecal N (Est_DFN) (No=99).

Model		Coefficient	SE[1]	*P*-value	R^2adj	SEP[2]
Measured predictor	Constant	–1.06	0.27	<0.001	0.948	0.25
DNI=const+DUN+DFN+	DUN (g/d)	0.74	0.15	<0.0010		
+MW^3+Animal+Error	DFN (g/d)	1.77	0.16	<0.0010		
	MW (kg $BW^{0.75}$)	0.75	0.27	0.003		
	Animal (1 growing/2 lactating does)	1.00	0.38	0.0050		
Estimated predictor	Constant	–0.14	0.10	0.089	0.994	0.03
DNI=const+Est_DUN+	Est_DUN (g/d)	0.76	0.062	<0.001		
+Est_DFN+MW+Animal+Error	Est_DFN (g/d)	2.78	0.072	<0.001		
	MW (kg $BW^{0.75}$)	0.16	0.097	0.056		
	Animal (1 growing/2 lactating does)	–0.0928	0.15	0.27		

[1]SE: standard error of the regression coefficient.

[2]SEP: standard error of prediction.

[3] Metabolic weight.

contribution of urinary analysis in the prediction of DFN and derived variables was of minor importance. The worst fit was found for apparent protein digestibility (CPAD: 0.18, not shown in Table 3), which could be explained by the independence of the 2 N excretory pathways, solid *vs.* liquid. As highlighted in Figure 2, elaborated from the correlation coefficients reported in Table 4, the daily N intake (DNI) was closest to faecal N (DFN and PFN) and this cluster was very far from the urinary excretion cluster (DUN-PUN). Noticeable the relative positions of 2 key variables: the F/U ratio, which is near the solid N phase (r: F/U, PFN=0.50) and distant from the liquid N phase, because of its opposition to the PUN (r=–0.55). The protein digestibility (CPAD) in turn is near the liquid N phase, as its strong negative correlation with the PFN (r=–0.98) and the high N digestibility are physiologically associated with a superior urinary N excretion.

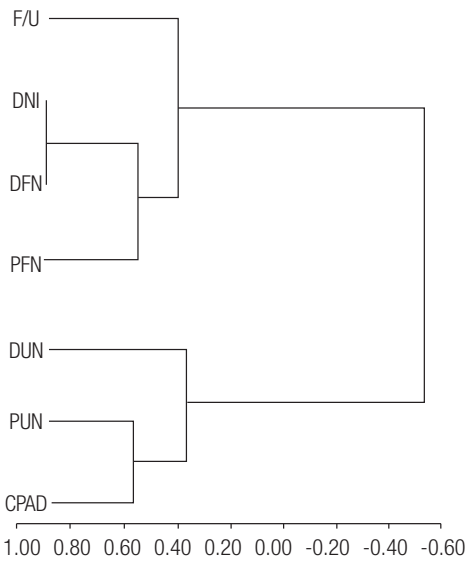

Figure 2: Ward's Cluster Hierarchical analysis of the variables correlation matrix reported in Table 4 (abscise: Euclidean distance). Daily N Intake (DNI). Daily Urinary N (DUN). Daily Faecal N (DFN). Faecal N – to urinary N ratio (F/U). Percentage urinary N (PUN). Percentage faecal N (PFN). Crude protein apparent digestibility (CPAD).

The focus of the proposed bottom-up method was on the goodness of fit of the second complete model (Table 5), which was built by regressing the daily N intake on the estimates obtained from the 2 urinary N (DUN) and faecal N (DFN) models, considering the metabolic weight and the type of animals. The individual DNI was fitted very accurately (R^2=0.994; SE=0.03), and was even better than for the real measurements (R^2=0.948; SE=0.25, respectively). This result depends mainly on the very close relationships (r=0.96; *P*<0.001) between the N intake and faecal N (Table 4). Digestibility of CP registered less variability (CV: 6%, not shown) than the urinary N percentage (CV: 39%) in all the experiments.

Averaging the estimates according to the groups reduced the prediction standard error and increased the r-square fit, as expected, to 0.91 for the PUN and 0.86 for the urine weight, but not so much for the faecal excretion (PFN: 0.45) or protein digestibility (CPAD: 0.44, data not shown). Ciszuk and Gebregziabher (1994) regressed UUN on the milk urea N content of dairy cows, and observed that the R^2 increased from 0.64 to 0.94 when it was calculated from the mean values (3 or 4 animals) of each experimental period or diet.

Urea is the main product of N catabolism and is synthesised in the liver as a result of deamination processes. The urea of rabbits is almost the only way of excretion for liquid N (Lebas, 2010). Rabbits have limited storage ability and increase urination in order to eliminate urea. Masoero *et al.* (2008) observed that blood and urine urea in growing rabbits appeared to be positively correlated, and a decrease in the level of dietary protein reduced blood urea (–32%) and urine urea (–37%). Amber *et al.* (2004), using yucca extracts, observed an improvement in N utilisation, because N urinary excretion was reduced in parallel with a strong reduction in urea and ammonia in the blood, and also in the caecum, while the F/U ratio was increased by 6%.

By combining the data obtained in this experiment with data from literature, it has been shown that the 19 groups of the present work overlapped 10 groups in the published references (Figure 3). Two relevant features emerge: 1) a very wide variation in the relative excretion of the N catabolite in liquid form (a 10-34% range of PUN) and 2) a close parabolic between-group relationship, which links the liquid and the solid N excretions expressed in relative form (R^2=0.82; P<0.001).

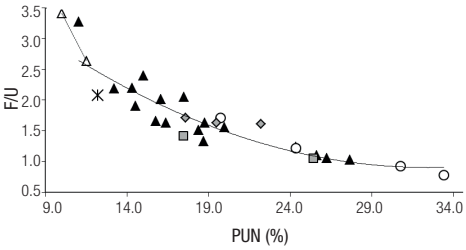

Figure 3: Relationship between the ratio of the Faecal-to-Urinary N excretion (F/U) and the Percentage of Urinary N compared to N intake (PUN) for the 19 groups of growing rabbits and rabbit does, compared with 10 groups from literature (▲: growing rabbits from this work; △: rabbit does from this work; ○: Gidenne *et al.*, 2002; ■: Calvet *et al.*, 2008; ◆: Amber *et al.*, 2004; ✳: Oluokun, 2005). General regression: $F/U=0.0020\times PUN^2-0.16\times PUN+3.97$; R^2: 0.81; SEP: 0.21; P<0.001.

Xiccato *et al.* (2005, 2007) calculated a yearly N excretion of 7.40 kg per doe, by means of ERM/AB-DLO (1999) criteria, based on the standard N content of the carcass yield, and highlighted that 66% of N was not retained in the commercial carcass, but was excreted into the environment. Maertens *et al.* (2005) evaluated a release of 7.42 kg N doe-cage/yr. Compared to the up-down ERM/AB-DLO method, this work, which deals with a partial and short test-period, proposes a bottom-up method which underestimates the N excretion at a 47.8% level (30.4 % from faeces and 17.4% from urine). As far as the productive system is concerned, if a holistic approach is adopted, the raw difference (47.8% *vs.* 66% official) should be attributed to the different unproductive steps of the herd system, namely: the dressing-out percentages at slaughtering (N in the digestive tract, blood, skin, etc.), the N losses due to mortality, and the N-feed maintenance cost of the non-milking does and of their young renewals.

CONCLUSION

The target N excretion and efficiency variables were properly predicted through the average ingested N, the protein level of the diet, and through 2 simple clinical tests of the urine for urea and creatinine. Progress in best practice management procedures could be checked, at a real scale, by adopting these cheap and non invasive measures. Further studies need to be conducted to verify whether spot sample collection of urine is effectively equivalent to a whole 24 h collection in terms of clinical parameters.

REFERENCES

Aarnink A.J.A , Verstegen M.W.A. 2007. Nutrition, key factor to reduce environmental load from pig production. *Livest. Sci., 109: 194-203. doi:10.1016/j.livsci.2007.01.112*

Amber K.H., Yakout H.M., Hamed Rawya S. 2004. Effect of feeding diets containing yucca extract or probiotic on growth, digestibility, nitrogen balance and caecal microbial activity of growing new zealand white rabbits. *In Proc.: 8th World Rabbit Congress, 7-10 September, 2004, Puebla, Mexico. 737-745.*

Calvet S., Estellés F., Hermida B., Blumetto O., Torres A.G. 2008. Experimental balance to estimate efficiency in the use of nitrogen in rabbit breeding. *World Rabbit Sci., 16: 205-211. doi:10.4995/wrs.2008.615*

Ciszuk P., Gebregziabher T. 1994. Milk Urea as an Estimate of Urine Nitrogen of Dairy Cows and Goats. *Acta Agriculturae Scandinavica, A, 44: 87-95. doi:10.1080/09064709409410187*

ERM/AB-DLO. 1999. Establishment of criteria for the assessment of the nitrogen content of animal manures, *European Commission, Final Report, Luxembourg.*

Gidenne T., Lapanouse A., Segura M., Bannelier C., Tartié V. 2002. Effect of the dietary ratio "digestible fibre/crude protein" on rabbit nitrogen balance and on health status. *COST Action 848, WG-1 and WG-4 joint meeting 2002, Ispra, Italy, 28.*

Lebas F. 2010. Les reins et l'excrétion urinarie. *In:* Lebas, F. (ed.) *La biologie du lapin. Available at: http:// www.cuniculture.info/ Docs/Biologie/biologie-06.htm*

Maertens L., Cavani C., Petracci M. 2005. Nitrogen and phosphorus excretion on commercial rabbit farms: calculations based on the input-output balance. *World Rabbit Sci. 13: 1-16.* doi:10.4995/wrs.2005.533

Maertens L., Luzi F. 1996. Effect of dietary dilution on the performance and N-excretion of growing rabbits. In: Lebas, F. (ed.) *Proceedings of 6th World Rabbit Congress, July 9-12, 1996, Toulouse, France, vol. 1. Association Francaise de Cuniculture, Lempdes, 237-241.*

Masoero G., Baricco G., Cherubini R., Barge P., Sala G., de Poi E. 2008. Very low protein, aminoacid-supplied diet for heavy broiler rabbits: effects on nitrogen metabolism, and digital evaluation of excreta and products. *In: Proc. 9th World Rabbit Congress, June 10-13, 2008, Verona, Italy, 729-734.*

Masoero G., Miniscalco B., Mattioda R., Chicco R., Solomita M. 2011a. Relazioni fra escrezione urinaria, creatininuria e urea nel coniglio in accrescimento e nelle coniglie allattanti. *Giornate di Coniglicoltura ASIC, 8-9 April, 2011, Forlì, Italy, 95-97.*

Masoero G., Miniscalco B., Mattioda R., Chicco R., Solomita M. 2011b. Diete miniazotate integrate con Aminoacidi: prove di crescita e bilancio azotato. *Giornate di Coniglicoltura ASIC, 8-9 April, 2011, Forlì, Italy.*

Meineri G., Peiretti P.G., Masoero G., 2009. Appraisal of ingestion and digestibility in growing rabbits using near infrared reflectance spectroscopy (NIRS) of feeds and faeces. *Ital. J. Anim. Sci., 8: 75-82*

Moen R., Del Giudice G., 1997. Simulating Nitrogen Metabolism and Urinary Urea Nitrogen: Creatinine Ratios in Ruminants. *J. Wildlife Manage., 61: 881-894. doi:10.2307/3802197*

Nahm K.H. 2007. Feed formulations to reduce N excretion and ammonia emission from poultry manure. *Bioresource Technol., 98: 2282-2300.* doi:10.1016/j.biortech.2006.07.039

Núñez-Sánchez N., Martínez Marín A.L., Hernández M.P., Carrion D., Castro G.G., Pérez Alba L.M. 2012. Faecal near infrared spectroscopy (NIRS) as a tool to asses rabbit's feed digestibility. *Livest. Sci., 150: 386-390. doi:10.1016/j.livsci.2012.07.030*

Oluokun J.A., 2005. Intake, digestion and nitrogen balance of diets blended with urea treated and untreated cowpea husk by growing rabbit. *Afr. J. Biotechnol. 4: 1203-1208, Available online at http://www.academicjournals.org/AJB*

Palomares J., Carabaño R., García-Rebollar P., De Blas J.C., García-Ruíz A.I 2006. Effects of dietary protein reduction during weaning on the performance of does and suckling rabbits. *World Rabbit Sci., 14: 23-26.* doi:10.4995/ wrs.2006.545

Parigi-Bini R., Xiccato G., Cinetto M., Dalle Zotte A. 1992. Energy and protein utilization and partition in rabbit does concurrently pregnant and lactating. *Anim. Prod., 55: 153-162. doi:10.1017/S0003356100037387*

Pascual J.J., Tolosa C., Cervera C., Blas E., Fernández-Carmona J. 1999. Effect of diets with different digestible energy content on the performance of rabbit does. *Anim. Feed Sci. Tech., 81: 105-117. doi:10.1016/S0377-8401(99)00052-8*

Peiretti P.G., Masoero G., Meineri G. 2011. Effects of replacing palm oil with maize oil and *Curcuma longa* supplementation on the performance, carcass characteristics, meat quality and fatty acid profile of the perirenal fat and muscle of growing rabbits. *Animal, 5: 795-801. doi:10.1017/S175173111000234X*

Perez J.M., Lebas F., Gidenne T., Maertens L., Xiccato G., Parigi-Bini R., Dalle Zotte A., Cossu M.E., Carazzolo A., Villamide M.J., Carabaño R., Fraga M.J., Ramos M.A., Cervera C., Blas E., Falção-e-Cunha L., Bengala Freire J. 1995. European reference method for *in vivo* determination of diet digestibility in rabbits. *World Rabbit Sci., 3: 41-43. doi:10.4995/ wrs.1995.239*

Rademacher M. 2000. How can diets be modified to minimize the impact of pig production on the environment?. *AminoNews, by Degussa-Huls AG, Feed Additives Division, Hanau, DE, 1: 3-10.*

Xiccato G., Trocino A., De Boever J.L., Maertens L., Carabaño R., Pascual J.J., Perez J.M., Gidenne T., Falção-e-Cunha L. 2003. Prediction of chemical composition, nutritive value and ingredient composition of European compound feeds for rabbits by near infrared reflectance spectroscopy (NIRS). *Anim. Feed Sci. Technol., 104: 153-168.* doi:10.1016/ S0377-8401(02)00294-8

Xiccato G., Schiavon S., Gallo L., Bailoni L., Bittante G. 2005. Nitrogen excretion in dairy cow, beef and veal cattle, pig, and rabbit farms in Northern Italy. *Ital. J. Anim. Sci., 4: 103-111.*

Xiccato G., Trocino A., Fragkiadakis M., Majolini D. 2007. Indagine sugli allevamenti di conigli in Veneto: risultati di gestione tecnica e stima dell'escrezione azotata. *Giornate di Coniglicoltura ASIC, 26-27 september, 2007, Forlì, Italy, 115-117.*

Zoccarato I., Gasco L., Schiavone A., Guo K., Barge P., Rotolo L., Savarino G., Masoero G. 2008. Effect of extract of chestnut wood inclusion (Enc®) in normal and low protein aminoacid supplemented diets on heavy broiler rabbits. *9th World Rabbit Congress, June 10-13, 2008, Verona, Italy, 873-878*

Zunino V., Meineri G., Peiretti P.G. 2010. *Curcuma longa* and dietary plant oils for growing rabbits: Effect on apparent digestibility. *J. Food Agric. Environ. 8: 435-438.*