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LENGTH OF COLLECTION PERIOD AND NUMBER OF RABBITS IN DIGESTIBILITY ASSAYS.

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SUMMARY :
The purpose of this work is to evaluate the influence of the methodology used (number of animals and length of the experiment) on the variability of digestibility coefficients, in order to propose a working guide line for these assays on rabbits. Seventeen New Zealand x Californian growing rabbits were fed a commercial diet *ad libitum*. After an adaptation period of 7 days, food intake and excreta were recorded daily and in periods of 2, 3 up to 10 consecutive days in order to determine dry matter digestibility (DMD).
Variabilities of DMD coefficients among animals and among periods were studied. The variance due to animals decreased with the number of days of the collection period up to 4-day periods and then it almost stabilized. The variance due to periods followed the same pattern. Comparing these two variances it can be noted that for 1-day periods the variance due to animals was similar (only 1.11 times higher) to that due to periods, but for 4 or 5-day periods this ratio increased (2.06 and 2.63 respectively). The number of animals necessary to detect significant differences ($P<0.05$) of 2 points between mean values of DMD was 10 rabbits for collection periods of 4 days, 8 rabbits for 7 days, or 7 rabbits for 10 days. Despite the residual effects, the correlation between DM ingested/DM excreted the same day was higher (0.939) than between DM ingested one day/DM excreted the day after (0.873). The feed intake and the growth rate measured in the adaptation period had a moderate correlation (- 0.54 and - 0.48, respectively) with the DMD.

RESUME : Longueur de la période de collecte et nombre de lapins dans les essais de digestibilité.
Le but de ce travail est d'évaluer l'influence de la méthodologie utilisée (nombre de lapins et durée de l'expérimentation) sur la variabilité des coefficients de digestibilité, afin de proposer un méthode de travail pour ces essais. Soixante dix lapins Néo-Zélandais x Californiens en croissance ont été nourris ad libitum *avec un aliment du commerce. Après un période d'adaptation de 7 jours, la consommation alimentaire et l'excreta ont été enregistrés chaque jour durant des périodes allant de 2, 3 jusqu'à 10 jours consécutifs, afin de déterminer la digestibilité de la matière sèche.*
La variabilité des coefficients de digestibilité de la matière sèche entre animaux et périodes de récolte ont été étudiés. La variance due aux animaux décroît avec le nombre de jours de la période de récolte jusqu'à un période de 4 jours puis se stabilise. La variance due aux périodes suit le même modèle. En comparant ces deux variances on peut noter que pour la période 1-jour la variance due aux animaux est identique (seulement 1.11 plus élevée) à celle des autres périodes, mais pour les périodes 4 ou 5-jours ce taux augmente (2.06 à 2.63, respectivement). Le nombre d'animaux nécessaire pour détecter des différences significatives ($P<0.05$) de deux points entre deux valeurs moyennes de digestibilité de la matière sèche est de 10 lapins pour une période de collecte de 4 jours, 8 lapins pour un période de 7 jours et 7 lapins pour un période de 10 jours. En dépit des effets résiduels, la corrélation entre Matière sèche ingérée/Matière sèche excrétée le même jour, est plus élevée qu'entre Matière sèche ingérée un jour/Matière sèche excrétée le lendemain (0.873). La consommation alimentaire et la vitesse de croissance mesurés pendant la période d'adaptation étaient modérément corrélées (- 0.54 et - 0.48, respectivement) avec le digestibilité de la matière sèche.

INTRODUCTION

Energy content of diets and feedstuffs is a very important topic for breeders and feed companies to assess the relation price-quality of feeds. An increase or decrease in the DE content of 100 kcal/kg would be associated to a decrease or increase in the feed conversion rate of nearly 0.2 points (MAERTENS and LEBAS, 1989 ; FERNANDEZ, 1993). So, it becomes important to establish a definite method to determine the energy content of feeds for rabbits.

Digestible energy content of feeds is determined by means of digestibility assays, the first step being the precise measure of dry matter (DM) ingested and dry matter excreted. The individual variation of the digestibility coefficients in rabbits is very high [mean variation coefficient 4.54 % for dry matter digestibility (DMD), n=115, data from our Department]. This could be the result of many factors of variation involved in the digestibility assays : breed, age, sex, origin of animals, level of intake, methodology, etc...However, the difference between the commercial strains used (New Zealand, Californian or their crossbreds), are negligible (MAERTENS and DE GROOTE, 1982 ; DESSIMONI, 1984) ; the influence of age (from 7 weeks to slaughter) is of limited importance (LEBAS, 1973 ; AUXILIA, 1980 ; PARTRIDGE, 1980 ; EVANS and GEBELIAN, 1982 ; MAERTENS and DE GROOTE, 1982 ; XICCATO and CINETTO, 1988) and the effect of sex is not relevant (MAERTENS and DE GROOTE, 1982 ; XICCATO *et al., 1992*). The influence of the feeding level has been shown by several authors when restricted feeding was compared to *ad libitum* feeding (LEDIN, 1984 ; XICCATO and CINETTO, 1988 ;

XICCATO *et al*, 1992), showing that the intake is one of the inherent variables of the digestibility coefficient.

There are different methodologies used for the digestibility assays on rabbits. Although efforts have been done to standardize these methodologies (i.e. Round Table n°IV in the IV World Rabbit Congress, which gave raise to several papers, i.e. MAERTENS and LEBAS, 1989), they have not had a great repercussion. This is shown by the disparity of methodologies of digestibility assays presented in the V World Rabbit Congress (Nutrition section, 1992) where the length of the collection period varied from 4 to 10 consecutive days or it was two periods of 4 days (two working weeks) being the most usual 7 days (57 % of the works). The number of animals per diet changed from 4 to 15 rabbits and presented a great dispersion ; in some cases it was not constant for all treatments because the aberrant or extreme data were removed in order to decrease the standard deviation. There are no experimental data on this subject at the moment, and the choice of the length of the collection period and the number of animals depends on the available facilities and habits of the research team.

The importance of the variability in the digestibility coefficients becomes more evident when single feedstuffs are evaluated because the error of determination increases inversely to the level of inclusion ; thus, when the level of inclusion increases from 15 to 45 %, the error of determination decreases 240 % (VILLAMIDE *et al.*, 1991).

The aim of this work was to evaluate the variability due to animals and to the length of feces collection period in digestibility assays, in order to propose a working guide line

MATERIAL AND METHODS

Seventeen New Zealand x Californian growing rabbits were used. The animals were chosen randomly from the growing rabbitry of the "Departamento de Producción Animal de la E.T.S.I. Agrónomos de Madrid", the live weight and physical conditions being the only criteria for the selection. The mean live weight at the beginning of the adaptation period was 1555 ± 42 g. No control of age, litter or sex was done. The rabbits were housed individually in metabolism cages allowing separation of feces and urine, in an environmentally controlled building.

A commercial pelleted diet (Dry matter : 89.7 % ; crude protein : 17.1 % of DM. ; crude fibre : 15.8 % of DM ; ash : 3.8 % of D.M.) was offered *ad libitum*. After an adaptation period of 7 days, food intake and total fecal output were recorded individually over a period of 10 days and dried daily in order to determine D.M.D. Coprophagy was not prevented. The live weight was also measured at the beginning and at the end of the experimental period.

Total DM ingested and DM excreted were measured daily and then in periods of 2, 3 up to 10 consecutive days to calculate DMD so that number of periods for the same animal decreased proportionally to the increase in the number of days per period. Therefore there were 10 possible periods of 1 day, 9 possible periods of 2 days (1–2, 3–4, 5–6, 7–8, 9–10, 2–3, 4–5, 6–7, 8–9), 8 possible periods of 3 days, and so on, 2 possible periods of 9 days (1–9 and 2–10) and only 1 period of 10 days. All these periods were used to study the variability among animals within each period. To study the variability among periods it was necessary to choose only the independent periods (those such that not one day is shared by two periods), so it was only possible to study this effect up to the 5–day periods ; thus the number of analyzed data for each animal was 10 of 1–day periods, 5 of 2–day periods, 3 of 3–day periods with 4 replications (first replication : 1–3, 4–6, 7–9 ; second replication : 2–4, 5-7, 8–10 ; third replication : 1–3, 5–7, 8–10 ; fourth replication : 1–3, 4–6, 8–10. Replications are possible when the number of periods multiplied by the number of days per period is not equal to 10), 2 of 4–day periods with 6 replications and 2 of 5–day periods for each animal. The differences among periods include all those environmental factors not controlled, but that affect all the animals.

Statistical analysis were performed using the general linear models procedure of Statistical Analysis System Institute (1986). The methodology of MEAD and CURNOW (1983) was used to calculate the number of animals needed in a digestibility trial to detect differences between DMD means according to the length of the collection period.

RESULTS AND DISCUSSION

The variance among animals and the DMD mean within each period according to the duration of the collection period in the 17 animals for all possible periods is shown in Figure 1a. In the second part of the figure (Figure 1b) we find the extreme individual values (minimum and maximum DMD for one animal in a determinated period), and the extreme mean values (minimum and maximum DMD mean of the 17 animals for the different periods). As we can observe, when the collection period increased, the variance

Table 1 : Variability of the Dry Matter Digestibility (% DMD) among independent collection periods according to their length within each animal.

Experim. period (d)	Mean (1)	Variances(1)	Range (2)		Variation Coefficients (2)	
			Minimum	Maximum	Minimum	Maximum
1	60.40	5.873	4.48	10.94	2.20	5.94
2	60.40	2.239	1.47	6.62	0.70	4.67
3	60.41	1.416	0.12	4.97	0.10	4.39
4	60.39	0.955	0.00	3.57	0.00	4.14
5	60.39	0.721	0.15	2.91	0.18	3.46

(1) Mean values of means and variances among periods.

(2) Extreme values of ranges (highest and lowest maximum differences of DMD between periods for the same animal) and of variation coefficient.

due to animals decreased, but this was more evident for the first 3–4 periods ; thus, from 1 to 5–day periods the variance decreased 3.5 times but from 5 to 10-day periods it only decreased 1.3 times. The range between DMD means for 1–day period was relatively low, 1.66 points, which is due to the high number of animals used (17) despite of the great dispersion of the individual data (maximum range of 11.52 points). That means that if we want to use a very short period of collection we must use high number of animals to get a good estimation of the mean, although the standard deviation will remain high. The individual extreme values followed the same tendency from 4 to 10–day periods (range between 5.16 and 6.44 points).

Table 1 shows the variability of DMD among independent collection periods within each animal for all 17 animals. This turned out to be very high for 1–day period as shown by the mean variance (5.873) and the mean variation coefficient (VC = 4.01 %) obtained ; moreover the highest range found in DMD from one day to another in the same animal was 10.94 points, and the lowest 4.48 points. This means that one day is a very short time to estimate the ratio DM ingested/DM excreted, and therefore the duration of the collection period must be long enough for this relation to be constant. When the number of days of the collection period increased, the variability decreased ; thus, the mean variation coefficient for 5–day periods is 2.9 times lower than for 1–day periods. Nevertheless it must be taken into account that the estimation of the variance is statistically less precise when a few number of periods (i.e. 5 days) is used. If we compare the variability due to animals (Figure 1) with that due to periods (Table 1) we can observe that for 1–day periods the variance due to animals was similar (only 1.11 times higher) than that due to periods, but for 4 or 5–days periods this ratio increased (2.06 and 2.63, respectively).

Table 2 shows the number of animals necessary to detect significant differences ($P<0.05$) between treatments (mean values of DMD) according to the duration of the collection period applying the methodology of MEAD and CURNOW (1983). To obtain the same significant differences we can use more animals in less days (i.e. 10 animals during 4 days) or less animals in more days (7 animals during 10 days). It would be more convenient to use long collection periods if we are trying to find very small differences, such as 1 to 1.5 points. But it must be taken into account that the longer the collection period, the higher will the risk of digestible disorders and residual effects be as well as the cost of the assay. So, although it would be useful to increase the collection period in order to decrease the error of the determination, a compromise with its practical use must be done.

Another method to carry out digestibility assays is to measure the intake and excretion during 2 consecutive weeks and then use, for each animal, the DMD mean of the 2 weeks. A similar method was tested in the current assay where the first week was from 1st to 4th day, the second week from 7th to 10th day and the total period from 1st to 10th day. The results are shown in Table 3. Neither the mean values nor the standard deviation varied significantly among methods, although the variability was a little higher for the first week with respect to the second. The mean value of the two weeks had a little lower variability than the individual weeks. An interesting result is the relatively low correlation found between the digestibility of the two weeks (0.567), which confirms the high variability due to periods mentioned in Table 1. Obviously, the DMD mean of the two weeks is highly correlated to the two components of this mean. The DMD total is highly correlated with all the calculated values, although the highest correlation is with the DMD mean. In this method the individual DMD is the mean value of two independent periods of 4 days, but this is not equivalent to the use of an 8–day period.

Another important subject in digestibility methodology is the variation due to residual effects.

Figure 1a.

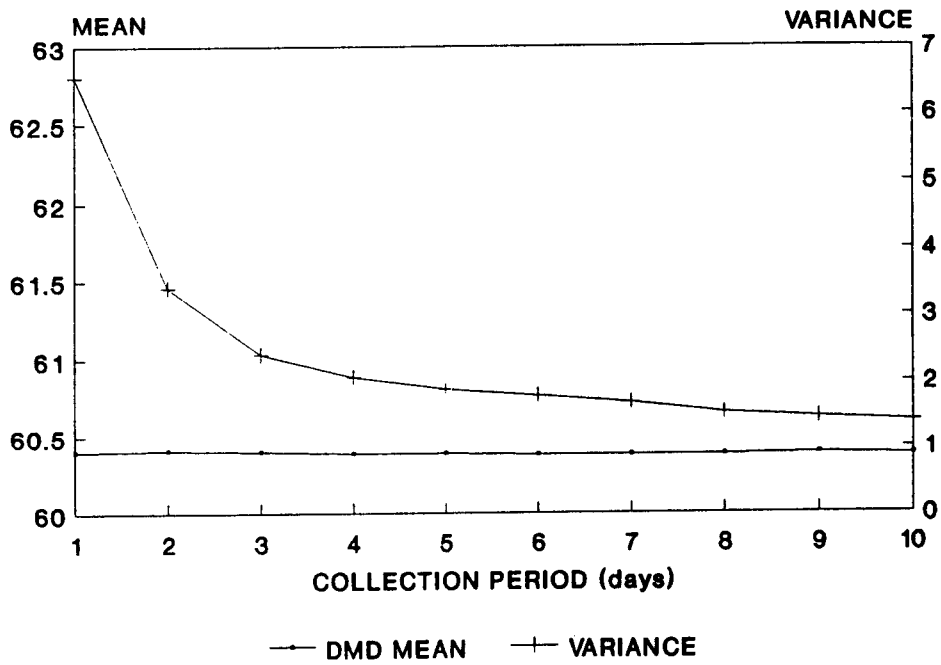

Figure 1b.

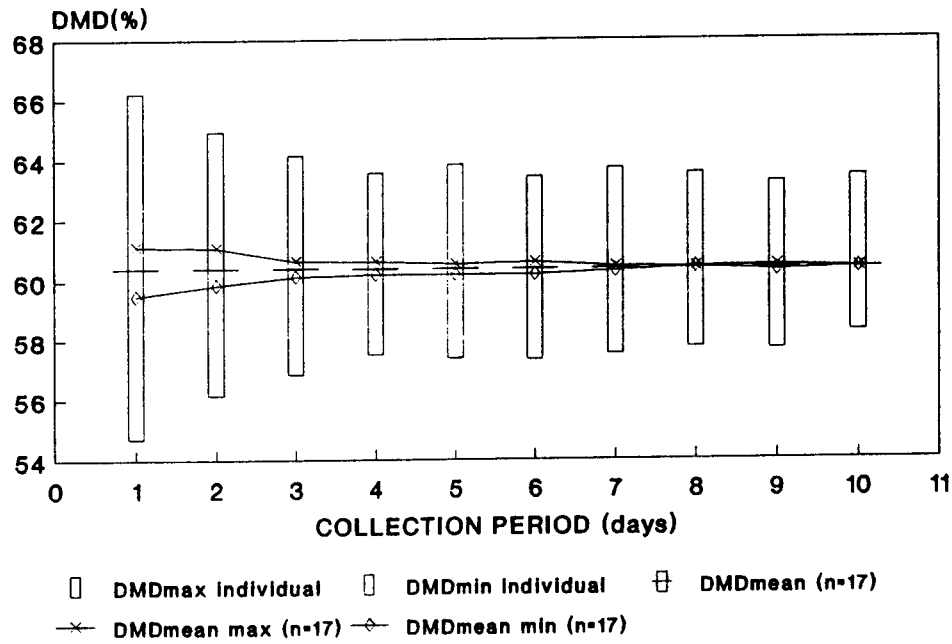

Figure 1 : Variability of dry matter digestibility among animals for all possible collection periods.

Table 2 : Number of animals necessary to detect significant differences (P<0.05) between treatments (mean values of DMD) from 1 to 4 points according to the length of the collection period.

Differences between means of DMD (points)	Number of animals		
	Collection period		
	4 days	7 days	10 days
4	3	2	2
3	5	4	3
2	10	8	7
1	38	30	26

Table 3 : Correlation analysis between DMD (%) calculated weekly (4-days period), mean of the two weeks and total (10-days period) (n = 17)

	Mean	Std. Dev.	Minimum	Maximum
DMD 1st week	60.30	1.47	57.51	63.46
DMD 2nd week	60.47	1.24	58.94	63.62
DMD mean	60.38	1.20	58.53	63.54
DMD total	60.39	1.18	58.23	63.45

Correlation analysis

	DMD 1st week	DMD 2nd week	DMD mean
DMD 2nd week	0.5674	–	–
	0.0175[1]	–	–
DMD mean	0.9046	0.8642	–
	0.0001	0.0001	–
DMD total	0.8501	0.8532	0.9610
F	0.0001	0.0001	0.0001

[1] Level of significance

The feces collected during the first day do not correspond to the intake registered the same day. However, it is assumed that the first feces collected are proportional to the feed consumed the last day of the assay. In other animal species, such as adult poultry, a fasting period is used to clear out the digestive tract, however this would cause a terrible problem in the rabbit because of its particular metabolism. As the transit time in the rabbit is about 17 hours (LEBAS and LAPLACE, 1977 ; FRAGA *et al.*, 1991) and it eats mainly during the evening and night, some authors suggest collecting feces the day after the intake has been controlled. In this assay the correlation found between DM ingested/DM excreted the same day was 0.939, but the DM ingested one day/DM excreted the day after was 0.873, so it could be concluded that the traditional method is more correct. Problems could appear when a very long periods are used or in the case of young rabbits just after weaning because they increase considerably their intake during the fifth week of life (BLAS *et al.*, 1991) ; this, together with the stabilization of the weight of the different parts of the digestive tract, could imply considerable variations in the digestibility values.

The selection of the animals in adaptation for the experimental period must also be taken into account. Rabbits must present normal intake and growth rate and no digestive disorders. However, both intake and growth rate during the adaptation period had a moderate correlation with the DMD (Table 4), so it is difficult to predict the digestive behaviour of rabbits during the collection period from the data obtained in the adaptation period. The management of rabbits during the digestibility trials affects their normal behaviour. For example, most of them begin to eat just after the feed is weighed, so their normal eating behaviour is altered. In fact, the variability of the intake is higher (VC : 11.6 *vs* 10.3 %) and the correlation between the intake and the growth rate is lower (0.79 *vs* 0.92) during the collection period than during the adaptation period. So, it could be better to measure the global intake during the experimental period (weight differences from the last day to the first) in order to disturb the animals as little as possible.

Table 4 : Correlation analysis between parameters measured in the adaptation period and in the collection period.

	Mean	Std. Dev.	Minimum	Maximum
DMD (%)	60.39	1.18	58.23	63.45
INTc (g/d)	131.47	15.28	104.40	159.57
INTa (g/d)	128.57	13.23	105.00	146.30
GRc (g/d)	33.08	6.78	20.60	40.70
GRa (g/d)	37.50	7.16	24.86	49.43

Correlation analysis

	DMD	INTc	INTa	GRc
INTc	– 0.6964	-	-	-
	0.0019[1]	-	-	-
INTa	– 0.5345	0.8290	-	-
	0.0271	0.0001	-	-
GRc	– 0.5327	0.7948	0.5424	-
	0.0277	0.0001	0.0245	-
GRa	– 0.4762	0.8298	0.9171	0.5388
	0.0533	0.0001	0.0001	0.0256

[1] Level of significance ; INTc : intake in the collection period ; INTa : intake in the adaptation period ; GRc : Growth rate in the collection period ; GRa : Growth rate in the adaptation period.

The main conclusion to be drawn from this work is that there is a higher variability of the DMD among animals than among periods and the relation between these two variances increases with the length of the collection period. So, it seems to be more convenient to use more animals during less days than the opposite. Thus the same significant differences can be found using 10 animals during 4 days of collection, 8 animals during 7 days or 7 animals during 10 days.

It is advisable to control the intake and the excretion the same day without taking into account the residual effects, mainly when short periods on collection or older animals are used.

The number of measures to be taken in the digestibility assays must be the least possible to allow a correct determination of the parameters under study in order to disturb the rabbit's behaviour as little as possible.

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