

DETERMINATION OF FAECAL DRY MATTER DIGESTIBILITY TWO WEEKS AFTER WEANING IN TWENTY FIVE DAY OLD WEANED RABBITS

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ABSTRACT: The aim of this work was to analyse the evolution from 26 to 40 d of age of apparent faecal dry matter digestibility (DMd) in rabbits weaned at 25 d of age to define how to determine nutrient digestibility in the post-weaning period. Fifteen New Zealand×Californian rabbits from 5 litters (3 rabbits/litter) weaned at 25 d of age and weighing 602 ± 75 g were fed *ad libitum* a commercial diet containing 20.0% crude protein and 33.5% neutral detergent fibre (on DM basis). Feed intake and faeces excretion were recorded daily from 25 to 40 d of age and DMd determined. Litter affected DM intake and excretion ($P=0.013$ and 0.014 , respectively), and tended to affect DMd ($P=0.061$), whereas age influenced all these traits ($P<0.001$). Dry matter intake and excretion increased from 26 to 40 d of age by 158 and 480%, respectively. During the first week after weaning, DM intake increased more slowly than DM excretion (55 vs. 245%), but in the second week after weaning both increased by 67%. The correlation between daily feed intake was higher with the faeces excretion of the same day than with faeces excretion of the next day, and the first values were used to determine daily DMd. A broken line regression model was fitted to daily DMd, which decreased linearly from weaning to 32 d of age (2.17 ± 0.25 percentage units per day), whereas from 32 to 40 d it remained constant ($69.4\pm 0.47\%$). Accordingly, for 25-d old weaned rabbits it would be advisable to begin a digestibility trial not before 32 d of age, using the first week after weaning as adaptation period. Average standard deviation of DMd decreased by 54% when the length of the collection period increased from 2 to 6 d. Consequently, the number of animals required to detect a significant difference among means depends on the length of the collection period. For a conventional collection period of 4 d, a difference of 2 percentage units could be detected by using 14 animals/treatment.

Key Words: dry matter digestibility, weaned rabbits.

INTRODUCTION

The post-weaning period is a critical time for rabbits, as their digestive function is not completely mature and important changes take place in terms of organ/mucosa development, enzyme activity, microbiota colonisation, gut barrier mechanisms, and feed intake (Carabaño *et al.*, 2010). Some of these changes also seem to depend on the composition and nutritional value of the weaning diet (Gallois *et al.*, 2005, 2008; Álvarez *et al.*, 2007; Gómez-Conde *et al.*, 2007). However, different dietary digestibility values may be obtained depending on the methodology used, the time after weaning and age at weaning (Parigi Bini *et al.*, 1991; Gallois *et al.*, 2008). In fact, recently weaned rabbits show a different digestive pattern than older animals (> 49 d) normally used in *in vivo* digestibility trials as recommended by the European

reference method (Perez *et al.*, 1995a). This method assumes that 49 d old rabbits have a steady feed intake and faecal excretion during the 4 d collection period. In contrast, at 25 d rabbits are changing from a mixed diet (milk + feed) to an exclusively solid diet and a progressive development of the caecum is taking place (Laplace and Lebas, 1972; Padilha *et al.*, 1995; Gutiérrez *et al.*, 2002; Xiccato *et al.*, 2003), resulting in changes in feed intake and faeces excretion throughout this period.

The aim of this work was to study the DM digestibility pattern in rabbits weaned at 25 days during the two weeks after weaning and define the best procedure to determine faecal nutrient digestibility by using total faecal collection.

MATERIAL AND METHODS

Experimental procedure

Fifteen New Zealand×Californian rabbits weaned at 25 d of age were taken at random from 5 litters (3 rabbits/litter). Feed intake and total faeces excretion were recorded daily from 25 to 40 d of age. Rabbit weight averaged 602±75 g at weaning, and 1405±156 g at 40 d of age. Animals were housed individually in metabolism cages (405×510×320 mm) and had *ad libitum* access to the same commercial feed (crude protein 20.0%, starch 20.8%, neutral detergent fibre 33.5%, DM basis, with no antibiotic) throughout the experiment. Faeces were collected daily, stored at -20°C and, once the trial finished, dried at 80°C for 3 d. Samples of faeces dried at 80°C and feed were dried at 103°C for 24 h (AOAC, 2000; method 934.01). Apparent faecal dry matter digestibility (DMd) was calculated as: $(\text{DM intake} - \text{DM excretion}) \times 100 / \text{DM intake}$.

Statistical analysis.

The effect of age on daily feed intake, faeces excretion and DMd was studied using a repeated measures analysis by the MIXED procedure of SAS (Littell *et al.*, 1998), including the litter effect as a block. The NLIN procedure from SAS was used to fit a one-slope broken line model (Robbins *et al.*, 2006) to the evolution of DMd with age. Number of replicates required to detect a significant difference were calculated according to Kaps and Lamberson (2004).

RESULTS AND DISCUSSION

Average daily gain, feed intake and feed efficiency from 25 to 40 d of age were 53.5±6.04 g/d, 79.7±9.40 g/d and 0.673±0.039, respectively, and no mortality was recorded. Dry matter intake and excretion were influenced by litter ($P=0.013$ and 0.014, respectively) and age ($P<0.001$ for both) and increased from 26 to 40 d of age by 158 and 480%, respectively (Figure 1). The first week after weaning, DM intake increased more slowly than DM excretion (55 vs. 245%), but during the second week after weaning both increased by 67%. DM excretion was low just after weaning (6.52±3.38 g at 26 d of age), likely because of the still high milk intake compared to feed intake (around 30 and 10 g, respectively, Nicodemus *et al.*, 2010) in the last 4 d of lactation (21 to 25 d of age). However, in the first day after weaning (26 d of age), feed intake seemed to increase sharply until 45.8±16.3 g DM/d, compared to feed intake before weaning (10 g/d from 21 to 25 d of lactation, reported previously by Nicodemus *et al.*, 2010), and kept on increasing until 29 d of age (81.3±10.8 g DM/d, Figure 1). The increase in feed intake was not constant, and rabbits decreased feed intake at 30 d of age and, although at a lower rate, at 33 and 37 d of age (Figure 1). This behaviour probably depended on the adaptation of rabbits to solid feed intake. With regard to faeces excretion, around weaning the caecum is developing and increasing its capacity (Padilha *et al.*, 1995; Gutiérrez *et al.*, 2002) which might lead to a higher mean retention time of digesta, with faeces

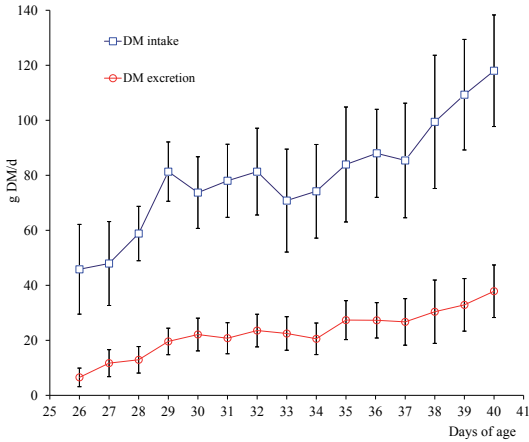


Figure 1: Daily dry matter (DM) intake and faeces excretion (g DM/d) from 26 to 40 d of age (n=15). (bars indicate standard deviation; $P_{age} < 0.001$ for DM intake and excretion).

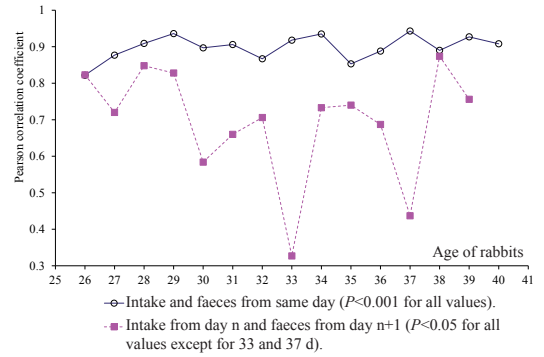


Figure 2: Correlation between feed intake and faeces excretion (both on dry matter basis, n=15).

excretion decreasing and DMd consequently increasing. This situation changed in the first week after weaning, as faeces excretion proportionally increased more than feed intake (Figure 1).

Based on the evolution of feed intake and faeces excretion after weaning, Gallois *et al.* (2008) compared DMd calculated either as in this work or by using the faeces excretion delayed by 1 d, as initially proposed by Parigi Bini *et al.* (1991), since young rabbits show a great increase in feed intake compared to a delayed faeces excretion due to the contemporary gut development. Gallois *et al.* (2008) found that DMd values were similar between 37 and 42 d of age but different between 23 and 28 d of age depending on the calculation procedure. Parigi Bini *et al.* (1991) did not find significant differences in DMd using the two methods, while changes were found in fibre fraction digestibility in young rabbits still nursing

(from 20 to 25 d of age). In this work, we studied the correlation between daily feed intake and daily faeces excretion of the same day or of the following day (Figure 2) to take into account that around 12-24 h are required by the feed to complete its transit through the gut. As the correlation was higher between feed intake and faeces excretion of the same day, these data were used to determine daily DMd (Figure 3).

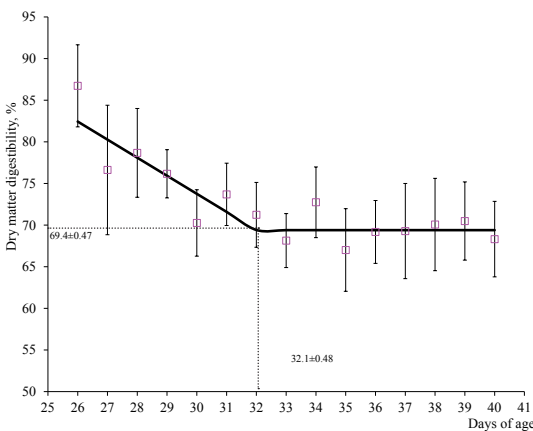


Figure 3: Daily dry matter digestibility (%) from 26 to 40 d of age (n=15). (bars indicate standard deviation. $P_{age} < 0.001$. Slope 2.17 ± 0.25 from 26 to 32 d of age).

DMd calculated daily tended to be influenced by litter ($P=0.061$) and was affected by age ($P < 0.001$) (Figure 3). DMd linearly decreased from weaning to 32 d of age (2.17 ± 0.25 percentage units per day), whereas it remained constant from 32 to 40 d ($69.4 \pm 0.47\%$). The changes in DMd with age are in agreement with the observed feed intake and excretion patterns. During the first week after weaning, DMd was reduced as DM excretion

Table 1: Dry matter digestibility (mean \pm standard deviation) determined on animals weaned at 25 d and on periods of different length (n=15).

No. consecutive days	Independent periods ¹							Averged standard deviation	
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	All periods	Only from day 32 onwards
2	26-27 d	28-29 d	30-31 d	32-33 d	34-35 d	36-37 d	38-39 d		
	81.6 \pm 5.68	77.2 \pm 3.62	72.0 \pm 3.16	69.8 \pm 3.01	69.7 \pm 2.43	68.6 \pm 2.66	70.3 \pm 4.19	3.68	3.15
3	26-28 d	29-31 d	32-34 d	35-37 d	38-40 d	-	-		
	80.2 \pm 4.03	73.4 \pm 2.71	70.6 \pm 2.00	68.4 \pm 2.35	69.6 \pm 3.76	-	-	3.08	2.81
4	26-29 d	30-33 d	34-37 d	-	-	-	-		
	78.7 \pm 3.38	70.9 \pm 2.43	69.3 \pm 1.80	-	-	-	-	2.62	1.8
5	26-30 d	31-35 d	36-40 d	-	-	-	-		
	76.7 \pm 3.26	70.5 \pm 1.70	69.1 \pm 2.11	-	-	-	-	2.45	2.11
6	26-31 d	32-37 d	-	-	-	-	-		
	76.0 \pm 2.81	69.5 \pm 1.45	-	-	-	-	-	2.24	1.45

¹ In the same row there is no overlapping between two consecutive periods.

Table 2: Number of rabbits required (n) per treatment group to detect a significant difference ($P=0.05$ or 0.001) between two means with a 80% power¹ for dry matter digestibility in a period between 32 and 40 d of age for rabbits weaned at 25 d of age.

Difference to be detected (percentage units)	Length of collection period (days) and standard deviation (in brackets) ²									
	$P=0.05$					$P=0.001$				
	2 [3.15]	3 [2.81]	4 [1.80]	5 [2.11]	6 [1.45]	2 [3.15]	3 [2.81]	4 [1.80]	5 [2.11]	6 [1.45]
5	8	7	4	4	3	17	14	8	9	6
4	11	9	5	6	4	24	20	10	13	8
3	19	15	7	9	5	41	33	16	20	11
2	40	32	14	19	10	88	71	31	41	21
1	157	125	52	71	34	342	273	114	155	75

¹For two-tailed tests with two-treatment experiments.

²The value of standard deviation used corresponds to periods from day 32 onwards.

increased faster than feed intake. During the second week after weaning, DM intake and excretion increased at a similar rate and therefore DMd remained unchanged. According to these results, it would be advisable in 25-d weaned rabbits to begin a digestibility trial no sooner than 32 d of age, using the first week after weaning as adaptation period. Similarly, Gallois *et al.* (2008) found lower organic matter digestibility in rabbits from 37 to 42 d of age (68.1%) compared to that determined from 23 to 28 d of age (74.5%), regardless of weaning age.

The effect of the collection period length (no overlapping periods from 2 to 6 d) and the initial day of the collection period is shown in Table 1. DMd values remained constant in those periods starting from day 32 of age onwards. Average standard deviation of DMd decreased by 20% when the first week after weaning was not considered. Moreover, average standard deviation decreased by 54% when the length of the collection period increased (3.15 vs. 1.45 from 2 to 6 d of collection), as previously observed by Villamide and Ramos (1994). The standard deviation obtained with a 4-d digestibility period (2.62) was higher than that reported by Perez *et al.* (1995b) for the European reference digestibility procedures obtained in 6 different laboratories (1.53) (using 49 d-old rabbits and 4 d collection period). It indicates more variable results with younger rabbits that are only compensated with a longer (6 d) collection period (reducing standard deviation until 1.45). Consequently, the number of animals required to detect a significant difference among means depends on the length of the collection period. In Table 2, the number of animals required per treatment to detect a difference among DMd means at a significant level ($P=0.050$ or 0.001) and 80% power was calculated using for each period the average standard deviation of periods from day 32 onwards. For a conventional collection period of 4 d, 5 rabbits per treatment would be needed to detect a significant difference of 4 percentage units, but at least 14 and 52 rabbits per treatment would be required to detect 2 or 1 percentage units of difference, respectively.

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