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EFFECT OF THE AGE AT INTRODUCTION OF A STARTER FEED**

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SUCKLING RABBIT DIGESTIBILITY: EFFECT OF THE AGE AT INTRODUCTION OF A STARTER FEED

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ABSTRACT

Early feeding in the nest is a promising biomimetic strategy to improve gut health and prevent digestive diseases. To formulate adequate starter feed, it is essential to determine the nutritional values of the pre-weaning diets. However, the assessment of suckling rabbits' digestion is challenging since the kits are raised together with their mother along with the fact that gut morphology and functionalities are deeply changing during the transition from milk to solid feed. To study the digestive capacity of the young rabbits, a 15-days digestibility trial was performed from 21 days of age to weaning on rabbits provided with early feeding or not. A mother-litter separate feeding system with controlled suckling was used to monitor intake and faecal excretion of 20 litters. All the litters had access to pellet feeds from 15 days. A starter feed in a form of a gel was provided as early as 3 days of age to half of the litters. Gut development dynamics and milk intake were taken into account to adjust faecal digestibility calculations. Digestibility coefficients of dry matter, crude protein, gross energy, and fibre fractions (NDF, ADF, Hemicelluloses) were high between 21 and 24 days (64%, 72%, 68%, 44%, 37% and 55%, respectively) and were followed by a decrease between 25 and 27 days (-16%, -10%, -16%, -32%, -37%, -7%). Starter feed supplementation did not modify faecal digestibility. Our original data revealed a short period where digestive capacity of the rabbit seemed to be overwhelmed by the sudden influx of dry matter in the gut. This could be implemented in the future for pre-weaning feed formulation.

Key words: Digestibility, Pre-weaning, Solid feed intake, Feed transition, Digesta increment.

INTRODUCTION

Pre-weaning period represents a sensitive dietary transition for the young rabbit, sometimes followed by enteropathies. From 21 to 31 days, dry feed intake is multiplied by 14, along with a sharp drop of milk intake (Fortun-Lamothe and Gidenne, 2000), and corresponds to a sharp shift in nutrient intake profile: milk-based diet rich in fats and proteins shifts to a plant-based diet rich in carbohydrates. Moreover, rabbit digestive functionalities before weaning are still developing (Gallois *et al.*, 2008). Recent studies highlighted significant solid feed ingestion of 8-days-old rabbits on-farm (Paës *et al.*, 2019) together with positive effects of pellet feed intake stimulation from 18 days on kits growth (Read *et al.*, 2015) and digestive ecosystem maturation (Read *et al.*, 2019). Thus, allowing adequate feed intake before weaning (time of introduction and feed quality) appears as a promising strategy to improve the health management of the young rabbit. With this study, we aimed to extend our knowledge on the young rabbit's digestion ability in relation with early solid feed intake stimulation. We performed a 15-days digestibility trial from 21 days of age to weaning for young rabbits fed in the nest a starter diet (in form of gel) or not.

MATERIALS AND METHODS

Animals and experimental design

Thirty-two crossbred litters (commercial lines Hyplus PS19 x Hyplus PS59; Hypharm, France) were raised with their doe in a wire mesh cage allowing a separate feeding of the litter (Paës *et al.*, 2019 adapted from Fortun-Lamothe *et al.*, 2000) INRAE experimental farm, Castanet-Tolosan, France).

Two days after parturition (d2) litter size was standardized to 10 kits. Does were fed a commercial diet separately from their kits. At d3, litters were allocated to two groups (kits body weight: 101±9 g). In the early feeding group (EF), the litters were fed from d3 to d17 a starter gel form, while no starter feed was supplied to the control group (C). Starter feed gels were processed with the kits pellets transformed into mash. This mash was supplemented with vanilla flavor (0.06%, Phodé, Terssac, France) before being thoroughly mixed with three times its weight of hot water and 0.6% of agar. Starter feed gels were daily processed and provided inside plastic cups into the nest. All the litters had access to 2.5-mm-diameter pellets formulated for kits (Table 1) as of d15. Weaning occurred at d36. Litters were weighed at days 3, 14, 21, 28 and 35. Mortality and health status of all the rabbits were registered daily.

Digestibility trial

Ten litters among the sixteen of each group were selected to assess faecal nutrient digestibility from d21 to d35. In half of those litters, one kit was sacrificed at days 21, 25, 30 and 35 to evaluate increment of digesta content. Meanwhile, kits from non-sampled litters were removed to maintain litter size. Digestibility was evaluated at the litter-level with controlled suckling. The faeces of kits were collected daily with a perforated aluminum plate placed under the kits' allotted space (38 x 62 cm) and then were weighed. Milk intake was assessed at days 21, 24, 28, 31 and 35. Feed intake and feed waste were recorded every 3 or 4 days. Milk consumption was considered to calculate dry matter (DM), crude protein (CP) and fat digestibility by using milk digestibility values determined by Gidenne *et al.* (2018). The increase of gut contents before weaning was taken into account for DM digestibility calculation by adding it to the faecal DM excretion (Debray *et al.*, 2003). Regarding the weight increment of the stomach content, we considered that 94% of the digesta was digestible (mainly milk) and only the remaining 6% were kept for correction. The formula to calculate DM faecal digestibility coefficient (FDC, %) was:

$$DM\ FDC = 100 \times \frac{DM\ intake\ (undigested\ milk + feed) - DM\ excreted\ (faeces + gut\ digesta\ increase)}{DM\ intake\ (undigested\ milk + feed)}$$

Table 1: Ingredients and chemical composition of the experimental diet

Ingredients (%)		Amino acids	1.22
Sunflower meal	25.00	Rapeseed oil	0.50
Barley	15.00	Mineral and vitamin premix	1.00
Wheat bran	12.80		
Alfalfa	6.90	Chemical composition (g/kg as fed-basis)	
Beet pulp	6.70	Dry matter	893
Apple pomace	6.00	Ash	67
Cane molasses	5.50	Crude protein	160
Wheat	5.20	NDF	356
Straw	5.00	ADF	215
Rapeseed meal	3.50	ADL	73
Rapeseed whole seed	2.30	Starch	119
Grape pulp	2.20	Fat content	28
Calcium carbonate	1.18	Gross energy (MJ/kg)	17

Chemical Analyses

Faeces were dried at 80°C for 24h, then half part was dried at 103°C for 24h to determine DM excreted per litter. Faeces dried at 80°C were grounded and pooled in 5 periods (d22-25, d26-28, d29-30, d31-32 and d33-35) to obtain sufficient quantities for further analysis: DM, ash, nitrogen, crude fat, gross energy, NDF, ADF and ADL (EGRAN, 2001). Feed starch content was determined with enzymatic method (AFNOR NF V03-606). DM content of starter feed gels was obtained by freeze-drying for 48h and was equal to 25%.

Statistical Analysis

Data were analyzed with R software by analysis of variance or linear mixed model to fit longitudinal data (age and experimental group as fixed effects and litter as random effects).

RESULTS AND DISCUSSION

The experiment was performed in good sanitary conditions since the pre-weaning mortality reached only 4.3%. Regarding EF group rabbits, we were able to quantify their ingestion of feed in the nest from 7 days of age (0.1 ± 0.1 g of gel consumed per kit). Their starter feed gel intake exponentially increased with age (1.6 ± 0.4 g of gel/kit at d17) to reach a total consumption of 5.0 ± 1.4 g of gel per kit (1.2 ± 0.3 g in DM) from d3 to d17. Pellets intake between d15 and d18 averaged 0.6 ± 1.1 g of DM/kit in the two groups, before a four-fold increase at d21. Feed and milk intake before weaning as well as growth performances of the 32 litters of the experiment were similar.

Table 2: Characteristics of the 20 litters monitored during the digestibility trial period (d21-35)

	Early feeding (n=10 litters)	Control (n=10 litters)	Prob.	RMSE
Initial body weight (d21, g/kit)	379	396	0.33	49
Final body weight (d35, g/kit)	952	947	0.84	102
Daily weight gain (g)	40	41	0.33	3
Total feed intake (g of DM/kit)	488	452	0.16	71
Daily feed intake between d21&d25 (g/kit/day)	11	10	0.86	4
Daily feed intake between d32&d35 (g/kit/day)	59	56	0.77	15
Milk intake at d21 (g of FM/kit)	30	34	0.57	6
Milk intake at d35 (g of FM/kit)	3	3	0.63	6
Digesta content increment (total tract, g of DM)	19	16	0.34	5

Rabbit gut tract rapidly evolves before weaning in response to solid feed intake stimulation (Gallois et al., 2008). Since the digestive content greatly increased during the digestibility trial measurements (+18 g of DM, see Table 2), raw data were corrected for caecal and intestinal contents as well as for the increase of stomach indigestible content. Digesta increase correction values (Table 3) contributed to a decrease of raw DM FDC estimates (-3.8% from d21 to d34). On the opposite, implementation of milk dry matter intake corrections increased raw DM FDC values (+0.8 % from d21 to d34), with stronger effects at the lactation peak. On overall, taking into account those two corrections prevented an overestimation of DM FDC.

Table 3: Digesta increment values used to correct DM FDC (based on n=10 rabbits/period).

	Raw DM FDC (before any corrections)	Digesta content growth (in g of DM)		
		Small intestine + colon	Caecum	Stomach (indigestible part only)
d21-24	72.2 ± 3.4^C	0.97	1.69	0.12
d25-27	53.4 ± 3.1^A	1.47	1.80	0.06
d28-29	65.4 ± 2.0^B	0.85	0.92	-0.01
d30-31	65.5 ± 2.6^B	0.66	1.19	-0.06
d32-34	65.5 ± 2.7^B	1.17	1.73	-0.12

Means \pm SD with different letters indicate means that significantly differ with the time

Strikingly, digestibility coefficients (Table 4) peaked at the onset of solid feed intake (d21-24), and dropped in the next period (d25-27; DM: -16%; CP: -10%; Gross energy: -16%; NDF: -32%; ADF: -37%; HCell: -6.5%). From d28 to d34, the digestibility increased again and stabilized, though a lower digestibility of NDF and hemicelluloses was observed at the end of the assay (d32-34). Debray *et al.* (2003) observed similar pattern with high digestibility between 25 and 27 days followed by a decrease in the next period (d28-31) with further decrease at weaning except for fat. In a few days, rabbit digestive system has to overcome important substrates changes: in our experiment, the pellet DM intake became equivalent to DM milk intake between d21 and d25 and quickly exceeded it. The decrease of digestibility observed at d25-27 can be explained by an adaptation process to solid feed diet. Limited enzymatic activities in pancreas and small intestine are indeed observed before 32 days (Debray et al., 2003), combined with the decline of the enzymes active in early life such as observed for trypsin at 28 days (Gallois et al., 2008). We also hypothesized that, since the caecotrophy starts around 25-28 days (Orengo and Gidenne, 2007), it could mathematically induce a digestibility drop. Indeed, the new intake of caecotrophes induces additional faecal excretion possibly leading to an

underestimation of digestibility coefficients. On the contrary, when caecotrophy behaviour is settled, the excretion of caecotrophes products is balanced by the intake of previous caecotrophes.

Table 4: Whole tract apparent digestibility of nutrients before weaning after corrections for dry matter milk intake and digesta content increment when applicable (n=10 litters/group/period)

	Digestibility coefficients (%)								
	DM (corrected)			CP (corrected)			Gross energy (corrected)		
	EF	C	Age effect	EF	C	Age effect	EF	C	Age effect
d21-24	64.4±3.4	65.6±2.0	D	71.6±2.3 ^a	74.1±2.4 ^b	B	68.1±2.9	70.4±3.3	C
d25-27	49.0±3.0	48.3±2.6	A	63.1±2.6	63.0±2.6	A	53.6±3.0	53.1±2.7	A
d28-29	60.8±2.0	60.9±2.5	B	72.4±2.1	72.5±2.5	B	64.3±2.1	64.6±2.2	B
d30-31	63.1±2.4	61.7±2.2	C	73.8±2.8	72.1±2.3	B	64.6±2.3	63.3±2.1	B
d32-34	61.0±4.9	63.2±0.9	BC	72.7±3.9	75.0±0.9	B	62.4±4.5	64.3±1.7	B
	NDF			ADF			Hemicelluloses		
	EF	C	Age effect	EF	C	Age effect	EF	C	Age effect
	d21-24	44.1±5.6	47.1±6.4	D	37.0±6.3	41.1±7.6	C	55.1±5.3	56.5±5.3
d25-27	13.5±6.0	12.9±4.7	A	1.3±7.7	4.0±6.2	A	32.3±5.5	26.6±5.7	A
d28-29	33.7±3.9	34.3±4.0	C	24.2±3.6	26.1±4.4	B	48.4±6.7	47.0±7.4	C
d30-31	34.1±3.6	31.6±3.5	C	25.9±3.9	25.0±3.8	B	46.6±4.7	41.7±4.7	C
d32-34	26.6±8.4	30.0±2.6	B	20.6±9.7	24.4±2.5	B	36.0±7.0	38.7±4.0	B

Means±SD with different letters on the same row indicate treatment effects (Benjamini-Hochberg test; P=0.045).

Early supply of solid feed did not affect faecal digestibility, whatever the nutrient. Limited adaptive response of enzymes activities to feed input was previously demonstrated with early weaning procedures (Gallois et al., 2008). Thus, ontogenic factors appear as main drivers of digestion of the young rabbit.

CONCLUSIONS

In this study, we provided original data on suckling rabbit digestion and we outlined the importance of ontogenic factor. Our starter feeding system did not induce significant changes on rabbit nutrients digestion.

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➤ Suckling rabbit digestibility: effect of the age at introduction of a starter feed

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Rabbit Experimental Group "GEC"

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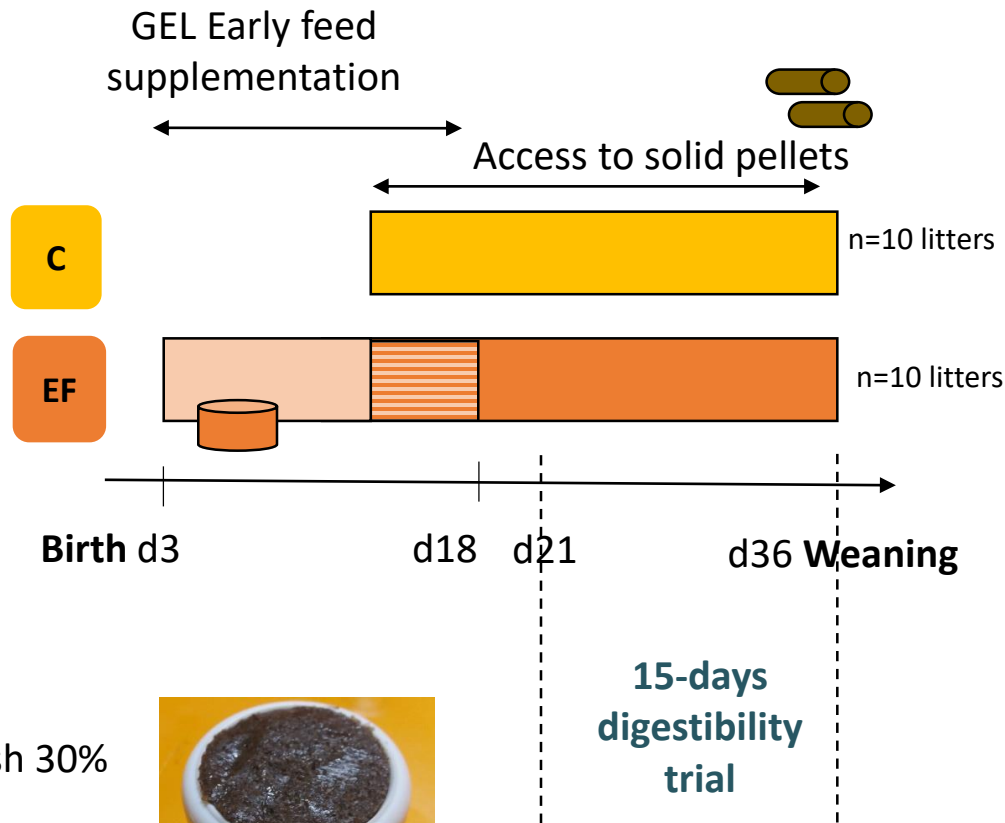
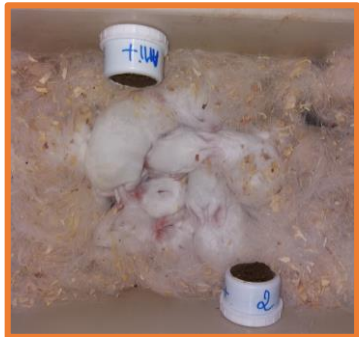
➤ Context



- **Pre-weaning period** = marked changes with modification of diet type and development of digestive functionalities (Gallois et al., 2008), sometimes followed by enteropathies
 - Ingestion of solid feed starts at 8 days of age (Paës et al 2019)
 - Positive effects of pellet feed intake stimulation from 18 days on kits growth and digestive ecosystem maturation (Read et al., 2019)
- Development of early feeding strategies as **biomimetic strategy** to improve the health management of the young rabbit

- **What is the young rabbit's digestion ability and can it be modified with early solid feed intake stimulation?**

➤ Material and methods



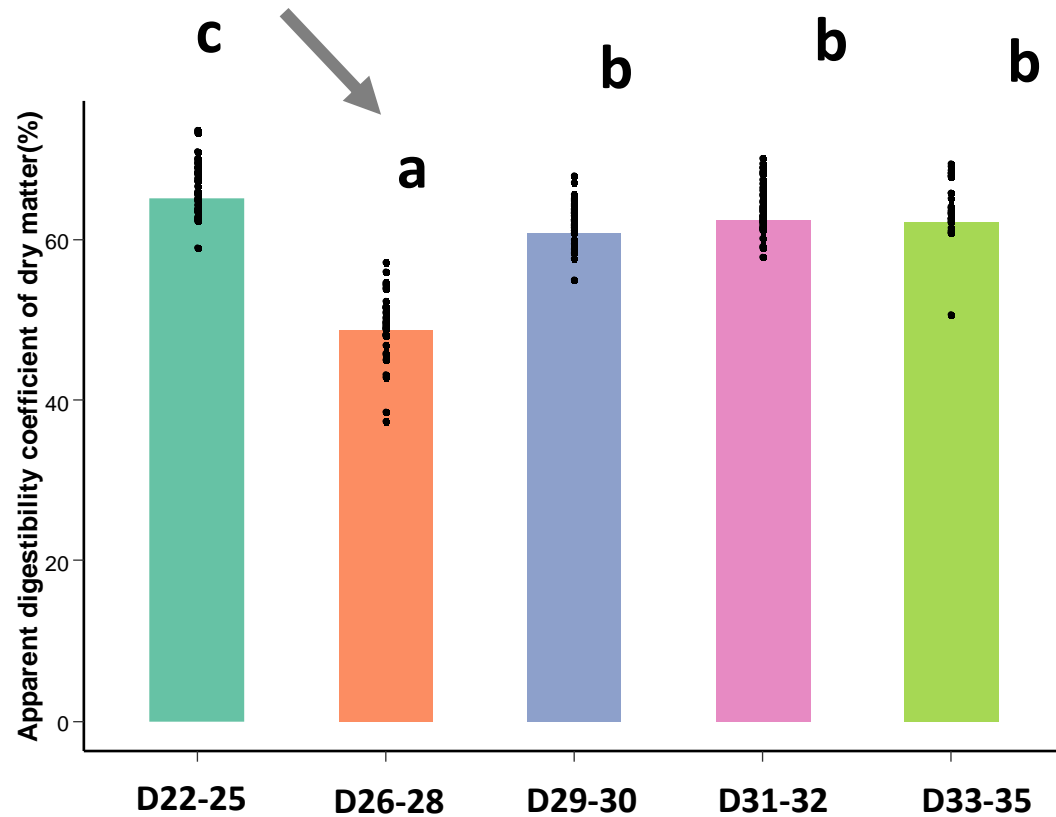
- Feed pellet mash 30%
- Vanilla 0,06%
- Agar 0,6%

- Use of a **mother-litter separate feeding system** with controlled suckling
- **Solid and milk intake monitoring** every 2-3d
- **Daily collection of faeces** at the litter level
- Milk digestibility and increase of gut contents taken into account for DM digestibility calculation

$$\text{DM Fecal Digestibility Coefficient} = \frac{\text{DM intake (undigested milk+feed)} - \text{DM excreted (faeces+gut digesta increase)}}{\text{DM intake (undigested milk+feed)}} \times 100$$

➤ Results

- Starter feed supplementation did not modify faecal digestibility.
- Strong modulation of apparent nutrient digestibility **over time** in suckling rabbits

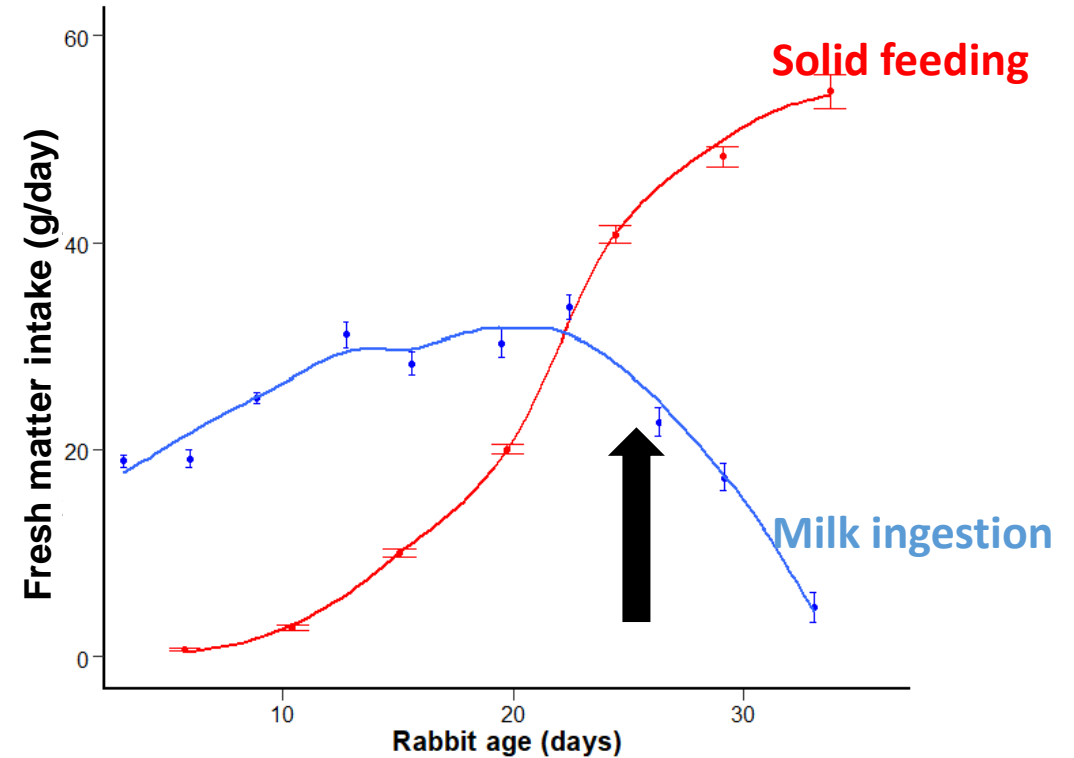
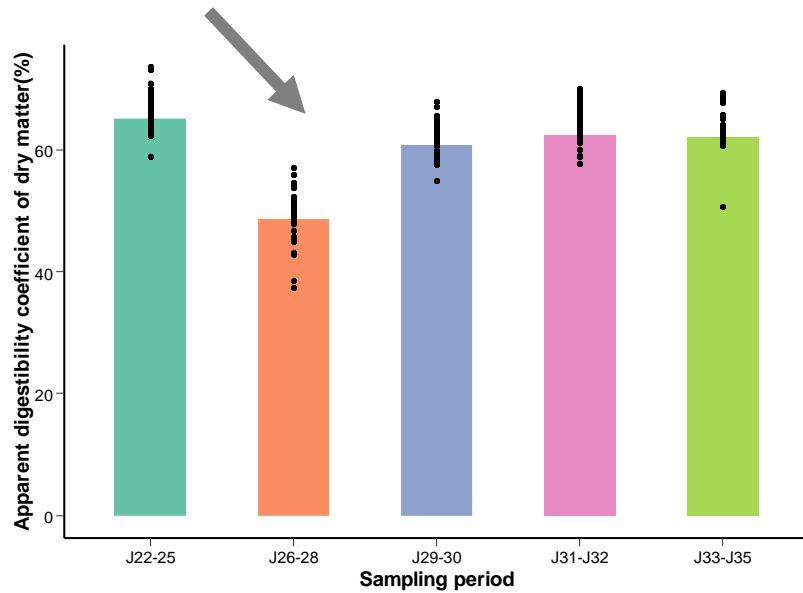


	Fecal Digestibility Coefficient D22-D25	Drop D22-D25 & D26-D28
DM	64 %	-16 %
Crude protein	72 %	-10 %
Energy	68 %	-16 %
NDF	44 %	-32 %
ADF	37 %	-37 %
Hemicellulose	55 %	-7 %

N=20 litters/period

➤ Results

- The drop in digestibility is coupled with an important **dietary shift** at the onset of solid feed intake



➤ Conclusion

- **The decrease of digestibility observed at d25-27** can be explained
 - ➔ by an adaptation process to solid feed diet. Limited enzymatic activities in pancreas and small intestine (*Debray et al., 2003*), combined with the decline of the enzymes active in early life such as observed for trypsin (*Gallois et al., 2008*).
 - ➔ the caecotrophy starts around 25-28 days (*Orengo and Gidenne, 2007*), could mathematically induce a digestibility drop (an underestimation of digestibility coefficients)
- **Our gel starter feeding system did not induce significant changes on rabbit nutrients digestion**
 - ➔ ontogenic factors appear as main drivers of digestion of the young rabbit.