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Impact of feed restriction and fragmented feed distribution on performance, intake behaviour and digestion of the growing rabbit

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ABSTRACT

Postweaning feed restriction preserves rabbit digestive health after weaning, but the underlying physiological mechanisms are not yet understood. To elucidate whether the feeding intake pattern modification related to feed restriction might be involved, we studied the effects of both feed intake quantity and intake frequency. Animals were allotted at weaning (28 d old) in a 2 × 2 factorial design: feed intake quantity (**AL** = *ad libitum* vs **R** = 75% of AL) and fragmented feed distribution (**FFD**) (1 vs 13 distributions), thus forming four groups (**AL1, AL13, R1** and **R13**). New Zealand White growing rabbits were used from weaning to slaughter (70 d old), to analyse mortality, morbidity, performance, intake behaviour, digestion and microbial activity. Seven days after starting feed restriction (35 d old, group R1), rabbits consumed 44% of the feed within 2 h, 65% in 4 h and in 7 h over 95%. Over the 28–70 d period, mortality was low (5.3%) while morbidity averaged 18.5% and neither was affected by treatment. However, FFD tended to decrease the morbidity rate during the first 14 days after weaning ($P = 0.06$). Feed conversion (28–70 d) was improved by restriction (+15%, $P < 0.001$) and by FFD (+5%, $P < 0.001$). Nutrient digestibility was improved by restriction (+10% for energy, $P < 0.01$), but not by FFD. Fragmented feed distribution led to a lower stomachal pH, in the antrum (1.48 vs 2.13, $P < 0.001$) and in the fundus (1.52 vs 2.63, $P < 0.001$), while a higher pH was found in the caecum (6.07 vs 5.86, $P < 0.001$). Butyrate proportion in the caecum was reduced by four units for restricted groups. Fragmented feed distribution reduced the caecal VFA concentration by 23% within restricted rabbit groups only. A similar interaction between intake level and FFD was observed for fibrolytic activity (cellulase and xylanase). The diversity of caecal bacterial community was not modified by either of the two factors studied. Globally, fragmented meals have no major impacts on the caecal microbial activity, diversity, and thus would not be implicated in the better resistance of restricted rabbit to digestive troubles.

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Implications

Postweaning feed restriction is a common strategy to improve feed efficiency and to preserve the digestive health of the young rabbit, and thus to reduce medication during critical phases (weaning) or in farms with poor health status. Feed restriction strongly affects the feeding behaviour, but seems to moderately impair the welfare too. Fragmented feed distribution tended to reduce morbidity rate, and contributed to improve the feed efficiency without major impact on the digestion. New insights on digestive physiology and feeding behaviour are provided, thus contributing to construct restricted intake strategies for the growing rabbit that optimize both welfare, digestive health and feed efficiency.

Introduction

Strategies to control and restrict feed intake have been initially explored in the growing rabbit to improve meat and carcass quality and digestive efficiency (Lebas, 1979; Ledin, 1984, Perrier and Ouhayoun, 1996; Diaz Arca et al., 1999). In 2002, they were first used to improve the resistance to digestive disease (Gidenne et al., 2012a). Further work on restriction strategies has been published recently often with a positive impact on health (Alabiso et al., 2017; Birolo et al., 2016), but sometimes with a negative effect (Romero et al., 2010; Birolo et al., 2020a) or a neutral effect when the mortality rate is already low (Birolo et al., 2020b). Pre-

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sently, intake limitation strategies are commonly used by French rabbit breeders, not only to improve the resistance to specific pathologies, such as Epizootic Rabbit Enteropathy (Larour et al., 2004; Boisot et al., 2003), but also to improve feed efficiency and thus to reduce feed costs. However, the physiological mechanisms underlying the positive impact of short-term feed restriction remain to be clarified, and we still puzzle whether the quantity of feed ingested is the main determinant, or the feed intake pattern could play a role. Besides, restricting feed intake might be detrimental to the rabbit's welfare, with daily transitory hunger periods and a strongly modified feeding behaviour. However, to our knowledge, very few literatures are available on this point.

Thus, we aimed to analyse the effect of the feed intake pattern on the digestive physiology of restricted animal, to differentiate whether the favourable effect of a restriction on digestive disorders originates from a strict impact of the quantity of feed ingested, or from a change in the intake pattern that may lead to circadian strong variations in nutrient supply. Since, rabbits freely fed have a biphasic intake pattern (night intake, Prud'hon et al., 1975) and restricted rabbits have a strong biphasic pattern, we choose to compare a fragmented pattern intake evenly distributed over the nycthemeron with the "classical" biphasic intake pattern. Therefore, we designed a 2 × 2 bifactorial experiment to independently analyse the effect of reducing the feed intake level (*ad libitum* vs. 75% of *ad libitum*) and that of the intake behaviour through a single or a fragmented feed distribution (1 vs. 13 feed deliveries).

Material and methods

Experimental designs and animals

Three trials were performed in April 2008 to study: growth, feed intake, feed efficiency and health status in trial 1; feeding behaviour and faecal excretion patterns in trial 2; and digestive physiology and digestive efficiency in trial 3. For all the trials, rabbits (New Zealand white × Californian) were produced in the INRAE experimental farm (PECTOUL unit). They were weaned at 28 d of age and allotted to four groups (**AL1**, **R1**, **AL13** and **R13**) arranged in a 2 × 2 bifactorial design: intake level "**IL**" ("**AL**" = *ad libitum* vs. "**R**" restricted at 75% of AL) and fragmented feed distribution "**FFD**" (1 vs. 13 feed deliveries over 24 h) with the same amount of feed at each delivery. Treatments were applied from 28 to 52 days of age, and then all groups were fed ad libitum. The R1 group was fed with one delivery per day at around 1100, and both groups R13 and AL13 were fed with eight deliveries during night (1300, 1500, 1630, 1800, 1930, 2100, 2230, 2400)) and five deliveries during light period (0200, 0400, 0630, 0900, 1100) to simulate a natural feed intake pattern of *ad libitum* rabbits (for review Gidenne et al., 2020a). Feed distribution was done by hand for all groups. To impose the rhythm of 13 deliveries, particularly for the rabbits fed *ad libitum*, the intake level of AL13 group was adjusted 5% lower than AL1 one. The R1, R13 and AL13 were fed with a quantity of feed, adjusted every three or four days according to the mean intake of AL1 group. Water was freely available for all groups throughout the trials. From 52 to 70 d of age, all animals were fed freely the same diet (Table 1) formulated to cover the nutritional requirements of the growing rabbit (Gidenne et al., 2015). The breeding unit was kept at a temperature of 20 °C (±2 °C) and under a 0200 to 1200 lighting schedule (without natural light entering the breeding rooms). This lighting schedule was adjusted to synchronize the digestion rhythm of the AL group, which eat more particularly during the night (Gidenne et al., 2020a), with the digestion rhythm of the R groups directly depending on the feed delivery time (around 1100). Animals were handled according to the care of animals in experimentation, in agreement with European legislation (European Union, 2003).

Table 1
Ingredients and chemical composition of the experimental diet for growing rabbits.

Ingredients, g/kg (as-fed)		Analysed composition, g/kg DM	
Wheat bran	215	DM	905
Dehydrated alfalfa	210	Ash	91
Sunflower oil	200	Crude Energy (MJ/kg)	16.35
Wheat	94	CP	179
Barley	89	NDF	401
Wheat straw	55	ADF	228
Dehydrated sugar beet pulp	50	ADL	61
Sucrose	40	Calculated composition,[2] g/kg DM	
Soybean oil	25	Starch	140
Calcium carbonate	10	Lysine	7.2
Vitamins and minerals mixture[1]	5	Methionine	3.8
Salt	5	Methionine + cysteine	6.5
L-lysine	1	Calcium	9.8
DL-methionine	1	Phosphorus	5.5

[1] Vitamins: A: 1 500 000 UI/kg; D_3: 200 000 UI/kg; E: 3 000 mg/kg; B_1: 200 mg/kg; K_3: 50 mg/kg and oligo elements: Cu^{2+}: 800 mg/kg; Fe^{2+}: 8 000 mg/kg; Zn^{2+}: 20 000 mg/kg; Mn^{2+}: 4 000 mg/kg and coccidiostat: robenidine.
[2] Calculated values according to Feedipedia (http://www.feedipedia.org/).

Intake level, growth and health status (trial 1)

Growth performance was measured along with two replicates of the same trial (with three week interval) counting first 200 rabbits and secondly 120 animals, equally shared in the four groups and collectively caged (five rabbits per cage, 46 × 70 × 33 cm, 4 × 10 and 4 × 7 cages). Feed intake was measured daily for each cage, except for control group (AL1: three times a week). Morbidity and mortality were checked daily: a control of clinical signs of digestive disorders such as diarrhoea, caecal impaction, suspicion of epizootic rabbit enteropathy or other pathophysiological/pathological disorders (respiratory problems, injuries, etc.) was carried out. A health risk index was then calculated as the sum of the morbid and the dead for the initial number of animals for a given period (Gidenne et al., 2009). The live weight was measured individually twice a week, and animals having very low live weight (under 2 SD below the mean) were declared morbid.

Feed intake and faecal excretion patterns (trial 2)

At 36 d and 46 d of age, the intake pattern was measured over a 24 h period for the four groups (4 × 40 cages of five rabbits). At each feed supply time (for AL13 and R13 groups), the feed refusal was weighed and intake calculated, while for AL1 and R1 groups, it was calculated at 0900 am. At 37 d and 47 d old, the excretion pattern for hard faeces was measured every 30 min over a 24 h period, on four further groups of five rabbits kept in individual metabolism cages (50 × 40 × 33 cm) equipped with an automatic faecal collector (Gidenne and Lapanouse, 1997), and under the same treatment. The number of rabbits studied was determined according to the variability of faecal excretion (low variability under feed restriction) between individuals within a group (5, 6, 3 and 6 rabbits respectively for the AL1, AL13, R1 and R13 groups). This variability was calculated from a first set of measurements on two groups of five rabbits at 34 d old and restricted or not. Each collection of hard faeces was dried (24 h at 103 °C) then weighed.

Measurements of growth, feed intake, digestibility and ratio hard faeces/soft faeces (trial 3)

Digestibility of diet (caecotrophy was not prevented) was measured from 41 to 47 d of age according to Perez et al. (1995) on 48

rabbits (four groups of 12), housed individually in metabolism cages (50 × 40 × 33 cm) from weaning (28 d), in a specific room with the same environmental conditions as for trials 1 and 2. This was followed by soft faecal collection, by preventing caecotrophy from 1700 h to 0900 h on rabbits equipped with a rigid plastic collar and as reported by Gidenne and Lapanouse (2000). Then, the ratio of soft faeces:hard faeces was calculated.

Digesta sampling, physicochemical parameters of caecum and stomach (trial 3)

Digesta sampling time was chosen between 1600 and 2000, during the phase of the active digestion in the caeco-colic segments, that is on average 5 h after feed delivery (for R1 group) as shown by Gidenne et al. (1993). At 49, 50 and 51 d of age, 12 rabbits of each group previously used for digestibility measurements (chosen healthy and in the mean weight of their group) were first anaesthetized using an intramuscular injection of ketamine (Imalgène™, Merial, Lyon, France; 20–25 mg/kg BW), then euthanized with an intracardiac injection of embutramide (T61™, Intervet International GmbH, Unterschleissheim, Germany; 0.3 mL/kg LW). pH was measured in the fundus and the antrum of the stomach, and in the caecum using a glass pH "Unitrode" electrode (Pt1000/B/2/3MKCl; Metrohm™) connected to a digital pH meter (Metrohm model 713 CH-9101, Herisau, Switzerland). Thereafter, full caecum was quickly removed and weighed, then emptied and weighed. DM of caecal content was determined by heating at 103 °C during 24 h.

Chemical analyses of feed and faeces

The following chemical analyses were carried out on feed (EGRAN, 2001) and faeces: DM (24 h at 103 °C), ash (5 h at 550 °C), fibrous fractions (**NDF**, **ADF** and **ADL**) according to the sequential method of Van Soest et al. (1991) with an amylolytic pretreatment, and crude fat according to the method described by Alstin and Nilsson (1990). Nitrogen was determined according to the DUMAS combustion method using the Leco auto-analyser (model FP-428, Leco Corporation, St Joseph, MI, USA) and converted to CP.

Characterization of the caecal bacterial community diversity

To address the diversity of the bacterial community, we analyse the bacterial profile of caecal samples using a Capillary Electrophoresis Single Strand Conformation Polymorphism (**CE-SSCP**) procedure. Total DNA from about 0.2 g of caecal sample was extracted and purified with QIAamp® DNA Stool Mini kit (Qiagen Ltd, West Sussex, England) as previously described (Michelland et al 2011). The V3 region of the 16S rRNA genes was used as a bacterial diversity marker with the primers w49 and 5′-6FAM-labelled w34, and PCR assays were performed as described previously (Michelland et al., 2011). The CE-SSCP was performed on an ABI Prism 3100 Genetic (Applied Biosystems, Branchburg, New Jersey, USA). CE-SSCP profiles were aligned and normalized using StatFingerprints program version 2.0 (Michelland et al., 2009) running on R version 2.8.3. The Simpson diversity index was estimated on each CE-SSCP profile with $-\log\sum(ai)2$ where *ai* is the relative area under the *i*th peak (Rosenzweig, 1995).

Fibrolytic activity of caecal bacteria

Portions of caecal content stored at −80 °C were defrosted and frozen three times to weaken cell membranes (Martin and Michalet-Doreau, 1995). Microorganism cells were then disrupted under a CO_2 stream by ultrasonic disintegration at 4 °C for 30 s. These two latter steps were repeated three times. Finally, homogenates were centrifuged (15 min at 20 000*g* and 4 °C) and supernatants containing released soluble proteins were stored at −80 °C under a CO_2 headspace (Gidenne et al., 2002). Fibrolytic activity of bacteria was determined with measurements of polysaccharidase activities (carboxymethylcellulase, xylanase and pectinase). Amount of reducing sugars released from purified substrates (carboxymethylcellulose, Sigma C5678; birchwood-xylan, Sigma-Aldrich X1500; and citrus-pectins, Sigma P9135) was measured after incubation of 0.1 mL of supernatants with 1 mL of substrate for 60 min at 39 °C and heating at 100 °C for 5 min. Reducing sugars were quantified with a spectrophotometer, using the p-hydroxybenzoic acid and hydrazide method (Lever, 1977). Enzymatic activity was expressed as µmol of reducing sugars released in 1 h by enzymes extracted from 1 g of digesta DM basis.

Caecal fermentative activity

Two samples of 1 g of fresh caecal content were diluted in storage solutions: one in $HgCl_2$ (2 mL, 2%w/v) and one in H_2SO_4 (3 mL, 2%w/v), for further analysis of volatile fatty acids (VFAs) and ammonia–nitrogen (NH_3-N), respectively. These caecal samples were stored at −20 °C until measurements were made. NH_3-N concentration was measured with the procedure of Verdouw et al. (1977) using an auto-analyser (Technicon, Domont, France). VFA concentrations were determined by gas chromatography (CP9000, Chrompack, Middelburg, the Netherlands). After thawing, the stored samples were centrifuged for 20 min at 7 700 *g*. One mL of the supernatant was mixed with 0.2 mL of HPO3 (25% w/v). This mixture was centrifuged at 20 000 *g* for 15 min, and 1 mL of the supernatant was transferred to a glass vial with extra 50 µL of an internal standard: 4-methyl valeric acid at 1%. 0.5 µL of this last mixture was injected into a gas chromatograph. The total quantity of VFA represented the sum of four components: C2 acetic acid, C3 propionic acid, C4 butyric acid, C5 valeric acid and was expressed in mM of water supernatant of the caecal content.

Statistical analysis

Categorical (morbidity, mortality and health risk index) and quantitative variables were analysed according to a model including two main effects: intake level and feed distribution, and considering their interaction. The effect of replicating the trial 1 with a 3 week interval was not significant (and did not interact with the two main factors) for any variable, and this effect was thus not presented in table. When treatment has a significant effect, we also conducted analyses using a model with one factor (groups) and four levels: AL1, AL13, R1 and R13. Categorical variables were analysed using the CATMOD procedure of SAS (SAS online guide), while quantitative one was analysed using R software. Concerning feed intake, since feed restriction led to a null intragroup variability, no statistics were performed for restricted groups.

Results

Growth performances and health

The level of feed intake from 28 to 52 d was slightly over the restriction level initially planned (−30% vs. −25%, table 2), since the AL feed intake was larger than expected. The AL13 group consumed about 4% less feed than the AL1 control group as intended, to impose the rhythm of 13 deliveries per 24 h without feed refusals. Accordingly, at 52 d, the restricted rabbits weighted 16% less (−310 g, $P < 0.001$) than the *ad libitum* fed rabbits, but feed conversion was improved by 5% for the restricted rabbits. Once all the

Table 2
Fattening performances of weanling rabbit, according to the intake level (AL, *ad libitum* or R, restricted) and to a fragmented feed distribution (1 or 13 meals per day) (Trial 1).

	Groups					*P*-value[1]		
Item	AL1	AL13	R1	R13	SEM	IL	FFD	ILxFFD
Restriction period (28–52 d old)								
Live weight, 28 d old, g	654	653	654	653	4	0.96	0.93	0.99
Feed intake, g/d/rabbit[2]	120.4[b]	115.5[a]	83.0	84.5	0.8[4]	ND[5]	ND[5]	ND[5]
Weight gain, g/d/rabbit[3]	52.1[b]	50.6[b]	37.5[a]	38.9[a]	0.5	<0.001	0.93	0.039
Feed to gain ratio[2]	2.30[a]	2.27[a]	2.17[b]	2.17[b]	0.03	<0.001	0.43	0.28
Ad libitum period (52–70 d old)								
Live weight, 52 d old, g	1 874[b]	1 845[b]	1 532[a]	1 561[a]	15	<0.001	0.99	0.20
Feed intake, g/d/rabbit [2]	192.1[b]	166.5[a]	163.8[a]	165.7[a]	3.0	0.010	0.035	0.015
Weight gain, g/d/rabbit[3]	42.1[a]	42.2[a]	49.3[b]	53.1[b]	0.6	<0.001	0.062	0.078
Feed to gain ratio [2]	4.44[a]	3.86[b]	3.30[c]	3.10[c]	0.06	<0.001	<0.001	0.19
Whole trial (28–70 d old)								
Live weight, 70 d old, g	2 661[c]	2 634[bc]	2 457[a]	2 550[ab]	15	<0.001	0.25	0.038
Feed intake, g/d/rabbit [2]	152.5[c]	138.8[b]	118.9[a]	121.5[a]	2.1	<0.001	0.035	<0.01
Weight gain, g/d/rabbit[3]	47.7[c]	47.0[bc]	42.9[a]	45.2[b]	0.3	<0.001	0.16	<0.01
Feed to gain ratio[2]	3.15[a]	2.91[b]	2.74[c]	2.67[c]	0.03	<0.001	<0.001	0.042

Abbreviations: IL = intake level; FFD = fragmented feed distribution.
[1] *P*-value for a bifactorial model, with effect of intake (AL vs. R) and FFD (1 vs. 13).
[2] *n* = 16 cages per group.
[3] *n* = 80 rabbits per group (16 cages as replicates).
[4] SEM calculated for AL1 and AL13 groups (only).
[5] ND: not determined, $\sigma^2 = 0$.
[a,b,c] Within a row, means without a common superscript differ ($P < 0.05$, for a monofactorial model: effect of groups).

groups were fed *ad libitum* (from 53 d), feed intake of the AL13 group was 12% lower than that of AL1, and was similar to that of both restricted groups, leading to a significant interaction among the two main factors. Restricted rabbits showed a compensatory growth compared to AL groups (+20%, $P < 0.001$). Consequently, the feed conversion was sharply reduced by restriction (−29%, $P < 0.001$) and also by a feed distribution in 13 meals (−10%, $P < 0.001$). Over the whole growth period, significant interactions were detected between intake level and FFD for intake, growth and feed conversion. For instance, FFD reduced feed intake only for *ad libitum* fed rabbits ($P < 0.05$) and without reduction of growth, thus leading to a better feed conversion (+10%, $P < 0.001$). In return, for restricted rabbits, FFD increased the weight gain (+5%, $P < 0.05$) and not the feed conversion. At 70 d of age, live weight of the AL1 and AL13 groups was similar, while that of R13 tended to be higher (+93 g, $P < 0.10$) than that of the R1 group.

Over the whole fattening period, growth rate was high (over 45 g/d) for AL groups (table 2) and the incidence of digestive troubles leading to mortality remained low (Table 3). Morbidity and mortality sourced only from digestive troubles (diarrhoea essentially). No significant effect of the intake level on mortality or morbidity rate was detectable. However, FDF tended ($P < 0.10$) to reduce the morbidity rate during the postweaning period (28–52 d), particularly for the AL13 group when compared to AL1 (18.8 vs 8.8, $P = 0.063$).

Feed intake pattern

The feeding behaviour of rabbits freely fed (AL1 group) differed significantly at 36 d (Fig. 1A) compared to 46 days (Fig. 1B) for several measurements ($P < 0.05$ for 4, 9, 11, 13, 15 h). However, at both ages, we observed two periods of low consumption (<8% of daily intake), in the middle of the dark period (1800–2200), and in the middle of the light period (0400–0600). From 0600 to 1400, the intake increased till 13% (20 g within 2 h) at 46 days, and 65–70% of the total daily feed intake happened during dark period (1200–0200). The feed delivery and peak of consumption of R1 (at 1100) were synchronized with the peak of feed consumption of the AL1 group. Within 2 h after delivery, the intake of R1 group peaked at 42% (31 g) and 33% (37 g) of the daily feed intake ($P < 0.001$) respectively at 36 and at 46 days. At 36 d, the R1 group consumed around 65% of its daily intake within 4 h (over 95% in 7 h) compared to 20% for AL1 group (36% in 7 h). Accordingly, R1 rabbits starved about 13 and 11 h respectively at 36 and 46 days, suggesting adaptation to the restriction programme with ageing.

The AL13 and R13 groups showed regular feed intake patterns, since they adapted rapidly to the 13 feed deliveries per day (Fig. 1A and B), without refusals between each delivery after 2 days.

Hard and soft faecal excretion pattern

Although feed was delivered in 13 meals, the faecal excretion pattern of AL13 and R13 groups was similar to that of the AL1 control group, with a period of high excretion during the dark period (Fig. 2A and B). Caecotrophy corresponds to very low hard faecal excretion, here occurring between 0400 and 0900, thus about 14–16 h after the intake peak (1400–1600). For the R1 group, caecotrophy occurred similarly about 12–14 h after the feed delivery, between 0200 and 0600 the day after. The R1 group hard faecal excretion pattern differed between both ages, since at 47 d old, we observed one main peak of faecal excretion (75% of the daily excretion between 1600 and 2100) about 5 h after the intake peak; while a larger faecal excretion period (1300–2300 h) was observed at 36 days.

The hard faecal/soft faecal ratio, the dry mass of soft faeces and the percentage of soft faeces/dry feed ingestion were not affected by the intake level neither by the FFD (Table 4).

Apparent total tract digestibility

Significant interactions were detected between intake level and FFD for the digestibility of all nutrients (Table 4). Indeed, digestibility between 41 and 47 days of age was sharply improved ($P < 0.05$) only for the R1 group and for all nutrients considered: proteins (+3.5% units), energy (+5% units) and fibrous fractions. Accordingly, the digestible energy content of the same diet was increased by about 8% (+5% for digestible protein, $P < 0.05$) when its intake was limited. For the 13 feed delivery groups, feed intake restriction (R13 vs AL 13) only slightly improved the digestibility. Globally, fragmented feed delivery did not change the nutrient digestibility, and even tended to decrease the fibre digestibility (particularly for

Table 3
Digestive health of weanling rabbit according to the intake level (AL, *ad libitum* or R, restricted) and to a fragmented feed distribution (1 or 13 meals per day) (Trial 1).

	Groups*				*P*-value[1]		
Item	AL1	AL13	R1	R13	IL	FFD	ILxFFD
First period (28–52 d old)[2]							
Morbidity, %	18.8	8.8	20.0	15.0	0.28	0.057	0.41
Mortality, %	5.0	6.3	2.5	5.0	0.39	0.39	0.67
Health risk index [3], %	23.8	15.0	22.5	20.0	0.62	0.20	0.46
Second period (52–70 d old)							
Morbidity, %	5.9	4.5	4.3	5.9	0.97	0.97	0.58
Mortality, %	0	0	2.9	0	0.59	0.59	0.59
Health risk index[3], %	5.9	4.5	7.1	5.9	0.64	0.64	0.94
Whole experiment (28–70 d old)							
Morbidity, %	22.5	12.5	21.3	17.5	0.58	0.107	0.43
Mortality, %	5.0	6.3	5.0	5.0	0.81	0.82	0.81
Health risk index[3], %	27.5	18.75	26.3	22.5	0.75	0.19	0.58

Abbreviations: IL = intake level; FFD = fragmented feed distribution.
* *n* = 16 cages per group, *n* = 80 rabbits per group.
[1] *P*-value for a bifactorial model, with effect of intake (AL vs. R) and FFD (1 vs. 13).
[2] Eighty rabbits per groups, at 28 d (weaning).
[3] Health risk index = mortality + morbidity (within the same period).

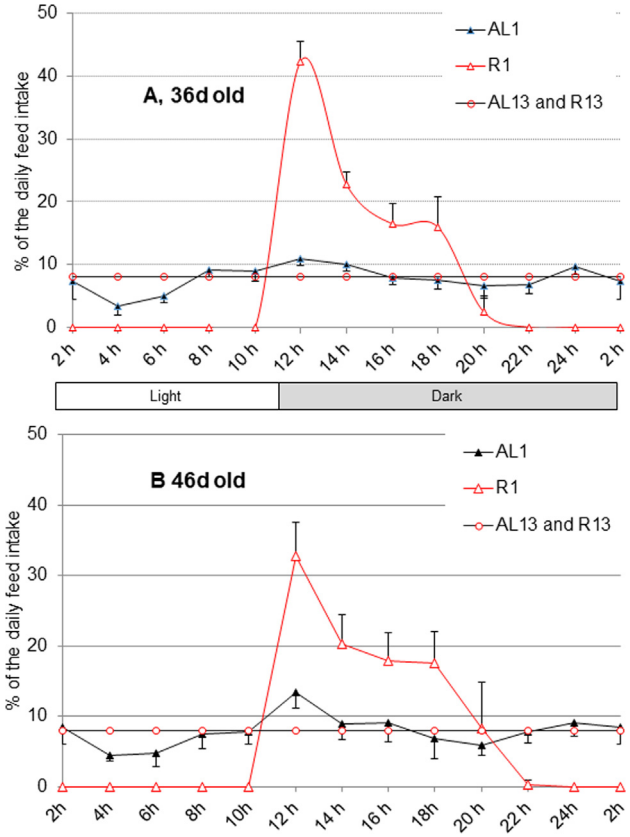

Fig. 1. Feed intake patterns of growing rabbits at 36 d (A) and 46 d old (B), according to the intake level (AL, *ad libitum* or R, restricted) and to a fragmented feed distribution (1 or 13 meals a day). (Trial 2).

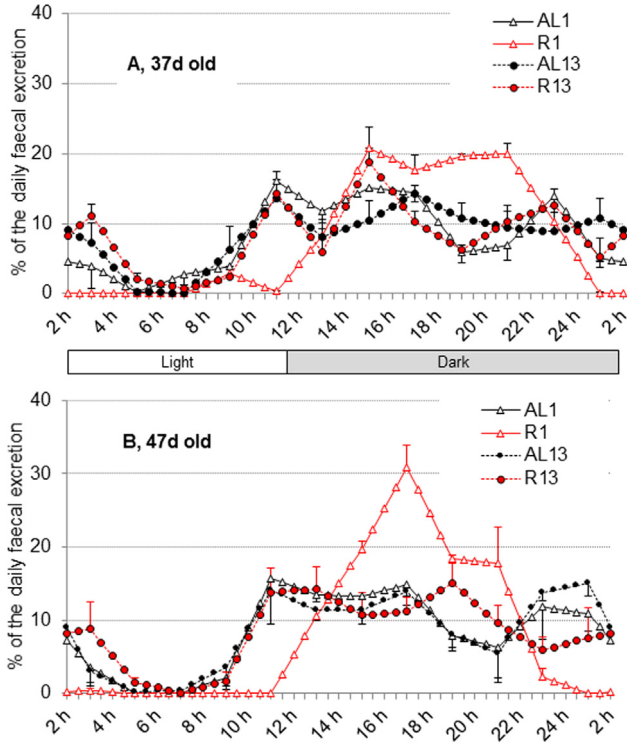

Fig. 2. Hard faecal excretion patterns of growing rabbits at 37 d (A) and 47 d (B) old, according to the intake level (AL, *ad libitum* or R, restricted) and to a fragmented feed distribution (1 or 13 meals a day). (Trial 2).

restricted groups). However, the 13 feed deliveries led to a higher DP/DE ratio (10.15 vs. 9.85, $P = 0.002$).

Gastric digesta parameters, and caecal microbial activity and diversity

Both fundus and antrum pH (Table 5) in the stomach contents decreased sharply for 13 meal delivery pattern. In addition, the intake restriction tended to increase the gastric pH ($P < 0.10$) in fundus and antrum, and also increased the caecal pH by 0.15 units ($P < 0.011$). Similarly, fragmented feed delivery also increased the caecal pH by 0.19 units ($P < 0.001$). The caecum empty weight (Table 5) was not modified by either of the two factors, while a significant interaction was observed for its fresh content which was increased in restricted group ($P < 0.05$).

A significant interaction was found for the global fermentative activity in the caecum (total VFA and ammonia concentrations). For instance, the VFA level was 25% lower in the R13 group compared to the three other groups (Table 6), and was associated with a highest caecal pH (Table 5).

Ammonia caecal concentration was similar among groups, except for rabbits restricted with one meal (R1 group) where it

Table 4
Faecal nutrient digestibility and caecotrophy production in the growing rabbit, according to feed intake level (AL, *ad libitum* or R, restricted, Intake) and to the fragmented feed distribution (1 or 13 meals per day) (Trial 3).

Item	Groups*					*P*-value[1]		
	AL1	AL13	R1	R13	SEM	IL	FFD	ILxFFD
Feed intake, g/d[2]	133.1	127.4	111.0	104.9	4.1[3]	ND[4]	ND[4]	ND[4]
Live weight[2], g	1 432[b]	1 439[b]	1 229[a]	1 314[a]	21	<0.001	0.083	0.12
Faecal digestibility, %								
Organic matter	60.9[a]	60.8[a]	66.2[b]	61.1[a]	0.5	<0.001	<0.001	<0.001
Energy	62.5[a]	62.7[a]	67.7[b]	63.2[a]	0.5	<0.001	0.002	<0.001
CP	77.0[a]	78.9[ab]	81.5[b]	79.2[ab]	0.5	<0.01	0.85	<0.01
NDF	34.2[a]	32.8[a]	43.1[b]	33.9[a]	1.0	<0.001	<0.001	<0.01
ADF	25.9[a]	23.6[a]	35.6[b]	25.2[a]	1.2	<0.001	<0.001	<0.01
Hemicelluloses[5]	44.7[a]	44.3[a]	52.6[b]	44.7[a]	1.0	0.022	0.022	0.032
Nutritive value of the diets								
DP, g/kg	101.7[a]	104.2[ab]	107.6[b]	104.5[ab]	0.6	<0.01	0.85	<0.01
DE, MJ/kg	10.21[a]	10.25[a]	11.06[b]	10.32[a]	0.08	<0.001	0.002	<0.001
DP:DE, g/MJ	9.96[ab]	10.16[b]	9.73[a]	10.13[b]	0.05	0.13	<0.01	0.25
Caecotrophy (48–49 and 52–53 d old)								
Caecotrophe excretion, g DM/day	16.6	23.1	18.2	17.2	1.0	0.37	0.19	0.070
Caecotrophe:intake, % DM basis	12.4	18.1	16.8	16.1	0.8	0.41	0.16	0.073
Hard faeces:soft faeces, DM basis	3.19	2.06	2.81	2.94	0.18	0.56	0.18	0.086

Abbreviations: IL = intake level; FFD = fragmented feed distribution.
* n = 12 rabbits per group.
[1] *P*-value for a bifactorial model, with effect of intake (AL vs. R) and FFD (1 vs. 13).
[2] Mean feed intake and live weight measured during the digestibility period (from 41 to 47 d).
[3] SEM calculated for AL1 and AL13 groups (only).
[4] ND: not determined, $\sigma^2 = 0$.
[5] Hemicelluloses calculated as "NDF-ADF".
[a,b,c] Within a row, means without a common superscript differ ($P < 0.05$, for a monofactorial model: effect of groups).

Table 5
Physico-chemical parameters of stomach and caecum in 50 day old rabbits according to feed intake level (AL, *ad libitum* or R, restricted) and to a fragmented feed distribution (1 or 13 meals per day) (Trial 3).

	Groups					*P*-value[1]		
Item	AL1	AL13	R1	R13	SEM[2]	IL	FFD	ILxFFD
Stomach pH								
Antrum	1.87[ab]	1.40[a]	2.39[b]	1.53[a]	0.11	0.09	<0.001	0.30
Fundus	2.15[ab]	1.43[a]	3.02[b]	1.61[a]	0.17	0.07	<0.001	0.24
Caecum								
Content mass, g	102[a]	98[a]	126[b]	102[a]	3	<0.01	<0.01	0.034
Content DM (%)	22.1[a]	21.8[a]	22.4[ab]	23.6[b]	0.2	<0.01	0.24	0.055
Organ mass, g	28.4	27.4	25.7	27.0	0.7	0.24	0.92	0.37
pH	5.84[a]	5.97[a]	5.92[a]	6.17[b]	0.03	0.011	0.001	0.26

Abbreviations: IL = intake level; FFD = fragmented feed distribution.
[1] *P*-value calculated with a bifactorial model, with effect of intake (AL vs. R) and effect of FFD (1 vs. 13).
[2] n = 12 rabbits per group.
[a,b,c] Within a row, means without a common superscript differ ($P < 0.05$, for a monofactorial model: effect of groups).

increased by 60% compared to the AL1 group. The fermentation pattern was globally not affected by fragmented feed delivery, whereas restricting intake strongly reduced the butyrate proportion by one-third. Accordingly, the butyrate/propionate ratio reduced by 30% in restricted groups, and increased the (acetate + propionate)/butyrate ratio. In parallel, the bacterial pectinolytic activity (Table 6) was impaired by approximately one-third in restricted groups. The diversity of the caecal bacterial community remained unaffected by the treatments (Table 6).

Discussion

Previous studies (Rantzer et al., 1996; Martignon et al., 2010) reported that a postweaning restriction managed in one meal per day improved the feed efficiency and the digestive health of the rabbit. We thus hypothesized that the feed intake pattern, and especially the time interval between two meals, rather than the feed quantity, could contribute to the positive effects of a feed restriction strategy on digestion and health of the growing rabbit. First, we more precisely described the feed intake pattern after weaning for rabbits fed freely or restricted, since feed intake and the hard faecal excretion patterns were described only for rabbits fed freely, where the lighting schedule is the main control factor (Horton et al., 1974). In agreement with Prud'hon et al. (1975), we confirmed that the feed intake pattern of the growing rabbit fed freely evolved progressively according to the lighting schedule, with a maximum intake at the beginning of the night, and minimum in the middle of the light period. Obviously, when intake is limited and given in one meal, the intake pattern is deeply changed, since 65% of the daily intake was reached within the 4 h after the meal. The hard faecal excretion was strongly correlated to the intake pattern in restricted animals with one meal, or for freely fed animals. When the feed distribution was evenly distributed along the day, faecal excretion maintained a classical circadian rhythm controlled by the lighting programme, as found previously

Table 6
Bacterial community activity and diversity in the caecum of 50 day old rabbits, according to feed intake level (AL, *ad libitum* or R, restricted) and to a fragmented feed distribution (1 or 13 meals a day) (Trial 3).

	Groups					*P*-value[1]		
Item	AL1	AL13	R1	R13	SEM[2]	IL	FFD	ILxFFD
Total VFA (mM)	85.9[b]	85.7[b]	92.4[b]	67.1[a]	2.0	0.046	<0.001	<0.001
VFA (mol/100 mol)								
Acetate (**Ac.**), %	80.9[a]	82.6[ab]	85.8[b]	83.9[ab]	0.6	<0.01	0.90	0.085
Propionate (**Prop.**), %	3.7	3.4	3.3	3.8	0.1	0.93	0.67	0.056
Butyrate (**But.**), %	15.0[c]	14.9[bc]	10.6[a]	11.2[ab]	0.5	<0.001	0.77	0.32
Butyrate/propionate	4.01[bc]	4.41[c]	3.29[ab]	3.06[a]	0.13	<0.001	0.71	0.17
(Ac. + Prop.)/But.	5.76[a]	6.30[ab]	8.63[ab]	9.01[b]	0.45	0.001	0.57	0.92
NH_3-N (mmol/L)	9.3[a]	7.6[a]	15.1[b]	10.1[a]	0.6	<0.001	<0.001	0.050
Bacterial Fibrolytic activity (μmol RS/g DM/h)								
Carboxymethylcellulase	12.1[a]	14.3[ab]	21.3[b]	11.6[a]	1.2	0.30	0.15	0.011
Xylanase	50.5[ab]	69.5[b]	56.9[ab]	40.7[a]	3.9	0.11	0.81	0.024
Pectinase	139.3[ab]	151.2[b]	100.9[a]	96.4[a]	6.8	<0.001	0.73	0.50
Bacterial community diversity[3]	5.5	5.5	5.5	5.3	0.1	0.77	0.67	0.61

Abbreviations: IL = intake level; FFD = fragmented feed distribution; VFAs = volatile fatty acids.
[1] *P*-value calculated with a bifactorial model, with effect of intake (AL vs. R) and FFD(1 vs. 13).
[2] *n* = 12 rabbits per treatment.
[3] Modified Simpson index.
[a,b,c] Within a row, means without a common superscript differ ($P < 0.05$, for a monofactorial model: effect of groups).

(Prud'hon et al., 1975; Bellier et al., 1995). Caecotrophy occurred mainly at the beginning of the light period, about 8 h after the period of high intake, as shown previously (Ruckebusch and Hörnicke, 1977; Bellier et al. (1995). However, we observed short period without faecal excretion during the night for rabbits fed freely, with a high inter-individual variability. This could be due to rabbits with a diphasic circadian caecotrophy rhythm as described by Jilge (1982). Restricted rabbits show a long diurnal period without faecal excretion that makes it difficult to identify the caecotrophy period and its importance. However, we measured during a 4 day period that caecotrophe production was not affected either by intake level or by a fragmented feed delivery.

The gap between intake and hard faecal excretion was bigger of few hours for restricted animals compared to control, suggesting that retention time could be longer. This hypothesis was supported by the results of Gidenne and Feugier (2009) indicating that retention time of particles was 50% longer for restricted rabbits, particularly in the mixing compartment such as the caeco-colic segment. This higher retention time would explain the improvement of the digestive efficiency showed here for restricted rabbits. In fact, most studies reported an improvement in the faecal digestibility, either during restriction or after restriction during re-feeding freely (Gidenne et al., 2012a). For a short delay of adaptation (one week or less) to the restriction, the improvement of digestibility was too little to be significant (Diaz Arca et al., 1999; Gidenne and Feugier, 2009).

We hypothesized a larger biodiversity (and activity) of the caecal microbiota that would explain the better resistance of the restricted rabbit to digestive trouble around weaning (Combes et al., 2013; Kieckhaven and Wolf, 2016). Since a quantitative restriction with one delivery a day improves the digestibility and increases the retention time in the hind gut, we expected a stimulated microbial activity in the caecum. For instance, Bellier et al. (1995) showed a higher fermentative activity in the caecum 6 h after a meal. In restricted growing pig, the faecal microbiota was affected by a feed restriction (Le Floc'h et al., 2014). However, the VFA concentration was not modified in restricted animals with a once a day meal, and the caecal bacteria diversity remained unaffected. In return, the microbial activity seemed qualitatively modified by restriction. Indeed, the butyrate proportion decreased, while the (acetate + propionate)/butyrate ratio increased clearly for restricted rabbits suggesting a stimulated fibrolytic activity to the expense of amylolytic activity. The higher carboxymethyl cellulase activity registered for restricted rabbits with one meal a day supports this hypothesis.

Most of the favourable effects of the restriction on feed efficiency are found during the period of free-feeding following a restriction, as previously reported (Gidenne et al., 2012a). A fragmented feed delivery improved the feed conversion in ad libitum fed rabbit, although rabbits fed "ad libitum" with a fragmented distribution were in fact slightly restricted (−5% compared to ad libitum). This improved feed efficiency was not supported by an improved digestibility of the nutrients. Even, the improvement of digestion detected for restricted rabbits was cancelled by the fragmentation of the feed delivery. The meal fragmentation modifies the digestive physiology, and for instance, the caecal pH was slightly higher, and in relation to a higher ammonia concentration. Also, the clear higher acidity of the stomach content was obtained in the fundus and the antrum of restricted and freely fed rabbits with 13 feed supplies per day, and suggests a modified gastric digestion. This could be explained by a regular and an overproduction of chlorydric acid by the gastric mucosa in comparison to the little quantity of food ingested within a meal, and may explain a better feed efficiency. This higher gastric acidity would correspond to a reinforcement of the barrier against pathogens (Gidenne et al. 2020b) that could be related to the little beneficial impact ($P = 0.11$) of fragmented meals on morbidity.

Previous large-scale studies demonstrated that an intake limitation after weaning reduced the mortality and morbidity rate (Gidenne et al., 2009, 2012b; Birolo et al., 2016; Knudsen et al., 2014, 2017) for a quantitative feed restriction of at least −20% applied during three weeks after weaning. In our study, sanitary conditions were good (mortality <6% over the whole fattening period) and we did not evidence a significant impact of our treatments on digestive health.

The compensatory growth observed after the restriction period once animals were fed freely has already been reported for the growing rabbit, but with various extent according to the length and/or strength of the restriction. Globally, the intensity of the compensatory growth is proportional to the level of restriction applied (Gidenne et al., 2012a; Knudsen et al., 2014, 2017). For instance, a strong restriction just after weaning (50 g/d from 35-42 d old) gave a 40% growth increase compared to a control group fed *ad libitum* (Tumova et al., 2016), while a more moderate restriction gave lower compensatory growth (Alabiso et al., 2017). However, we here found that a feed delivery in 13 meals gave a higher

feed efficiency, either for freely fed or restricted rabbits. This was not supported by a higher digestibility of higher microbial activity, but more related with a higher acidity of the content suggesting a more active gastric digestion.

In conclusion, we confirm a better feed conversion of the growing rabbit after a postweaning feed restriction; and that fragmented meals would contribute to this improvement. Similarly, the improvement in the nutrient digestion under restriction would be provided only by the intake level and not by the number of meals. The restriction or fragmented meals have no major impacts on the caecal microbial activity, diversity, and thus would not be implicated in the better resistance of restricted rabbit to digestive troubles. Further knowledge on the feeding behaviour and digestive physiology of the growing rabbit was provided. This will contribute to construct restricted intake strategies that optimize both welfare, digestive health and feed efficiency.

Ethics approval

The study was conducted in agreement with INRAE and ANSES regulation about use of animal for experimentation. Moreover, animals were handled according to the care of animals in experimentation, in agreement with European legislation (European Union, 2003). At the date of the study (April 2008), requirement for an ethics committee was not applicable.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available upon request.

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Sylvie Combes: Conceptualization, draft preparation, Methodology.
Thierry Gidenne: Supervision of the study, methodology, writing and article preparation.

Declaration of interest

None.

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