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# FEEDING BEHAVIOUR OF THE GROWING RABBIT FED FREELY OR RESTRICTED, AND IMPACT ON PERFORMANCE AND DIGESTIVE ORGANS

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Abstract: This study aimed to determine how rabbits' feeding and drinking behaviour was influenced by a feed restriction programme, and how performance and the morphometry of the digestive tract and lymphoid organs were influenced. At weaning (28 d old), 432 rabbits were housed in cages of 6, and allotted to 2 groups according to feed intake level: *ad libitum* feeding (AL group) from 28 to 72 d old, and feed intake (R group) restricted to 70% of AL intake from 28 till 49 d old, followed by *ad libitum* feeding from 50 till 72 d old. During the restriction, the R group intake was 36% lower than that of the AL group. When returning to an *ad libitum* feeding, the R group intake increased by 270%, thus exceeding the AL intake by 26% ($P$=0.03). The daily weight gain was reduced by 28% for R group during the restriction (40.0 *vs.* 55.7 g/d; $P$<0.001), whereas the feed conversion was improved (–11%, 1.86 *vs.* 2.09; $P$<0.001). The restriction led to a shorter intestine (–15%, 202 *vs.* 233; $P$<0.05) and lighter spleen (–15%, 4.8 *vs.* 5.9; $P$<0.05), whereas the number of Peyer patches was not influenced. Most of the growth delays of lymphoid tissues observed at the end of the restriction period in the R rabbits remained until the end of the experiment. The feeding activity of AL rabbits mainly occurred during the dark period (19:00-09:00), with 16% of rabbits eating. The R group strongly and massively started their feeding activity at feed distribution time (8:30-09:00), with 65% of rabbit eating at the start, then 35% still eating half an hour later. Feeding activity of R group remained high for 8 h after the feed distribution, with 28% of rabbits having a feeding activity between 9:30 and 17:00. R group had a higher number of meals (+30%) and drinks (+28%), and a longer meal duration (+30%) compared to AL group. R group consumed 63% of the intake within 6-7 h compared to *ad libitum* fed rabbits, which spread their intake over 15 h. No changes in social behaviour (access to feed or drinking, resting, aggressiveness) were detected, suggesting that this restriction programme did not impair welfare compared to that of *ad libitum* fed animals.

Key Words: rabbits, feed intake limitation, feeding behaviour, lymphoid organs, digestive tract.

## INTRODUCTION

Post-weaning feed restriction has been used in French rabbit breeding systems for 15 yr as an efficient method to reduce the incidence of digestive disorders in the growing rabbit (for review, Gidenne *et al.*, 2012) and to improve feed efficiency, and finally the economical results of the farms (Knudsen *et al.*, 2017; Gidenne, 2019). Recently, several authors studied various feed restriction programmes (Alabiso *et al.*, 2017; Birolo *et al.*, 2017, 2020) to optimise

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performance or carcass quality. However, till now, no clear mechanism has been identified to explain the favourable effects of a quantitative feed restriction on the digestive efficiency and health of the growing rabbit.

The feeding and drinking behaviour of the growing rabbit was studied in the past with adult or laboratory rabbits (Prud'hon *et al.*, 1972; Horton *et al.*, 1974), but no recent studies have addressed the feeding behaviour of growing rabbits under conventional collective housing, similar to commercial farms. As feed restriction programmes are commonly used, studies on their impact on the feeding and drinking behaviour of restricted-fed rabbits and how they re-adapt to *ad libitum* feeding continue to be of interest. Moreover, as feed restriction modifies the feeding rhythm, this may impair the welfare of farmed rabbits, as recently suggested by EFSA (EFSA report, 2020).

Therefore, we studied the effects of a 3-week feed restriction after weaning, followed by three weeks of free feeding, on the feeding and drinking behaviour in comparison to rabbits always fed *ad libitum*. We also described the morphometry of the digestive tract and lymphoid organs, assuming that it may be associated with favourable effects on digestive efficiency and health.

## MATERIAL AND METHODS

Animals were handled according to the guidelines for animals used in experiments, according to EU 2010/63/EU and in agreement with French legislation (NOR:AGRG1238753A 2013). The local ethic committee (ANSES, Ploufragan) also approved the protocol.

### *Experimental design, animals and housing*

This study involved 432 hybrid rabbits (Hycole®) produced at the ANSES experimental farm (Ploufragan, France). The trial started at weaning (28 d old) and finished at 72 d old. Only rabbits weighing more than 510 g at weaning were included in the trial. At weaning, rabbits from the same litter were randomly distributed in cages and the cages were then allocated to two treatments, with the same weight at weaning and without considering rabbit gender. The two groups corresponded to two feed intake levels: a control group "AL" fed *ad libitum* and a restricted-fed "R" group, for which the intake was limited to 70% of the AL intake from 28 to 49 d old. The R group was fed daily with one meal between 08:30 and 09:00, with a quantity of feed adjusted once a week according to the mean feed intake of the AL group, using an intake prediction curve. Simultaneously, rabbits of AL groups were visited daily, to have the same level of contact with human as the R group. From 50 d old to the end of the experiment, the rabbits of the two groups (AL & R) were fed *ad libitum*. Free access to drinking water (nipple drinker) was provided throughout the trial.

Rabbits were housed in two rooms (same unit), each containing 2 rows of 18 collective cages (77×50 cm) with 6 animals (216 rabbits per room), and the density was kept at 15.6 rabbits per $m^2$. Each group of rabbits was equally assigned to one of the two rooms of the same unit. Each row housed one group (AL or R). The breeding unit was kept at a temperature of 20±2°C and under a 09:00 to 19:00 lighting schedule alternated with 14 h of darkness.

The experimental diet (Table 1) was formulated (and pelleted) to cover the nutritional requirements of the growing rabbit fed *ad libitum* (Gidenne *et al.*, 2015). No antibiotic was given either in feed or through drinking water throughout the trial.

### *Feed intake and growth measurements*

Feed intake of the AL group was measured once a week, whereas feed intake of restricted rabbits (R) was checked daily during the first period of the trial, then once a week during the second period (49-72 d old) when fed *ad libitum*.

Rabbits were individually weighed at weaning (28 d old) then at 49, 56 and 72 d old. Daily growth, feed intake and feed conversion ratio were determined for the first period (28-49 d old), for the first week of the second period (49-56 d old) to look at adaptation to free intake for R group, and for the remaining part of the second period (56-72 d old).

### *Lesions and health status*

All rabbits were individually examined at weaning (28 d old), at 49 and 72 d old, for severity of body scratches, lesions and injuries. The lesions score would enable us to determine whether a restriction of the feed intake induces more

aggressiveness than an *ad libitum* feeding. Morbidity and mortality were also checked daily.

### *Behavioural traits: feeding and other activities*

The first week (28-35 d old) was not considered for the behavioural study, as it was an adaptation period to maternal separation and new cages and new pelleted feed. Then, four nychthemera of video were recorded at 35, 37, 42 and 44 d old. Then, only the first week of the second period (free feeding) was studied for behavioural traits, at 50 and 52 d old, to study the adaptation to free access to food of previously restricted rabbits. Each camera filmed 1 or 2 cages. At each nychthemeron, 6 cages per group, equally shared in both rooms, were filmed for 24 h. Cameras were moved along rows of cages, filming 24 cages per group for the first period. The videos of the 50$^{th}$ and 52$^{nd}$ d of life recorded the same cages and rabbits (for focal sampling) as those recorded at 35 and 44 d old, respectively, for comparison of the rabbits' behaviour for restriction and *ad libitum* periods.

Instantaneous scans were carried out using all the video recordings to study the rabbits' feeding and resting activities over 24 h. Feeding activities were determined by instantaneous scans of 30 s at 30 min intervals, i.e. 48 video-scans per nychthemeron. At each scan, the number of rabbits eating (head in the feeder or in front of it, or chewing food) and resting (lying on belly or flank) were evaluated in the 6 cages filmed per group (AL and R). Results are given averaging the number obtained for the 24 cages considered per group for the restriction period, and for the 6 cages observed at 50 d old then 52 d old for R group, and for the 12 cages considered for the second period for AL group.

One rabbit per cage was identified using a coloured pencil. It was followed over 24 h by focal sampling. In addition to frequency of meals and drinking behaviours, average duration of the meals and total meal duration were registered. This was performed at 35, 42, 50 and 52 d old, i.e. 2 d per period. Our first observations by scan sampling showed high variations of eating activity in the course of a nychthemeron, so we decided to split the 24 h of focal observation into 2 phases: 1$^{st}$ phase from 08:30 to 21:30 (restricted rabbits' feed consumption phase) and 2$^{nd}$ phase from 21:30 to 08:30 (phase of no feed intake for restricted rabbits).

Table 1: Ingredients and chemical composition of the experimental diet.

| Ingredients (g/kg as fed) | |
|---|---|
| Wheat bran | 216.0 |
| Sunflower meal | 177.0 |
| Dehydrated alfalfa | 177.0 |
| Dehydrated sugar beet pulp | 137.0 |
| Barley | 61.0 |
| Wheat middling | 65.0 |
| Apple pomace | 28.0 |
| Sugar cane molasses | 21.6 |
| Citrus pulp | 20.0 |
| Kaolin | 20.0 |
| Oat | 20.0 |
| Rapeseed 00 | 20.0 |
| Grape pulp | 12.0 |
| Soybean oil | 12.0 |
| Premix[1] | 10.0 |
| Lysine (0.5, liquide) | 2.2 |
| Methionine (0.9, liquide) | 0.4 |
| Choline chloride (0.75, liquide) | 0.8 |
| Analysed composition (g/kg as fed) | |
| Dry matter | 905 |
| Ash | 91 |
| Crude protein | 179 |
| Neutral detergent fibre | 367 |
| Acid detergent fibre | 210 |
| Acid detergent lignine | 54 |
| Calculated composition[2] (g/kg as fed) | |
| Starch | 74 |
| Crude fibre | 169 |
| Lysine | 6.7 |
| Methionine | 2.8 |
| Cysteine | 2.4 |

[1] Vitamins: A: 1 500 000 UI/kg; $D_3$: 200 000 UI/kg; E: 3000 mg/kg; $B_1$: 200 mg/kg; $K_3$: 50 mg/kg and oligo elements: $Cu^{2+}$: 800 mg/kg; $Fe^{2+}$: 8000 mg/kg; $Zn^{2+}$: 20 000 mg/kg; $Mn^{2+}$: 4000 mg/kg; coccidiostat: robenidine.

[2] Calculated values according to Feedipedia (http://www.feedipedia.org/).

### *Digestive tract and lymphoid tissues morphometry*

Eight rabbits per group were sacrificed by cervical dislocation (in agreement with AVMA, 2001) at the end of the restriction period (49 d old) and at the end of the trial (72 d old). Rabbits were selected from the cages not videotaped.

Digestive tract growth was compared between the R and AL rabbits by measuring the lengths of small intestine (including duodenal ampoule, without *sacculus rotundus*), caecum (including *sacculus rotundus* and caecal appendix) and colon (with rectum). Some lymphoid tissues growth were also assessed, considering spleen mass and length, caecal appendix mass and length, *sacculus rotundus* mass, number of Peyer's patches and unit mass. These organs have various implications in the immune system (Drouet-Viard and Fortun-Lamothe, 2002). Caecal appendix is a primary lymphoid organ allowing production and maturation of B lymphocytes of young rabbits. Moreover, it is a secondary lymphoid organ inducing specific immune response, just as Peyer's patches and *sacculus rotundus*. Spleen is a lymphoid organ implicated in cell immunity.

### *Statistical analyses*

All statistical analyses were performed using the R software. The data concerning feed intake, growth and feed conversion ratio were analysed using a bifactorial model (ANOVA) including the effects of the breeding room and of the group (AL or F) and considering their interaction. Since the effect of the breeding room was never significant, we did not present this factor in the tables. Organ growth was analysed by ANOVA, using a model including two effects (feed intake level and animal age and considering their interaction). In addition, because of the feed restriction strategy, the variances of some criteria (live weight, intake or feed conversion) were logically lower in the restricted group compared to the *ad libitum* one (Bartlett test). In these cases, a mixed model (FLM procedure) using the "repeated" option (for the variable "cage within group) to consider the differences in variance between R and AL groups was applied.

For behavioural data from scan sampling, data obtained for the 6 rabbits of the 6 cages per group observed per day (36 rabbits/group day) were averaged for the 4 d of restriction period that were videotaped. Regarding the second period (*ad libitum*), to determine whether animals kept the activity rhythms they had shown during the first period (restriction) or if they spontaneously adopted the feed intake and resting habits of *ad libitum* rabbits, the data collected from the AL group during two days of the second period were averaged, whereas both days recorded during the second period were considered separately for R rabbits. Data obtained by focal sampling for the 12 rabbits observed per period and per group were averaged. These data were analysed by ANOVA using a model with two effects (feed intake level and phase of the day) and considering their interaction. Moreover, data were presented according to both periods.

## RESULTS

### *Health status and lesions*

No morbidity or mortality were observed over the five weeks of the trial. Whatever the group considered, no severe lesions (only a few scratched ears) were found at the end of the first period, as only one rabbit from R group showed small lesions at one ear. At the end of the second period (72 d) only 8 rabbits (on 216) of the R group had slight lesions on the ears. Three observations of aggressiveness were noticed only during the last week of fattening, for 3 cages of the R group. Moreover, no aggressive behaviour was observed during the scan or focal observations.

### *Feed intake and growth*

Over the first period (from 28 to 49 d old) the feed intake of the R group was 36% lower than the control AL group, thus slightly over the expected restriction rate of –30% (Table 2). Over the first week after the restriction (49-56 d old), the R group intake strongly increased by 270% to average 206 g/d, exceeding the intake of AL group by 26% ($P$=0.03). This over-intake of the R group persisted for the following weeks, with a 9% higher intake between 56 and 72 d old for R group compared to AL ($P$<0.001). Finally, over the whole fattening period, the feed intake of the R group was only 6% lower than for AL ($P$<0.001). Accordingly, the weight gain was reduced, whereafter we observed a compensatory growth for R group compared to AL, with a 24% higher weight gain between 49 and 72 d old ($P$<0.001). The feed conversion of restricted rabbits was improved (-11%; $P$<0.001) during the restriction period, and this favourable effect was reinforced between 49 and 56 d old (-16%; $P$<0.001), when the compensatory growth was the highest. At the end of the period, feed conversion of both groups was similar.

Table 2: Intake and growth of rabbits fed *ad libitum* (AL) or restricted (R).

| | AL | R | SEM | *P*-value[3] |
|---|---|---|---|---|
| Feed intake (g/d rabbit)[1] | | | | |
| Period 28 to 49 d old | 116.3 | 74.1 | 2.5[4] | ND |
| Period 49 to 56 d old | 162.5 | 205.9 | 2.8 | <0.001 |
| Period 56 to 72 d old | 151.1 | 165.1 | 1.3 | <0.001 |
| Period 49 to 72 d old | 154.6 | 177.5 | 1.6 | <0.001 |
| Period 28 to 72 d old | 136.3 | 128.2 | 0.7 | <0.001 |
| Live weight (g)[2] | | | | |
| 28 d old (weaning) | 593 | 593 | 2 | NS |
| 49 d old | 1763 | 1433 | 10 | <0.001 |
| 56 d old | 2095 | 1934 | 7 | <0.001 |
| 72 d old | 2686 | 2571 | 9 | <0.001 |
| Daily weight gain (g/d rabbit)[2] | | | | |
| Period 28 to 49 d old | 55.7 | 40.0 | 0.5 | <0.001 |
| Period 49 to 56 d old | 47.1 | 71.6 | 0.8 | <0.001 |
| Period 56 to 72 d old | 36.9 | 39.8 | 0.3 | <0.001 |
| Period 49 to 72 d old | 40.0 | 49.5 | 0.37 | <0.001 |
| Period 28 to 72 d old | 47.5 | 45.0 | 0.19 | <0.001 |
| Feed conversion ratio[1] | | | | |
| Period 28 to 49 d old | 2.09 | 1.86 | 0.02 | <0.001 |
| Period 49 to 56 d old | 3.45 | 2.92 | 0.05 | <0.001 |
| Period 56 to 72 d old | 4.12 | 4.17 | 0.04 | NS |
| Period 49 to 72 d old | 3.87 | 3.54 | 0.04 | <0.001 |
| Period 28 to 72 d old | 2.87 | 2.85 | 0.01 | NS |

R: Intake restricted at 70% of the AL group; SEM: standard error of the mean; NS: no significant, $P<0.10$.

[1]Measured on 2×36 cages of 6 rabbits.

[2]Measured on 2×216 rabbits, and then on 2×208 rabbits (8 rabbits per group sacrificed).

[3]*P* level of the group effect, but resulting from bifactorial variance analysis, including the effect of the breeding room and the interaction, those two latter effects being non-significant for all parameters.

[4] Standard deviation value for the group AL only; ND: Not Determined, $\sigma^2 = 0$ for R group.

### Feeding and drinking behaviour

During the two weeks after weaning, most of the feeding activity of rabbits fed freely (AL group, Figure 1A) occurred during the dark period (19:00–09:00), as the proportion of rabbits eating averaged 16% during dark compared to light periods (11%). Moreover, two peaks of feeding activity were detected, first around the light switching off (19:00) with 20% of rabbit eating, and the second before the light switched on (05:00-08:30) with 17% of rabbits eating. In contrast, the lowest feeding activity occurred just after the light switched on (09:00-14:00), with only 5% of rabbits presenting feeding activity (Figure 1A). Correspondingly, for AL group, the number of meals and drinks did not differ according to the phase time (A *vs.* B, Table 3), and neither did the meal duration nor the cumulative time for feeding.

In return, restricted-fed rabbits (group R) strongly began their feeding activity at feed distribution time (8:30-09:00) with 65% of rabbit eating, with 35% still eating half an hour later. Their feeding activity remained high for 8 h after the feed distribution, with 28% of rabbits showing feeding activity between 9:30 and 17:00 (Figure 1A). This led to a higher number of meals (+30%) and drinks (+28%), and a longer meal duration during this phase (+ 2 min = +30%, Table 3) compared to the AL group. Then, from 21:00 to 8:30, we recorded a low feeding activity consisting of only very short visits to the feeder without effective intake, as feeders were emptied from 15:30 and the meal duration was null (Table 3). In parallel, the number of drinks was very low (n=5) for R rabbits compared to the AL group (n=22). Over a nychthemeron, rabbits of R groups spent 150 min on feeding, compared to 197 min for AL groups ($P<0.001$). From our feeding patterns (Figure 1), we also observed that the timing of feed distribution to R group did not interfere (e.g. intake stimulation) with feeding behaviour of the AL group.

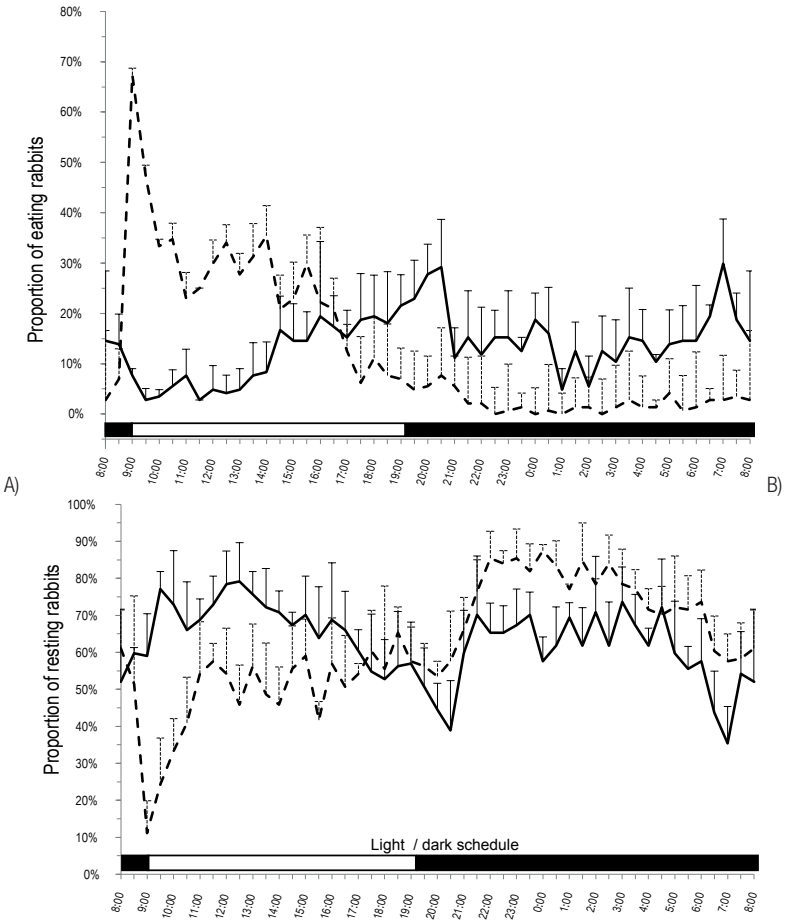


Figure 1: Proportion* of rabbits eating (A) and resting (B) over 24 h, during the restriction period (28-49 d old) for restricted (R) and *ad libitum* (AL) fed rabbits. *mean proportions for 6 rabbits per cage, and 12 cages per group. Feed distribution time for R group (8:30-9:00), ——: AL, – – –: R.

The 47 drinks/d rabbit of freely fed rabbits did not differ from the 38 drinks of the restricted group during the restriction period (Table 3, $P=0.107$). However, the drinks time allocation was different, with only 5 drinks during the dark period for R group compared to 22 for the AL group.

Once R rabbits were fed freely (49-72 d), they showed a strong feeding activity during the light period, with a 60% higher number of drinks and meals ($P<0.01$), and a 30% higher meal duration and feeding time ($P<0.01$). Moreover, during the first week of the second period, we observed that the R rabbits still expressed the main part of their feeding activity during the 1st phase of the nychthemeron (36 *vs.* 12 meals, $P<0.001$), as they did throughout the first period (restriction), whereas AL rabbits presented an activity equally shared between both phases (Table 3). Scan sampling data (Figure 2) showed that during the first nychthemeron of free feeding (50 d old) 90% of R rabbits were found eating around 09:00 h, which was the feed supply time during the first period. Two days later, the circadian feeding pattern of R group (Figure 2A, Rb) became similar to that of the AL group. On average for 50 and 52 d old rabbits (Table 3, period 2), the number of daily meals was higher for R group compared to AL (48 *vs.* 38), still with a different time allocation for R group at light period: twice longer meal duration for R group and 36 *vs.* 20 meals for R and AL group, respectively).

Table 3: Characteristics of drinks and meals according to feed intake level (AL, *ad libitum vs.* R, restricted) and to day phase (08:30-21:30 and 21:30-08:30) during a restriction programme (first period) or after returning to *ad libitum* feeding (second period).

| | Phase A: 08:30-21:30 | | Phase B: 21:30-08:30 | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Intake level: | AL | R | AL | R | SEM | Intake | Phase | Interaction |
| First period (Restriction program, average of 35 and 42 d old) | | | | | | | | |
| Number of drinks[1] | 25 [bc] | 33 [c] | 22 [b] | 5 [a] | 1.9 | NS | <0.001 | <0.001 |
| Number of meals[2] | 21 [b] | 27 [b] | 20 [b] | 0 [a] | 1.8 | 0.003 | <0.001 | <0.001 |
| Meal duration (min) | 04.57 [b] | 06.47 [b] | 04.56 [b] | 00.00 [a] | 00.27 | 0.003 | <0.001 | <0.001 |
| Cumulative feeding time (min) | 102.14 [b] | 150.09 [c] | 95.04 [b] | 00.00 [a] | 08.14 | <0.001 | <0.001 | <0.001 |
| Second period (*ad libitum* feeding, average of 50 and 52 d old) | | | | | | | | |
| Number of drinks[1] | 20 [b] | 32 [c] | 16 [b] | 8 [a] | 1.5 | NS | <0.001 | <0.001 |
| Number of meals[2] | 20 [a] | 36 [b] | 18 [a] | 12 [a] | 1.7 | 0.038 | <0.001 | <0.001 |
| Meal duration (min) | 03.53 [a] | 06.06 [b] | 04.26 [a] | 04.50 [ab] | 00.14 | 0.002 | NS | 0.028 |
| Cumulative feeding time (min) | 75.35 [a] | 202.20 [b] | 76.46 [a] | 54.51 [a] | 09.26 | <0.001 | <0.001 | <0.001 |

[1]With 12 replicates per period and per group; SEM: standard error of the mean.
[2]Number of meals or drinks per rabbit and per day.
[abc]Within a row, means not sharing superscript differ ($P<0.05$, for a monofactorial model holding both factors: intake level and phase).
NS: no significant, $P<0.10$.

### *Resting and other behaviours*

As expected, the resting activity patterns evolved inversely (with half an hour delay) to the feeding pattern, whatever the group considered (Figure 1B and 2B). For instance, a high feeding activity occurred at the onset of darkness (Figure 1A and 2A) and concurrently with a drop in the resting activity (Figure 1B and 2B), likewise for R rabbits with the very high feeding activity around 09:00 (Figure 1A) corresponding to a strong drop in resting activity (Figure 1B). Similarly, we observed that around 80% of R rabbits were resting throughout the darkness time, either during the first period (Figure 1B) or at 50 d old (Figure 2B). Over the day, the time dedicated to resting reached 2/3 of the whole day. In parallel with the feeding activities, we noted that for rabbits fed *ad libitum*, the resting periods were spread over the whole day, with a decrease at the end of the day. In R group, the resting behaviour was more "concentrated" on the "non-food" period. More rabbits were reported to be active in R group, especially for 6 h after emptying of the feeder.

We did not observe adverse behaviour or aggressiveness due to feed restriction, such as competitive access to the feeder. We also did not see a significantly higher number of lesions in the restricted group. No abnormal behaviours were noticed either in R or AL groups.

### *Digestive tract and lymphoid tissues growth*

Restricted feed intake for three weeks after weaning did not affect the hindgut morphometry (Table 4, caecum or proximal colon), while a 15% growth was observed at 72 d compared to 49 d old, as expected. In turn, the small intestine length of the R rabbits was smaller (–15%) at 49 d old ($P=0.003$), but this deviation disappeared three weeks later, causing a significant interaction between intake and age (Table 4).

All lymphoid tissues were affected by the feed intake level (R *vs.* AL, for the first period), except for the *Sacculus rotundus*' mass, which was only age-dependent ($P<0.001$, Table 4). The restriction programme led to a shorter (–15%) and lighter (–15%) spleen ($P=0.003$ and 0.045, respectively). Unit mass of Peyer patches decreased by almost 1/6 with feed restriction ($P=0.006$), whereas their number was not significantly influenced. Most of the growth delays of lymphoid tissues observed at the end of the first period in the R rabbits remained until the end of the experiment. In contrast, the caecal appendix mass of the AL rabbits showed a decrease of around 1/3 between 49 and 72 d old ($P<0.001$), which was not found in the R group.

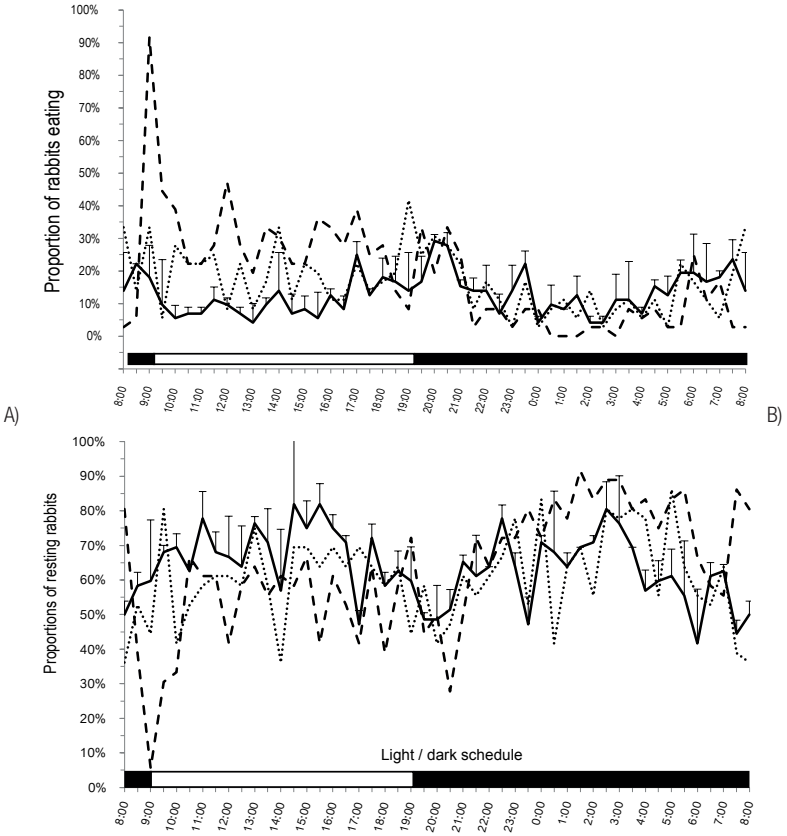


Figure 2: Proportion* of rabbits eating (A) and resting (B) over 24 h, once restricted rabbits were fed freely (Ra, 50 d) and 2 d after (Rb, 52 d old), and for *ad libitum* rabbits (AL, 50 and 52 d old). * mean proportions for 6 rabbits per cage, and 6 cages per group. Feed distribution time for R group (8:30-9:00), ——: AL, – – –: R, ·····: Rb.

## DISCUSSION

### *Feeding behaviour of the growing rabbit*

No study has described the circadian pattern of rabbit feeding behaviour, in conventional housing system for fattening (i.e. collective cages) and for modern commercial hybrid rabbit lines. In the past, some studies have described the pattern of drinking and feed intake for some individually housed adult or growing laboratory rabbits (Prud'hon *et al.*, 1972, 1975; Horton *et al.*, 1974; Jolivet *et al.*, 1983), or more recently for 6 growing rabbits housed individually in metabolism cages (Bellier *et al.*, 1995).

Our study thus provided original detailed description of the feeding and drinking behaviour of growing hybrid rabbits housed similarly to those in commercial farms and fed freely. In agreement with the first descriptions (Prud'hon *et al.*, 1975; Bellier *et al.*, 1995), the lighting schedule is the key driver of the feed intake, with an increasing feeding activity during the 3 to 4 h before light switch off and also at the end of the dark period interval one or two hours before light switch on. Restricted rabbits consumed within 6-7 h 63% of the intake of freely fed rabbits, which was spread over 15 h from 28 to 49 d old. Then, the low feeding activity in the "morning" corresponded to a resting and caecotrophy period. This is coherent with the behaviour of the wild rabbit having a "crepuscular" activity for foraging and eating (Gidenne *et al.*, 2020).

Table 4: Digestive tract and lymphoid tissues morphometry to the feed intake[1] (AL, *ad libitum vs.* R, restricted) and to age (49 or 72 d old).

| | 49 d old (n=8) | | 72 d old (n=8) | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Group: | AL | R | AL | R | SEM | Intake | Age | Interaction |
| Digestive tract length | | | | | | | | |
| Small intestine (cm) | 233 [b] | 202 [a] | 293 [c] | 294 [c] | 7 | 0.021 | <0.001 | 0.017 |
| Caecum (cm) | 44 [ab] | 40 [a] | 49 [b] | 49 [b] | 1 | NS | 0.001 | NS |
| Colon (cm) | 94 [a] | 91 [a] | 116 [b] | 117 [b] | 3 | NS | <0.001 | NS |
| Lymphoid tissues | | | | | | | | |
| Spleen | | | | | | | | |
| Length (cm) | 5.9 [ab] | 4.8 [a] | 6.4 [b] | 5.5 [ab] | 0.2 | 0.002 | 0.062 | NS |
| Mass (g) | 1.3 [a] | 0.9 [a] | 2.3 [b] | 1.4 [ab] | 0.1 | 0.007 | 0.003 | NS |
| Caecal appendix | | | | | | | | |
| Length (cm) | 9.5 [a] | 8.6 [a] | 12.1 [b] | 11.5 [b] | 0.3 | 0.08 | <0.001 | NS |
| Mass (g) | 5.7 [b] | 3.9 [a] | 3.9 [a] | 4.0 [a] | 0.2 | 0.013 | 0.015 | 0.006 |
| *Sacculus rotundus* | | | | | | | | |
| Mass (g) | 1.7 [a] | 1.4 [a] | 9.7 [b] | 9.2 [b] | 0.7 | NS | <0.001 | NS |
| Peyer patch | | | | | | | | |
| Number | 5.9 [ab] | 4.9 [a] | 6.0 [ab] | 7.5 [b] | 0.3 | NS | 0.004 | 0.009 |
| Unit mass (g) | 0.17 [a] | 0.14 [a] | 0.26 [b] | 0.23 [b] | 0.01 | 0.006 | <0.001 | NS |

[1]Intake was restricted by 30% for R group between 28 and 49 d old.
[abc]Within a row, means without a common superscript differ ($P<0.05$, for a monofactorial model with 4 levels (intake×age)).
SEM: standard error of the mean (8 replicates).
NS: no significant, $P<0.10$.

Besides, we observed relatively large variability (20 to 50%) in the feeding behaviour circadian pattern, indicating that each individual probably had its own circadian clock. In fact, the domestic rabbit no longer has prolonged periods without eating, as it has over 20 meals of dry feed a day (Gidenne *et al.*, 2020) and consumes caecotrophes (early in the light period). Past studies using commercial hybrid rabbit lines observed a high frequency of meals and drinks averaging 40 per day, with a time budget of almost 3 h for eating activity (Prud'hon *et al.*, 1972; Horton *et al.*, 1974). Only 13 to 19 drinks and 23 to 33 meals per day, with a time budget of 2 h for eating, were observed in old studies with laboratory or domestic rabbits. As reported by Boisot *et al.* (2003), a quantitative feed restriction increased the water intake. We here found that it also strongly increased the frequency of access to drinkers. However, we were not able to measure the water intake, as large quantities of water were wasted (about 2 to 3 times the expected water consumption) with nipple drinkers.

Contrarily to rabbits fed *ad libitum*, whose feed intake pattern depended on lighting schedule, the feeding behaviour of restricted animals depended strictly on the time of the meal. Within 8 h, restricted rabbits emptied their feeder, as observed previously for a similar restriction level (Gidenne *et al.*, 2009; Gidenne and Feugier, 2009). A feed distribution in the evening would certainly have led to a synchronisation of the restricted animals with freely fed ones, and thus closer to the natural cycle. However, within two days after returning to a free feeding regime, previously restricted rabbits have a feeding behaviour similar to those constantly fed freely.

### *Resting and other behaviours*

The resting activity decreased simultaneously to the increase in eating activity, corresponding to a "classical" transfer of activity. For freely fed growing rabbit, the time budget for resting (66% of the day) was close to the range of 57-60% reported previously (Morisse *et al.*, 1999; Postollec *et al.*, 2006; Jordan *et al.*, 2008; Princz *et al.*, 2008). This was regularly spread over the whole day, whereas for restricted rabbits the resting phase was more "concentrated" after the acute feeding period just after the food was supplied. Moreover, caecotrophy, which corresponds to resting and low feeding activity, occured around 8 h after the main period of feed intake, either for restricted or freely fed

animals and as described previoulsy (Hörnicke *et al.*, 1984). This suggests that our restriction programme did not greatly perturb the digestive processes of the rabbit. Besides, we observed no abnormal behaviours (such repetitive movements, aggressiveness, etc.) either in restricted or freely fed rabbits. Thus, we assumed that a quantitative feed restriction did not fundamentally modify major behaviours (feeding, drinking, resting), but rather separated them over time. However, under the favourable environmental conditions of our experimental farm, it remains difficult to affirm that the modification of the feeding pattern of restricted rabbits could have a negative effect on the welfare compared to that of freely fed animals.

### *Morphometry of the digestive tract and of lymphoid organs*

Between 7 and 10 wk of age, intestinal length grew by 40% in freely fed rabbits to reach 3 metres, as previously observed by Lebas and Laplace (1972). For the same period, caecum and proximal colon length increased by 10 and 20%, respectively. Our restriction programme mainly affected the development of the small intestine (–10% at 7 wk old), but no further impact was detectable at the end of the fattening and whatever the digestive segment. Thus, the improved feed conversion and digestion found in previous studies (Gidenne *et al.*, 2012) would not be associated with changes in digestive morphometry.

Similarly, the caecal appendix development was similar to that reported in previous studies (Lebas and Laplace, 1972), although we noticed a 12% decrease in the mass of this organ at 10 wk compared to 7 wk of age for freely fed animals, while the restriction reduced the caecal appendix length and mass without a compensatory growth at 10 wk after returning to a free feeding. Spleen was also impaired by feed restriction. Therefore, lymphoid organs development seemed not to be implicated in the favourable effect of a feed restriction on health status of the young rabbit (Gidenne *et al.*, 2012).

Besides, a feed intake limitation influenced the circadian timing system that interacted between the circadian molecular clock and a variety of metabolic networks. Thus, further studies would be pertinent to describe the metabolic changes associated with an intake limitation that could explain the favourable effects on health cited in the literature.

### *Feed restriction and fattening performance*

Since a 36% feed restriction for 3 wk after weaning led to a 28% lower growth rate, we assumed that the impact of the restriction on growth was not strictly proportional to the intake level, in agreement with the meta-analysis of Gidenne *et al.* (2012). Returning to *ad libitum* feeding produced a "classical" compensatory growth (+24% for weight gain), already described by several studies (Perrier, 1998; Gidenne *et al.*, 2003, Tumová *et al.,* 2003; Knudsen *et al.*, 2017). As shown by Gidenne *et al.* (2012) this compensatory growth is proportional to level of the feed restriction, but did not fully compensate the loss of growth, since at 10 wk of age, the live weight remained 4.3% lower. Compensatory growth was associated with a better feed conversion (–8.6 units between 49-72 d old), in agreement with previous studies using a similar restriction programme (Gidenne *et al.*, 2009; Martignon *et al.*, 2010).

## CONCLUSION

Feed restriction slowed the growth of the young rabbit, but improved its feed efficiency. In parallel, development of the digestive tract and lymphoid organs was impaired and thus could hardly be implicated in the improved digestion or health status as mentioned in the literature.

The feeding behaviour of the growing rabbit, housed conventionally in collective cages, followed a circadian rhythm depending on the lighting schedule, and thus similar to that of the wild rabbit. Under a restricted feeding programme, the feeding behaviour is locked to the time of the meal. However, the feeding and drinking behaviours were slightly modified and separated throughout the day but not fundamentally modified. As no changes in social behaviour (access to feed or drinking, resting, aggressiveness) were detected, we assumed that our restriction programme would not impair the welfare compared to that of freely fed animals. Further studies more focused on the social and feeding behaviour should be undertaken to detail the welfare status of the restricted rabbit.

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