

## Time-based restriction and refeeding programmes in growing rabbits: Effects on feeding behaviour, feed efficiency, nutrient digestibility, and caecal fermentative activity

M. Birolo<sup>a</sup>, A. Trocino<sup>b,\*</sup>, A. Zuffellato<sup>c</sup>, G. Xiccato<sup>a</sup>

<sup>a</sup> Department of Agronomy, Food, Natural resources, Animal and Environment (DAFNAE), University of Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy

<sup>b</sup> Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy

<sup>c</sup> A.I.A. Agricola Italiana Alimentare S.p.A., Piazzale Apollinare Veronesi, I-37036 San Martino Buon Albergo, Verona, Italy

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### ABSTRACT

The present study evaluated the effect of five feeding programmes (AL, *ad libitum* feeding; DF, daylight access to feed followed by fast full refeeding; NF, nightly access to feed and fast full refeeding; NS, nightly access to feed and slow full refeeding; NI, night access to feed until the end of the trial) on growth, nutrient digestibility, caecal fermentative activity, and carcass and meat quality of 400 crossbred rabbits housed in 40 open-top pens (10 rabbits/pen) from weaning to slaughter (28–70 days of age). In all feed-restricted rabbits, feeding time decreased from 14 to 9 h/day during the 1st week, it stood at 8 h/day during the 2nd week, and it increased from the beginning of the 3rd week according to three refeeding systems: *i*) fast until *ad libitum* (+4 h/day until 24 h/day) for the DF and NF groups; *ii*) slow until *ad libitum* (+1 h/day until 24 h/day) for the NS group; and *iii*) very slow and until 12-h-restriction (+30 min/day until 12 h/day) for the NI group. During the restriction period, DF and NF programmes compared with the AL programme, improved the coefficient of total tract apparent digestibility (CTTAD) for crude protein (+4.6%) and ether extract (+2.7%) ( $P < 0.001$ ); NF programmes increased caecal volatile fatty acids (VFA) (79.7 vs 67.7 mmol/L;  $P = 0.034$ ) and decreased pH (5.32 vs 5.46;  $P = 0.013$ ) compared with AL. During the refeeding period, the NI programme increased the CTTAD of dry matter (+2.9%;  $P = 0.001$ ), crude protein (+3.9%;  $P = 0.003$ ), and gross energy (+2.6%;  $P = 0.001$ ) compared with the DF and NF groups. The AL, DF, and NF treatments decreased caecal VFA compared with NS and NI (81.0 mmol/L vs. 95.4 mmol/L;  $P < 0.001$ ). In the whole trial, the NI programme decreased feed intake (−7.5%;  $P < 0.001$ ) and feed conversion (−6.7%;  $P = 0.002$ ) compared with the AL programme, without differences in growth, final live weight, and slaughter traits among groups. Compared to AL feeding, feed restriction increased the mortality rate due to digestive disorders (9.0 vs 1.6%;  $P = 0.036$ ), without affecting morbidity and health risk index. Slaughter weight (on average: 2608 g), carcass yield (60.2 g/100 g live weight at slaughter), and

**Abbreviations:** ADF, Acid detergent fibre; AL, *ad libitum* feeding; aNDF, neutral detergent fibre; CC, cold carcass; CP, crude protein; CTTAD, coefficient of total tract apparent digestibility; DE, digestible energy; DF, daylight access to feed followed by fast full refeeding; DM, dry matter; DP, digestible protein; ERE, epizootic rabbit enteropathy; LW, live weight; NF, nightly access to feed and fast full refeeding; NI, night access to feed until the end of the trial; NS, nightly access to feed and slow full refeeding; RC, reference carcass; SW, slaughter weight; VFA, volatile fatty acids.

\* Corresponding author.

E-mail address: [angela.trocino@unipd.it](mailto:angela.trocino@unipd.it) (A. Trocino).

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carcass and meat quality traits were not affected. In conclusion, the NI programme improved feed efficiency without impairing growth, slaughter, and carcass traits.

## 1. Introduction

Feed restriction can be used to reduce mortality and morbidity due to digestive problems in growing rabbits (Gidenne et al., 2009, 2012; De Blas et al., 2012) and to enhance farm feed efficiency (Knudsen et al., 2014; Gidenne et al., 2017; Birolo et al., 2020a). However, effective protection against the occurrence of enteric diseases such as epizootic rabbit enteropathy (ERE) has not been definitively proven. Indeed, during weaning and post-weaning periods, growing rabbits are subjected to important changes in digestive physiology, including caecal microflora, fermentation patterns, and gut enzymatic activity (Gidenne and Fortun-Lamothe, 2002; Gallois et al., 2008; Combes et al., 2017). Severe feed restriction (about 75–80% of *ad libitum*) during the first weeks after weaning can protect young rabbits against digestive disorders (Gidenne et al., 2009, 2012). Nevertheless, during the refeeding phase, digestive disturbances often reoccur, increasing morbidity and mortality rates during the fattening period, with even greater economic losses (Alabiso et al., 2017; Birolo et al., 2020a). In addition, feed conversion in rabbits can be impaired during the *ad libitum* feeding phase (Birolo et al., 2020b) depending on the intensity, timing, and duration of the restriction, as well as the technique and duration of refeeding. Nonetheless, to our knowledge, only one study compared different refeeding systems (Birolo et al., 2016), and a few studies have evaluated the effects of restricted access to feed during the entire rearing period on the health and growth performance of rabbits (Weissman et al., 2009; Salaun et al., 2011).

The intake pattern of domestic rabbits fed *ad libitum* comprises several small meals over a 24-h period (Bellier et al., 1995; Gidenne et al., 2010). The amount of ingested feed is not constant throughout the day, increasing during the afternoon, reaching a maximum during the evening and night hours, and becoming minimal in the morning (Gidenne et al., 2010; Birolo et al., 2020b). Accordingly, withdrawing feed during the dark period could increase the restriction rate (Romero et al., 2010) and positively affect feed efficiency (Weissman et al., 2009).

Thus, the present study aimed to evaluate the effect of feed restriction programs based on daily or nightly access to feed and different refeeding systems (fast, slow, very slow, or incomplete) on growth performance, nutrient digestibility, and caecal activity in growing rabbits. The effects of feed restriction programmes on slaughter traits, carcass, and meat quality were also evaluated.

## 2. Materials and methods

The study was approved by the Ethical Committee for Animal Experimentation (Organismo Preposto al Benessere degli Animali) of the University of Padova (Padua, Italy) (project 16/2016; Prot. no. 147681). All animals were handled according to the principles stated by the EU Directive 2010/63/EU regarding the protection of animals used for experimental and other scientific purposes (EU, 2010). The research staff involved in animal handling were animal specialists (PhD or MS in Animal Science) and veterinary practitioners.

### 2.1. Animals and experimental conditions

The trial was conducted on the rabbit farm of the University of Padova (Legnaro, Padova, Italy), in a closed building, from February to March under a natural photoperiod (approximately 12 h of light/12 h of darkness). Extraction fans and an automatic heating system guaranteed air quality and maintained the temperature between 20 and 23 °C.

At 28 days of age, a total of 400 crossbred rabbits (Hypharm, Groupe Grimaud, Roussay, France), 200 females and 200 males, were selected on a commercial farm from healthy litters of multiparous does ( $\geq 3$  kindling) and moved to the experimental farm. The animals were individually identified using ear marks and allocated to five experimental groups, homogenous by sex, average live weight (LW) and standard deviation, according to a completely randomised design: AL, *ad libitum* feeding throughout the trial period; DF, daylight access to feed followed by fast refeeding until *ad libitum*; NF, night access to feed and fast refeeding until *ad libitum*; NS, night access to feed and slow refeeding until *ad libitum*; NI, night access to feed and very slow refeeding until 12-h-access to feed maintained until slaughter (incomplete refeeding).

The rabbits were housed in 40 open-top pens (0.64 m  $\times$  0.78 m; area: 0.5 m<sup>2</sup>) with 10 animals (5 females and 5 males) per pen (8 pens, 80 animals per group) and monitored from 28 to 70 days of age. The pen floor was made of plastic slats (hole dimensions: 70 mm long  $\times$  10 mm wide; distance between holes: 7 mm), which guaranteed the complete removal of faeces from the pens. All pens were equipped with automatic nipple drinkers (two per pen) and a feeder (600 mm wide) for the manual distribution of feed and an automatic continuous recording system of feed consumption.

### 2.2. Diets and feeding programs

All animals had *ad libitum* access to water during the trial which lasted from 28 to 70 days of age. Due to the occurrence of a severe enteric disease at the commercial farm where the rabbits were born, and according to the therapeutic programme adopted at the farm, from 28 to 53 days of age, all rabbits were fed with a post-weaning diet (diet P) supplemented with an antibiotic (active substance oxytetracycline, 1450 mg/kg diet; obtained by the inclusion over feed mixture preparation of 7250 mg/kg diet of Oxiter 200 BMP –

200 mg oxytetracycline/g - Dox-Al Italia S.p.A., Milan, Italy) and a coccidiostat (active substance diclazuril, 1 mg/kg diet; obtained by the dietary inclusion of 0.5 g/kg diet of Coxirill® 0.2% - Huvepharma, Sofia, Bulgaria). From 54 days of age until slaughter at 70 days of age, a fattening diet (diet F) without antibiotics and coccidiostats was provided to all groups. The pelleted diets (diameter: 3.5 mm; length: 10–11 mm) were formulated to satisfy the nutritional requirements of rabbits during the post-weaning and fattening periods (De Blas and Mateos, 2010). The ingredients and chemical composition of the experimental diets are listed in Table 1.

Feed restriction was implemented by varying the access time to the feeders according to the programs shown in Fig. 1. The programs were designed based on previous studies (Foubert et al., 2007; Birolo et al., 2020a, 2020b) to decrease the feeding level of all feed-restricted groups from 80% to 70% of the *ad libitum* level in the 1st week of the trial and then increased from 70% to 80% in the 2nd week of the trial. In detail, the feeding time for all restricted rabbits decreased from 14 to 9 h/day during the 1st week (28–34 days of age) and was set at 8 h/day during the 2nd week (35–42 days of age). Subsequently, the access time to feed increased from the 43rd day of age onward as follows: *i*) fast refeeding (+4 h/day) until 24 h/day of access to the feeders (groups DF and NF); *ii*) slow refeeding (+1 then +2 h/day) until 24 h/day of access to the feeders (group NS); *iii*) very slow refeeding (+30 min/day) until 12 h/day of access to the feeders (group NI) and then maintained until slaughter at 70 days of age (incomplete refeeding). Thus, the DF and NF groups reached 24 h/day of access to feed at 46 days of age, the NS group at 56 days, and the NI group at a rate of 12 h/day at 50 days of age. The feeding programmes were implemented by shortening and then extending the access time to feeders during the day (NF, NS, and NI groups) or the night (DF group). Access to feeders was managed by closing them off with a removable partition.

### 2.3. Growth performance, mortality, and morbidity rates

During the trial, individual live weights were recorded once a week; feed intake (at a pen level) was measured daily through a computerised weighing system connected to all feeders, from the rabbits' arrival until the day before commercial slaughtering. In fact, the last recordings for growth performance was set at 69 days of age since recordings on the slaughtering day (at 70 days) were made under different conditions (*i.e.* earlier in the morning and after 4-h fasting period before animal loading).

During the trial, morbidity and mortality were monitored daily, and rabbits were considered ill when they had diarrhoea and/or mucus in faeces or a live weight loss during a week. In the morbidity calculation, the ill rabbits were counted once, even if symptoms lasted several days. The dead animals were considered only in the calculation of mortality. The health risk index was calculated as the

**Table 1**  
Ingredients and chemical composition of the experimental diets.

Items	Diets	
	Post-weaning (28–53 days)	Fattening (54–70 days)
Ingredients, g/kg		
Dehydrated alfalfa meal (160 g CP/kg)	340	253
Wheat bran	190	240
Barley meal (6 row)	120	160
Dried beet pulp	190	160
Soybean meal (490 g CP/kg)	50	40
Sunflower meal (300 g CP/kg)	70	100
Sunflower oil	10	15
Cane molasses	15	15
Calcium carbonate	1.0	4.0
Dicalcium phosphate	3.5	2.5
Salt	4.0	4.0
L-lysine HCl (70 g lysine/100 g)	1.0	1.0
DL-methionine (95 g methionine/100 g)	1.0	1.0
Vitamin–mineral premix <sup>a</sup>	4.0	4.0
Coccidiostat <sup>b</sup>	0.5	–
Oxytetracycline <sup>b</sup> , mg/kg	1450	–
Chemical composition, g/kg as fed		
Gross energy, MJ/kg as fed	16.5	16.6
Dry matter	902	902
Crude protein	159	160
Ether extract	32	38
aNDF	376	366
ADF	210	196
Lignin (sa)	59	53
Starch	107	132
Ash	78	73

<sup>a</sup> Premix provided per kg of complete diet: vit. A, 12000 UI; vit. D3, 1000 UI; vit. E acetate, 50 mg; vit. K<sub>3</sub>, 2 mg; Biotin, 0.1 mg; Thiamine, 2 mg; Riboflavin, 4 mg; vit. B<sub>6</sub>, 2 mg; vit. B<sub>12</sub>, 0.1 mg; Niacin, 40 mg; Pantothenic acid, 12 mg; Folic acid, 1 mg; Fe, 100 mg; Cu, 20 mg; Mn, 50 mg; Co, 2 mg; I, 1 mg; Zn, 100 mg; Se, 0.1 mg.

<sup>b</sup> Coccidiostat: Coxirill® 0.2% (Huvepharma, Sofia, Bulgaria) (active substance diclazuril, 2 g/kg product; 1 mg diclazuril/kg diet). Oxytetracycline (active substance): 7250 mg Oxiter 200 BMP (200 mg oxytetracycline/g; DOX-AL Italia S.p.A., Milano, Italy)/kg diet included over feed mixture preparation corresponding to 1450 mg oxytetracycline/kg diet.

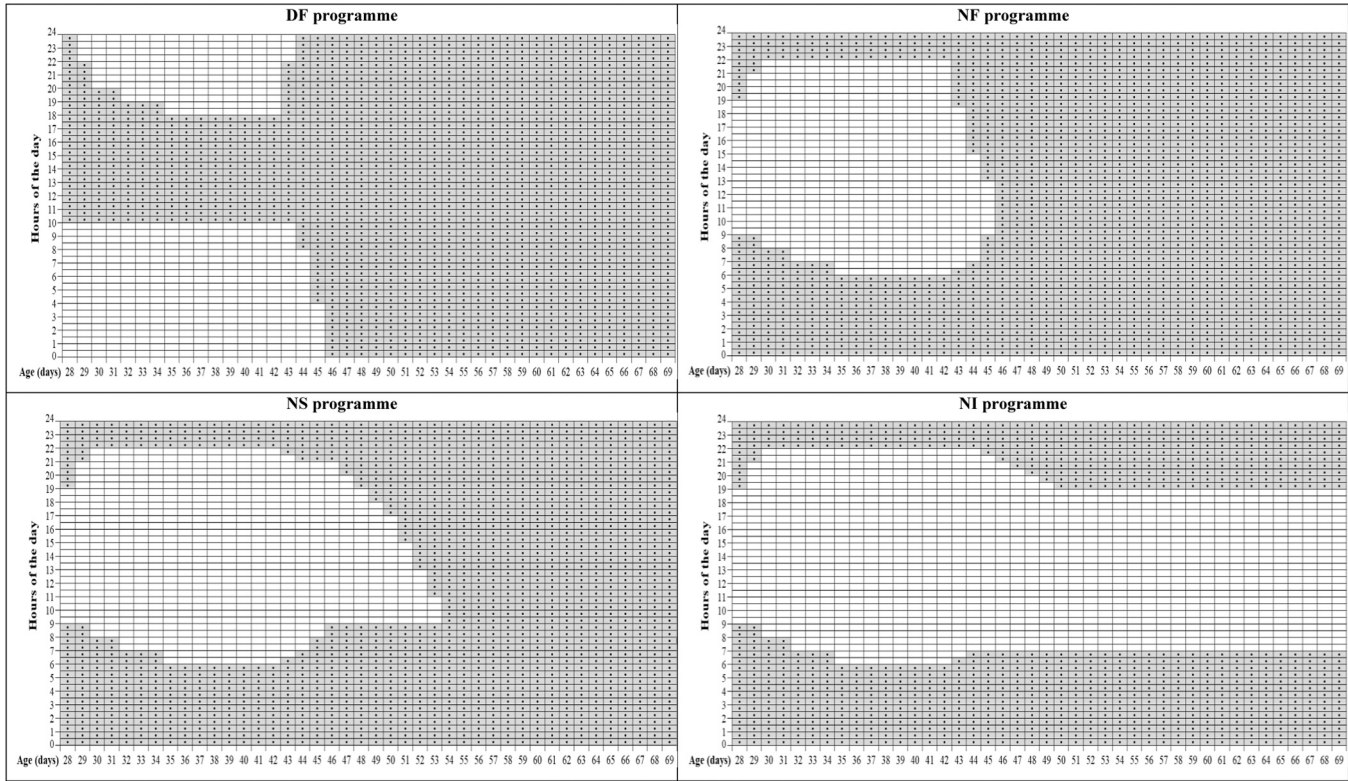


Fig. 1. Daily access time to the feeders in rabbits subjected to daylight access to feed with fast refeeding until *ad libitum* (DF programme), night feed access with fast refeeding until *ad libitum* (NF programme), night access to feed with slow refeeding until *ad libitum* (NS programme), night access to feed with very slow refeeding and 12 h/day of restriction until the end of fattening (NI programme).

sum of morbidity and mortality rates (Gidenne et al., 2009). To prevent ill animals from affecting the digestibility trial, growth performance recordings, and carcass and meat quality, when the symptoms of digestive diseases (diarrhoea, presence of mucus, constipation, loss of weight) occurred for more than five days, ill rabbits were moved from the pens to infirmary cages (0.64 m × 0.78 m; area: 0.5 m<sup>2</sup>) and excluded from the trial. In total, 90 rabbits (17 in group AL, 22 in group DF, 14 in group NF, 20 in group NS, and 17 in group NI) were excluded (Table 2). Only data of animals that reached the end of the trial were retained for evaluating performance at any time of the trial.

#### 2.4. Digestibility trials

The coefficients of total tract apparent digestibility (CTTAD) of dry matter (DM) and nutrients and the digestible energy (DE) contents of the P and F diets were measured *in vivo* in two digestibility trials. The pens (where rabbits were housed) were equipped with a wire net (2 mm mesh) under the floor to collect all hard faeces excreted daily by rabbits.

Digestibility trials were performed according to the European standardised method (Perez et al., 1995). The first trial was carried out in 12 pens (10 animals per pen, 5 females and 5 males; 4 pens per group; AL, DF, and NF groups) from 38 to 42 days of age when rabbits were fed diet P. Only the NF rabbits were subjected to the digestibility trial because all the groups with nightly access to feed (NF, NI, and NS groups) had the same feeding schedule from 28 to 42 days of age. The second trial was performed in 20 pens (8 animals per pen, 4 females and 4 males; 4 pens per group; AL, DF, NF, NS, and NI groups) from 60 to 64 days of age when rabbits were fed diet F. Animals showing any sign of disease were promptly taken out of the pens.

#### 2.5. Sampling of caecal content

At 42 days, when the restricted groups had 8 h/day of access to the feeders, 24 rabbits (8 rabbits per group, 4 females and 4 males; 1 per pen; AL, DF, and NF groups), representative of the corresponding experimental groups in terms of average live weight and standard deviation, were slaughtered to sample their caecal content. Only NF rabbits were used out of those subjected to nightly access to feed (as explained above) for the first digestibility trial. Slaughtering was performed twice daily, one hour after the closure of feeders for both DF and NF groups (to limit the variability of the weight and composition of caecal content depending on the previous fasting period). In detail, eight rabbits belonging to the NF group were slaughtered in the morning (between 07:00 and 08:00), while eight rabbits belonging to the DF group were slaughtered in the evening (between 19:00 and 20:00). The eight rabbits belonging to the AL group were divided into two groups of four and slaughtered in the morning or evening just after the restricted animals.

The rabbits were weighed immediately before slaughter and executed by cervical dislocation. The stomach and gut were then removed and weighed. The caecum was removed, weighed, and the pH of the caecal contents was immediately measured. The caecal contents were then diluted with a 15% HPO<sub>3</sub> solution (25% wt/wt) and stored at −20 °C until further chemical analyses (Trocino et al., 2011). In the NS and NI groups, one rabbit per pen was removed and excluded from the trial to maintain the same group size (9 rabbits/pen) and stocking density in all experimental groups.

At 51 days of age, when the DF, NF, NS, and NI groups reached 24 h/day, 24 h/day, 18 h/day, and 12 h/day of access to the feeders, respectively, an additional 40 rabbits (8 rabbits per group, 4 females and 4 males; AL, DF, NF, NS, and NI groups) were slaughtered to sample caecal contents as described above. The rabbits in the NI group were slaughtered between 08:00 and 08:30 (one h after the closure of the feeders); AL, DF, and NF rabbits (now *ad libitum*) were slaughtered between 08:30 and 09:30. NS rabbits were slaughtered between 10:00 and 10:30 (one h after the closure of the feeders).

**Table 2**

Number of rabbits at the beginning, excluded, and slaughtered during the trial—mortality, morbidity, and health risk.

	Feeding programmes					Total	P-value
	AL	DF	NF	NS	NI		
Rabbits, no.							
Housed at 28 days of age	80	80	80	80	80	400	
Slaughtered/excluded at 42 days of age	8	8	8	8	8	40	
Slaughtered at 51 days of age	8	8	8	8	8	40	
On trial	64	64	64	64	64	320	
Dead	1	2	8	7	6	24	
Excluded due to illness	17	22	14	20	17	90	
Slaughtered at 70 days of age	46	40	42	37	41	206	
Mortality <sup>a</sup> , %	1.56	3.12	12.5	10.9	9.38		0.137
Morbidity, %	26.6	34.4	21.9	31.3	26.5		0.612
Health risk index <sup>b</sup> , %	28.2	37.5	34.4	42.2	35.9		0.667

AL, rabbits fed *ad libitum*; DF, rabbits subjected to daylight access to feed with fast refeeding until *ad libitum*; NF: rabbits subjected to night feed access with fast refeeding until *ad libitum*; NS rabbits subjected to night feed access with slow refeeding until *ad libitum*; NI: rabbits subjected to night feed access with very slow refeeding and 12 h/day of restriction until the end of fattening.

<sup>a</sup> Contrast: AL vs. DF + NF + NS + NI; P = 0.04.

<sup>b</sup> Calculated as Mortality + Morbidity (Gidenne et al., 2009).

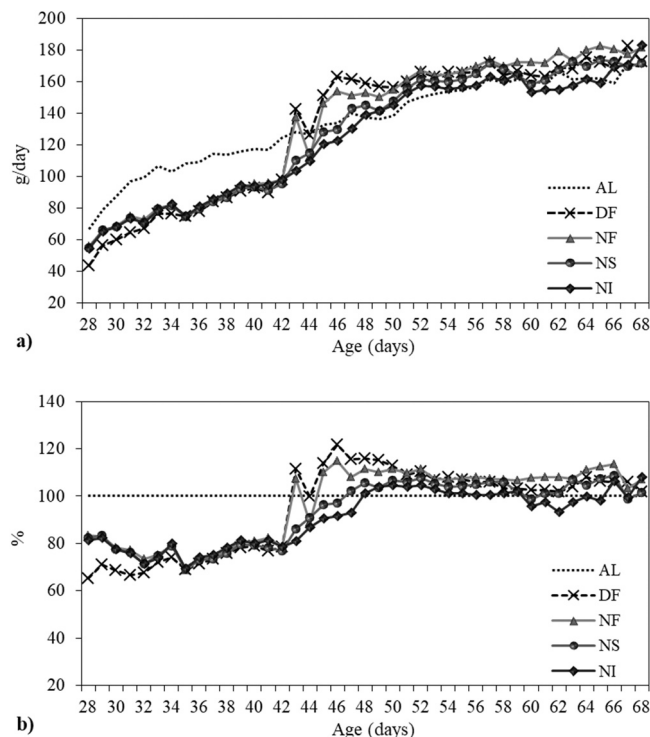
## 2.6. Commercial slaughter and carcass and meat quality recordings

At 70 days of age, the rabbits in the trial [206 overall (105 females and 101 males); 46 from group AL (22 females and 24 males), 40 from DF (21 females and 19 males), 42 from NF (23 females and 19 males), 37 from NS (19 females and 18 males), and 41 from NI (20 females and 21 males)] were weighed at the experimental farm after a 4-h fasting period, according to slaughterhouse practices, properly caged and transported to a commercial slaughterhouse in an authorised truck, which took approximately 1 h. Slaughter took place approximately 1 h after arrival at the slaughterhouse, where the rabbits were individually weighed, stunned by electroanaesthesia, and killed by jugulation. After 2.5 h of chilling, the commercial carcasses were weighed to calculate the individual carcass yield. The carcasses were then transported to the department laboratory and stored at 3–4 °C for 24 h. The reference carcass was obtained by removing the head, the thoracic organs, and the kidneys according to the procedures described by [Blasco et al. \(1993\)](#). Later, the carcasses were dissected ([Blasco and Ouhayoun, 1996](#)).

The pH value of the right *Longissimus lumborum* muscles was measured in duplicate using a pH meter (Basic 20; Crison Instruments Sa, Carpi, Italy) equipped with a specific electrode (cat. 5232; Crison Instruments Sa, Carpi, Italy). Duplicate measurements of the L\*a\*b\* colour indices ([Commission Internationale de l'Éclairage, 1976](#)) were obtained from the same muscle using a Minolta CM-508 C spectrophotometer (Minolta Corp., Ramsey, NJ, USA). The hind legs and *L. lumborum* muscles were dissected, and the meat-to-bone ratio of the hind legs was measured ([Blasco and Ouhayoun, 1996](#)). The right *L. lumborum* muscles were stored at –18 °C in vacuum-sealed plastic bags until downstream meat analyses, wherein thawing and cooking losses were measured. After thawing, the *L. lumborum* muscles were kept in plastic bags and cooked in a water bath until an internal temperature of 80 °C was achieved. After 1 h of cooling, the *L. lumborum* was cut into three parts (length: 40 mm; thickness: 10–30 mm). The maximum shear force was measured on these sections with a TA.HDI dynamometer (Stable Micro Systems Ltd., Godalming, Surrey, UK) using an Allo-Kramer (10 blades) probe (load cell: 500 kg; distance between the blades: 5 mm; thickness: 2 mm; cutting speed: 500 mm/min) ([Bianchi et al., 2007](#)).

## 2.7. Chemical analyses

The diets and the faeces were analysed to determine the dry matter (method 934.01), ash (967.05), crude protein (2001.11), and starch contents (amylglucosidase  $\alpha$  amylase method, 996.11) using [AOAC \(2000\)](#) methods following harmonised procedures ([EGRAN, 2001](#)). The ether extract was analysed after acid hydrolysis ([EC, 1998](#)). Neutral detergent fibre (aNDF) was analysed according to [Mertens \(2002\)](#), assayed using a heat-stable amylase without sodium sulphite and expressed inclusive of residual ash. Acid detergent fibre (ADF), expressed inclusive of residual ash, was analysed according to the AOAC method (2000, method 973.187).



**Fig. 2.** Daily feed intake (a) and feeding level (b) (% of *ad libitum* rabbits) in rabbits fed *ad libitum* (AL); rabbits subjected to daylight access to feed with fast refeeding until *ad libitum* (DF); rabbits subjected to night feed access with fast refeeding until *ad libitum* (NF); rabbits subjected to night feed access with slow refeeding until *ad libitum* (NS); rabbits subjected to night feed access with very slow refeeding and 12 h/day of restriction until the end of fattening (NI).

Lignin (determined by the solubilisation of cellulose with sulphuric acid) was analysed according to Van Soest et al. (1991). The sequential procedure and filter bag system (Ankom Technology, Macedon, NY, USA) were used in the analyses. The gross energy contents of the diets and faeces were measured using an adiabatic bomb calorimeter (IKAC200, Staufen, Germany).

The thawed caecal content samples were centrifuged at 9000 rpm for 10 min. Caecal N ammonia was determined in the supernatant using a pH meter (Crison GLP 22, Barcelona, Spain) equipped with an ammonia-specific electrode (mod. 9663, combined with the reference electrode mod. 5044) (Crison Instruments S.A., Barcelona, Spain). Volatile fatty acid (VFA) molar contents were measured in the supernatant by gas chromatography (Agilent 7820 A, equipped with a flame ionisation detector FID, split-splitless injection system, programmable oven) on an Agilent J&W cross bond capillary column DB-FFAP (30 m × 0.25 mm I.D., 0.25 µm film thickness) (Agilent Technologies, Santa Clara, CA, USA) according to the method described by Osl (1988).

## 2.8. Statistical analyses

Individual data of live weight, daily growth, caecal VFA content, slaughter results, and meat quality were analysed by two-way ANOVA using the PROC MIXED of SAS (2013). The model used the animal as experimental unit and included the feeding programme set as a fixed effect and the pen as a random effect. Pen data on feed intake, feed conversion, and CTAD were analysed using the PROC GLM of SAS, with the feeding programme as a fixed effect and the pen as experimental unit. As for mortality and morbidity, the PROC CATMOD was used, with the feeding programme as a fixed effect. Least-squares means were compared using the Bonferroni test. Differences among means were considered statistically significant at  $P \leq 0.05$ .

## 3. Results

### 3.1. Health and growth performance

During the first four weeks of the trial, no health problems were recorded. In the last two weeks, a severe enteric disease, characterised by the typical symptoms of ERE (distension of the gastrointestinal tract and diarrhoea with the presence of mucus), appeared and spread in all pens. Mortality in the whole trial was 7.5% and was not affected by the feeding programme (Table 2), but it was significantly lower in the AL group (1.6%) than in all other feed-restricted groups (9.0% on average) (contrast AL vs feed-restricted

**Table 3**  
Growth performance from weaning to the day before slaughter (28–69 days of age).

	Feeding programmes					P-value	RMSE <sup>1</sup>
	AL	DF	NF	NS	NI		
Rabbits <sup>2</sup> , no.	46	40	42	37	41		
Live weight, g							
At 28 days	647	633	638	638	634	0.785	66
At 42 days	1387 <sup>a</sup>	1172 <sup>c</sup>	1276 <sup>b</sup>	1249 <sup>b</sup>	1258 <sup>b</sup>	<0.001	122
At 56 days	2113 <sup>a</sup>	2014 <sup>c</sup>	2088 <sup>ab</sup>	2027 <sup>bc</sup>	2035 <sup>bc</sup>	0.029	172
At 69 days	2696	2645	2727	2645	2673	0.279	202
Restriction period (28–42 days)							
Weight gain, g/day	52.8 <sup>a</sup>	38.7 <sup>c</sup>	45.9 <sup>b</sup>	43.6 <sup>b</sup>	44.5 <sup>b</sup>	<0.001	5.6
Feed intake, g/day	102 <sup>a</sup>	74 <sup>c</sup>	79 <sup>b</sup>	78 <sup>b</sup>	79 <sup>b</sup>	<0.001	2
Feed conversion <sup>2</sup>	1.93 <sup>a</sup>	1.96 <sup>a</sup>	1.71 <sup>c</sup>	1.81 <sup>bc</sup>	1.78 <sup>c</sup>	<0.001	0.09
Refeeding period (43–56 days)							
Weight gain, g/day	51.8 <sup>c</sup>	60.0 <sup>a</sup>	57.7 <sup>ab</sup>	55.4 <sup>b</sup>	55.4 <sup>b</sup>	<0.001	5.7
Feed intake, g/day	140 <sup>b</sup>	152 <sup>a</sup>	148 <sup>a</sup>	140 <sup>b</sup>	135 <sup>b</sup>	<0.001	5
Feed conversion <sup>2</sup>	2.70 <sup>a</sup>	2.57 <sup>ab</sup>	2.56 <sup>ab</sup>	2.56 <sup>ab</sup>	2.44 <sup>b</sup>	0.025	0.15
Fattening period (57–69 days)							
Weight gain, g/day	45.3 <sup>b</sup>	49.0 <sup>a</sup>	49.7 <sup>a</sup>	48.0 <sup>ab</sup>	49.5 <sup>a</sup>	0.019	7.0
Feed intake, g/day	162 <sup>b</sup>	169 <sup>ab</sup>	176 <sup>a</sup>	168 <sup>ab</sup>	162 <sup>b</sup>	<0.001	5
Feed conversion <sup>2</sup>	3.58	3.54	3.58	3.52	3.31	0.101	0.26
Whole period (28–69 days)							
Weight gain, g/day	49.9	49.1	49.7	48.9	51.0	0.164	4.2
Feed intake, g/day	134 <sup>a</sup>	131 <sup>ab</sup>	133 <sup>ab</sup>	128 <sup>bc</sup>	124 <sup>c</sup>	<0.001	3
Feed conversion <sup>2</sup>	2.68 <sup>a</sup>	2.71 <sup>a</sup>	2.60 <sup>ab</sup>	2.63 <sup>ab</sup>	2.50 <sup>b</sup>	0.002	0.10

The experimental unit is the animal for live weight and weight gain (number of replicate per group = number of rabbits per group). The experimental unit is the pen for feed intake and feed conversion (number of replicates per group = 8).

Means with different superscript letters on the same row differ significantly ( $P < 0.05$ ; Bonferroni test).

AL, rabbits fed *ad libitum*; DF, rabbits subjected to daylight access to feed with fast refeeding until *ad libitum*; NF: rabbits subjected to night feed access with fast refeeding until *ad libitum*; NS rabbits subjected to night feed access with slow refeeding until *ad libitum*; NI: rabbits subjected to night feed access with very slow refeeding and 12 h/day of restriction until the end of fattening.

<sup>1</sup> Root mean square error.

<sup>2</sup> All data in the table refer to rabbits that reached the end of the trial and were slaughtered at 70 days of age [206 overall (105 females and 101 males); 46 from group AL (22 females and 24 males), 40 from DF (21 females and 19 males), 42 from NF (23 females and 19 males), 37 from NS (19 females and 18 males), and 41 from NI (20 females and 21 males)].

rabbits,  $P < 0.05$ ). Morbidity and health risk indices did not differ between groups (Table 2).

Regarding feeding behaviour, the daily feed intake of rabbits in the different groups and the corresponding feed restriction levels are shown in Fig. 2. During the first two weeks of the trial (28–42 days of age), feed intake was 79% of AL on average in rabbits subjected to nightly feed restriction programmes, which reached 74% in the DF group. At the start of refeeding (43 days), with an access time to the feeders of 12 h/day, DF and NF rabbits exceeded the intake level of the AL group by 11% and 7%, respectively. In the following two days, the DF and NF groups showed several fluctuations in feed intake, which remained higher than that of the AL group (+8% on average) until the end of the trial. Slow refeeding in the NS and NI groups allowed a more progressive transition from restricted to *ad libitum* feeding. The NS rabbits reached the feeding level of AL rabbits at 47 days of age and 13 h feeding/day, while NI rabbits did so at 48 days of age and 11 h feeding/day.

Regarding growth performance, in the first two weeks of the trial, AL rabbits exhibited higher daily weight gain (52.8 vs 43.2 g/day on average;  $P < 0.001$ ) and feed intake (102 vs 78 g/day on average;  $P < 0.001$ ) compared with rabbits subjected to feed restriction (Table 3) for which the former reached a heavier live weight at 49 days of age compared with the latter (1387 vs 1239 g on average;  $P < 0.001$ ). The DF rabbits exhibited the lowest weight gain and feed intake, and the worst feed conversion (1.96), whereas nightly restriction programs improved feed conversion (1.77 for NF, NS, and NI groups) compared with the AL group (1.93;  $P < 0.001$ ).

During the refeeding period (3rd and 4th week of trial), previously restricted rabbits exhibited higher daily weight gain (+10.3%;  $P < 0.001$ ) than the AL ones, with the highest values seen in DF and NF groups (58.9 g/day on average). The DF and NF groups also showed a higher feed intake than the AL group and the other feed restriction programmes (150 vs 138 g/day, respectively;  $P < 0.001$ ). Feed conversion was lower in NI than AL rabbits (−9.6%;  $P = 0.025$ ).

In the last two weeks of fattening, the DF, NF, NS, and NI groups still had a higher daily weight gain (+8.3%;  $P = 0.019$ ) than the AL group. NF rabbits showed the highest feed intake, while AL and NI rabbits showed the lowest feed intake (176 vs 162 g/day, respectively;  $P < 0.001$ ).

In the whole trial, the feeding programmes did not affect daily weight gain (49.7 g/day on average) for a similar final live weight (2677 g). The NS and NI groups had a lower feed intake than the AL group (−6.0% on average;  $P < 0.001$ ), whereas the NI programme had the lowest feed conversion ratio compared with the AL and DF groups ( $P = 0.002$ ).

### 3.2. Digestibility and nutritive value of experimental diets

Eight days after the beginning of the trial, with 8 h feeding/day for the restricted programmes, both DF and NF programmes increased the CTTAD of crude protein (+4.6%;  $P < 0.001$ ) and ether extract (+2.7%;  $P < 0.001$ ) as well as dry matter and gross energy (+2.5 and +2.9%, respectively;  $P \leq 0.05$ ) with respect to the AL group (Table 4). The CTTAD of starch was higher in the NF group than in the AL group ( $P = 0.030$ ).

During the refeeding period, when DF, NF, and NS rabbits had 24 h feeding/day and NI rabbits had 12 h/day, the DF and NF groups showed the highest feed intake and the lowest CTTAD of dry matter, crude protein, and gross energy (Table 5). The NI group had the lowest feed intake ( $P = 0.001$ ) and the highest CTTAD for dry matter ( $P = 0.001$ ), crude protein ( $P = 0.003$ ), and gross energy ( $P = 0.001$ ), whereas intermediate values were recorded in the AL and NS groups.

### 3.3. Caecal content and caecal fermentative activity

At 42 days of age, the proportion of full gut (including its content) was lower in AL rabbits than in feed-restricted rabbits (22.1 vs

**Table 4**

Coefficients of total tract apparent digestibility (CTTAD) and nutritive value of diet P measured during the restriction period (digestibility trial from 38 to 42 days of age).

	Feeding programmes			P-value	RMSE <sup>1</sup>
	AL	DF	NF		
Pens, no.	4	4	4		
Feed intake, g/day	117 <sup>a</sup>	93 <sup>b</sup>	96 <sup>b</sup>	< 0.001	3
Gross energy	0.619 <sup>b</sup>	0.639 <sup>a</sup>	0.635 <sup>ab</sup>	0.046	0.010
Dry matter	0.612 <sup>b</sup>	0.632 <sup>a</sup>	0.623 <sup>ab</sup>	0.044	0.010
Crude protein	0.769 <sup>b</sup>	0.807 <sup>a</sup>	0.801 <sup>a</sup>	< 0.001	0.008
Ether extract	0.814 <sup>b</sup>	0.838 <sup>a</sup>	0.834 <sup>a</sup>	< 0.001	0.004
aNDF	0.311	0.337	0.334	0.086	0.013
ADF	0.201	0.224	0.221	0.311	0.017
Starch	0.989 <sup>b</sup>	0.991 <sup>ab</sup>	0.993 <sup>a</sup>	0.030	0.001
Digestible protein (DP), g/kg	120	126	125	–	–
Digestible energy (DE), MJ/kg	10.03	10.36	10.29	–	–
DP to DE ratio, g/MJ	11.96	12.13	12.11	–	–

The experimental unit is the pen (number of replicates per group = 4).

Means with different superscript letters on the same row differ significantly ( $P < 0.05$ ; Bonferroni test).

AL, rabbits fed *ad libitum*; DF, rabbits subjected to daylight access to feed with fast refeeding until *ad libitum*; NF: rabbits subjected to night feed access with fast refeeding until *ad libitum*.

<sup>1</sup> Root mean square error.



**Table 5**

Coefficients of total tract apparent digestibility (CTTAD) and nutritive value of diet F measured during the fattening period (digestibility trial from 60 to 64 days of age).

	Feeding programmes					P-value	RMSE <sup>1</sup>
	AL	DF	NF	NS	NI		
Pens, no.	4	4	4	4	4		
Feed intake, g/day	165 <sup>bc</sup>	178 <sup>a</sup>	178 <sup>a</sup>	170 <sup>ab</sup>	157 <sup>c</sup>	0.001	5
Gross energy	0.615 <sup>ab</sup>	0.607 <sup>b</sup>	0.605 <sup>b</sup>	0.615 <sup>ab</sup>	0.622 <sup>a</sup>	0.001	0.005
Dry matter	0.606 <sup>ab</sup>	0.595 <sup>b</sup>	0.597 <sup>b</sup>	0.607 <sup>ab</sup>	0.613 <sup>a</sup>	0.001	0.005
Crude protein	0.729 <sup>ab</sup>	0.714 <sup>b</sup>	0.715 <sup>b</sup>	0.731 <sup>ab</sup>	0.743 <sup>a</sup>	0.003	0.010
Ether extract	0.808	0.810	0.811	0.812	0.825	0.335	0.013
aNDF	0.308	0.306	0.308	0.319	0.317	0.231	0.009
ADF	0.201	0.191	0.193	0.201	0.199	0.892	0.015
Starch	0.987	0.988	0.988	0.988	0.990	0.062	0.001
Digestible protein (DP), g/kg	113	111	111	113	115	–	–
Digestible energy (DE), MJ/kg	9.86	9.70	9.69	9.86	9.96	–	–
DP to DE ratio	11.43	11.44	11.41	11.47	11.54	–	–

The experimental unit is the pen (number of replicates per group = 4).

Means with different superscript letters on the same row differ significantly ( $P < 0.05$ ; Bonferroni test).

AL, rabbits fed *ad libitum*; DF, rabbits subjected to daylight access to feed with fast refeeding until *ad libitum*; NF: rabbits subjected to night feed access with fast refeeding until *ad libitum*; NS rabbits subjected to night feed access with slow refeeding until *ad libitum*; NI: rabbits subjected to night feed access with very slow refeeding and 12 h/day of restriction until the end of fattening.

<sup>1</sup> Root mean square error.

26.4% on average;  $P < 0.001$ ), as was the proportion of full stomach (6.08 vs 9.21 g/100 g LW on average;  $P < 0.001$ ) and full caecum (6.98 vs 8.37 g/100 g LW on average;  $P = 0.001$ ) (Table 6). The total caecal content of VFA was the lowest ( $P = 0.034$ ), and the caecal pH highest (5.46 vs 5.34 on average;  $P = 0.013$ ) in the AL group compared with feed-restricted ones. The N-ammonia content in the caecum was higher in AL than in DF rabbits (6.32 vs 3.74 mmol/L;  $P = 0.053$ ). NF rabbits showed the lowest molar proportion of valeric acid ( $P = 0.001$ ).

At 51 days, when DF, NF, NS, and NI rabbits had 24, 24, 18, and 12 h/day feedings, respectively, the proportion of full gut was lower in AL rabbits than in feed-restricted rabbits (20.6 vs 23.6 g/100 g LW, on average;  $P < 0.001$ ; Table 7). The proportion of the full stomach was lower in the AL, DF, and NF groups than in the NS and NI groups (7.20 vs 8.61 g/100 g LW, on average;  $P < 0.001$ ), whereas the proportion of full caecum was the highest in NI rabbits and the lowest in AL rabbits (7.65 vs 6.57 g/100 g LW;  $P = 0.021$ ). The caecal content had similar pH and N-ammonia values in all groups, whereas the total VFA content in the cecum was higher in the NS and NI groups than in the AL, DF, and NF groups (+17.8%;  $P < 0.001$ ).

### 3.4. Slaughter results, carcass traits and meat quality

All experimental groups achieved similar slaughter weights (2667 g on average), cold carcass weights (1571 g on average), and

**Table 6**

Caecal fermentative activity measured during the restriction period (42 days of age).

	Feeding programmes			P-value	RMSE <sup>1</sup>
	AL	DF	NF		
Rabbits, no.	8	8	8		
Live weight (LW), g	1446 <sup>a</sup>	1310 <sup>b</sup>	1337 <sup>ab</sup>	0.042	106
Full gut, g/100 g LW	22.1 <sup>b</sup>	26.4 <sup>a</sup>	26.3 <sup>a</sup>	< 0.001	1.2
Full stomach, g/100 g LW	6.08 <sup>b</sup>	9.36 <sup>a</sup>	9.05 <sup>a</sup>	< 0.001	1.03
Full caecum, g/100 g LW	6.98 <sup>b</sup>	8.41 <sup>a</sup>	8.32 <sup>a</sup>	0.001	0.74
Caecal content					
pH	5.46 <sup>a</sup>	5.36 <sup>b</sup>	5.32 <sup>b</sup>	0.013	0.08
N-NH <sub>3</sub> , mmol/L	6.32 <sup>a</sup>	3.74 <sup>b</sup>	5.28 <sup>ab</sup>	0.053	2.22
Total VFA, mmol/L	67.7 <sup>b</sup>	75.4 <sup>ab</sup>	79.7 <sup>a</sup>	0.034	8.6
Acetic acid, mol/100 mol total VFA	83.8	84.9	85.1	0.480	2.3
Propionic acid, mol/100 mol total VFA	5.30	4.54	4.54	0.139	0.84
Butyric acid, mol/100 mol total VFA	10.25	9.98	10.02	0.939	1.68
Valeric acid, mol/100 mol total VFA	0.62 <sup>a</sup>	0.56 <sup>a</sup>	0.36 <sup>b</sup>	0.001	0.12
Propionic acid/butyric acid ratio	0.52	0.46	0.46	0.159	0.08

The experimental unit is the animal (number of replicates per group = 8, 4 females and 4 males).

Means with different superscript letters on the same row differ significantly ( $P < 0.05$ ; Bonferroni test).

AL, rabbits fed *ad libitum*; DF, rabbits subjected to daylight access to feed with fast refeeding until *ad libitum*; NF: rabbits subjected to night feed access with fast refeeding until *ad libitum*.

<sup>1</sup> Root mean square error.

**Table 7**  
Caecal fermentative activity measured during the refeeding period (51 days of age).

	Feeding programmes					P-value	RMSE <sup>1</sup>
	AL	DF	NF	NS	NI		
Rabbits, no.	8	8	8	8	8		
Live weight (LW), g	1844	1721	1778	1761	1779	0.734	159
Full gut, g/100 g LW	20.6 <sup>b</sup>	22.9 <sup>a</sup>	22.7 <sup>a</sup>	24.0 <sup>a</sup>	24.7 <sup>a</sup>	<0.001	1.5
Full stomach, g/100 g LW	6.31 <sup>b</sup>	7.23 <sup>b</sup>	7.20 <sup>b</sup>	8.75 <sup>a</sup>	8.47 <sup>a</sup>	<0.001	0.92
Full caecum, g/100 g LW	6.57 <sup>b</sup>	7.53 <sup>ab</sup>	7.50 <sup>ab</sup>	7.03 <sup>ab</sup>	7.65 <sup>a</sup>	0.021	0.71
Caecal content							
pH	5.85	5.84	5.79	5.64	5.56	0.277	0.33
N-NH <sub>3</sub> , mmol/L	6.42	8.18	8.29	10.7	8.22	0.150	3.50
Total VFA, mmol/L	76.5 <sup>b</sup>	83.0 <sup>b</sup>	83.4 <sup>b</sup>	96.2 <sup>a</sup>	94.6 <sup>a</sup>	<0.001	7.2
Acetic acid, mol/100 mol total VFA	83.8	83.5	84.0	83.1	82.0	0.078	1.6
Propionic acid, mol/100 mol total VFA	4.86	4.66	4.60	4.58	4.41	0.644	0.71
Butyric acid, mol/100 mol total VFA	10.66 <sup>b</sup>	10.78 <sup>b</sup>	10.80 <sup>b</sup>	11.68 <sup>ab</sup>	12.97 <sup>a</sup>	0.031	1.63
Valeric acid, mol/100 mol total VFA	0.54	0.54	0.53	0.52	0.49	0.619	0.08
Propionic acid/butyric acid ratio	0.45	0.43	0.42	0.39	0.34	0.808	0.10

The experimental unit is the animal (number of replicates per group = 8, 4 females and 4 males).

Means with different superscript letters on the same row differ significantly ( $P < 0.05$ ; Bonferroni test).

AL, rabbits fed *ad libitum*; DF, rabbits subjected to daylight access to feed with fast refeeding until *ad libitum*; NF: rabbits subjected to night feed access with fast refeeding until *ad libitum*; NS rabbits subjected to night feed access with slow refeeding until *ad libitum*; NI: rabbits subjected to night feed access with very slow refeeding and 12 h/day of restriction until the end of fattening.

<sup>1</sup> Root mean square error.

carcass yield (60.2 g/100 g LW at slaughter on average) (Table 8). The liver proportion was higher in feed-restricted rabbits than in AL and reached the highest value in the NI group ( $P = 0.001$ ). The feeding program did not affect carcass muscularity, fatness, or meat quality traits (Table 8).

#### 4. Discussion

The nightly feeding restriction programs tested in the present trial were designed considering the feeding behaviour of rabbits, which show a low feeding activity during the day (Gidenne et al., 2010; Birolo et al., 2020b), especially in the morning when caecotrophy occurs (Gidenne and Lebas, 2005). In fact, during the first two weeks of the trial, the nightly feeding restriction programmes had a less severe feed intake reduction compared with the DF programme, for which rabbits required a longer adaptation period (about one week) after the start of the trial to reach the intake level of the other feed-restricted groups (Fig. 2). Unexpectedly, the nightly programmes improved feed efficiency compared with the AL and DF groups. For the latter, a lower feed intake resulted in a proportionally lower growth with no positive effect on feed conversion.

Indeed, during the restriction phase, a positive effect on feed efficiency has been previously reported by applying either quantitative or time-based restriction programmes ranging from 63% to 75% of *ad libitum* (Foubert et al., 2007; Knudsen et al., 2014). Increased digestibility of most nutrients could partially account for this result (Knudsen et al., 2014, 2017), whereas the mean retention time of digesta increased in rabbits subjected to quantitative feed restriction compared with *ad libitum* feeding regimes (Gidenne and Feugier, 2009). Duperray and Guyonvarch (2013) reported that feed efficiency in growing rabbits fed 14 h/day was better than that in rabbits fed *ad libitum*.

In the present trial, this worked only in the case of nightly restriction programs, indicating that these programmes better fit rabbit feeding behaviour and digestive physiology compared with a daily restriction programme. This result is consistent with the findings of Weissman et al. (2009), who measured a better feed efficiency (during the first three weeks after weaning) in rabbits fed during the night rather than during the day.

As for the refeeding phase, previously feed-restricted rabbits usually exhibit compensatory growth, which is linearly related to the previous feed restriction rate (Gidenne et al., 2009) and is associated with improved feed efficiency (Gidenne et al., 2012, 2017). In the present study, the compensatory growth of restricted rabbits was confirmed both in the refeeding and fattening periods, whereas DF rabbits, showing the greatest compensatory growth during refeeding, decreased growth rate during fattening. However, a better feed efficiency was observed only in the group restricted until the end of the trial (NI). In fact, during refeeding, the diet digestibility did not differ between the *ad libitum* and previously restricted groups (present trial; Tůmová et al., 2016; Knudsen et al., 2014; Uhlřřová et al., 2015; Birolo et al., 2017) because the mean retention time of digesta in the gut, the enzyme/substrate ratio, and nutrient absorption are expected to be the same in all groups (Gidenne and Feugier, 2009).

Over the entire rearing period, the effect of feed restriction on the performance and feed efficiency of growing rabbits can vary widely depending on the restriction rate and the duration of both restriction and refeeding phases (Xiccato, 1999; Gidenne et al., 2012). Compared with *ad libitum* feeding, quantitative feed restriction from 70% to 90% of AL during the first three weeks after weaning, followed by two weeks of refeeding, decreased the growth rate from 5% to 10%, but improved feed conversion from 10% to 15% (Gidenne et al., 2012, 2017). Consistent with the results observed for NI rabbits in the present study, Salaun et al. (2011) reported that adhering to 12 h feeding/day from weaning to 66 days of age helped decrease the feed conversion by 4.4% compared with *ad*

**Table 8**

Slaughter data, main characteristics of 24 h chilled carcasses and meat quality of rabbits slaughtered at 70 days of age.

	Feeding programmes					P-value	RMSE <sup>1</sup>
	AL	DF	NF	NS	NI		
Rabbits, no.	46	40	42	37	41		
Live weight at farm <sup>2</sup> , g	2680	2684	2705	2629	2637	0.345	179
Slaughter weight (SW), g	2628	2621	2643	2574	2574	0.261	174
Transport losses, %	1.64	2.02	1.90	1.88	2.16	0.186	1.01
Cold carcass (CC), g	1588	1572	1600	1550	1546	0.172	113
Carcass yield, g/100 g SW	60.4	60.0	60.6	60.2	60.0	0.525	1.85
Liver, g/100 g CC	4.07 <sup>b</sup>	4.36 <sup>ab</sup>	4.48 <sup>ab</sup>	4.28 <sup>b</sup>	4.78 <sup>a</sup>	0.001	0.66
Reference carcass <sup>3</sup> (RC), g	1351	1316	1346	1316	1292	0.207	101
Dissectible fat, g/100 g RC	2.08	2.19	2.11	2.10	1.71	0.310	0.68
Hind leg muscle/bone ratio	5.65	5.44	5.57	5.44	5.64	0.542	0.47
<i>Longissimus lumborum</i>							
pH	5.67	5.66	5.62	5.63	5.64	0.581	0.09
<i>L</i> *	51.7	51.7	52.4	51.9	51.9	0.809	2.4
<i>a</i> *	-1.64	-1.81	-1.86	-2.05	-1.92	0.059	0.55
<i>b</i> *	1.33	2.02	1.31	1.52	1.86	0.493	1.92
Thawing losses, %	8.12	8.29	9.06	8.51	8.26	0.699	1.98
Cooking losses, %	36.6	36.6	36.1	36.2	36.4	0.877	1.5
Shear force, kg/g	6.38	6.15	6.07	6.36	6.55	0.805	1.22

The experimental unit is the animal (number of replicates per group = number of rabbits per group).

[206 overall (105 females and 101 males); 46 from group AL (22 females and 24 males), 40 from DF (21 females and 19 males), 42 from NF (23 females and 19 males), 37 from NS (19 females and 18 males), and 41 from NI (20 females and 21 males)]

Means with different superscript letters on the same row differ significantly ( $P < 0.05$ ; Bonferroni test).

AL, rabbits fed *ad libitum*; DF, rabbits subjected to daylight access to feed with fast refeeding until *ad libitum*; NF: rabbits subjected to night feed access with fast refeeding until *ad libitum*; NS rabbits subjected to night feed access with slow refeeding until *ad libitum*; NI: rabbits subjected to night feed access with very slow refeeding and 12 h/day of restriction until the end of fattening.

<sup>1</sup> Root mean square error.

<sup>2</sup> Live weight measured after 4 h of fasting.

<sup>3</sup> Reference carcass obtained by removing the head, the thoracic organs and the kidneys (Blasco et al., 1993).

*libitum* feeding, whereas growth rates and final live weights were not affected. Foubert et al. (2007) observed that feed conversion significantly decreased in rabbits fed from 6 to 10 h/day in the first three weeks after weaning and then fed *ad libitum* during the last two weeks. However, in the latter study, the growth rate and final live weight of feed-restricted rabbits were also reduced. In contrast, in a previous study (Birolo et al., 2020b), growth performance and feed conversion were not affected when feeding time increased from 10 to 24 h/day during the first four weeks post-weaning, as observed in the present trial for rabbits allowed *ad libitum* feeding (24 h feeding/day) after restriction.

Surely, either quantitative or time-based feed restriction strategies produce significant changes in the feeding behaviour of rabbits, which ingest a high quantity of feed in a short time as soon as feed is provided (Gidenne et al., 2010; Birolo et al., 2020b). Consequently, a peak in fermentation is expected to occur in the caecum because of the high flow of digesta (Bellier et al., 1995). In the present trial, an increased fermentation rate was also observed when feeding was prevented both during the restriction and refeeding periods when the caecal sampling was performed one hour after closing the feeders.

Gidenne and Feugier (2009) observed that caecal VFA concentration was significantly higher in severely restricted rabbits (60% of *ad libitum*) than in the *ad libitum* group and that caecal pH values also decreased with a moderate feed restriction (80% of *ad libitum*). According to De Blas et al. (2012), a higher caecal VFA concentration and a lower caecal pH might limit the proliferation of pathogens in the gut and protect feed-restricted rabbits against digestive diseases. However, how the changes in the fermentation activity produced by feed restriction can affect rabbit health has not been elucidated.

Undoubtedly, the claimed protective effect of feed restriction on gut health has been found to disappear during the refeeding phase (Alabiso et al., 2017; Knudsen et al., 2017). Additionally, Tůmová et al. (2016) reported no residual effects on the caecal VFA concentration in previously feed-restricted rabbits, as was seen in the comparison between AL and NF groups in the present trial. Feed intake peaks are usually observed in feed-restricted rabbits when the restriction is abruptly interrupted for *ad libitum* feeding (Foubert et al., 2007; Knudsen et al., 2014, 2017), as evident in the present trial for rabbits subjected to fast refeeding. Peaks are usually associated with the appearance of digestive disorders. In contrast, a very slow (+30 min/day) and incomplete refeeding (until 12 h feeding/day), such as that used in the present trial, would allow rabbits to gradually reach the intake level of the AL group, preventing ingestion peaks and overconsumption during the refeeding and fattening phases, as previously reported (Birolo et al., 2020a). These facts did not reduce digestive troubles, however, under the conditions of the present trial and despite the use of a post-weaning diet containing antibiotics, the occurrence of ERE in the last two weeks rapidly increased and impaired health in more than one-third of the rabbits. Despite the absence of a significant effect on the feeding programme, a numerically higher mortality was observed in NF, NS, and NI rabbits compared to the other two groups, whereas the contrast analysis showed higher mortality in the restricted than in the non-restricted groups, which needs to be confirmed using a higher number of animals.

Several authors have reported an increased proportion of the full gastrointestinal tract and the liver in feed-restricted rabbits,

mainly because of the speedy filling up of the digestive organs during the refeeding phase (Knudsen et al., 2014, 2017; Crespo et al., 2020). Tümová et al. (2016) described that the large intestine and caecum achieved a higher development and weight in feed-restricted rabbits, either during the restriction or refeeding phase. According to Knudsen et al. (2014), the higher proportion of liver in feed-restricted rabbits, which was also observed in our study, could be associated with an increased demand for glycogen storage. Additionally, the digestive tract's increased weight may lead to an overestimation of growth and feed efficiency in the first refeeding period (Knudsen et al., 2014), as well as a decreased slaughter yield in feed-restricted rabbits (Xiccato, 1999). Nonetheless, none of the feed restriction programmes used in the present study affected the growth rate over the entire period and final live weight, for which neither slaughter results nor carcass and meat quality differed among groups. These findings agree with those of other studies in which mild feed restriction levels or prolonged refeeding phases were applied (Birolo et al., 2016, 2017; Alabiso et al., 2017). Moreover, Salaun et al. (2011) reported that rabbits fed for 12 h from 35 to 66 days of age reached similar slaughter yields as those of rabbits always fed *ad libitum*, as observed for NI rabbits in the present study.

## 5. Conclusions

Under the conditions of the present trial (that is, collective housing systems for growing rabbits), time-based feed restriction was confirmed to affect performance during the restriction period, but rabbits quickly adapted their feed intake to the available feeding time. Thus, at the end of the trial, differences among groups disappeared, with no residual effects on slaughter results, carcass, and meat quality. Nightly access to feed appeared closer to the natural feeding behaviour of rabbits and can be used until the end of the trial with positive results for feed conversion. The pattern of caecal fermentation changes during the day owing to feed restriction with the different programmes must be investigated further because of its possible relationship with rabbit gut health.

## CRedit authorship contribution statement

**Marco Birolo:** Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Angela Trocino:** Conceptualization, Supervision, Validation, Writing – review & editing. **Andrea Zuffellato:** Conceptualization, Validation. **Gerolamo Xiccato:** Conceptualization, Supervision, Validation, Writing – review & editing, Funding acquisition.

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## References

- Alabiso, M., Di Grigoli, A., Mazza, F., Maniaci, G., Vitale, F., Bonanno, A., 2017. A 3-week feed restriction after weaning as an alternative to a medicated diet: effects on growth, health, carcass and meat traits of rabbits of two genotypes. *Animal* 11, 1608–1616. <https://doi.org/10.1017/S175173111600269X>.
- AOAC (Association of Official Analytical Chemists), 2000. *Official Methods of Analysis*, 17th ed. Ass. Off. Analyst. Chemists, Arlington, VA, USA.
- Bellier, R., Gidenne, T., Vernay, M., Colin, M., 1995. In vivo study of circadian variations of the cecal fermentation pattern in post-weaned and adult rabbits. *J. Anim. Sci.* 73, 128–135. <https://doi.org/10.2527/1995.731128x>.
- Bianchi, M., Petracci, M., Pascual, M., Cavani, C., 2007. Comparison between Allo-Kramer and Warner-Bratzler devices to assess rabbit meat tenderness. *Int. J. Anim. Sci.* 6, 749–751. <https://doi.org/10.4081/ijas.2007.1s.749>.
- Birolo, M., Trocino, A., Tazzoli, M., Xiccato, G., 2017. Effect of feed restriction and feeding plans on performance, slaughter traits and body composition of growing rabbits. *World Rabbit Sci.* 25, 113–122. <https://doi.org/10.4995/wrs.2017.6748>.
- Birolo, M., Trocino, A., Zuffellato, A., Xiccato, G., 2016. Effect of feed restriction programs and slaughter age on digestive efficiency, growth performance and body composition of growing rabbits. *Anim. Feed Sci. Technol.* 222, 194–203. <https://doi.org/10.1016/j.anifeedsci.2016.10.014>.
- Birolo, M., Trocino, A., Zuffellato, A., Xiccato, G., 2020a. Effects of time-based feed restriction on morbidity, mortality, performance and meat quality of growing rabbits housed in collective systems. *Animal* 14, 626–635. <https://doi.org/10.1017/S1751731119002283>.
- Birolo, M., Trocino, A., Zuffellato, A., Xiccato, G., 2020b. Time-based feed restriction and group composition in growing rabbits: effects on feed intake pattern, growth performance, carcass traits and meat quality. *Livest. Sci.* 239, 104086 <https://doi.org/10.1016/j.livsci.2020.104086>.
- Blasco, A., Ouhayoun, J., 1996. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. *World Rabbit Sci.* 4, 93–99. <https://doi.org/10.4995/wrs.1996.278>.
- Blasco, A., Ouhayoun, J., Masoero, G., 1993. Harmonization of criteria and terminology in rabbit meat research. *World Rabbit Sci.* 1, 3–10.
- Combes, S., Massip, K., Martin, O., Furbeyre, H., Cauquil, L., Pascal, G., Bouchez, O., Le Floc'h, N., Zemb, O., Oswald, I.P., Gidenne, T., 2017. Impact of feed restriction and housing hygiene conditions on specific and inflammatory immune response, the cecal bacterial community and the survival of young rabbits. *Animal* 11, 854–863. <https://doi.org/10.1017/S1751731116002007>.
- Commission International de l'Éclairage, 1976. *Official recommendations on uniform colours spaces, colour differences equations and metric colours terms*. Supplement No. 2, Publication No. 15, Colorimetry, Paris, France.
- Crespo, R., Alfonso, C., del Barrio, A.S., García-Ruiz, A.I., Marco, M., Nicodemus, N., 2020. Effect of feed restriction on performance, carcass yield and nitrogen and energy balance in growing rabbits. *Livest. Sci.* 241, 104278 <https://doi.org/10.1016/j.livsci.2020.104278>.
- De Blas, J.C., Chamorro, S., García-Alonso, J., García-Rebollar, P., García-Ruiz, A.I., Gómez-Conde, M.S., Menoyo, D., Romero, C., Carabaño, R., 2012. Nutritional digestive disturbances in weaner rabbits. *Anim. Feed Sci. Technol.* 173, 102–110. <https://doi.org/10.1016/j.anifeedsci.2011.12.016>.
- De Blas, J.C., Mateos, G.G., 2010. Feed formulation. In: De Blas, C., Wiseman, J. (Eds.), *Nutrition of the Rabbit*, 2nd edition... CAB International, Wallingford, Oxon, UK, pp. 222–232. <https://doi.org/10.1079/9781845936693.0222>.
- Duperray, J., Guyonvarch, A., 2013. Intérêt d'une mise à jeun quotidienne pour améliorer les performances des lapins en engraissement. *Proc. 15èmes Journ. Rech. Cunic. Le Mans, France.* 43–46.
- EC, 1998. Commission Directive 98/64/EC of 3 September 1998 establishing Community methods of analysis for the determination of amino acids, crude oils and fats, and olaquinox in feeding stuffs and amending Directive 71/393/EEC. *J. Eur. Union.* (<http://publications.europa.eu/en/publication-detail/-/publication/856db9b7-6fd4-4d6e-a778-c766c9fa1776/language-en>). Accessed: November, 2020.

- European Group on Rabbit Nutrition, 2001. Technical note: attempts to harmonize chemical analyses of feeds and faeces, for rabbit feed evaluation. *World Rabbit Sci.* 9, 57–64. <https://doi.org/10.4995/wrs.2001.446>.
- EU, 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes Text with EEA relevance. *J. Eur. Union* 276, 33–79. (<http://data.europa.eu/eli/dir/2010/63/oj>). Accessed: September 2021.
- Foubert, C., Boisot, P., Duperray, J., Guyonvarch, A., 2007. Intérêt d'un accès limité à la mangeoire de 6h, 8h et 10h par jour pour engendrer un rationnement alimentaire chez le lapin en engraissement. *Proc. 12èmes Journ. Rech. Cunic. Le Mans, France.* 123–126.
- Gallois, M., Huërôu-Luron, I.L., Fortun-Lamothe, L., Lallès, J.P., Gidenne, T., 2008. Adaptability of the digestive function according to age at weaning in the rabbit: I. Effect on feed intake and digestive functionality. *Animal* 2, 525–535. <https://doi.org/10.1017/S1751731108001729>.
- Gidenne, T., Combes, S., Feugier, A., Jehl, N., Arveux, P., Boisot, P., Briens, C., Corrent, E., Fortune, H., Montessuy, S., Verdelhan, S., 2009. Feed restriction strategy in the growing rabbit. 2. Impact on digestive health, growth and carcass characteristics. *Animal* 3, 509–515. <https://doi.org/10.1017/S1751731108003790>.
- Gidenne, T., Combes, S., Fortun-Lamothe, L., 2012. Feed intake limitation strategies for the growing rabbit: effect on feeding behaviour, welfare, performance, digestive physiology and health: a review. *Animal* 6, 1407–1419. <https://doi.org/10.1017/S1751731112000389>.
- Gidenne, T., Feugier, A., 2009. Feed restriction strategy in the growing rabbit. 1. Impact on digestion, rate of passage and microbial activity. *Animal* 43, 501–508. <https://doi.org/10.1017/S1751731108003789>.
- Gidenne, T., Fortun-Lamothe, L., 2002. Feeding strategy for young rabbits around weaning: a review of digestive capacity and nutritional needs. *Anim. Sci.* 75, 169–184. <https://doi.org/10.1017/S1357729800052942>.
- Gidenne, T., Garreau, H., Drouilhet, L., Aubert, C., Maertens, L., 2017. Improving feed efficiency in rabbit production, a review on nutritional, technico-economical, genetic and environmental aspects. *Anim. Feed Sci. Technol.* 225, 109–122. <https://doi.org/10.1016/j.anifeedsci.2017.01.016>.
- Gidenne, T., Lebas, F., 2005. Le comportement alimentaire du lapin. *Proc. 11èmes Journ. Rech. Cunic. Paris, France.* 29–32.
- Gidenne, T., Lebas, F., Fortun-Lamothe, L., 2010. Feeding behaviour of rabbits. In: De Blas, C., Wiseman, J. (Eds.), *Nutrition of the Rabbit*, 2nd edition., CAB International, Wallingford, Oxon, UK, pp. 233–252. <https://doi.org/10.1079/9781845936693.0222>.
- Knudsen, C., Combes, S., Briens, C., Coutelet, G., Duperray, J., Rebours, G., Salaun, J.M., Travel, A., Weissman, D., Gidenne, T., 2014. Increasing the digestible energy intake under a restriction strategy improves the feed conversion ratio of the growing rabbit without negatively impacting the health status. *Livest. Sci.* 169, 96–105. <https://doi.org/10.1016/j.livsci.2014.08.015>.
- Knudsen, C., Combes, S., Briens, C., Coutelet, G., Duperray, J., Rebours, G., Salaun, J.M., Travel, A., Weissman, D., Gidenne, T., 2017. Substituting starch with digestible fiber does not impact on health status or growth in restricted fed rabbits. *Anim. Feed Sci. Technol.* 226, 152–161. <https://doi.org/10.1016/j.anifeedsci.2017.01.002>.
- Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *J. AOAC Int.* 85, 1217–1240.
- Osl, F., 1988. Bestimmung der niederen freien Fettsäuren im Hart-und Schnittkäse mit der Head-Space Gaschromatographie. *Dtsch. Mol. Ztg.* 109, 1516–1518.
- Perez, J.M., Lebas, F., Gidenne, T., Maertens, L., Xiccato, G., Parigi Bini, R., Dalle Zotte, A., Cossu, M.E., Carazzolo, A., Villamide, M.J., Carabaño, R., Fraga, M.J., Ramos, M.A., Cervera, C., Blas, E., Fernández, J., Falcão-e-Cunha, L., Bengala Freire, J., 1995. European reference method for in vivo determination of diet digestibility in rabbits. *World Rabbit Sci.* 3, 41–43. <https://doi.org/10.4995/wrs.1995.239>.
- Romero, C., Cuesta, S., Astillero, J.R., Nicodemus, N., De Blas, J.C., 2010. Effect of early feed restriction on performance and health status in growing rabbits slaughtered at 2 kg live-weight. *World Rabbit Sci.* 18, 211–218. <https://doi.org/10.4995/wrs.2010.778>.
- Salaun, J.M., Renouf, B., Bourdillon, A., Picot, A., Perdriau, A., 2011. Comparaison d'un accès nocturne à la mangeoire à un rationnement progressif et à une alimentation *ad libitum* sur les composantes du rendement carcasse des lapins en engraissement. *Proc. 14èmes Journ. Rech. Cunic. Le Mans, France.* 101–104.
- SAS (Statistical Analysis System Institute, Inc.), 2013. SAS/STAT(R) 9.2 User's Guide, second ed. SAS Institute Inc., Cary, NC, USA (Available at). ([http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#glm\\_toc.htm](http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#glm_toc.htm)).
- Trocino, A., Fragkiadakis, M., Majolini, D., Carabaño, R., Xiccato, G., 2011. Effect of the increase of dietary starch and soluble fibre on digestive efficiency and growth performance of meat rabbits. *Anim. Feed Sci. Technol.* 165, 265–277. <https://doi.org/10.1016/j.anifeedsci.2011.03.008>.
- Tůmová, E., Volek, Z., Chodová, D., Hártilová, H., Makovickya, P., Svobodová, J., Ebeid, T.A., Uhlřřová, L., 2016. The effect of 1-week feed restriction on performance, digestibility of nutrients and digestive system development in the growing rabbit. *Animal* 10, 1–9. <https://doi.org/10.1017/S1751731115001810>.
- Uhlřřová, L., Volek, Z., Marounek, M., Tůmová, E., 2015. Effect of feed restriction and different crude protein sources on the performance, health status and carcass traits of growing rabbits. *World Rabbit Sci.* 23, 263–272. <https://doi.org/10.4995/wrs.2015.3229>.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2).
- Weissman, D., Troislouches, G., Picard, E., Davoust, C., Leroux, C., Launay, C., 2009. Amélioration de l'indice de consommation de lapins en engraissement par une distribution nocturne de l'aliment. *Proc. 13èmes Journ. Rech. Cunic. Le Mans, France.* 47–49.
- Xiccato, G., 1999. Feeding and meat quality in rabbits: a review. *World Rabbit Sci.* 7, 75–86. <https://doi.org/10.4995/wrs.1999.383>.