


Effects of time-based feed restriction on morbidity, mortality, performance and meat quality of growing rabbits housed in collective systems

M. Birolo¹ , A. Trocino^{2†} , A. Zuffellato³ and G. Xiccato¹ 

¹Department of Agronomy Food Natural Resources Animal and Environment (DAFNAE), University of Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy;

²Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy; ³A.I.A. Agricola Italiana Alimentare S.p.A., Piazzale Apollinare Veronesi, I-37036 San Martino Buon Albergo, Verona, Italy

(Received 29 December 2018; Accepted 21 August 2019)

In rabbit farms, quantitative feed restriction in the post-weaning period is widely used with the aim of reducing the impact of digestive diseases, whereas less information is available about feed restriction strategies based on the reduction of access time to feeders in different housing systems. This study compared morbidity, mortality, growth performance, carcass characteristics and meat quality of 368 crossbred rabbits fed ad libitum (L) or subjected to a time-based feed restriction programme (R) and housed from 31 to 73 days of age in cages or pens with different dimensions and group sizes, that is, eight conventional cages (0.33 m², six rabbits/cage), eight small open-top pens (0.50 m², eight rabbits/pen), eight medium open-top pens (1.00 m², 16 rabbits/pen) and four large open-top pens (2.00 m², 32 rabbits/pen). Feed restriction was attained by progressively reducing the access time to feeders in the 1st week from 14 to 8 h/day, maintaining 8 h in the 2nd week and then by increasing access time by 1 h/day during the 3rd and 4th week up to 24 h/day. In the first 2 weeks, R rabbits showed a lower ($P \leq 0.001$) daily weight gain, feed intake and feed conversion as compared with L rabbits. During the 3rd and 4th weeks, R rabbits exhibited a greater daily weight gain and better feed conversion ($P \leq 0.001$). In the last 2 weeks of trial, daily weight gain tended ($P = 0.06$) to be greater in the R than L rabbits. In the whole trial, R rabbits manifested a lower daily weight gain, feed intake and feed conversion, as well as lower final live weight and the carcass dressing percentage at slaughter ($0.05 \leq P \leq 0.01$). During feed restriction, R rabbits did not show digestive problems, which, however, appeared in the following 2 weeks of refeeding. Thus, R rabbits had a higher health risk index in the whole trial as compared with L rabbits ($P \leq 0.05$). The housing system did not affect growth performance, characteristics at slaughter, and carcass and meat quality. Mortality tended to increase with group size ($P = 0.06$). In conclusion, the time-based feed restriction significantly improved feed efficiency of growing rabbits housed collectively but had somewhat negative effects on characteristics at slaughter and on morbidity and mortality rate.

Keywords: cage, pen, feeding system, mortality rate, carcass quality

Implications

This study shows that time-based feed restriction can improve feed efficiency of growing rabbits during the whole growth period. Nevertheless, it also increases the risk of digestive diseases during the refeeding phase and impairs slaughter weight and the dressing percentage, thus reducing the convenience of this approach as compared to *ad libitum* feeding. Time-based feed restriction yields consistent results in different types of group housing, cages or pens regardless of group size.

Introduction

Over the last decade, feed efficiency on rabbit farms was greatly enhanced through progress in genetics, management and feeding techniques, but further improvements are required in view of farm economic sustainability (Gidenne *et al.*, 2017). Among different strategies, feed restriction in the post-weaning period is widely used with the aim of reducing the impact of digestive diseases, in particular epizootic rabbit enteropathy (ERE) (Gidenne *et al.*, 2012). The physiological mechanisms underlying these effects have not yet been clarified. Nevertheless, in growing rabbits, feed restriction impacts the caecal fatty acids content, the pH of the caecal content and the abundance of some genera in the

[†] E-mail: angela.trocino@unipd.it

caecal microbiota (Combes *et al.*, 2017). Nonetheless, severe restriction levels ($\leq 80\%$ of the *ad libitum* level) during 3 or 4 weeks after weaning are generally necessary to reduce mortality and morbidity rates (Gidenne *et al.*, 2009 and 2012), which unavoidably reduce final live weight and carcass weights as well as dressing percentages and main cut proportions (Xiccato, 1999; Knudsen *et al.*, 2017). Nevertheless, even mild rationing ($\sim 90\%$ of the *ad libitum* level) followed by a gradual refeeding phase can be effective in enhancing digestive health without detrimental effects on growth performance and characteristics at slaughter (Birolo *et al.*, 2016). Indeed, the restriction technique itself plays a major role in its success and its implementation in the field. In the case of a quantitative feed restriction, the quantity of feed to be distributed may require several adjustments according to animal genotypes and live weights, diet composition and environmental conditions. Moreover, the refeeding technique after the restriction phase (whether gradual or sudden) may affect a rabbit's feed intake and its digestive equilibrium (Birolo *et al.*, 2016).

Thus, to overcome these limits, restriction strategies may be based on a reduction of access time to feeders. In fact, some authors successfully restricted rabbit feeding by this technique with positive effects on health status (Romero *et al.*, 2010) and feed efficiency (Foubert *et al.*, 2007; Salaun *et al.*, 2011). On the other hand, more information is needed to identify the optimal parameters of the time-based feed restriction in different field conditions and housing systems. To our knowledge, data about the use of feed restriction and its effects in growing rabbits kept in different types of group housing with different group sizes represent a gap in the literature, whereas the use of these alternative housing systems is required to improve rabbit welfare.

Thus, the present study was aimed at evaluating morbidity and mortality rates, growth performance, carcass characteristics and meat quality of rabbits subjected to two feeding programmes (*ad libitum* feeding *v.* time-based feed restriction) reared in standard conventional cages (6 rabbits/cage, 18 rabbits/m²) or in collective pens at different group sizes (8, 16, and 32 rabbits/pen), but at the same stocking density (16 rabbits/m²).

Materials and methods

Animals and experimental conditions

The trial was conducted on the rabbit farm of the University of Padova, in a closed shelter from February to April during a natural photoperiod (about 12 h of lightness, 0600 to 1800 h and 12 h of darkness, 1800 to 0600 h). Extraction fans and an automatic heating system controlled air circulation and maintained temperature within 20°C to 23°C. A total of 368 cross-bred rabbits (Hypharm, Groupe Grimaud, Roussay, France), half males and half females, were selected on a commercial farm from healthy litters of multiparous does (three to six kindling) and were moved to the experimental farm. The animals were identified by an ear mark and were allocated to eight experimental groups, balanced for mean and SD of live weight, according to a bi-factorial arrangement with two feeding programmes (L: *ad libitum* feeding, 184 rabbits; R: time-based feed restriction, 184 rabbits) and four housing systems (C06: conventional cages, 0.33 m², six rabbits/cage; P08: small open-top pens, 0.50 m², eight rabbits/pen; P16: medium open-top pens, 1.00 m², 16 rabbits/pen; P32: large open-top pens, 2.00 m², 32 rabbits/pen). The housing conditions are detailed in Table 1.

The conventional cages contained a wire net floor (hole dimensions: 70 mm long \times 10 mm wide; wire diameter: 3 mm). The pens had a plastic slat floor (hole dimensions: 70 mm long \times 10 mm wide; distance between the holes: 7 mm). The plastic slat floor guaranteed the complete dropping of faeces out of the pens. Cages and pens were equipped with automatic nipple drinkers (two per cage; two, four or eight in the small, medium and large pens, respectively) and feeders for the manual distribution of feed (60 mm of access space per rabbit in standard cages; 75 mm of access space per rabbit in the pens). The male-to-female ratio in both cages and pens was 1 : 1. Rabbits were monitored from weaning to commercial slaughter (31 to 73 days of age).

Diets and feeding programmes

The animals had free access to fresh water during the whole trial. Two diets were used, which were formulated to satisfy the requirements of rabbits during post-weaning and fattening (De Blas and Mateos, 2010). Due to the presence of

Table 1 Housing conditions

Housing system	Replicates (n)	Cage or pen dimensions w \times l \times h (m)	Rabbits per cage or pen (n)	Total surface (m ²)	Stocking density (animals/m ²)
C06 – standard cage	8	0.87 \times 0.38 \times 0.42	6	0.33	18
P08 – small open-top pen	8	0.64 \times 0.78	8	0.50	16
P16 – medium open-top pen	8	1.28 \times 0.78	16	1.00	16
P32 – large open-top pen	4	2.56 \times 0.78	32	2.00	16

Table 2 *Ingredients and chemical composition (g/kg) of the experimental diets*

Diets	Post-weaning	Fattening
Age (d)	31 to 58	59 to 73
<i>Ingredients</i>		
Dehydrated alfalfa meal	340	253
Wheat bran	190	240
Barley	120	160
Dried beet pulp	190	160
Soybean meal 49%	50	40
Sunflower meal 30%	70	100
Sunflower oil	10	15
Molasses	15	15
Calcium carbonate	1.0	4.0
Dicalcium phosphate	3.5	3.0
Sodium chloride	4.0	4.0
L-lysine HCl	1.0	1.0
DL-methionine	1.0	1.0
Vitamin–mineral premix ¹	4.0	4.0
Drugs ²	0.5	–
<i>Chemical composition</i>		
DM	879	880
CP	153	152
Ether extract	31	37
Crude fibre	166	151
Ash	68	66
NDF	357	348
ADF	197	188
ADL	50	50
Starch	112	131
Lysine ³	7.8	7.5
Methionine + cystine ³	6.2	6.4
Ca ³	8.3	7.9
P ³	5.2	5.7
Na ³	2.3	2.2
Digestible energy ³ (MJ/kg)	10.13	10.50

¹ Supplementation per kilogram of feed: vitamin A, 12 000 IU; vitamin D₃, 1000 IU; vitamin E acetate, 50 mg; vitamin K₃, 2 mg; biotin, 0.1 mg; thiamine, 2 mg; riboflavin, 4 mg; vitamin B₆, 2 mg; vitamin B₁₂, 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.

² Coccidiostat: diclazuril, 1 mg/kg; antibiotic: oxytetracycline, 1450 mg/kg.

³ Calculated values (Maertens *et al.*, 2002).

ERE on the commercial farm where the rabbits were born, in the first 4 weeks of the trial (from 31 to 58 days of age), all rabbits received the post-weaning diet supplemented with an antibiotic (oxytetracycline, 1450 mg/kg) and a coccidiostat (diclazuril, 1 mg/kg). In the last 2 weeks (from 59 to 73 days of age), the fattening diet without antibiotics and coccidiostats was provided. The ingredients and chemical composition of the two experimental diets are listed in Table 2. The experimental diets were pelleted (3.5 mm diameter and 10 to 11 mm length of pellets) and supplemented with L-lysine-HCl and DL-methionine, vitamins and macro- and micro-minerals.

Feed restriction for R rabbits was realised by varying the access time to the feeders according to the following programme: a gradual decrease from 14 to 8 h/day during

the 1st week of the trial (31 to 37 days of age); constant access time of 8 h/day during the 2nd week (up to 44 days of age); a gradual increase (+1 h/ to 24 h/day) of access time from the beginning of the 3rd week until the beginning of the 5th week (59 days of age; Figure 1). After that, all the rabbits had free access to feed until the end of the trial. To address the natural feeding behaviour of the rabbit, the access to feed was permitted mainly during the hours of darkness by manually closing the feeders in the morning (0600 to 0900 h) and re-opening these in the evening (1900 to 2200 h) during the first 2 weeks; from the beginning of the 3rd week, the time of closing was progressively postponed and the time of opening progressively anticipated. This programme was designed on the basis of our previous observations (not published) to decrease the feeding level of R rabbits from 80% to 70% of the *ad libitum* level in the 1st week, then to increase it from 70% to 80% in the 2nd week, and from 80% to the *ad libitum* level during the 3rd and 4th week, without sharp fluctuations in feed intake.

Growth performance and morbidity and mortality rates

During the trial, individual live weight was recorded once a week, whereas feed intake in cages and pens was measured daily. Morbidity and mortality were monitored daily: rabbits were considered ill when showing diarrhoea and/or mucus in the faeces or a live weight loss during a week. In the calculation of morbidity, the ill rabbits were taken into account 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 sum of morbidity and mortality rates (Gidenne *et al.*, 2009).

Commercial slaughter and determination of carcass and meat quality

At 73 days of age, the rabbits were weighed on the experimental farm after 6 h fasting, according to current slaughterhouse practices. Then they were transported to a commercial slaughterhouse by an authorised truck (~1 h of transport). The slaughtering took place after ~1 h lairage in the slaughterhouse; rabbits were individually weighed, stunned by electroanaesthesia and killed by throat slitting. After 2.5 h chilling, the commercial carcasses were weighed to calculate the individual dressing percentages. A total of 112 carcasses (8 L and 8 R carcasses for cage-housed rabbits and 16 L and 16 R carcasses for each pen-housing system), representative of their experimental groups in terms of mean and SD of live weight, were selected, transported to the department laboratory and stored at 3°C to 4°C. After 24 h, the chilled carcasses were dissected and meat quality was analysed. To obtain the reference carcass weight, the main cuts for meat quality analyses and the meat-to-bone ratio of the hind leg, the harmonised dissection procedures described by Blasco and Ouhayon (1996) were used.

Next, pH of the *longissimus lumborum* muscles was measured in duplicate with a pH meter (Basic 20; Crison Instruments SA, Carpi, Italy) equipped with a specific electrode (cat. # 5232; Crison Instruments SA). CIE L*, a* and b* colour indexes were measured in duplicate in the

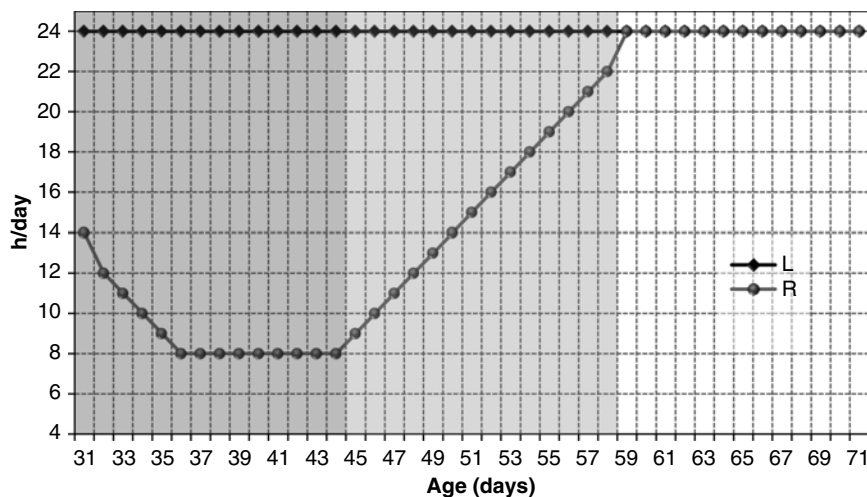


Figure 1 Daily access time to the feeders of *ad libitum*-fed (L) and feed-restricted rabbits (R) from weaning to the day before slaughter. Dark grey, light grey and white areas correspond to phases 'feed restriction', 'refeeding' and '*ad libitum* feeding', respectively.

same muscles using a Minolta CM-508 C spectrophotometer (Minolta Corp., Ramsey, NJ, USA). The hind legs were dissected, and the muscles were separated from the bones to measure the meat-to-bone ratio (Blasco and Ouhayoun, 1996). The *longissimus lumborum* muscles were also separated from the carcasses and vacuum packaged and stored at -18°C until water holding capacity determined as thaw and cooking loss and shear force was assessed. After thawing for 12 h at room temperature, the whole muscles were kept in plastic bags and cooked in a water bath for 1.0 h at 80°C . After a 1 h cooling period at room temperature, the *longissimus lumborum* was cut to collect the middle part (length: 70 mm). On this section, the maximum shear force was measured by means of a TA.HDI dynamometer (LS5; Lloyd Instruments Ltd., Bognor Regis, UK) with the Allo-Kramer (10 blades) probe (load cell: 500 kg, distance between the blades: 5 mm, thickness of blades: 2 mm, and cutting speed: 500 mm/min).

Chemical analyses

The diets were analysed to determine the concentrations of DM (934.01), ash (967.05), CP (2001.11) and starch (amylglucosidase α amylase method, 996.11) by Association of Official Analytical Chemists (AOAC, 2000) methods. Ether extract was analysed after acid hydrolysis (European Commission, 1998). The fibre fractions, that is, NDF (without sodium sulphite and inclusive of residual ash), ADF (inclusive of residual ash) and ADL (obtained by solubilisation of cellulose with sulphuric acid), were analysed according to Mertens (2002), AOAC (2000, 973.187) and Van Soest *et al.* (1991), respectively, using the sequential procedure and the filter bag system (Ankom Technology, Macedon, NY, USA).

Statistical analyses

The individual data on performance, characteristics at slaughter and carcass and meat quality were analysed by two-way ANOVA using PROC MIXED of SAS (Statistical Analysis System Institute, 2013). The model included the

feeding programme (L and R), the housing system (C06, P08, P16 and P32) and their interaction as main effects with the cage or pen as a random effect. The cage and pen data for feed intake and feed conversion were analysed by two-way ANOVA via the PROC GLM of SAS and the feeding programme, housing system, and their interaction as the main effects. The data of daily feed intake were analysed by the PROC MIXED of SAS with a repeated measures model which included the feeding programme, housing system, day and their interactions as main effects. A heterogeneous autoregressive model was chosen after checking the goodness of fit criteria compared to other covariance structures. Mortality, morbidity and health risk were analysed with an ordinal repeated measures model by PROC GENMODE of SAS, with the feeding programme, housing system, day and their interactions as the main effects. Least-square means are given in tables. The Bonferroni's test was used to compare means. Differences among means with $P < 0.05$ were assumed to be statistically significant. Differences among means with $0.05 < P < 0.10$ were regarded as approaching significance.

Results

Morbidity and mortality rates

During the trial, 32 animals died: 1 from group L-C06, 1 from group L-P08, 5 from group L-P16, 5 from group L-P32, 2 from group R-P08, 9 from group R-P16, and 9 from group R-P32. An average mortality rate of 8.7% was registered. All cases of illness and death were caused by digestive diseases characterised by the typical symptoms of ERE. Mortality and morbidity rates were similar between the L and R groups, but the latter showed a higher health risk index compared to the former (+4.4 percentage points; $P \leq 0.05$; Table 3). With respect to the housing system, mortality tended to increase from rabbits housed in conventional cages or pens in a small group to those kept in pens in large groups (2.1% and 4.7% in C06 and P08, 10.9% in P16 and P32; $P = 0.06$). The health

Table 3 Effects of a feeding programme and housing system on the sanitary status of rabbits from weaning (31 days of age) to slaughter (73 days of age)

	Feeding programme (F)		Housing system (H)				Probability		
	L	R	C06	P08	P16	P32	F	H	F × H
Initial rabbits (n)	184	184	48	64	128	128			
Final rabbits (n)	172	164	47	61	114	114			
Mortality (%)	6.5	10.9	2.1	4.7	10.9	10.9	0.13	0.06	0.55
Morbidity ¹ (%)	5.4	5.4	10.4	1.6	3.9	7.0	0.31	0.20	0.23
Health risk index ² (%)	11.9	16.3	12.5	6.3	14.8	18.0	0.05	0.08	0.38

L = *ad libitum*-fed rabbits; R = feed-restricted rabbits; C = multifunctional cages with 6 rabbits/cage (stocking density: 18 animals/m²); P = open-top pens with 8, 16 and 32 rabbits/pen (stocking density: 16 animals/m²).

¹In the calculation of morbidity, the ill rabbits were taken into account once, even if symptoms lasted several days.

²Health risk index = mortality rate + morbidity rate (Gidenne *et al.*, 2009).

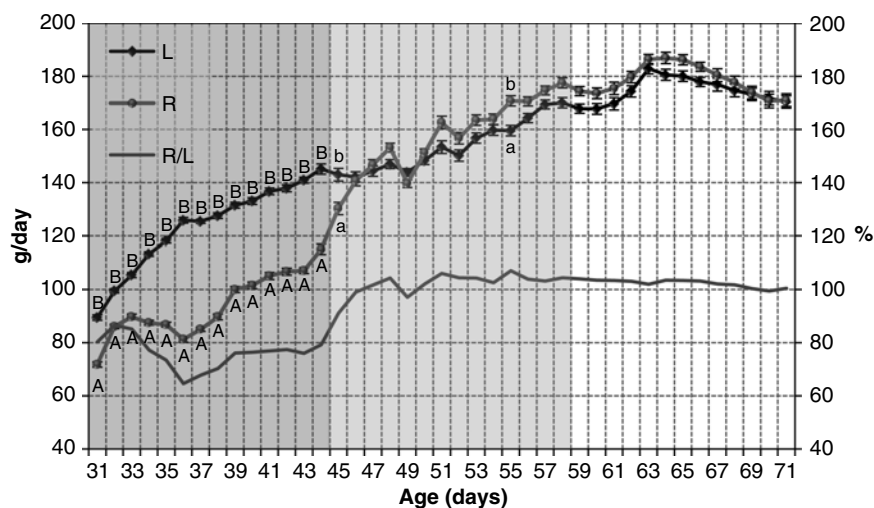


Figure 2 Measured daily feed intake (Least-square-means \pm standard error) of *ad libitum* (L) and restricted (R) rabbits and feed restriction level (R/L, %) from weaning to the day before commercial slaughter. Dark grey, light grey and white areas correspond to feed restriction, refeeding and *ad libitum* phases, respectively. Means with different superscript letters are statistically different (a, b: $P < 0.05$; A, B: $P < 0.001$).

risk index varied from 12.5% to 6.3%, 14.8% and 18.0% from C06 to P08, P16 and P32 rabbits ($P = 0.08$).

Growth performance, slaughter data, carcass characteristics and meat quality

In our trial, the gradual reduction in access time in the 1st week from 14 to 8 h/day decreased feed intake from 80% to 70% of the *ad libitum* level; maintenance of 8 h/day access time in the 2nd week raised the feeding level to 80%, whereas the increase in access time by 1 h/day during the 3rd week changed the intake level from 80% to the *ad libitum* level only after 3 days (11 h/day access time) (Figure 2). Therefore, the present trial involved a rather severe, but progressive feed restriction programme (from 80% to 70% of the *ad libitum* level) followed by gradual refeeding (+1 h/day) to avoid abrupt changes in rabbits' feed intake from one day to another. The feed restriction programme affected ($P \leq 0.001$) growth performance during the restriction period (first 2 weeks of the trial) when R rabbits showed a smaller daily weight gain (-15.7%), feed intake (-23.6%) and live weight (-7.9%) and improved feed

conversion (-10.9%) as compared with L rabbits (Table 4). During the refeeding period (3rd and 4th weeks of the trial), R rabbits showed a better feed conversion (-6.9% ; $P \leq 0.001$) and higher daily weight gains ($+10.1\%$; $P \leq 0.001$) as compared with L rabbits with a similar feed consumption. The gap in live weight at the end of refeeding (59 days of age) remained significant (2266 v. 2206 g for the L and R groups, respectively; $P \leq 0.05$; Table 4). When all rabbits had free access to feed (5th and 6th week of the trial), daily growth tended ($P = 0.06$) to be greater in the R than L rabbits. In the whole trial, the restriction programme decreased the daily weight gain (-1.2 g/day; $P \leq 0.05$) and feed intake (-8 g/day; $P \leq 0.001$), but feed conversion was enhanced (3.12 v. 3.04; $P \leq 0.001$), and final live weight only tended to decrease (-1.8% ; $P = 0.06$) relative to the *ad libitum* feeding.

Regarding the housing system, during the first 2 weeks, rabbits kept in standard cages achieved a higher daily weight gain ($P \leq 0.05$) and better feed conversion ($P \leq 0.01$) in comparison with rabbits in pens (Table 4). In the last 2 weeks of the trial, an opposite trend was observed: a higher weight

Table 4 Effects of a feeding programme and housing system on rabbit growth performance from weaning (31 days age) to slaughter (73 days age)

	Feeding programme (F)		Housing system (H)				Probability			RSD
	L	R	C06	P08	P16	P32	F	H	F × H	
Rabbits (n)	172	164	47	61	114	114				
Cages/pens (n)	14	14	8	8	8	4				
Live weight ¹ (g)										
At 31 days	815	812	817	816	810	812	0.57	0.84	0.91	50
At 45 days	1602	1475	1570	1538	1528	1519	<0.001	0.07	0.43	115
At 59 days	2266	2206	2246	2249	2221	2228	0.02	0.83	0.31	192
At 73 days	2819	2767	2759	2812	2801	2799	0.06	0.64	0.30	203
Restriction period (31 to 44 days)										
Weight gain ¹ (g/day)	56.2	47.4	53.8 ^b	51.6 ^{ab}	51.3 ^{ab}	50.5 ^a	<0.001	0.05	0.19	6.8
Feed intake ² (g/day)	123	94	109	109	108	107	<0.001	0.34	0.22	3
Feed conversion ² (g/g)	2.21	1.97	2.03 ^a	2.11 ^b	2.11 ^b	2.12 ^b	<0.001	<0.01	0.28	0.04
Refeeding period (45 to 58 days)										
Weight gain ¹ (g/day)	47.4	52.2	48.3	50.7	49.6	50.7	<0.001	0.48	0.20	9.0
Feed intake ² (g/day)	151	152	150	155	151	152	0.53	0.50	0.08	6
Feed conversion ² (g/g)	3.19	2.97	3.14	3.07	3.08	3.01	<0.001	0.25	0.17	0.10
Ad libitum period (59 to 73 days)										
Weight gain ¹ (g/day)	39.5	40.0	36.6 ^a	40.3 ^b	41.4 ^b	40.8 ^b	0.06	0.01	0.95	6.7
Feed intake ² (g/day)	170	171	161 ^a	171 ^{ab}	173 ^b	175 ^b	0.69	0.01	0.67	7
Feed conversion ² (g/g)	4.56	4.55	4.56	4.51	4.48	4.67	0.92	0.77	0.47	0.30
Whole period (31 to 73 days)										
Weight gain ¹ (g/day)	47.7	46.5	46.2	47.5	47.4	47.3	0.05	0.49	0.21	4.5
Feed intake ² (g/day)	149	141	143	145	145	147	<0.001	0.24	0.36	3
Feed conversion ² (g/g)	3.12	3.04	3.09	3.05	3.07	3.11	0.001	0.33	0.12	0.05

L = *ad libitum*- rabbits; R = feed-restricted rabbits; C = standard cages with six rabbits/cage (stocking density: 18 animals/m²); P = small, medium and large open-top pens with 8, 16 and 32 rabbits/pen (stocking density: 16 animals/m²).

^{a,b}Means with different superscript letters are statistically different ($P < 0.05$).

¹Weight gain was determined from each individual rabbit.

²Feed intake and feed conversion were determined on a pen basis; the average weight gain of the pen was used for the calculation of the feed conversion.

gain and feed intake ($P \leq 0.01$) in rabbits in pens as compared to those in standard cages. Nevertheless, considering the whole trial, the housing system had no effect on rabbit growth performance regardless of the feeding programme. The feed restriction reduced rabbit slaughter weight (-2.0% ; $P \leq 0.05$), cold carcass weight (-2.8% ; $P \leq 0.01$) and the dressing percentage (-0.5 percentage points; $P \leq 0.01$) relative to the *ad libitum* feeding but did not affect carcass and meat quality (Table 5).

Discussion

Effects of the feeding programme

The restriction rate, timing and duration as well as refeeding duration may affect the final results in terms of growth performance, carcass characteristics and meat quality in growing rabbits (Xiccato, 1999). Time-based feed restriction has been previously used in programmes with restricted access time (6 to 10 h/day) to feeders in the first 2 to 3 post-weaning weeks and free access in the last 2 to 3 fattening weeks (Foubert *et al.*, 2007; Romero *et al.*, 2010). Other authors (Salaun *et al.*, 2011) restricted access time to feeders to 12 to 14 h/day during the whole rearing period or permitted

free access only a few days before slaughter. Nevertheless, to our knowledge, no data have been published on restriction programmes based on gradual restriction and refeeding.

According to Gidenne *et al.* (2010), when access time is lower than 14 to 16 h/day, feed intake decreases, and 8 h/day access time yields a $\sim 80\%$ restriction rate compared to *ad libitum* feeding. Moreover, the reduction is greater in younger than in older rabbits, whereas the latter progressively adjust their ingestion to the available time therefore making feed restriction less severe. Indeed, Foubert *et al.* (2007) observed that a reduction in the access time to 6, 8 and 10 h/day during the first 3 post-weaning weeks led to restriction rates 63%, 74% and 80% of the *ad libitum* level, respectively. These changes confirm the ability of the rabbits to rapidly adapt its hourly feeding rate to the effective time available for feeding (Gidenne *et al.*, 2010).

Both in the study by Foubert *et al.* (2007) and in the present one, time-based feed restriction significantly improved feed conversion during the restriction as well as in the refeeding period, which had residual positive effects during the whole trial, whereas growth and final live weight decreased. These results are consistent with those of Knudsen *et al.* (2014 and 2017) on rabbits subjected to quantitative feed restriction (75% of the *ad libitum* level) during

Table 5 Effects of a feeding programme and housing system on characteristics at slaughter, main characteristics of carcasses chilled for 24 h, and meat quality parameters of growing rabbits

	Feeding programme (F)		Housing system (H)				Probability			RSD
	L	R	C06	P08	P16	P32	F	H	F × H	
Rabbits (n)	172	164	47	61	114	114				
SW (g)	2750	2695	2689	2741	2731	2729	0.03	0.61	0.24	196
CW ¹ (g)	1694	1646	1654	1677	1677	1673	0.01	0.80	0.14	134
Dressing percentage ² (% SW)	61.6	61.1	61.5	61.2	61.4	61.3	0.01	0.78	0.40	1.7
Carcasses (n)	56	56	16	32	32	32				
Head (% CW)	7.7	7.8	7.7	7.8	7.7	7.9	0.38	0.58	0.15	0.5
Liver (% CW)	4.3	4.4	4.2	4.2	4.6	4.4	0.35	0.06	0.89	0.7
RC ³ (g)	1438	1399	1434	1413	1415	1413	0.08	0.92	0.18	109
Dissectible fat (% RC)	2.7	2.6	2.9	2.5	2.5	2.7	0.38	0.29	0.13	0.8
Hind leg muscle-to-bone ratio	5.88	5.79	6.05	5.81	5.69	5.78	0.30	0.11	0.11	0.48
<i>Longissimus lumborum</i>										
pH	5.54	5.56	5.53	5.55	5.54	5.57	0.27	0.52	0.54	0.09
L*	53.3	52.6	53.2	52.1	53.4	53.0	0.14	0.19	0.28	2.2
a*	-1.91	-2.02	-1.84	-2.06	-1.98	-1.98	0.34	0.63	0.92	0.56
b*	1.23	1.21	1.08	1.60	1.35	0.84	0.98	0.54	0.97	1.68
Thawing losses (%)	7.4	6.9	6.4	7.0	7.3	7.8	0.26	0.18	0.48	2.1
Cooking losses (%)	34.9	35.1	35.7	34.4	34.7	35.1	0.52	0.21	0.66	1.8
Shear force (kg/g)	5.08	4.98	5.33	5.08	5.01	4.70	0.61	0.18	0.93	0.98

L = *ad libitum* rabbits; R = feed-restricted rabbits; C = multifunctional cages with 6 rabbits/cage (stocking density: 18 animals/m²); P = open-top pens with 8, 16 and 32 rabbits/pen (stocking density: 16 animals/m²); SW = slaughter weight; CW = cold carcass weight; RC = reference carcass; L*, a* and b* = CIE colour indexes.

¹Weight of the carcass after 2.5 h of chilling in a ventilated cold room (0°C to 2°C).

²Cold carcass weight divided by slaughter weight × 100.

³Cold carcass weight minus the weight of liver, kidneys, thymus, trachea, oesophagus, lungs and heart.

4 post-weaning weeks and refeeding in the last week before slaughter. Surely, in our study, restriction rates were not too severe, and the long refeeding duration allowed the animals to partially recover their performance with an overall improvement of feed conversion (−2.6%) despite a decrease in final live weight (−1.8%) as compared to L groups. These results are in agreement with those reported in other studies involving different restriction strategies, but similar feed restriction rates (Salaun *et al.*, 2011; Birolo *et al.*, 2016 and 2017).

The improvement of feed conversion in the feed-restricted animals has been associated with increased nutrient digestibility in the restriction period and with compensatory growth during refeeding (Knudsen *et al.*, 2014 and 2017). In fact, as for broiler chickens, in the latter period, maintenance costs are likely lower and the proportion of nutrients available for growth increases (Sahraei, 2012). Indeed, some studies on rabbits have detected no changes in digestive efficiency when previously feed-restricted rabbits returned to *ad libitum* feeding (Knudsen *et al.*, 2014 and 2017; Birolo *et al.*, 2016).

Under field conditions, among the strategies for ERE control, farmers have successfully used feed restriction to reduce morbidity and mortality of growing rabbits (Gidenne *et al.*, 2017). By contrast, sudden changes in feed intake from restricted to *ad libitum* feeding might negatively affect the health of rabbits by increasing the risk of digestive diseases in the whole rearing period (Knudsen *et al.*, 2017). Nevertheless, in our trial, the gradual increase of feed access time did not completely prevent ingestion peaks, and R

rabbits sharply increased their feed intake since the first days of refeeding (Figure 2). Thus, R rabbits started to fall ill a few days after refeeding and reached a morbidity peak after 10 days (Figure 3A and 3B) despite the administration of a medicated diet. Then, in the last period, when all rabbits received a non-medicated fattening diet, mortality of R rabbits continued to increase until the end of the trial. On the other hand, L rabbits fell ill few days after the start of the trial, reached the morbidity peak within the 2nd week, but their health recovered by the 4th week, with small increases in mortality during the last 2 weeks of the trial. Indeed, severe feed restriction rates (≤80% of the *ad libitum* level) generally succeed in reducing digestive diseases in growing rabbits (Gidenne *et al.*, 2009; Knudsen *et al.*, 2014 and 2017), but the protective effect often disappears when animals return to *ad libitum* feeding (Gidenne *et al.*, 2012; Alabiso *et al.*, 2016). This fact must be considered unfavourable because antibiotic use is not permitted during the last period of growth, and the economic impact of animal losses is higher in the last rearing phase (Gidenne *et al.*, 2017).

Regarding carcass quality, restricted feeding levels (from 50% to 80% of the *ad libitum* level) during post-weaning are generally responsible for decreased final live weight, carcass weight, dressing percentage and carcass fatness as compared to *ad libitum* feeding (Xiccato, 1999). The lower carcass dressing percentage in feed-restricted rabbits could be related to greater gut fill as found by Knudsen *et al.* (2014 and 2017). Furthermore, our findings confirmed the weak effect of feed

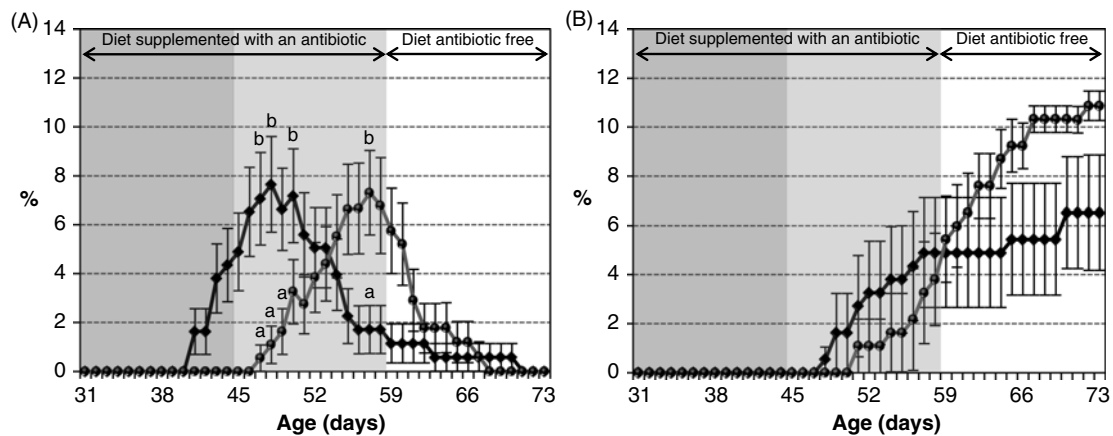


Figure 3 The daily morbidity rate (Least-square-means \pm standard error) calculated as a proportion of ill rabbits among live rabbits according to the feeding programme (L (♦): *ad libitum*-fed rabbits; R (●): feed-restricted rabbits; A). The cumulative mortality rate (Least-square-means \pm standard error) calculated from the initial number of 184 animals for L and R groups (B). Dark grey, light grey and white areas correspond to phases feed restriction, refeeding and *ad libitum* feeding, respectively. Means with different superscript letters are statistically different ($P < 0.05$).

restriction on meat quality parameters (pH values, the meat-to-bone ratio and colour indexes) as previously observed by other authors after application of different restriction techniques (Dalle Zotte *et al.*, 2005; Gidenne *et al.*, 2009).

Effects of the housing system

No interaction between the feeding system and the housing system was detected, that is, under our conditions, the implementation of the time-based feed restriction programme provided similar results both in collective cages and in pens with different group sizes. According to Gidenne *et al.* (2010), when quantitative feed restriction is applied to rabbits kept in groups, no real competition for feed is expected if one place per four to eight animals is available at feeders, and feed restriction is not below 85%. In contrast, severer restriction rates (60%) are likely to cause some competition among animals and to increase live weight variability (Gidenne *et al.*, 2010).

Regardless of feeding strategies, some studies have revealed that pen housing might impair growth performance and meat quality characteristics when compared with rabbits kept in small groups (2 to 6 rabbits/cage; Hamilton and Lukefhar, 1993; Maertens and Van Oeckel, 2001; Szendrő *et al.*, 2009). Under our conditions, no significant differences in weight gain and final live weight were registered among rabbits kept in collective cages or pens with the increasing group size, in line with the results of Rommers and Meijerhof (1998). Nonetheless, during the 1st weeks, rabbits housed in cages showed a higher daily growth rate and better feed efficiency as compared with rabbits housed in pens, regardless of group size, whereas an opposite trend was noted during the last 2 weeks. Differences in growth in the first period are difficult to explain, but they could be related to lower physical activity of rabbits kept in the smaller cages, thus increasing the energy available for growth at the same feed intake (Maertens and Van Oeckel, 2001; Szendrő and Dalle Zotte, 2011). In the last 2 weeks, the lower growth rate of caged rabbits likely depended on the higher live weight load compared to pen-housed rabbits. Indeed, both

in pens and especially in cages, stocking density at slaughter (45 and 50 kg/m²) exceeded the recommended value (40 kg/m²; European Food and Safety Authority, 2005), above which both growth performance and the behavioural pattern might develop some deficits when rabbits are reared in cages.


Regarding health, the literature does not provide clear evidence of the relationship between the housing system (cages *v.* pens) and the mortality rate (Rommers and Meijerhof, 1998; Princz *et al.*, 2009; Szendrő *et al.*, 2015). In our trial, differences in mortality and health risk according to the housing system only approached statistical significance ($P \leq 0.10$) and did not reveal a clear-cut trend. Consistently with other studies (Xiccato *et al.*, 2013) and in the absence of large differences in slaughter live weight, the housing system had no relevant effects on parameters at slaughter and on carcass characteristics. On the other hand, the absence of any difference in meat rheological properties linked to differences in physical exercise in pens may be explained by the early slaughter age of the animals (73 days), which likely prevented any stress caused by aggression among animals (Xiccato *et al.*, 2013; Trocino *et al.*, 2015).


Conclusions


Under our conditions, a progressive time-based feed restriction improved feed efficiency in growing rabbits with some reductions in performance and carcass characteristics, but without affecting meat quality. Moreover, this restriction programme reduced morbidity due to digestive troubles during the restriction period, but thereafter worsened morbidity and mortality even though gradual refeeding was applied. Accordingly, further research is necessary to identify the optimal way to manage the refeeding phase. Lastly, the absence of interactions between the feeding programmes and the housing systems proved that time-based feed restriction can be applied both to conventionally caged and pen-housed growing rabbits.

Acknowledgements

This study was funded by the University of Padova (DOR1891387/18).

 A. Trocino 0000-0001-9045-6156

 G. Xiccato 0000-0003-4572-3635

 M. Birolo 0000-0002-1236-4456

Declaration of interest

The authors declared no conflicts of interest.

Ethics committee

The study protocol was approved by the Ethical Committee for Animal Experimentation of the University of Padova. All the animals were handled in compliance with the principles stated by the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

Software and data repository resources

None of the data were deposited in an official repository. The data sets analysed in the current study are available from the corresponding author on reasonable request.

References

- Alabiso M, Di Grigoli A, Mazza F, Maniaci G, Vitale F and Bonanno A 2016. 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.
- Association of Official Analytical Chemists 2000. Official methods of analysis, 17th edition. AOAC, Gaithersburg, MD, USA.
- Birolo M, Trocino A, Tazzoli M and Xiccato G 2017. Effect of feed restriction and feeding plans on performance, slaughter traits and body composition of growing rabbits. *World Rabbit Science* 25, 113–122.
- Birolo M, Trocino A, Zuffellato A and Xiccato G 2016. Effect of feed restriction programs and slaughter age on digestive efficiency, growth performance and body composition of growing rabbits. *Animal Feed Science and Technology* 222, 194–203.
- Blasco A and Ouhayoun J 1996. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. *World Rabbit Science* 4, 93–99.
- Combes S, Massip K, Martin O, Furbeyre H, Cauquil L, Pascal G, Bouchez O, Le Floc'h N, Zemb O, Oswald IP and 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.
- Dalle Zotte A, Rémignon H and Ouhayoun J 2005. Effect of feed rationing during post-weaning growth on meat quality, muscle energy metabolism and fibre properties of Biceps femoris muscle in the rabbit. *Meat Science* 70, 301–306.
- De Blas JC and Mateos GG 2010. Feed formulation. In *The nutrition of the rabbit*, 2nd edition (ed. C De Blas and J Wiseman), pp. 222–232. CABI Publishing, Wallingford, UK.
- European Commission 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. Off. J. Eur. Union 19.9.1998, L257/14. Retrieved on 28 December 2018 from <http://publications.europa.eu/en/publication-detail/-/publication/856db9b7-6f1d-4d6e-a778-c766c9fa1776/language-en>
- European Food and Safety Authority 2005. Scientific Report: The impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbit. EFSA-Q-2004-023. pp. 1–137. Annex to EFSA Journal 267, 1–31.
- Foubert C, Boiso P, Duperray J and 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. In *Proceedings of the 12èmes Journées de la Recherche Cunicole*, 27–28 November 2007, Le Mans, France, pp. 123–126. Retrieved on 28 December 2018 from http://www.journees-de-la-recherche-avicole.org/JRC/Contenu/Archives/12_JRC/4-Session%20ASFC/2-ASFC.pdf
- Gidenne T, Combes S, Feugier A, Jehl N, Arveux P, Boiso P, Briens C, Corrent E, Fortune H, Montessuy S and Verdelhan S 2009. Feed restriction strategy in the growing rabbit. 2. Impact on digestive health, growth and carcass characteristics. *Animal* 3, 509–515.
- Gidenne T, Combes S and 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.
- Gidenne T, Garreau H, Drouilhet L, Aubert C and Maertens L 2017. Improving feed efficiency in rabbit production, a review on nutritional, technico-economical, genetic and environmental aspects. *Animal Feed Science and Technology* 225, 109–122.
- Gidenne T, Lebas F and Fortun-Lamothe L 2010. Feeding behaviour of rabbits. In *The nutrition of the rabbit*, 2nd edition (ed. C De Blas and J Wiseman), pp. 233–252. CABI Publishing, Wallingford, UK.
- Hamilton HH and Lukefahr S 1993. Influence of pen rearing system and stocking density on post-weaning performance of two breed types of rabbits. *Animal Science* 56, 129–134.
- Knudsen C, Combes S, Briens C, Coutelet G, Duperray J, Rebours G, Salaun JM, Travel A, Weissman D and 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. *Livestock Science* 169, 96–105.
- Knudsen C, Combes S, Briens C, Coutelet G, Duperray J, Rebours G, Salaun JM, Travel A, Weissman D and Gidenne T 2017. Substituting starch with digestible fiber does not impact on health status or growth in restricted fed rabbits. *Animal Feed Science and Technology* 226, 152–161.
- Maertens L, Perez JM, Villamide M, Cervera C, Gidenne T and Xiccato G 2002. Nutritive value of raw materials for rabbits: EGRAN tables 2002. *World Rabbit Science* 10, 157–166.
- Maertens L and Van Oeckel MJ 2001. Effet du logement en cage ou parc et de son enrichissement sur les performances et la couleur de la viande des lapins. In *Proceedings of the 9èmes Journées de la Recherche Cunicole*, 28–29 November 2001, Paris, France, pp. 31–34.
- Mertens DR 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC International* 85, 1217–1240.
- Princ Z, Dalle Zotte A, Metzger Sz, Radnai I, Biró-Németh E, Orova ZSzendrő Zs and 2009. Response of fattening rabbits reared under different housing conditions. 1. Live performance and health status. *Livestock Science* 121, 86–91.
- Romero C, Cuesta S, Astillero JR, Nicodemus N and De Blas JC 2010. Effect of early feed restriction on performance and health status in growing rabbits slaughtered at 2 kg live-weight. *World Rabbit Science* 18, 211–218.
- Rommers J and Meijerhof R 1998. Effect of group size on performance, bone strength and skin lesions of meat rabbits housed under commercial conditions. *World Rabbit Science* 6, 299–302.
- Sahraei M 2012. Feed restriction in broiler chickens production: a review. *Global Veterinaria* 8, 449–458.
- Salaun JM, Renouf B, Bourdillon A, Picot A and 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. In *Proceedings of the 14èmes Journées de la Recherche Cunicole*, 22–23 November 2011, Le Mans, France, ITAVI, pp. 101–104. Retrieved on 28 December 2018 from <http://www.cuniculture.info/Docs/Magazine/Magazine2012/Fichiers-pdf/JRC/101-Salaun.pdf>
- Statistical Analysis System Institute 2013. SAS/STAT(R) 9.2 user's guide, 2nd edition. SAS Institute Inc., Cary, NC, USA. Retrieved on 28 December 2018 from http://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#glm_toc.htm
- Szendrő K, Szendrő Zs, Matics Zs, Dalle Zotte A, Odermatt M, Radnai I and Gerencsér Zs and 2015. Effect of genotype, housing system and hay supplementation on performance and ear lesions of growing rabbits. *Livestock Science* 174, 105–112.
- Szendrő Zs and Dalle Zotte A 2011. Effect of housing conditions on production and behaviour of growing meat rabbits. A review. *Livestock Science* 137, 296–303.

Szendrő Zs, Princz Z, Romvári R, Locsmándi L, Szabó A, Bázár Gy, Radnai I, Biró-Németh E, Matics Zs and Nagy I 2009. Effect of group size and stocking density on productive, carcass, meat quality and aggression traits of growing rabbits. *World Rabbit Science* 17, 153–162.

Trocino A, Filiou E, Tazzoli M, Birolo M, Zuffellato A and Xiccato G 2015. Effects of floor type, stocking density, slaughter age, and gender on productive and qualitative traits of rabbits reared in collective pens. *Animal* 9, 855–861.

Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597.

Xiccato G 1999. Feeding and meat quality in rabbits: a review. *World Rabbit Science* 7, 75–86.

Xiccato G, Trocino A, Majolini D, Tazzoli M and Zuffellato A 2013. Housing of growing rabbits in individual, bicellular and collective cages: growth performance, carcass traits and meat quality. *Animal* 7, 627–632.