

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

M. Alabiso, A. Di Grigoli, F. Mazza, G. Maniaci, F. Vitale and A. Bonanno[†]

Dipartimento Scienze Agrarie e Forestali, unità di Produzioni Animali, Università degli Studi di Palermo, viale delle Scienze, 90128 Palermo, Italy

(Received 3 June 2016; Accepted 18 November 2016)

Feed restriction after weaning is widely used in meat rabbit farms to promote health and reduce mortality, but this practice impacts negatively on rabbit growth and slaughter performance. This study compared a 3-week post-weaning feed restriction with ad libitum medicated feeding, evaluating effects on feed intake, growth, health, carcass and meat quality of rabbits of two genotypes: Italian White pure breed and Hycole hybrid × Italian White crossbred. A total of 512 rabbits at 36 days of age, of both sexes and two genotypes, were divided into four homogeneous groups assigned, from 36 to 57 days of age, to different feeding programmes (FP): restricted non-medicated (R-N), ad libitum non-medicated (L-N), restricted medicated (R-M) and ad libitum medicated (L-M). The diets were medicated with oxytetracycline (1540 mg/kg) and colistin sulphate (240 mg/kg). The restriction, performed by giving 70, 80 and 90 g/day of feed for the 1st, 2nd and 3rd week, was followed by ad libitum feeding in the successive 5 weeks, up to slaughter at 92 days of age. Restricted feeds were ingested at a level of 64% of the feed intake recorded in the ad libitum fed rabbits; it was significantly associated, regardless of medication and rabbit genotype, with a lower feed intake (−22 to −24 g dry matter/day) during the entire experiment, compensatory growth and a lower feed conversion ratio in the ad libitum period, and a lower final live weight (−150 g) than ad libitum feeding (P < 0.001). During restriction, mortality was lower in the restricted rabbits (6.25%, 5.47% v. 12.5%, 14.8% for R-N, R-M, L-N and L-M; P < 0.05), whereas in the ad libitum period mortality did not differ among the groups (9.23%, 9.90%, 11.0% and 4.59% for R-N, R-M, L-N and L-M). Dressing out percentage was not affected by FP or genotype; heavier carcasses were produced by rabbits fed ad libitum (+100 g; P < 0.001) and crossbred rabbits (+122 g; P < 0.001). Restriction did not alter meat quality, except for a tendency towards a higher cooking loss and less fat; crossbred meat was higher in L (+1.3; P < 0.01) and b* (+0.51; P < 0.05) colour indexes and tenderness (−0.14 kg/cm²; P < 0.05) than pure breed meat. Under the conditions of this study, a 3-week restricted feeding after weaning resulted to be a suitable alternative, also for high growth potential genotypes, to the antibiotics to preserve rabbit health. The production of lighter carcasses could be compensated partly by the lower feed conversion ratio showed by restricted rabbits.*

Keywords: feed restriction, genotypes, growth, health, meat

Implications

Limiting feed intake after weaning is a nutritional strategy commonly used in meat rabbit farms as an alternative to antibiotics to prevent the frequent occurrence of digestive disorders in the post-weaning period, which are responsible for considerable production losses. By comparing the health and productivity responses of rabbits of two genotypes to 3-week post-weaning restricted or *ad libitum* medicated or non-medicated feedings, this study showed that short-term feed restriction may be beneficial, also for high growth potential genotypes, for promoting rabbit health. Its negative

impact on rabbit growth and carcass weight could be counterbalanced partly by improved feed utilisation.

Introduction

In most livestock sectors today there is great interest in developing models of sustainable production to provide consumers with safe products of animal origin. This is true also in rabbit production, which aims to enhance the value of rabbit meat as a functional and safe food, especially through proper feeding strategies (Hernández 2008; Cavani *et al.*, 2009; Dalle Zotte and Szendrő, 2011).

A short feed restriction after weaning is commonly used in meat rabbit farms (Gidenne *et al.*, 2012) to prevent the frequent

[†] E-mail: adriana.bonanno@unipa.it

occurrence of digestive disorders in the post-weaning period, as such disorders are responsible for high mortality rates and consequently considerable losses in productivity. This technique represents an alternative to the use of antibiotics to control rabbit enteropathies, reducing feed and drug costs and resulting in the production of meat by healthy animals without serious risk of the development of microbial resistance to antibiotics.

Moreover, the lower growth rate and dressing out percentage associated with limited post-weaning intake seems to be counterbalanced by the improvement in feed efficiency and compensatory growth supported by the subsequent *ad libitum* feeding (Xiccato, 1999; Gidenne *et al.*, 2012).

In the past few years, several studies (Gidenne *et al.*, 2012; Knudsen *et al.*, 2014; Read *et al.*, 2015) have verified the impact of various restricted feeding programmes (FP) on the health, physiological and productive traits of growing rabbits. As reported in the review of Gidenne *et al.* (2012), the methods used to control rabbit feed intake differ in terms of duration and intensity, but commonly involve a limited feeding time or a reduced supply of feed; studies have also examined energy (Szendrő *et al.*, 2008; Metzger *et al.*, 2009; Pascual *et al.*, 2014) and water restriction (Bovera *et al.*, 2013). Nevertheless, only Matics *et al.* (2008) studied the effects of a post-weaning feed restriction compared directly with the dietary use of antibiotics, whereas there is a lack of information regarding these effects in relation to the growth potential of rabbits genotypes.

The object of this research was to promote the health of growing rabbit through a 3-week feed restriction after weaning. This restricted feeding was compared with *ad libitum* feeding and with the consumption of diets that did or did not contain antibiotics. The effects of the feeding strategies on feed intake, growth performance, health status and carcass and meat quality have been assessed on rabbits of two different genotypes.

Material and methods

Animals, housing and feeding programmes

This experiment was conducted on a commercial rabbit farm in Sicily from March to June 2014 using a total of 512 rabbits of both sexes, weaned at 31 days of age.

The rabbits were of two genotypes, the Italian White pure breed (IWP), derived by Italian genetic selection of the New Zealand White breed, with an adult weight of 4.3 to 4.7 kg, and the Hycole hybrid × Italian White crossbred (HIWC), achieved inseminating IWP does with semen of bucks of a coloured Hycole hybrid line with an adult weight of 6 to 7 kg.

The rabbits were kept in an indoor unit under natural photoperiod at a temperature of 18°C to 24°C and a relative humidity of 60% to 75% maintained by a forced heating and cooling system. During the experiment, all rabbits were managed according to EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes and Recommendation 2007/526/EC.

At 36 days of age, rabbits were equally singled out from two batches of litters born at a 42-day interval and divided into four groups ($n = 128$ per group) according to genotype and sex, and homogeneous in terms of weaning weight. In each group, eight rabbits of the same sex and genotype were pair-housed in a wire net, flat-deck cage formed by four cells of 28 × 40 × 30 cm (length × width × height) equipped with nipple drinkers (1 per cell) providing drinking water *ad libitum* and a common, central round feeder.

For 3 weeks, from 36 to 57 days of age, the rabbits in each group were assigned to a different FP: restricted non-medicated (R-N), *ad libitum* non-medicated (L-N), restricted medicated (R-M) or *ad libitum* medicated (L-M). The experimental non-medicated and medicated diets had the same formulation and similar chemical compositions (Table 1). The medicated diet was supplemented with 1540 mg/kg oxytetracycline and 240 mg/kg colistin sulphate. The feed restriction was achieved by giving an individual feed amount equal to 70, 80 and 90 g/day (as-fed basis) during the 1st, 2nd and 3rd week, respectively, in accordance with the restriction plan practised in the farm. In the successive 5 weeks of the experiment, all groups received the same farm diet supplied *ad libitum*. Table 1 shows the commercial diets fed to rabbits during the weaning period and the successive growth phases and their chemical compositions.

Intake, growth, health and slaughter

Feed intake per cage was measured by weighing the feed supplied and refused daily with restricted feeding and weekly with *ad libitum* feeding. Individual live weight (LW) was recorded at 36, 57 and 92 days of age. Mortality was checked daily, whereas morbidity was recorded when the rabbits were weighed. A rabbit was considered morbid when it showed signs of digestive disorders or weight loss or had a growth rate -2 SD below the mean for the period, as suggested by Gidenne *et al.* (2012). Mortality was attributed to enteropathies when external signs of diarrhoea were evident. The health risk index was calculated as the sum of morbid and dead rabbits (Gidenne *et al.*, 2009).

At the end of the 8-week growth period, at 92 days of age, 32 rabbits per FP were selected to represent the composition, in terms of sex and genotype, and the variability of their experimental group. On the next day, after 12 h fasting from solids, applied to reduce the gastrointestinal content and facilitate its post-slaughter removal, they were weighed and then slaughtered by cutting the carotid arteries and jugular veins after electric stunning and individually processed in the commercial slaughterhouse annexed to the farm.

Commercial carcasses were obtained as recommended by Blasco and Ouhayoun (1996) by removing the skin, the distal part of the tail, the distal parts of fore and hind legs (HL), the urogenital organs and the gastrointestinal tract. To estimate the gastrointestinal content, the weight of full and empty gastrointestinal tract was recorded; to remove its content, the full gastrointestinal tract was washed under running water, dripped and then got dry with absorbent paper. The hot carcasses were weighed, then stored at 4°C for 24 h.

Carcass and meat quality measurements

At 24 h after slaughter, the chilled carcasses were weighed and then dissected, as indicated by Blasco and Ouhayoun (1996), by removing the head and internal organs (thymus, trachea, oesophagus, lungs, heart, liver and kidneys) to obtain the reference carcasses; cutting the anatomical joints, such as fore (cut between the last thoracic and the first lumbar vertebrae), intermediate and hind parts (cut between the sixth and seventh lumbar vertebrae); separating perirenal and scapular fat, and both HL from the hind part. Each left HL was dissected to weigh the tissue components, such as fat (inguinal and intramuscular), bone and meat.

pH and colour were measured 24 h after slaughter. pH was measured in duplicate on the *Longissimus lumborum* (LL) and right HL (*Biceps femoris*) muscles using a Hanna FC 200 pH meter (Hanna Instruments, Baranzate, Milan, Italy) equipped with a penetrating probe. Colour was assessed at ambient temperature in duplicate on the surface of the right HL muscles and the transversal section of the muscle LL at level of the sixth lumbar vertebrae, with a Minolta chroma meter CR300 (Minolta, Osaka, Japan) measuring lightness (L^*), redness (a^*) and yellowness (b^*) according to the CIE $L^*a^*b^*$ system (CIE, 1986).

Each right HL was vacuum packed, frozen at -20°C , and then thawed at 4°C for 24 h. The weights of frozen and thawed HL were recorded to determine thawing loss. Cooking loss was measured on right HL wrapped in polyethylene bags, cooked in a water bath at 80°C for 2.5 h, cooled for 1 h, and then reweighed to determine moisture loss, as reported by Xiccato *et al.* (2013).

Warner–Bratzler (WB) shear force was measured in triplicate on rectangular samples of cooked right HL meat 2×1 cm in cross-section, excised along the muscle fibre direction, using an Instron 5564 (Instron, Trezzano sul Naviglio, Milan, Italy).

Feed and meat chemical analyses

Samples of diets offered during the experiment were analysed for dry matter (DM), ash, ether extract (EE) and CP ($N \times 6.25$) using Association of Official Analytical Chemists (AOAC, 2000) methods; starch using the Ewers polarimetric method (ISO 10520, 1997); and structural carbohydrates, such as NDF, ADF and ADL, together with acid insoluble ash (AIA), following the sequential procedure of Van Soest *et al.* (1991). Accordingly, cellulose (ADF – (ADL + AIA)) and hemicellulose (NDF – ADF) were calculated. Digestible energy was estimated as (MJ kg/DM) = $14.2 - 0.205 \text{ ADF}\% + 0.218 \text{ EE}\% + 0.057 \text{ CP}\%$, according to Fernández-Carmona *et al.* (1996).

The meat dissected from the left HL was freeze dried and analysed for water, lipids (EE) and ash content using AOAC (2000) methods, whereas protein was calculated by difference ($100 - \text{water} - \text{lipids} - \text{ash}$), in accordance with Dalle Zotte *et al.* (2015). The total collagen content in HL meat was determined by measuring hydroxyproline according to the colorimetric NMKL-AOAC method (Kolar, 1990) using a multiplication factor of 7.14 (Etherington and Sims, 1981).

Statistical analysis

The experimental unit was the 8-rabbit cage for the feed intake and feed conversion ratio and a single rabbit for the other parameters. Data were analysed statistically using the GLM procedure in SAS 9.2 (Statistical Analysis Systems Institute, 2010). For the analysis, morbidity, mortality and health risk index were considered Bernoulli variables (0 or 100). The statistical model included the fixed effects of FP (R-N, L-N, R-M or L-M), genetic type (IWP or HIWC), sex and litter batch (1, 2). The interactions among the effects were removed from the model when not significant. Tukey's test was used to compare means when the effects of FP or interactions were significant ($P < 0.05$).

Results

Feed intake, growth performance and health

The chemical composition of the commercial diets that the growing rabbits received in sequence, according to the feeding plan, is shown in Table 1. The diets offered to the groups in the 3 weeks post-weaning, from 36 to 57 days of age, had the same nutrient and energy contents, but differed in antibiotics. Compared with the diets offered *ad libitum* in the growth and fattening phases, until 71 and 92 days of age, the post-weaning diets were characterised by lower starch content, whereas they had a lower CP content especially in comparison with the fattening diet.

Table 2 summarises feed intake and growth performance. The R-N and R-M rabbits, compared with the L-N and L-M rabbits, maintained a similarly low feed intake during the entire experiment, then also after 57 days, when they were fed *ad libitum*, although to a lesser extent. In contrast to the restriction period, the reduced ingestion of both restricted groups from days 58 to 92 was associated with a higher weight gain ($P < 0.001$) and, as a consequence, a lower and more favourable feed conversion rate ($P < 0.001$). In spite of their compensatory growth, at 92 days of age the restricted groups had a lower final LW by about 150 g than the rabbits fed *ad libitum* ($P < 0.001$), regardless of diet medication. With regard to genotype, the high growth potential allowed the HIWC rabbits to show higher initial LW at 36 days of age, and better growth and feed conversion rate, especially from days 58 to 92, reaching a higher final LW (+238 g) than the pure breed rabbits. A significant FP \times GT interaction emerged for total feed intake because of the significantly greater intake of HIWC rabbits than IWP rabbits in the L-N regime (145 v. 134 g DM/day, respectively; $P < 0.05$), that did not emerge with R-N (118 v. 120 g DM/day), R-M (118 v. 115 g DM/day) or L-M diets (141 v. 139 g DM/day). No difference due to the rabbits' sex emerged for feed intake or growth performance.

Table 3 shows the health of the rabbits during the experiment. Health conditions on the farm were generally poor, as total mortality and the health risk index reached 17.6% and 21.1%, respectively. The incidence of morbidity and the health risk index were not affected by the FP; however, mortality

Table 1 Chemical composition (% dry matter (DM)) of commercial diets fed to rabbits by phase

Phase	Around weaning		Post-weaning		Growth	Fattening
Age (days)	21 to 35		36 to 56		57 to 70	71 to 92
Additive	Coccidiostatic ¹ + antibiotic ²		Coccidiostatic ¹	Coccidiostatic ¹ + antibiotic ³	Coccidiostatic ¹	
Groups	All	R-N, L-N	R-M, L-M	All	All	
DM	89.3	89.4	90.0	89.8	89.6	
CP (N × 6.25)	20.3	17.6	17.9	18.3	19.0	
Ether extract (EE)	4.84	4.84	4.73	5.19	3.08	
Ash	8.07	8.76	8.51	8.38	11.0	
Starch	16.2	11.6	11.2	16.2	14.2	
NDF	29.5	34.4	33.9	34.1	31.3	
ADF	20.1	23.7	23.5	22.1	23.5	
ADL	4.58	5.08	4.95	4.56	5.10	
Acid insoluble ash	0.54	1.15	1.23	1.23	1.89	
Cellulose	14.9	17.5	17.3	16.4	16.5	
Hemicellulose	9.47	10.7	10.4	12.0	7.87	
Digestible energy (DE) ⁴ , (MJ/kg DM)	12.3	11.4	11.4	11.8	11.1	

R-N = restricted non-medicated feeding; L-N = *ad libitum* non-medicated feeding; R-M = restricted medicated feeding; L-M = *ad libitum* medicated feeding.

¹Diclazuril E771 (Clinacox 0.5% Premix Janssen) 1 mg/kg.

²Apramicyn 60 mg/kg, tylosyn 120 mg/kg.

³Oxytetracycline 1540 mg/kg, colistin sulphate 240 mg/kg.

⁴DE (MJ kg/DM) = 14.2 – 0.205 ADF% + 0.218 EE% + 0.057 CP% (Fernández-Carmona *et al.* 1996).

Table 2 Effects of feeding programme (FP) and genetic type (GT) on growth, feed intake and feed conversion of rabbits by phase

	FP				GT		Sex		RMSE	P-value ¹		
	R-N	L-N	R-M	L-M	IWP	HIWC	Females	Males		FP	GT	Sex
Cage (n)	16	16	16	16	32	32	32	32				
Feed restriction (36 to 57 days of age)												
Rabbits (n)	118	111	117	108	235	219	226	228				
Feed intake (g DM/day)	70.5 ^b	110 ^a	70.7 ^b	112 ^a	89.6	92.0	89.7	91.8	7.06	<0.001	0.175	0.232
Live weight at 36 days (g)	904	914	905	901	881	932	898	915	84.3	0.709	<0.001	0.035
Live weight at 57 days (g)	1513 ^b	1854 ^a	1502 ^b	1801 ^a	1634	1701	1657	1678	245	<0.001	0.005	0.389
Weight gain (g/day)	29.0 ^b	44.7 ^a	28.4 ^b	42.8 ^a	35.8	36.6	36.2	36.3	10.4	<0.001	0.420	0.885
Feed conversion ratio	2.60	2.90	2.76	2.96	2.77	2.83	2.79	2.81	1.27	0.185	0.639	0.864
<i>Ad libitum</i> feeding (58 to 92 days of age)												
Rabbits (n)	105	96	108	103	212	200	203	209				
Feed intake (g DM/day)	147 ^b	158 ^a	143 ^b	157 ^a	149	154	151	151	8.21	<0.001	0.020	0.969
Live weight at 92 days (g)	2839 ^b	2977 ^a	2826 ^b	2971 ^a	2784	3022	2909	2897	288	<0.001	<0.001	0.691
Weight gain (g/day)	37.8 ^a	32.6 ^b	37.9 ^a	33.2 ^b	32.9	37.8	35.9	34.8	6.19	<0.001	<0.001	0.115
Feed conversion ratio	4.08 ^b	5.07 ^a	3.91 ^b	4.90 ^a	4.74	4.24	4.43	4.55	0.94	<0.001	<0.001	0.197
Whole period (36 to 92 days of age)												
Rabbits (n)	105	96	108	103	212	200	203	209				
Feed intake ² (g DM/day)	118 ^b	140 ^a	116 ^b	140 ^a	127	131	128	129	5.37	<0.001	0.005	0.586
Weight gain (g/day)	34.3 ^b	36.6 ^a	34.1 ^b	36.6 ^a	33.8	37.1	35.7	35.2	4.60	<0.001	<0.001	0.269
Feed conversion ratio	3.57 ^b	3.88 ^a	3.49 ^b	3.92 ^a	3.85	3.57	3.70	3.73	0.56	<0.001	<0.001	0.537

DM = dry matter. R-N = restricted non-medicated feeding; L-N = *ad libitum* non-medicated feeding; R-M = restricted medicated feeding; L-M = *ad libitum* medicated feeding. IWP = Italian White pure breed; HIWC = Hycole × Italian White crossbred.

^{a,b}P < 0.05.

¹The interactions among the effects were removed from the model because not significant (P > 0.05).

²Significant interaction FP × GT (P = 0.0225).

Table 3 Effects of feeding programme (FP) and genetic type (GT) on the health and mortality of rabbits by phase

	FP				GT		Sex		P-value ¹			
	R-N	L-N	R-M	L-M	IWP	HIWC	Females	Males	RMSE	FP	GT	Sex
Feed restriction (36 to 57 days of age)												
Rabbits (n)	128	128	128	128	256	256	256	256				
Morbidity (%)	1.56	0.78	3.13	0.78	1.17	1.95	2.34	0.78	12.4	0.387	0.477	0.155
Mortality (%)	6.25 ^b	12.5 ^a	5.47 ^b	14.8 ^a	7.03	12.5	9.38	10.2	29.5	0.025	0.036	0.765
Health risk index ² (%)	7.81	13.3	8.59	15.6	8.20	14.5	11.7	10.9	31.6	0.147	0.026	0.780
<i>Ad libitum</i> feeding (58 to 92 days of age)												
Rabbits (n)	120	112	121	109	238	224	232	230				
Morbidity (%)	3.30	3.33	0.82	0.93	3.30	0.90	2.12	2.07	14.5	0.354	0.077	0.969
Mortality (%)	9.23	11.0	9.90	4.59	7.48	9.90	10.2	7.15	28.2	0.341	0.357	0.240
Health risk index ² (%)	12.5	14.4	10.7	5.52	10.8	10.8	12.4	9.22	31.1	0.176	0.996	0.280
Whole period (36 to 92 days of age)												
Rabbits (n)	128	128	128	128	256	256	256	256				
Morbidity (%)	4.69	3.91	3.91	1.56	4.30	2.73	4.30	2.73	18.5	0.557	0.337	0.337
Mortality (%)	14.8	21.9	14.8	18.8	14.1	21.1	18.8	16.4	38.0	0.377	0.036	0.484
Mortality for enteropathies (%)	7.81	9.38	5.47	10.9	7.81	8.98	8.98	7.81	27.8	0.443	0.634	0.634
Health risk index ² (%)	19.5	25.8	18.8	20.3	18.4	23.8	23.0	19.1	40.8	0.506	0.131	0.280

R-N = restricted non-medicated feeding; L-N = *ad libitum* non-medicated feeding; R-M = restricted medicated feeding; L-M = *ad libitum* medicated feeding. IWP = Italian White pure breed; HIWC = Hycole × Italian White crossbred.

^{a,b} $P < 0.05$.

¹The interactions among the effects were removed from the model because not significant ($P > 0.05$).

²Health risk index = morbidity + mortality.

decreased in both restricted groups in the first 3-week period ($P < 0.05$), but did not differ significantly among groups in the second period. About half of the total losses in each group were rabbits showing signs of diarrhoea, for which death was attributed to enteropathies. Compared with the IWP rabbits, the HIWC rabbits showed higher levels of mortality and a higher health risk index during the restricted period; this between-genotype difference was maintained for total mortality only. Also with regard to health, no difference was recorded between female and male rabbits during the growth period.

Carcass and meat quality

Table 4 shows results related to carcass traits. At slaughter, which was performed at 92 days of age, the restricted rabbits had a lower body weight and, as a consequence, lighter chilled carcasses (-100 g; $P < 0.001$) than the rabbits fed *ad libitum*. The FP did not affect the dressing out percentages, although, among the removed body parts, the gastrointestinal content and internal organs had lower incidence in the rabbits on *ad libitum* diets ($P < 0.01$). Diet influenced some carcass characteristics: the percentage incidence of fore parts was lower in carcasses of R-N and R-M rabbits than those fed *ad libitum* ($P < 0.05$). However, the lower weight of the dissected HL in the restricted groups did not imply differences in terms of tissue composition.

With regard to genotype, the higher body weight at slaughter of the HIWC rabbits resulted in heavier carcasses that showed a lower drip loss and were characterised by a lower perirenal and scapular fat contents. At dissection, the heavier left HL of the crossbred rabbits resulted in higher

fat and bone, thus decreasing the meat content and the meat-to-bone ratio.

Also, sex affected some carcass traits. Indeed, at equal weights, the carcasses of females showed a higher drip loss, lower dressing out percentage and a greater incidence of empty gastrointestinal tracts. In addition, female carcasses had a different cut and tissue composition due to the greater contributions of intermediate and hind parts and of fat and bone tissue, respectively.

Meat quality was not affected by the FP, as reported in Table 5. Only tendencies towards a higher cooking loss and a lower lipid content were recorded for the meat from restricted rabbits compared with that from rabbits fed *ad libitum*, together with tendencies towards higher pH in LL and HL meat from L-M rabbits. Genotype affected some meat traits: indeed, the HIWC genotype showed higher L^* and b^* colour indexes of the LL muscle; a tendency towards higher thawing loss; major meat tenderness, as indicated by a lower value of WB shear force; as well as higher water and lower protein contents in the HL meat. The HL meat of female rabbits had a lower a^* colour index, a tendency to a lower water content, but higher lipid and lower collagen contents.

No significant interaction of FP with genotype or sex emerged for carcass traits and meat quality.

Discussion

Effects on feed intake, growth performance and health

This study has investigated the potential of post-weaning feed restriction as an alternative to the use of antibiotics in

Table 4 Effects of feeding programme (FR) and genetic type (GT) on carcass traits of rabbits slaughtered at 92 days of age

	FP				GT		Sex		RMSE	P-value ¹		
	R-N	L-N	R-M	L-M	IWP	HIWC	Females	Males		FP	GT	Sex
Rabbits (n)	32	32	32	32	64	64	64	64				
Slaughter body weight (SBW) (g)	2835 ^b	2958 ^a	2823 ^b	2992 ^a	2798	3006	2912	2892	140	<0.001	<0.001	0.435
Chilled carcass weight (g)	1672 ^b	1762 ^a	1680 ^b	1786 ^a	1664	1786	1721	1729	98.0	<0.001	<0.001	0.633
Drip loss (%)	3.25	3.12	3.07	2.91	3.55	2.63	3.23	2.95	0.48	0.062	<0.001	0.002
Reference carcass weight (RCW) (g)	1426 ^b	1510 ^a	1429 ^b	1530 ^a	1422	1526	1474	1474	92.0	<0.001	<0.001	0.995
Left hind leg weight (HLW) (g)	249 ^b	258 ^a	247 ^b	259 ^a	243	264	254	252	18.0	0.015	<0.001	0.473
Percentage on SBW												
Dressing out percentage	59.0	59.6	59.5	59.7	59.5	59.4	59.1	59.8	1.79	0.412	0.823	0.035
Gastrointestinal content	10.6 ^a	9.57 ^b	10.2 ^a	9.48 ^b	9.81	10.1	10.2	9.68	1.50	0.009	0.258	0.035
Empty gastrointestinal tract	6.42	6.33	6.53	6.54	6.49	6.42	6.63	6.28	0.65	0.538	0.581	0.004
Skin	13.2	13.3	13.4	13.9	13.5	13.4	13.3	13.6	1.46	0.169	0.851	0.317
Head	5.17	5.15	5.23	5.10	5.16	5.16	4.98	5.34	0.36	0.562	0.929	<0.001
Internal organs ²	3.54 ^{ab}	3.36 ^b	3.66 ^a	3.46 ^b	3.52	3.49	3.52	3.49	0.30	0.001	0.659	0.612
Percentage on RCW												
Fore part	36.1 ^b	36.9 ^a	36.3 ^b	36.4 ^{ab}	36.3	36.5	35.9	36.9	1.17	0.036	0.459	<0.001
Intermediate part	23.9	23.7	24.0	24.0	24.0	23.7	24.1	23.7	1.27	0.652	0.212	0.089
Hind part	36.9	36.4	36.8	36.3	36.4	36.8	36.9	36.3	1.29	0.180	0.122	0.003
Perirenal fat	2.27	2.33	2.15	2.46	2.46	2.15	2.32	2.29	0.82	0.489	0.031	0.842
Scapular fat	0.49	0.51	0.53	0.50	0.55	0.46	0.52	0.49	0.22	0.905	0.022	0.455
Perirenal and scapular fat	2.76	2.84	2.68	2.96	3.01	2.61	2.84	2.78	0.93	0.660	0.014	0.721
Percentage on HLW												
Hind leg meat	79.1	78.7	76.7	79.1	79.9	76.9	77.4	79.4	5.02	0.167	<0.001	0.310
Hind leg fat	2.03	2.09	2.21	1.87	1.91	2.19	2.32	1.79	0.77	0.347	0.042	<0.001
Hind leg bone	18.8	19.2	21.1	19.0	18.2	20.9	20.2	18.8	4.90	0.228	0.002	0.097
Hind leg meat-to-bone ratio	4.53	4.35	3.90	4.40	4.62	3.97	4.13	4.47	1.14	0.142	0.001	0.094

R-N = restricted non-medicated feeding; L-N = *ad libitum* non-medicated feeding; R-M = restricted medicated feeding; L-M = *ad libitum* medicated feeding. IWP = Italian White pure breed; HIWC = Hycole × Italian White crossbred.

^{a,b}P < 0.05.

¹The interactions among the effects were removed from the model because not significant (P > 0.05).

²Thymus, trachea, oesophagus, lungs, heart, liver and kidneys.

the diet for improving the digestive health of growing rabbits of two different genotypes.

The commercial medicated and non-medicated diets offered to the experimental rabbit groups in the 3 weeks post-weaning, from 36 to 57 days of age, differed only in the supplement of antibiotics. The diets were formulated to meet the nutritional needs of weaned rabbits, preventing digestive disorders and, at the same time, growth reduction, in line with Gidenne (2000). Nevertheless, compared with the suggestions of Gidenne (2000) expressed on as-fed basis, the post-weaning diets resulted adequate in starch (<140 g/kg) and ADF (>190 g/kg), quite close to CP (<160 g/kg) and NDF (>300 g/kg), but slightly lower in ADL (>55 g/kg). Moreover, especially because of their high EE content, the diets had slightly more digestible energy (10.3 MJ/kg) than the maximum recommended level (9.8 MJ/kg; Gidenne, 2000) so that the restricted rabbits could compensate for their lower feed intake. In this regard, Knudsen *et al.* (2014) and Read *et al.* (2015) observed enhanced nutrient utilisation in rabbits on restricted diets eating a diet rich in energy.

The restricted feeding plan, which offered 70, 80 and 90 g/day of feed in the 1st, 2nd and 3rd week after weaning, respectively, equalled 64% of the voluntary intake recorded in the rabbits fed *ad libitum*, resulting in a stronger restriction than the 75% in other experiments (Gidenne *et al.*, 2009; Knudsen *et al.*, 2014), and was responsible for a marked decrease in growth without a significant improvement in the feed conversion ratio. The effects of feed restriction on the growth of rabbits were independent of the dietary use of the antibiotics supplement, as was also reported by Matics *et al.* (2008), as well as did not change in relation to the rabbit genotype.

From days 58 to 92, when all experimental groups were fed freely, rabbits that were previously restricted continued to maintain a slightly lower feed intake compared with those fed *ad libitum*, also due to their lower LW, as feed intake resulted in similar percentages of LW with restriction (5.12%) and *ad libitum* diets (5.30%). It is interesting that, contrary to expectations, this lower feed intake of restricted rabbits was associated with compensatory growth and, as a

Table 5 Effects of feeding programme (FP) and genetic type (GT) on physical and chemical traits of *Longissimus lumborum* (LL) and right hind leg (HL) meat

	FP				GT		Sex		RMSE	P-value ¹		
	R-N	L-N	R-M	L-M	IWP	HIWC	Females	Males		FP	GT	Sex
LL												
pH	5.81	5.79	5.81	5.85	5.80	5.83	5.80	5.83	0.089	0.070	0.109	0.133
Colour												
<i>L</i> [*]	59.0	59.4	58.8	59.4	58.5	59.8	59.4	58.9	2.78	0.748	0.010	0.332
<i>a</i> [*]	4.23	4.78	4.32	3.97	4.51	4.14	4.38	4.27	1.50	0.192	0.163	0.663
<i>b</i> [*]	1.83	2.06	1.61	1.69	1.54	2.05	1.99	1.60	1.36	0.560	0.038	0.105
HL												
pH	5.95	5.92	5.96	5.99	5.94	5.97	5.94	5.97	0.090	0.064	0.123	0.143
Colour												
<i>L</i> [*]	52.8	52.3	53.3	52.7	52.8	52.8	52.8	52.7	2.37	0.368	0.985	0.751
<i>a</i> [*]	6.69	6.51	6.43	6.49	6.72	6.34	6.10	6.96	1.49	0.910	0.160	0.001
<i>b</i> [*]	2.27	1.93	2.28	1.90	2.21	1.98	2.10	2.10	1.48	0.588	0.380	0.999
Thawing loss (%)	1.25	1.32	1.52	1.46	1.24	1.54	1.45	1.33	0.87	0.588	0.060	0.414
Cooking loss (%)	16.7	15.2	16.2	15.2	16.1	15.6	15.8	15.9	2.82	0.085	0.384	0.924
Total loss (%)	17.8	16.3	17.5	16.4	17.1	16.9	17.0	17.0	2.93	0.128	0.716	0.906
WB shear force (kg/cm ²)	1.23	1.19	1.20	1.23	1.28	1.14	1.23	1.19	0.29	0.932	0.012	0.520
Water (% meat)	75.0	74.6	75.2	74.9	74.8	75.1	74.8	75.1	0.98	0.126	0.034	0.099
Protein (% meat)	21.4	21.5	21.2	21.1	21.5	21.1	21.3	21.3	0.70	0.101	0.004	0.775
Lipids (% meat)	2.30	2.54	2.28	2.66	2.45	2.44	2.59	2.30	0.63	0.058	0.947	0.009
Ash (% meat)	1.26	1.28	1.33	1.28	1.29	1.29	1.30	1.27	0.15	0.250	0.921	0.309
Collagen (mg/g DM)	26.1	26.5	24.8	25.5	25.8	25.7	23.9	27.6	3.63	0.240	0.794	<0.001

DM = dry matter; WB = Warner–Bratzler; R-N = restricted non-medicated feeding; L-N = *ad libitum* non-medicated feeding; R-M = restricted medicated feeding; L-M = *ad libitum* medicated feeding. IWP = Italian White pure breed; HIWC = Hycole × Italian White crossbred.

¹The interactions among the effects were removed from the model because not significant ($P > 0.05$).

consequence, a lower and more favourable feed conversion ratio, in line with the observations of other authors (Gidenne *et al.*, 2009; Gidenne *et al.*, 2012; Knudsen *et al.*, 2014). Because this improvement in feed utilisation was not found to be linked to an increase in nutrient digestibility, Knudsen *et al.* (2014) suggested that it could be of metabolic origin through reduced extra-heat production, or the higher relative weight of a full gastrointestinal tract could have led to an overestimation of rabbits' growth. In accordance with this latter hypothesis, in this study the restricted rabbits showed a higher incidence of content in the digestive tract at slaughter. However, the incidence of empty gastrointestinal tracts did not differ among groups, and the small differences in the weights of the content of the tracts (about 1%) do not seem to be enough to justify the differences in the rabbits' growth and LW. It is possible that, in the current study, the more intense feed restriction (to 64% of the voluntary intake) could have increased the digestibility of the feed, mainly as a consequence of the longer retention time of the digesta in the intestinal tract (Gidenne and Feugier, 2009).

On the whole, from days 36 to 92, in front of a consistent feed intake reduction (of 16% to 17%), the restricted rabbits reduced less markedly the growth rate (by 6% to 7%) and final LW (by about 5%) in comparison with the rabbits fed freely. Whereas, the higher growth performance of HIWC than IWP rabbits were in line with the differences observed by Szendrő *et al.* (2010) comparing Hycole line × maternal line crossbred

rabbits with purebred rabbits of the maternal line, although for both genotypes the weight gain found in the present study were lower by about 10 g/day than those reported by these authors, only in part due to the contribution of the restricted feeding.

The results also demonstrate that this short-term (3-week) and intense limited feed intake (64%) after weaning, applied on a rabbit farm with poor health conditions, reduced rabbit mortality compared with the *ad libitum* medicated (by 8.6 percentage points) and non-medicated (by 6.2 percentage points) diets with the same nutrient composition, and had a mortality analogous than that with the restricted medicated diet (R-M). Instead, this favourable effect of lower intake on the health of rabbits was not maintained during the following period when rabbits were fed *ad libitum* with the growth and fattening diets. However, probably due to the poor health level of the farm, the diet medication with antibiotic did not result very effective during the first 3-week period; instead, during the second period, the mortality recorded in the L-M group (4.6%) was lower by 6.4 percentage points than L-N, although not significantly, suggesting a delayed effect of the antibiotics, ingested *ad libitum* with the post-weaning diet, in preserving the health status of rabbits. Nevertheless, this trend implies that, on the whole, mortality losses and the health risk index for R-N (14.8% and 19.5%) were lower than those for L-N (21.9% and 25.8%), although these differences did not reach significance, and comparable with those for L-M (18.8% and 20.3%). Accordingly, other than the comparable results in terms of mortality, the feeding restriction

(R-N), in respect to an *ad libitum* medicated diet, ensures the advantage of avoiding the use of antibiotics and the risks connected to their release in the environment contributing to the spread of antibiotic resistance.

During the whole growth period, the health of female and male rabbits did not differ; however, the crossbred (HIWC) rabbits exhibited a higher mortality than the pure breed (IWP) rabbits from days 36 to 57 and over the entire period (+5.5 and +7.0 percentage points, respectively). These results are consistent with observations by Dal Bosco *et al.* (2009), who recorded lower immune resistance in commercial hybrid rabbits selected for rapid growth compared with rabbits of a more rustic local population, and also with results reported by Szendrő *et al.* (2008), who found an analogous higher mortality (+9.4 percentage points) in rabbits selected for low body fat deposition (and more rapid growth) than in those characterised by high body fat deposition, even though that difference did not reach statistical significance.

Effects on carcass and meat quality

In this study, the carcass weight of the rabbits on the restricted diets were 5% to 6% lower than that of the rabbits fed freely. But, unlike in other studies (Xiccato, 1999; Knudsen *et al.*, 2014), the feed restriction did not reduce the dressing out percentages because of the lack of differences in the incidence of major body parts removed, such as skin and empty gastrointestinal tracts and the slight differences emerged for digestive content. Knudsen *et al.* (2014), who did not apply a pre-slaughter fasting, justified the lower slaughter yield of rabbits on restricted diets by the higher weight of the full digestive tract caused by overeating when the rabbits were fed freely. In the current study, this overeating behaviour of the restricted rabbits did not occur from days 58 to 92, as proved by the lower feed intake and the analogous incidence of feed intake on LW, as well as of the empty gastrointestinal tracts in comparison with the rabbits fed *ad libitum*.

With regard to carcass traits, the feed restriction was responsible for only a lower incidence of fore parts and a higher incidence of internal organs that would indicate differences in the growth rate of these body parts. In this regard, Gidenne *et al.* (2012) and Bovera *et al.* (2013) reported that compensatory growth associated with free intake following a period of limited feed intake favours the rapid growth of digestive organs, such as the liver.

In line with other authors (Tumova *et al.*, 2006; Gidenne *et al.*, 2009; Bovera *et al.*, 2013) who observed no effect of limited feed intake on physical and chemical characteristics of meat, in this study the feed restriction did not influence meat quality and tended only to reduce the meat fat content, which corresponded with slightly higher cooking losses, whereas the tendency towards a higher pH in L-M was so weak as to be considered negligible.

Instead, the effect of genotype on carcass and meat quality was more relevant than that of feeding treatment, as it affected several traits, in particular carcass weight, perirenal and scapular fat content, HL tissue composition, the L^* and b^* colour indexes and tenderness. These results

can depend on the different degree of maturity of the two genotypes, linked to the different adult body weight of their respective paternal lines, that influences greatly the carcass and meat traits (Dalle Zotte, 2002); indeed, a lower maturity at slaughtering, as for the HIWC rabbits, can reduce fat deposition and the meatiness of the carcass. However, in accordance with Metzger *et al.* (2009), genotype never interacted with the feeding treatment, meaning that restricted feeding did not interfere differently with the reared breed in terms of carcass or meat quality traits.

Lastly, the gender of rabbits is confirmed to be one of the factors influencing the carcass traits and meat quality, although in this regard the data reported in literature are conflicting (Dalle Zotte *et al.*, 2016). On the whole, the effects of gender depend on the differences between sexes in the relative growth of organs and tissues. Indeed, for the females, the lower dressing out percentage has to be related to the greater incidence of the empty gastrointestinal tract, whereas the higher HL fat could be responsible of the lower collagen content.

Conclusions

Under the conditions of this study, a 3-week post-weaning feed restriction to a level of 64% of voluntary intake has showed that could represent, regardless of the reared genotype and its growth potential, a suitable strategy for preserving rabbits' digestive health while avoiding the use of antibiotics in the diet. Indeed, applied in a farm characterised by high incidence of mortality, restricted feeding maintained mortality and the health risk index at levels comparable with those recorded with a medicated diet offered *ad libitum* without markedly impairing rabbits' productive performance or affecting the quality of the meat. The negative impact of the restricted feeding on rabbits' growth rate and carcass weight could be counterbalanced partly by the use of a drug-free diet and improved feed utilisation by rabbits inducing compensatory growth and a lower and more favourable feed conversion ratio.

Acknowledgements

The authors would like to thank Stefania, Marina and Paolo Intravaia of the CUNISUD farm for their technical collaboration and valuable assistance provided throughout the experiment.

References

- Association of Official Analytical Chemists (AOAC) 2000. Official methods of analysis, 17th edition. AOAC, Gaithersburg, MD, USA.
- Blasco A and Ouhayoun J 1996. Harmonization of criteria and terminology in rabbit meat research. *World Rabbit Science* 4, 93–99.
- Bovera F, Lestingi A, Piccolo G, Iannaccone F, Attia YA and Tateo A 2013. Effects of water restriction on growth performance, feed nutrient digestibility, carcass and meat traits of rabbits. *Animal* 7, 1600–1606.
- Cavani C, Petracci M, Trocino A and Xiccato G 2009. Advances in research on poultry and rabbit meat quality. *Italian Journal of Animal Science* 8, 741–750.
- Commission International de l'Eclairage (CIE) 1986. Colorimetry – official recommendations of the international commission on illumination. CIE Publication No. 15.2, CIE Central Bureau, Vienna, Austria.

- Dal Bosco A, Mugnai C, Mourvaki E, Cardinali R, Moscati L, Paci G and Castellini C 2009. Effect of genotype and rearing system on the native immunity and oxidative status of growing rabbits. *Italian Journal of Animal Science* 8, 781–783.
- Dalle Zotte A 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livestock Production Science* 75, 11–32.
- Dalle Zotte A and Szendrő Zs 2011. The role of rabbit meat as functional food. *Meat Science* 88, 319–331.
- Dalle Zotte A, Szendrő K, Gerencsér Zs, Szendrő Zs, Cullere M, Odermatt M, Radnai I and Matics Zs 2015. Effect of genotype, housing system and hay supplementation on carcass traits and meat quality of growing rabbits. *Meat Science* 110, 126–134.
- Dalle Zotte A, Cullere M, Rémignon H, Alberghini L and Paci G 2016. Meat physical quality and muscle fibre properties of rabbit meat as affected by the sire breed, season, parity order and gender in an organic production system. *World Rabbit Science* 24, 145–154.
- Etherington DE and Sims TJ 1981. Detection and estimation of collagen. *Journal of the Science of Food and Agriculture* 32, 359–546.
- Fernández-Carmona J, Cervera C and Blas E 1996. Prediction of the energy value of rabbit feeds varying widely in fibre content. *Animal Feed Science and Technology* 64, 61–75.
- Gidenne T 2000. Recent advances in rabbit nutrition: emphasis on fibre requirements. A review. *World Rabbit Science* 8, 23–32.
- Gidenne T and Feugier A 2009. Feed restriction strategy in the growing rabbit. 1. Impact on digestion, rate of passage and microbial activity. *Animal* 3, 501–508.
- Gidenne T, Combes S, Feugier A, Jehl N, Arveux P, Boisot 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.
- Hernández P 2008. Enhancement of nutritional quality and safety in rabbit meat. In *Proceeding of 9th World Rabbit Congress*, 10–13 June 2008, Verona, Italy, pp. 1287–1299.
- International Standard Organization (ISO) 10520 1997. Native starch – determination of starch content – Ewers polarimetric method. International Standard Organization (ISO) 10520, Geneva.
- 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.
- Kolar K 1990. Colorimetric determination of hydroxyproline as measure of collagen content in meat and meat products: NMKL collaborative study. *Journal-Association of Official Analytical Chemists* 73, 54–57.
- Matics Zs, Dalle Zotte A, Radnai I, Kovács M, Metzger S and Szendrő Zs 2008. Effect of restricted feeding after weaning on the productive and carcass traits of growing rabbits. In *Proceedings of the 9th World Rabbit Congress*, 10–13 June 2008, Verona, Italy, pp. 741–746.
- Metzger S, Szendrő Zs, Bianchi M, Hullár I, Fébel H, Maertens L, Cavani C, Petracci M, Radnai I and Biró-Németh E 2009. Effect of energy restriction in interaction with genotype on the performance of growing rabbits: II. Carcass traits and meat quality. *Livestock Science* 126, 221–228.
- Pascual M, Soler MD, Cervera C, Pla M, Pascual JJ and Blas E 2014. Feeding programmes based on highly-digestible fibre weaning diets: effects on health, growth performance and carcass and meat quality in rabbits. *Livestock Science* 169, 88–95.
- Read T, Combes S, Gidenne T, Destombes N, Grenet L and Fortun-Lamothe L 2015. Stimulate feed intake before weaning and control intake after weaning to optimise health and growth performance. *World Rabbit Science* 23, 145–153.
- Statistical Analysis Systems Institute (SAS) 2010. SAS/STAT qualification tools user's guide, version 9.2. SAS Institute Inc., Cary, NC, USA.
- Szendrő Zs, Metzger S, Febel H, Hullar I, Maertens L, Bianchi M, Cavani C, Petracci M, Biró-Németh E and Radnai I 2008. Effect of energy restriction in interaction with genotype on the performance of growing rabbits I: productive traits. *Livestock Science* 118, 123–131.
- Szendrő Zs, Matics Zs, Gerencsér Zs, Nagy I, Lengyel M, Horn P and Dalle Zotte A 2010. Effect of dam and sire genotypes on productive and carcass traits of rabbits. *Journal of Animal Science* 88, 533–543.
- Tumova E, Zita L and Stolc L 2006. Carcass quality in restricted and ad libitum fed rabbits. *Czech Journal of Animal Science* 51, 214–219.
- 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, Filiou E, Majolini D, Tazzoli M and Zuffellato A 2013. Bicultural cage vs. collective pen housing for rabbits: Growth performance, carcass and meat quality. *Livestock Science* 155, 407–414.