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***ROLE OF FEED RESTRICTION PROGRAMS ON ENHANCING
GUT HEALTH, FEED EFFICIENCY AND MEAT QUALITY IN
GROWING RABBITS***

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Abbreviations

a*	=	redness
AAs	=	amino acids
ADF	=	acid detergent fibre
ADL	=	acid detergent lignin
AL	=	<i>ad libitum</i>
aNDF	=	amylase-treated neutral detergent fibre
b*	=	yellowness
CF	=	crude fibre
CP	=	crude protein
CW	=	carcass weight
DE	=	digestible energy
DM	=	dry matter
DP	=	digestible protein
EB	=	empty body
EPEC	=	enteropathogenic <i>Escherichia coli</i>
ERE	=	epizootic rabbit enteropathy
FC	=	feed conversion
GC	=	gas chromatography
GE	=	gross energy
L*	=	lightness
RC	=	reference carcass
SW	=	slaughter weight
TDF	=	total dietary fibre
VFAs	=	volatile fatty acids
vs	=	<i>versus</i>

Riassunto

Ruolo dei programmi di razionamento alimentare nel miglioramento della salute intestinale, dell'efficienza alimentare e qualità della carne nei conigli in accrescimento

L'applicazione di strategie di restrizione alimentare nei conigli in accrescimento si focalizza su due obiettivi principali: 1) il miglioramento dello stato di salute con la riduzione di mortalità e morbilità causate da patologie digestive; 2) l'aumento dell'efficienza globale delle aziende con l'ottimizzazione dell'indice di conversione e la diminuzione dei costi legati alla somministrazione di antibiotici.

Entrambi questi temi sono di grande interesse al fine di garantire la sostenibilità economica per gli allevatori e rispettare le normative Europee in tema di sicurezza alimentare. Per questi motivi, nel corso degli ultimi decenni, gli effetti delle strategie di restrizione alimentare nei conigli in accrescimento sono stati ampiamente studiati. Nella maggior parte degli studi, il razionamento è stato attuato durante le prime tre o quattro settimane successive allo svezzamento, con l'obiettivo di ottenere livelli di alimentazione compresi tra il 60% e il 90% dell'ingestione *ad libitum*, seguito da un periodo di alimentazione a volontà durante le ultime fasi dell'ingrasso (da una a tre settimane).

Restrizioni quantitative sono state ottenute direttamente, riducendo la quantità di mangime somministrato agli animali, o indirettamente, limitando il tempo di accesso al cibo o all'acqua. Inoltre, restrizioni qualitative sono state ottenute riducendo il contenuto energetico delle diete fornite agli animali. La scelta del metodo per applicare la restrizione alimentare deve tenere in considerazione le attrezzature disponibili in allevamento, le condizioni ambientali e di stabulazione nonché l'impatto sul benessere degli animali.

La letteratura scientifica riporta che i programmi di razionamento rappresentano un valido strumento per ridurre l'incidenza dei problemi digestivi, aumentare l'utilizzazione digestiva delle diete e migliorare l'efficienza alimentare nei conigli in accrescimento. Tuttavia, l'effetto protettivo del razionamento sulla salute intestinale degli animali si limita alla fase di restrizione, mentre il rischio per i conigli precedentemente razionati di contrarre malattie digestive spesso aumenta durante la fase di re-alimentazione. Pertanto, la gestione di quest'ultima fase rappresenta un punto critico, ma pochi studi hanno trattato questa problematica. Inoltre, l'origine multifattoriale dell'enteropatia epizootica del coniglio rende difficile l'interpretazione dei risultati diversi e talora contraddittori ottenuti nei vari studi. Se da un lato il miglioramento dell'indice di conversione sembra essere un effetto abbastanza ripetibile, i vantaggi in termini economici potrebbero essere

limitati considerando la necessità di allungare la fase di ingrasso per permettere un completo recupero delle performance produttive (pesi vivi finali) ed evitare considerevoli diminuzioni delle rese di macellazione. Infatti, gli aspetti economici legati all'utilizzo di strategie di riduzione alimentare nella produzione del coniglio da carne devono essere attentamente valutati, specialmente nei paesi dove il mercato richiede pesi di macellazione elevati come l'Italia.

La presente tesi ha avuto lo scopo di definire programmi di razionamento efficaci nel migliorare l'efficienza alimentare e la salute intestinale nei conigli in accrescimento, senza compromettere i risultati di macellazione, le caratteristiche della carcassa e la qualità della carne. Lo scopo è stato perseguito focalizzandosi su tre obiettivi principali: (i) valutare l'effetto di un sistema di restrizione quantitativo in conigli alloggiati individualmente; (ii) sviluppare un'adeguata restrizione basata sul tempo di accesso al cibo in conigli stabulati in recinti collettivi; (iii) ottimizzare la gestione della fase di re-alimentazione. Questi punti sono stati affrontati in quattro contributi sperimentali la cui organizzazione e principali risultati sono riassunti di seguito:

Primo contributo: *Effetto del sistema di restrizione alimentare e dell'età di macellazione in conigli in accrescimento*

L'effetto del sistema di alimentazione (AL: *ad libitum* vs. D: sistema di razionamento progressivo con variazioni giornaliere vs. W: sistema di razionamento scalare con variazioni settimanali) su efficienza digestiva, stato di salute, performance produttive, caratteristiche della carcassa e bilancio dell'azoto è stato valutato utilizzando 282 conigli ibridi commerciali allevati in gabbie monocellulari dallo svezzamento (36 d di età) alla macellazione avvenuta a due età (73 vs. 80 d). La restrizione alimentare era basata sulla somministrazione di ridotte quantità di mangime crescenti dall'80% del consumo AL allo svezzamento al 100% alla fine della terza settimana dopo lo svezzamento in funzione di due curve di alimentazione caratterizzate da piccoli incrementi giornalieri (+4 g/d; gruppo D) o da grandi aumenti settimanali (+23 g/settimana in media; gruppo W). Dall'inizio della quarta settimana la quantità di mangime fornito in entrambi i gruppi razionati è stata aumentata di 4 g/d per 9 giorni consecutivi al fine di prevenire bruschi picchi di ingestione alimentare, dopodiché tutti i conigli sono stati alimentati a volontà fino alla macellazione commerciale. Nessun trattamento antibiotico è stato effettuato durante la prova.

I coefficienti di digeribilità apparente della dieta da post-svezzamento sono stati misurati su 36 conigli (12 per sistema di alimentazione) da 49 a 53 d di età (ultima settimana di restrizione), mentre i coefficienti di digeribilità della dieta da ingrasso sono stati misurati su 36 conigli (12 per sistema di alimentazione) da 64 a 68 d di età (durante la fase di consumo volontario).

Considerato il periodo di realizzazione della prova di digeribilità, quanto il livello nutritivo era pari al 95% del *ad libitum*, solo la digeribilità della proteina grezza è stata aumentata ($P < 0,001$) nei gruppi razionati. Durante il secondo periodo, nessun effetto residuo del precedente trattamento di restrizione è stato osservato sulla digeribilità della dieta. Nell'intero periodo di prova, mortalità, morbilità e rischio sanitario hanno raggiunto rispettivamente 12,7%, 11,3% e 24,0% in media. Mortalità e rischio sanitario sono stati significativamente ($P < 0,05$) inferiori nei conigli razionati rispetto a quelli alimentati *ad libitum* (-12,0 punti percentuali), in particolare quando è stato applicato il sistema progressivo (-14,2 punti percentuali). Nelle prime tre settimane, il livello di restrizione è stato pari al 91% nel gruppo D e al 93% nel gruppo W; l'accrescimento giornaliero è diminuito in entrambi i gruppi razionati (-7,5% rispetto al gruppo AL) mentre l'indice di conversione non è stato modificato dal sistema di alimentazione. Nel secondo periodo, solo il gruppo W ha mostrato consumi (+4,2%) e accrescimenti (+14,2%) superiori e migliori indici di conversione (-3,2%) rispetto al gruppo AL. Il peso vivo finale (2748 g in media), l'indice di conversione (3,07), la resa di macellazione (59,8%) e le caratteristiche della carcassa non sono stati influenzati dal trattamento alimentare. L'escrezione giornaliera di azoto è stata ridotta (-0.08 g/d per coniglio; $P < 0,05$) nel gruppo D. L'aumento dell'età di macellazione da 73 a 80 d di età ha significativamente aumentato pesi vivi finali (+7,5%; $P < 0,001$) e consumi (+4 g/d; $P < 0,05$); ha ridotto gli accrescimenti (-3,1 g/d; $P < 0,001$) e peggiorato gli indici di conversione (2,92 vs. 3,22; $P < 0,001$) degli animali. Le caratteristiche della carcassa sono state fortemente ($P < 0,01$) modificate ritardando la macellazione di una settimana: il peso medio delle carcasse è aumentato (da 1563 g a 1705 g) così come la resa di macellazione (da 59,3% a 60,5%), l'adiposità e la muscolosità, mentre l'incidenza dell'intestino pieno è diminuita (-0,7 punti percentuali). L'escrezione di azoto è aumentata con l'età di macellazione (+0.21 g/d per coniglio).

In conclusione, la restrizione alimentare ha migliorato lo stato di salute senza compromettere le performance produttive e le caratteristiche della carcassa. La curva di razionamento progressiva ha rispecchiato in modo migliore il comportamento alimentare dei conigli rispetto alla curva scalare, permettendo di ottenere un livello alimentare in linea con quello atteso. Tuttavia, l'applicazione di questo tipo di programmi di razionamento alimentare appare difficile nelle aziende commerciali in quanto richiede molto tempo e lavoro quando non sono presenti sistemi di alimentazione automatici e potrebbe non essere adeguato per conigli allevati in colonia. L'aumento dell'età di macellazione ha incrementato la resa in carcassa, ma ha peggiorato la conversione alimentare ed aumentato l'escrezione di azoto.

Secondo contributo: *Restrizione alimentare basata sul tempo e composizione del gruppo in base al sesso: effetti su prestazioni produttive, comportamento alimentare e qualità della carne in conigli in accrescimento*

L'effetto del sistema alimentare (AL: *ad libitum* vs. R: razione) e della composizione del gruppo in base al sesso (X: sole femmine vs. Y: soli maschi vs. Z: sessi misti) su prestazioni produttive, comportamento alimentare, risultati di macellazione e qualità della carne è stato valutato su 288 conigli ibridi (144 femmine e 144 maschi), allevati dallo svezzamento (33 d di età) alla macellazione (75 d) in 18 recinti collettivi (1 m²; 16 animali per recinto).

La restrizione basata sul tempo è stata realizzata aumentando il tempo di accesso alle mangiatoie (principalmente durante le ore di buio) in modo graduale (+1 h ogni 3–4 d) da 10 h/d a 16 h/d nelle prime tre settimane. Dopodiché, il tempo di alimentazione è stato aumentato gradualmente (+1 h/d) fino al raggiungimento del libero accesso al cibo. Durante le prime tre settimane, è stato ottenuto un livello di restrizione mediamente pari al 95% dell'ingestione *ad libitum*. Nel primo periodo di prova, il consumo è diminuito (128 g/d vs. 122 g/d; P<0,01) e l'indice di conversione migliorato (2,40 vs. 2,29; P<0,001) nei conigli razionati rispetto a quelli alimentati a volontà. Nel secondo periodo di prova, i conigli razionati hanno mostrato un peggiore indice di conversione (2,70 vs. 2,83; P<0,001) rispetto ai conigli AL. Nell'intero periodo, le prestazioni produttive non sono state influenzate dal sistema alimentare. Diversamente, la restrizione ha fortemente modificato il comportamento alimentare dei conigli R, che mostravano un consumo medio orario superiore rispetto ai conigli AL per tutto il periodo di accesso all'alimento. Tuttavia, una volta raggiunto il libero accesso alle mangiatoie, nell'arco di pochi giorni, i conigli R hanno adottato un comportamento alimentare simile a quello degli animali sempre alimentati a volontà. Il razione non ha modificato il peso di macellazione (2860 g in media), la resa di macellazione (61,0%) e la qualità della carne dei conigli.

Il sesso degli animali non ha influenzato le prestazioni produttive nel primo periodo, mentre nel secondo periodo i recinti con sole femmine o a sessi misti hanno mostrato accrescimenti superiori rispetto a quelli di soli maschi (47,0 g/d vs. 43,8 g/d vs. 46,1 g/d per i gruppi X, Y e Z rispettivamente; P<0,01). Allo stesso tempo, l'indice di conversione è diminuito nei recinti X e Z rispetto ai recinti Y (3,66 vs. 3,91 vs. 3,72; P<0,01). Considerando l'intero periodo, accrescimenti, consumi ed indici di conversione non sono cambiati in funzione della composizione del gruppo e tutti gli animali hanno raggiunto pesi vivi simili alla fine della prova.

Le perdite di trasporto sono risultate superiori nei gruppi X rispetto ai gruppi Y e Z (3,00% vs. 2,45% vs. 2,80%; P<0,01), mentre la resa di macellazione (60,2% vs. 61,8% vs. 61,0%; P<0,001) e l'incidenza della testa (7,41% vs. 7,65% vs. 7,60%; P<0,05) sono risultate più alte nei

maschi. Il pH della carne, le perdite di scongelamento e lo sforzo di taglio misurati sul muscolo *l. lumborum* sono state significativamente ($0,05 < P < 0,01$) più bassi nei gruppi Y e Z rispetto ai gruppi X. La frequenza di ferite riscontrate alla fine della prova è aumentata nei recinti di soli maschi e, in misura inferiore, in quelli a sesso misto (5,2% vs. 18,3% vs. 13,3%; $P < 0,05$).

In conclusione, prestazioni produttive, risultati di macellazione e qualità della carne non sono state influenzate dal sistema di alimentazione. Il progressivo aumento delle ore di accesso alle mangiatoie da 10 h/d a 16 h/d nelle prime tre settimane non ha permesso un controllo preciso del livello di restrizione in quanto i conigli sono stati in grado di adattare molto rapidamente la loro capacità di ingestione alimentare al periodo di accesso alle mangiatoie.

Le prestazioni produttive, i risultati di macellazione e qualità della carne non sono cambiati in funzione della composizione del gruppo in base al sesso. Tuttavia, alloggiare i conigli in gruppi di soli maschi ha aumentato l'incidenza di ferite al termine della prova.

Terzo contributo: *Effetto del programma alimentare e della numerosità del gruppo su prestazioni produttive, stato di salute e qualità della carne in conigli in accrescimento*

L'effetto del programma alimentare (AL: *ad libitum* vs. R: razione) e della numerosità del gruppo (8 vs. 16 vs. 32 conigli per recinto) su prestazioni produttive, stato di salute, caratteristiche della carcassa e qualità della carne è stato valutato su 320 conigli ibridi (160 femmine e 160 maschi) allevati dallo svezzamento (31 d di età) alla macellazione (73 d) in 20 recinti collettivi a pari densità (16 animali/m²).

Il programma di razione ha previsto una progressiva diminuzione del tempo di accesso alle mangiatoie durante la prima settimana (da 14 h/d a 8 h/d), una fase stabile a 8 h/d durante la seconda settimana e un progressivo aumento (+1 h/d) durante la terza e la quarta settimana (fase di re-alimentazione), al fine di ottenere un livello di restrizione pari al 85% del *ad libitum* nel primo periodo di prova. Nelle ultime due settimane, tutti i conigli hanno avuto libero accesso all'alimento.

La restrizione alimentare ha significativamente ($P < 0,001$) ridotto il consumo (-15%) e l'indice di conversione (-12%) nelle prime tre settimane. Nelle ultime tre settimane, i conigli precedentemente razionati hanno mostrato accrescimenti superiori rispetto ai conigli AL (47,2 g/d vs. 48,8 g/d; $P < 0,05$), mentre consumi e indici di conversione non differivano a livello statistico tra i gruppi AL ed R. Nell'intero periodo, l'indice di conversione è stato significativamente migliore nel gruppo R rispetto al gruppo AL (3,05 vs. 2,93; $P < 0,001$). I problemi digestivi sono apparsi nei conigli AL nelle prime settimane di prova, nonostante la somministrazione di una dieta medicata nel primo periodo. Durante la fase di re-alimentazione, la morbilità è aumentata nei

conigli precedentemente razionati. Alla fine della prova, la mortalità è stata tendenzialmente superiore nei conigli R (6,3% vs. 11,9%; $P < 0,10$). Peso di macellazione (2734 g in media), resa di macellazione (61,3%), caratteristiche della carcassa e qualità della carne non sono stati influenzati dal programma alimentare.

Nessuna differenza rilevante sulle prestazioni produttive, i risultati di macellazione e la qualità della carne è stata osservata in funzione della numerosità del gruppo. Tuttavia, i conigli stabulati in gruppi di maggiori dimensioni sono stati esposti ad un rischio sanitario più elevato rispetto a quelli accasati in gruppi più piccoli (6,3% vs. 14,8% vs. 18,0% per i gruppi da 8, 16 e 32 animali/recinto rispettivamente; $P < 0,05$).

In conclusione, l'applicazione di un programma di razionamento caratterizzato da una progressiva riduzione del tempo di accesso al cibo durante la prima settimana, una fase di accesso costante durante la seconda e un progressivo aumento durante la terza e la quarta settimana ha permesso di raggiungere il livello di restrizione atteso nel primo periodo di prova (85% del *ad libitum*). Il razionamento alimentare ha significativamente migliorato l'indice di conversione senza modificare il peso vivo finale, i risultati di macellazione e la qualità del prodotto finale. Tuttavia, nonostante la re-alimentazione progressiva, il tasso di mortalità è aumentato nei conigli precedentemente razionati durante il secondo periodo di prova. La stabulazione degli animali in gruppi numerosi ha peggiorato le condizioni sanitarie.

Quarto contributo: *Effetto di differenti programmi di restrizione e re-alimentazione su prestazioni produttive, fisiologia digestiva, stato di salute, risultati di macellazione e qualità della carne in conigli allevati in colonia*

L'effetto del sistema di alimentazione (AL: *ad libitum* vs. R: razionamento) e del metodo di razionamento (accesso al cibo diurno vs. notturno) su prestazioni produttive, fisiologia digestiva, stato di salute, risultati di macellazione e qualità della carne è stato valutato su 400 conigli ibridi allevati dallo svezzamento (28 d di età) alla macellazione (70 d) in recinti collettivi (8 conigli per recinto, densità 16 animali/m²). Inoltre, sono stati valutati gli effetti della velocità di re-alimentazione (veloce: +4 h/d vs. lenta: +1/+2 h/d di accesso al cibo) e della tecnica di re-alimentazione (completa: fino a 24 h/d vs. incompleta: fino a 12 h/d di accesso al cibo). I seguenti gruppi sperimentali sono stati formati: *ad libitum* (AL); razionamento diurno con re-alimentazione veloce e completa (DF); razionamento notturno con re-alimentazione veloce e completa (NF); razionamento notturno con re-alimentazione lenta e completa (NS); razionamento notturno con re-alimentazione molto lenta ed incompleta (NI).

I programmi di razionamento hanno previsto una fase di riduzione del tempo di accesso alle mangiatoie durante la prima settimana (da 14 a 9 h/d), una fase stabile durante la seconda settimana (8 h/d) e un periodo di re-alimentazione variabile a partire dalla quarta settimana (fase di re-alimentazione).

Il razionamento diurno (ossia l'accesso diurno alle mangiatoie) ha inizialmente comportato un livello di restrizione più severo rispetto a quello ottenuto con i programmi di razionamento notturno (accesso alle mangiatoie durante le ore di buio), ma dopo una settimana di adattamento i conigli D hanno raggiunto i livelli di ingestione dei conigli N. La riduzione del tempo di alimentazione (fino a 8 h/d) nelle prime due settimane ha diminuito il consumo alimentare (-24%) e l'accrescimento medio giornaliero (-7%), portando a pesi vivi significativamente inferiori alla fine della terza settimana negli animali razionati rispetto a quelli alimentati a volontà (1761 g vs. 1671 g; $P < 0,001$). I conigli razionati hanno mostrato un indice di conversione migliore rispetto agli *ad libitum* (2,11 vs. 1,95; $P < 0,001$) nel primo periodo di prova. Infatti, la prova di digeribilità effettuata durante la seconda settimana, ha evidenziato un significativo ($0,01 < P < 0,001$) aumento della digeribilità della sostanza secca (+3,5%) e della maggior parte dei nutrienti della dieta nei conigli razionati rispetto a quelli *ad libitum*, indipendentemente dal metodo di restrizione adottato. A 43 d di età, il pH del contenuto ciecale era inferiore nei conigli razionati rispetto ai conigli alimentati a volontà (5,46 vs. 5,34 in media; $P < 0,01$). In accordo, la produzione totale di acidi grassi volatili (AGV) era superiore nei conigli razionati (+15% rispetto al gruppo *ad libitum*), mentre l'azoto ammoniacale è diminuito nei gruppi razionati (-29%).

Nel secondo periodo di prova, a seguito della fase di re-alimentazione, l'accrescimento giornaliero è significativamente aumentato nei conigli precedentemente razionati rispetto ai conigli AL (47,1 g/d vs. 51,1 g/d; $P < 0,001$) come conseguenza dell'aumento del consumo alimentare (157 g/d vs. 165 g/d per i gruppi AL e razionati rispettivamente; $P < 0,001$). I conigli sottoposti a re-alimentazione incompleta hanno mostrato una maggiore efficienza alimentare rispetto a quelli completamente re-alimentati ($P < 0,05$). Infatti, alla fine della fase di re-alimentazione (51 d di età), la digeribilità dell'energia lorda e della proteina grezza è risultata superiore nel gruppo NI rispetto ai gruppi sottoposti a re-alimentazione completa ($0,05 < P < 0,01$). Alla stessa età, la produzione totale di VFA era ancora maggiore nei conigli ancora sottoposti a razionamento rispetto a quelli *ad libitum* (+19% in media; $P < 0,001$) ed aumentava con la diminuzione della velocità di re-alimentazione ($P < 0,01$).

Lo stato di salute degli animali non è stato influenzato né dal sistema di alimentazione né dal metodo di razionamento o dal programma di re-alimentazione. I pesi di macellazione (2607 g in

media), le rese di macellazione (60,2%), le caratteristiche della carcassa e la qualità della carne non sono stati modificati dal programma alimentare.

In conclusione, un programma di razionamento notturno che preveda la graduale diminuzione del tempo di accesso alle mangiatoie nella prima settimana del post-svezzamento e di aumentare lentamente il tempo di alimentazione a partire dalla quarta settimana fino a raggiungere un massimo di 12 h/d di accesso alla mangiatoia potrebbe rappresentare la strategia migliore per ottimizzare l'efficienza alimentare nei conigli in accrescimento senza influenzare negativamente le prestazioni produttive, i risultati di macellazione e la qualità della carne. Tuttavia, nemmeno l'applicazione di un programma di razionamento con re-alimentazione incompleta è risultata efficace nel controllo dello stato di salute.

I livelli di restrizione alimentare testati nelle nostre prove (85-90% del *ad libitum* durante le prime tre settimane dopo lo svezzamento) sono stati adottati per trovare un equilibrio tra i supposti effetti positivi sulla salute digestiva e quelli negativi riscontrati spesso da vari autori sulle prestazioni produttive. In generale, una restrizione alimentare severa ($\leq 80\%$ del *ad libitum*) applicata durante la fase di post-svezzamento permette di ottenere un importante miglioramento dello stato di salute degli animali che rimane evidente anche quando si considera l'intero periodo di allevamento; tuttavia restrizioni simili possono compromettere le prestazioni complessive dei conigli diminuendo il peso vivo finale e la resa di macellazione e peggiorando le caratteristiche della carcassa.

Nel primo contributo sperimentale, con conigli stabulati individualmente, il tasso di complessivo mortalità è stato significativamente ridotto applicando un livello di restrizione attorno al 90% durante il post svezzamento, in particolare quando è stata adottata la curva di restrizione progressiva con variazioni giornaliere. È possibile perciò ipotizzare che anche un razionamento non troppo spinto potrebbe esercitare un effetto protettivo sulla salute intestinale quando i contatti tra animali siano limitati dalla stabulazione individuale (o in bicellulare). D'altra parte, nel terzo contributo, un programma di restrizione più severo ha salvaguardato lo stato di salute degli animali, ma tale effetto è sparito quando i conigli razionati hanno iniziato la fase di re-alimentazione. In questo modo, l'adozione di programmi di razionamento potrebbe avere addirittura un effetto negativo sullo stato di salute dei conigli, determinando ad un aumento di morbilità e mortalità durante la fase di ingrasso (quando avviene la re-alimentazione) con un incremento dell'impatto economico delle perdite dovute agli animali morti e scartati dalla macellazione. Nemmeno l'utilizzo di una re-alimentazione molto lenta ed incompleta (fino a un massimo di 12 h/d di accesso all'alimento) si è dimostrato efficace nel limitare l'incidenza di

patologie enteriche una volta terminata la copertura del trattamento antibiotico somministrato nella dieta da post-svezzamento.

Nelle prime due sperimentazioni, nessuna differenza in termini di efficienza alimentare è stata rilevata tra animali razionati o alimentati *ad libitum*, in quanto il lieve miglioramento osservato nel periodo di razionamento veniva annullato dal peggioramento dell'efficienza alimentare del secondo periodo di re-alimentazione. Diversamente, nel terzo e nel quarto contributo, l'adozione di programmi di razionamento più severi rispetto a quelli testati nelle prime due prove, ha diminuito l'accrescimento degli animali nel primo periodo, ma alla fine della prova i conigli razionati hanno raggiunto pesi vivi simili a quelli dei conigli alimentati a volontà, grazie agli accrescimenti compensativi che si sono verificati durante la fase di re-alimentazione. Inoltre è stata rilevata una significativa riduzione dell'indice di conversione nei conigli razionati, sia nel primo che nell'intero periodo di prova, soprattutto quando è stata applicata una re-alimentazione incompleta. Il miglioramento dell'efficienza alimentare nei conigli razionati può essere legato ad un aumento dell'utilizzazione digestiva dei nutrienti. Tuttavia, questo effetto avviene solamente durante la fase di restrizione. Infatti, solo l'applicazione di una re-alimentazione incompleta ha permesso ai conigli di mantenere una maggiore efficienza digestiva durante il secondo periodo di allevamento. Non va poi dimenticato l'effetto della restrizione alimentare sulla composizione corporea, con una tendenziale riduzione dell'accrescimento lipidico e un miglioramento della ritenzione proteica.

Nessuno dei programmi di razionamento testati nelle quattro prove sperimentali ha modificato in modo sostanziale e con ricadute commerciali i risultati macellazione, le caratteristiche della carcassa e la qualità della carne di coniglio. I moderati livelli di razionamento applicati e la lunga durata della fase di re-alimentazione possono spiegare i risultati ottenuti.

Il comportamento alimentare dei conigli cambia in modo considerevole quando gli animali sono sottoposti a programmi di razionamento. Tuttavia, nelle nostre condizioni, i conigli hanno ripreso un comportamento alimentare simile a quello degli animali *ad libitum* entro pochi giorni dal raggiungimento del libero accesso all'alimento. Un accesso al cibo permesso durante le ore di luce ha comportato cambiamenti più profondi nel comportamento alimentare dei conigli, che hanno impiegato circa una settimana per raggiungere i livelli di ingestione degli animali alimentati durante il periodo di buio.

In conclusione, i nostri risultati confermano la possibilità di applicare programmi di razionamento alimentare anche nel sistema produttivo italiano con l'obiettivo principale di migliorare l'efficienza alimentare. Al contrario, il controllo dello stato di salute appare ancora difficile da ottenere e la relazione tra restrizione alimentare e salute digestiva necessita di ulteriori

approfondimenti. Una restrizione alimentare temporale, caratterizzata da un tempo di alimentazione inferiore a 10 h/d durante le prime tre settimane di post-svezzamento e seguita da una re-alimentazione incompleta (fino a 12 h/d) potrebbe essere il metodo più efficace e nello stesso tempo più facile da applicare in campo e il più adeguato per conigli allevati in gruppo. La valutazione di possibili relazioni sinergiche tra programmi di restrizione alimentare e diverse composizioni delle diete come pure le relazioni con la genetica animale (sesso, tipo genetico) potrebbero essere oggetto di ulteriori studi.

Abstract

Role of feed restriction programs on enhancing gut health, feed efficiency and meat quality in growing rabbits

The application of feed restriction strategies to meat rabbit production is focused on two main aspects: 1) the improvement of animal health status by reducing mortality and morbidity due to digestive troubles; 2) the increase in the global farm efficiency by enhancing feed conversion and decreasing the costs related to antibiotic treatments. Both of these issues are of great interest in view of guaranteeing an economic sustainability to farmers and meeting the European regulations in terms of food safety. Hence, over the last decades, the effects of feed restriction strategies in growing rabbits have been widely investigated. Most of the studies has carried out feeding restriction during the first three or four weeks after weaning, with the aim to obtain feeding levels ranging from 60% to 90% of *ad libitum*, and free feeding during the last phase of fattening (from one to three weeks). Quantitative feed restrictions have been obtained directly by reducing the amount of diet supplied or indirectly by limiting the feeding time or the drinking time. Furthermore, qualitative feed restriction has been performed by reducing the energy level in the diets supplied to animals. The choice of restriction method should take into account the rearing facilities and equipment, the environmental conditions, and the impact on animal welfare.

The literature reported that rationing programs could represent a valid tool to reduce the incidence of digestive troubles, to increase diet utilization and to improve feed efficiency in growing rabbits. However, the protective effect of feed rationing on gut health is only limited to the restriction phase, while the incidence of digestive disease could even increase during fattening in rabbits previously restricted. Therefore, the re-feeding technique represents a critical point, but few studies have been focused on this issue. Moreover, the multifactorial origin of ERE makes difficult the interpretation of the variable and sometimes contrasting results among studies. On the other hand, the improvement of feed conversion seems to be a constant effect, but the economic benefits can be limited because of a longer fattening period is often required to achieve the complete recover of the animals in terms of live weight and avoid a substantial decrease in slaughter yield. Indeed, the economic aspects of feed restriction techniques should be carefully evaluated especially in the markets that require high slaughter weights (e.g. Italian market).

The present thesis aimed to define an effective feed restriction program for enhancing feed efficiency and gut health in growing rabbits, without impairing slaughter traits, carcass and meat quality. The goal was pursued by focusing on three specific objectives (*i*) to evaluate the effect of

quantitative feed restriction systems in individually-housed rabbits; *ii*) to develop an adequate time-based feed restriction program in group-housed rabbits; *iii*) to optimize the management of the re-feeding phase. These points were developed in four experimental contributions whose working plan and main results are summarized below:

First contribution: *Effects of feed restriction system and slaughter age in growing rabbits*

The effect of the feeding system (AL: *ad libitum* vs. D: day-by-day restriction system vs. W: week-by-week restriction system) on digestive efficiency, sanitary status, growth performance, carcass traits, and nitrogen excretion was evaluated on 282 commercial crossbred rabbits kept in individual cages from weaning (36 d of age) to slaughter. The effect of slaughter age (73 vs. 80 d of age) was also assessed. The feed restriction was based on the administration of a restricted amount of the diet which increased from 80% to 100% of AL intake in the first three weeks after weaning according to two feeding curves characterized by either small daily increments (+4 g/d; D group) or large weekly increments (+23 g/week on average; W group). From the beginning of the 4th week, the amount of feed supplied for both restricted groups was increased by 4 g/d for 9 consecutive d in order to prevent high picks in food ingestion, then all rabbits were fed *ad libitum* until commercial slaughter. No antibiotic treatment was administered during the trial.

The apparent digestibility of the post-weaning diet was measured on 36 rabbits (12 per feeding system) from 49 to 53 d of age (during the restriction phase), while the digestibility of the fattening diet was measured on 36 rabbits (12 per feeding system) from 64 to 68 d of age (during the *ad libitum* period).

At the moment of the first digestibility trial, the feed ingestion of the restricted group was equal to 95% of AL group and therefore only the digestibility of crude protein was increased ($P < 0.001$). During the second period, no residual effect of the previous restriction treatment was observed on diet digestibility. In the whole trial, mortality, morbidity and sanitary risk reached respectively 12.7%, 11.3% and 24.0% on average. Mortality and sanitary risk were significantly ($P < 0.05$) reduced in restricted rabbits compared to those fed *ad libitum* (-12.0 percentage points), in particular when a day-by-day restriction curve was applied (-14.2 percentage points). In the first three weeks the feed restriction level was equal to 91% in D group and 93% in W group; daily growth decreased in both restricted groups (-7.5% with respect to AL) whereas feed conversion did not differ among groups. In the second period only W rabbits showed higher feed intake (+4.2%) and weight gain (+14.5%), and a better feed conversion (-3.2%) compared to AL rabbits. Final live weight (on average 2748 g), feed conversion ratio (3.07), cold dressing percentage (59.8%) and other carcass traits were not affected by the feeding treatment. The daily nitrogen

excretion was reduced (-0.08 g/d per rabbit; $P < 0.05$) in the D group respect to AL group. The increase of slaughter age from 73 to 80 d significantly increased final live weight (+7.5% g; $P < 0.001$) and feed intake (+4 g/d, $P < 0.05$), reduced daily weight gain (-3.1 g/d; $P < 0.001$) and impaired feed conversion (2.92 vs. 3.22; $P < 0.001$). All carcass traits were greatly ($P < 0.01$) modified by delaying slaughter age; carcass weight increased (1563 g to 1705 g) as well as dressing percentage (59.3% to 60.5%), carcass fatness and muscularity, whereas the incidence of full gut decreased (-0.7 percentage points). Nitrogen excretion was also increased (+0.21 g/d per rabbit) in older rabbits.

In conclusion, feed restriction improved rabbit health status without impairing growth performance and carcass traits. The day-by-day restriction curve reflected better the feeding behaviour of growing rabbits compared to the week-by-week curve, allowing to obtain a feeding level similar to that expected. However, the application of this type of restriction appears difficult in commercial farms because it is time and labour consuming if automatic feeding systems are not available and could not to be adequate with group-housed rabbits. The delay of slaughter increased dressing percentage, but impaired feed conversion and increased environmental nitrogen excretion.

Second contribution: *Time-based feed restriction and group composition for gender: effects on growth performance, feeding behaviour and meat quality of growing rabbits*

The effect of the feeding system (AL: *ad libitum* vs. R: feed restriction) and the sex-based group composition (X: only females vs. Y: only males vs. Z: mixed sexes) on growth performance, feeding behaviour, slaughter traits and meat quality was evaluated on 288 crossbred rabbits (144 females and 144 males), housed from weaning (33 d of age) to slaughter (75 d of age) in 18 collective pens (1 m²; 16 animals per pen). A time-based feed restriction was realized by increasing the access time to the feeders (allowed mainly during the hours of darkness) step-by-step (+1 h every 3–4 d) from 10 h/d to 16 h/d in the first three weeks. After that, feeding time increased gradually (+1 h/d) until reaching the free access to the feeders. During the first three weeks, an average restriction level equal to 95% of *ad libitum* was obtained. During the first three weeks, feed intake decreased (128 vs. 122 g/d; $P < 0.01$) and feed conversion improved (2.42 vs. 2.29; $P < 0.001$) in restricted rabbits in comparison with rabbits fed *ad libitum*. In the second trial period, the previously restricted rabbits showed a worse feed conversion (2.70 vs. 2.83; $P < 0.001$) compared to rabbits always fed *ad libitum*. In the whole trial, growth performance were not affected by the feeding system. The feed restriction greatly modified the feeding behaviour of R rabbits, which showed a higher hourly feed consumption compared to AL ones throughout the

time of access to the feeders. Once fed freely, R rabbits showed an intake pattern similar to that of AL rabbits within two days. The feed restriction did not affect slaughter weight (on average 2860 g), dressing percentage (61.0%) or meat quality.

In the first trial period growth performance did not change according to the sex of the animals, while in the second period the pens with only females or with mixed sexes showed higher daily growths compared to those with only males (47.0 g/d vs. 43.8 g/d vs. 46.1 g/d for X, Y and Z groups, respectively; $P < 0.01$). At the same time, the feed conversion ratio decreased in pens X and Z in comparison to Y ones (3.66 vs. 3.91 vs. 3.72; $P < 0.01$). Considering the whole rearing period, daily weight gain, feed intake and feed conversion did not change according to the group composition and the animals reached similar live weights at the end of trial. Transport losses were higher in X pens compared to Y ones (3.00% vs. 2.45% vs. 2.80%; $P < 0.01$), while the cold dressing percentage (60.2% vs. 61.8% vs. 61.0%; $P < 0.001$) and the head proportion on carcass (7.41% vs. 7.65% vs. 7.60%; $P < 0.05$) were higher in males. Meat pH, thawing losses and shear force of *L. lumbrorum* were significantly ($0.05 < P < 0.01$) lower in Y and Z rabbits compared to X ones. The frequency of injuries increased in pens Y, with only males, and Z, mixed-sexes (5.2% vs. 18.3% and 13.3%; $P < 0.05$). In conclusion, growth performance and meat quality were not significantly affected by the feeding system. The progressive increase of the access time to the feeders from 10 to 16 h/d in the first three weeks did not allow to control accurately the feed restriction level because rabbits adjusted very quickly their ingestion capability. Growth performance, slaughter traits and meat quality were not affected by the sex-group composition. Housing animals in groups of only males increased the risks of aggressiveness.

Third contribution. *Effect of the feeding program and group size on growth performance, health status and meat quality in growing rabbits*

The effect of the feeding program (AL: *ad libitum* vs. R: feed restriction) and the group size (8, 16 or 32 rabbits per pen) on growth performance, health status, carcass traits and meat quality was evaluated on 320 crossbred rabbits (160 females and 160 males) reared from weaning (31 d of age) to slaughter (73 d) in 20 open-top pens with different group size (8, 16 or 32 animals) at the same stocking density (16 animals/m²).

Feed restriction provided a progressive decrease of the access time to the feeders during the first week (from 14 h/d to 8 h/d), a stable time of 8 h/d in the second week and a progressive increase (+1 h/d) during the third and fourth week (re-feeding phase), thus achieving a restriction level equal to 85% of *ad libitum*. Throughout the last two weeks, all rabbits had free access to the feeders.

Feed restriction significantly ($P < 0.001$) reduced feed intake (-15%) and feed conversion (-12%) in the first three-week period. During the last three-week period, the previously restricted rabbits showed higher daily growth rate compared to the AL ones (47.2 g/d vs. 48.8 g/d; $P < 0.05$), whereas feed intake and feed conversion did not differ between groups. In the whole period, feed conversion was significantly lower in R group compared to AL group (3.05 vs. 2.93; $P < 0.001$). Digestive troubles appeared before in AL rabbits, but morbidity soon raised in R group during the re-feeding phase. At the end of the trial, mortality rate tended to be higher in the restricted rabbits (6.3% vs. 11.9%; $P < 0.10$). Slaughter weight (2734 g), dressing percentage (61.3%), carcass traits and meat quality were not affected by the feeding program.

No significant difference in growth performance, slaughter results and meat quality was observed according to the group size. Nevertheless, rabbits housed in larger groups were subjected to increased sanitary risk (6.3% vs. 14.8% and 18.0% for groups with 8, 16 and 32 rabbits/pen, respectively; $P < 0.05$).

In conclusion, the application of a feed restriction program characterized by a progressive reduction of the access time to the feed during the first week, a constant phase in the second week, and a progressive increase during the third and fourth week allowed to obtain the expected restriction level in the first trial period (85% of *ad libitum*). Feed restriction significantly improved feed conversion without affecting final live weight, slaughter traits and meat quality. However, even if a progressive re-feeding phase was performed, morbidity and mortality rate increased in the previously restricted rabbits during the second trial period. Housing animals in large groups impaired sanitary conditions.

Fourth contribution. *Effect of different rationing and re-feeding programs on growth performance, digestive physiology, health status, slaughter traits and meat quality in group-housed rabbits*

The effects of feeding system (*ad libitum* vs. restriction) and restriction method (diurnal vs. nocturnal access to feed) were evaluated on growth performance, digestive physiology, health status, slaughter traits and meat quality of 400 growing rabbits housed from weaning (28 d of age) to slaughter (70 d) in collective pens of 8 animals (stocking density of 16 animals/m²). Moreover, the effects of re-feeding speed (fast: +4 h/d vs. slow: +1/+2 h/d of access to feed) and re-feeding level (complete: up to 24 h/d vs. incomplete: up to 12 h/d of access to feed) were also assessed. The following groups were formed: *ad libitum* (AL); diurnal rationing with fast and complete re-feeding (DF); nocturnal rationing with fast and complete re-feeding (NF); nocturnal rationing with

slow and complete re-feeding (NS) and nocturnal rationing with very slow and incomplete re-feeding (NI).

The rationing programs previewed a first week of decreasing access time to feed until 9 h/d; a second week of stable daily access time (8 h/day) and a re-feeding period starting from the beginning of the third week.

The diurnal access to the feed led to an initial restriction level more severe than that obtained with the nocturnal rationing programs, but after one week of adaptation DF group reached the feed intake of nocturnal restricted groups (N groups). Feeding time reduction (until 8 h/d) in the first two weeks decreased feed intake (-24% of *ad libitum*) and weight gain (-7% with respect to the AL group) of restricted rabbits leading to a significantly lower live weight at the end of the third week (1761 g vs. 1671 g for AL and restricted rabbits, respectively; $P < 0.001$). However, restricted rabbits showed a feed conversion significantly lower compared to that of *ad libitum* ones (2.11 vs. 1.95; $P < 0.001$) in the first period. Indeed, during the second week, the feed restriction significantly ($0.01 < P < 0.001$) enhanced the digestibility of dry matter (+3.5%) and most of nutrients compared to the *ad libitum* system, irrespective of the feed restriction method. At 43 d of age, the pH of the caecal content was lower in DF and NF rabbits compared to AL ones (5.46 vs. 5.34 on average; $P < 0.01$). Accordingly, the total production of volatile fatty acids (VFA) increased in DF and NF groups with respect to AL group (+15% on average), while the N-ammonia decreased in restricted groups (-29% on average). In the second rearing period, with the re-feeding phase, the weight gain was higher ($P < 0.001$) in previously restricted rabbits compared to AL ones (47.1 g/d vs. 51.1 g/d) because of the increase ($P < 0.001$) in feed intake (157 g/d vs. 165 g/d). The NI group, characterized by an incomplete re-feeding, showed higher feed efficiency with respect to the groups submitted to complete re-feeding curves ($P < 0.05$). Indeed, at the end of re-feeding (51 d of age), the digestibility of gross energy and crude protein of fattening diet was higher in NI group compared to the fast re-fed groups ($0.05 < P < 0.01$). At the same age, the total VFA production was still higher in the restricted groups compared to AL one (+19% on average; $P < 0.001$) and increased with the decrease of re-feeding speed ($P < 0.01$).

Animal health status was not affected either by the feeding system (*ad libitum* or feed restriction) or by the rationing method (diurnal or nocturnal) or by the re-feeding program (fast, slow or incomplete).

Slaughter weight (2607 g on average), dressing percentage (60.2% on average), carcass traits and meat quality did not change according to the feeding program.

In conclusion, a nocturnal rationing program performed by decreasing gradually the access time to feed in the first week of post-weaning and increasing slowly the feeding time up to 12 h/d

at the fourth week onwards represented the best strategy to optimize feed efficiency in growing rabbits without impairing health status, growth performance, slaughter traits and carcass and meat quality.

The restriction levels applied in our trials (85–90% of *ad libitum* during the first three weeks after weaning) have been set to find a balance between the positive effects on gut health and the negative impact on the growth performance of rabbits. Generally, a more severe feed restriction ($\leq 80\%$ of *ad libitum*) applied during post-weaning allows to obtain a relevant enhancement of animal health status, but it may compromise the overall productive performance of rabbits by decreasing final live weight, slaughter yield and impairing carcass traits. In the first contribution, with rabbits housed individually, the total mortality rate was significantly reduced by applying a moderate restriction level (90% of *ad libitum*) during post-weaning especially when a progressive and gradual feed restriction curve was adopted. In this regards, a slight feed restriction may exert a protective effect on gut health in conditions of limited contacts among animals. Nonetheless, in the third contribution, a strict rationing program preserved the digestive health of rabbits housed collectively in the first two weeks, but its positive effect disappeared when the animals started the re-feeding phase. Feed restriction can lead to a relevant increase of mortality in previously restricted rabbits during the last weeks of fattening, thus increasing the economic impact of animal losses. Indeed, in the fourth contribution neither the application of a very slow and even incomplete (12 h/d) re-feeding programs was effective in reducing the incidence of digestive troubles once an antibiotic-free diet was supplied.

In the first two trials, no differences in terms of feed efficiency and growth performance were detected among restricted and *ad libitum* groups. On the other hand, in the third and fourth contributions, with the implementation of more severe feed restrictions, daily growth decreased in the first period, but restricted rabbits reached live weights similar to those of AL ones at the end of trial, thanks to the occurrence of a compensatory growth during the re-feeding period. Moreover, a significant reduction of feed conversion was detected in restricted groups especially when an incomplete re-feeding was used. The rationing programs did not affected slaughter results, carcass traits and meat quality: the restriction levels tested and the length of re-feeding phase could explain these results. The improvement of feed efficiency in restricted rabbits could be related to the increase in diet digestibility. However, this effect occurred only during the restriction phase. Indeed, an incomplete re-feeding until the end of trial allowed to maintain a higher diet digestibility compared to the complete re-feeding techniques.

The feeding behaviour of rabbits greatly changed when the animals were subjected to limited access time to the feeders. However, in our conditions, the rabbits adopted a feeding behaviour similar to that of rabbits always fed *ad libitum* within few days after reaching the free access to the feed. In the fourth contribution, a diurnal access to the feed involved a deeper change in the feeding behaviour of rabbits, which need approximately one week to reach the intake level of rabbits fed during the night.

In conclusion, our results confirm the possibility to apply feed restriction programs in the Italian production system with the main objective of enhance feed efficiency. The control of digestive diseases still appear difficult to be achieved and the relationship between feed restriction and gut health needs further investigations. A time-based feed restriction, characterized by a feeding time shorter than 10 h/d during the first three weeks after weaning and followed by an incomplete re-feeding (up to 12 h/d) could be the most suitable technique to be used in field conditions and in group-housed rabbits. Further investigations may be also carried out to prove a possible synergistic effect by combining feed restriction programs with different diet compositions.

Introduction

Rabbit digestive physiology and feeding

Summarizing the digestive system

The core of the rabbit digestive system is represented by the caecum where the main fermentation processes develop. The microbial activity in the caecum plays a key role in the digestive utilization of nutrients and contributes in maintaining the digestive health of rabbits (Carabaño *et al.*, 2010). Moreover, a particular behaviour known as caecotrophy, during which the rabbit ingests the soft faeces formed in the caecum, improves the digestive utilization of the diet and ensures to the rabbit an additional supply of protein and vitamin from microbial source (Xiccato and Trocino, 2008).

Soft faeces, or caecotrophes, derive from the fermentation of small particles (<0.3 mm) and water-soluble substances that are brought back to the caecum through the anti-peristaltic movements of the proximal colon, while the coarser particles (>0.3 mm) are rapidly moved to the distal part of the colon and then excreted as hard faeces. The chemical composition of soft faeces is similar to that of the caecal content including high proportions of protein, minerals and vitamins, while hard faeces are very rich in fibre as a consequence of the mechanical separation of digesta at the caecum and proximal colon. The protein ingested with soft faeces represents 10% to 23% of the total protein intake per day in growing rabbits. In addition, the microbial activity contributes in enriching soft faeces in vitamin K and B, and in essential amino acids including lysine, sulphur amino acids and threonine (Carabaño *et al.* 2010).

The caecotrophes (groups of 3–6 small beads of 5–mm diameter) are identified by the rabbit at the moment of their excretion and ingested without gnawing. Soft faeces remain undamaged in the stomach fundus for a 3–6 hours thanks to the cover of a mucous envelope secreted at the proximal colon that protects the faecal material from enzymatic degradation. At the same time, the microflora activity and volatile fatty acids (VFAs) production continue inside the caecotrophe. The final digestion of soft faeces occurs in the small intestine in the absence of mucous envelope (Carabaño *et al.* 2010).

The symbiotic microbial population in the caecum is composed mainly by *Bacteroides* (10^9 – 10^{10} bacteria/g) and *Bifidobacterium*, *Clostridium*, *Streptococcus* and *Enterobacter* at a lower bacterial load (10^1 – 10^2 bacteria/g). However, the real composition of the microflora in the gut seems to be much more complex and almost yet unknown. In addition, the microbiota composition varies during the life of the rabbit and it is strongly affected by the age at weaning, the beginning of solid feed consumption and caecotrophy (Carabaño *et al.* 2010; Gidenne *et al.* 2010).

The microbial fermentation processes of carbohydrates and proteins produce VFAs and ammonia, respectively. The VFAs profile is represented by 60 to 80% of acetate, 8 to 20% of butyrate, 3 to 10% of propionate and 0.5 to 1% of caprylic, besides a very low rate of valeric acid. The VFAs can be rapidly absorbed in the hindgut tissues. Propionate and butyrate are mainly metabolized in the liver, while the acetate is available for extrahepatic tissue metabolism (Marty and Vernay, 1984; Carabaño *et al.* 2010). Marty and Vernay (1984), estimated that the rabbit achieves up to 40% of its maintenance energy requirement from VFAs produced by fermentation processes in the hindgut.

Caecal VFAs concentrations are higher during the excretion period of hard faeces rather than during the excretion of caecotrophes. The increment of VFAs in the excretion period of hard faeces may be related to the greater flow of substrate in the caecum as a consequence of the raise in feed intake or the development in the microbial population associated to the anti-peristaltic movements of the proximal colon (Bellier *et al.*, 1995). Caecal pH varies inversely to the increase in VFAs concentration showing higher values during soft faeces excretion (Carabaño *et al.* 2010).

The digestive efficiency of rabbits does not depend only on endogenous enzyme activities and digestion by the symbiotic microbial population, but also on the transit time of the particles through the digestive tract. The transit of feed through the stomach (3 h to 6 h) and caecum (4 h to 9 h) is relatively slow, while the transit times in the jejunum (10 min to 20 min) and ileum (30 min to 60 min) are shorter. Mean transit time through the entire digestive tract ranges from 9 h to 30 h with an average of 19 h. The application of feed restriction strategies as well the use of diets with smaller particle size increase the mean transit time of feed. Rising the transit time of digesta in the gut nutrients digestibility increase, but undesirable fermentation patterns could take place in the caecum and digestive troubles may appear. On the contrary, a decrease of the total mean transit time should be expected in presence of fibrous diets (Gidenne *et al.* 2010).

Nutritional requirements and feeding plans

When rabbit is fed *ad libitum*, dietary energy concentration and the ingestion capacity of animals are the two main factors regulating the daily feed intake. The energy system adopted in rabbit is based on digestible energy (DE) (Xiccato and Trocino, 2010). The appetite in rabbits is regulated by a physical mechanism such as the repletion of the digestive tract when the dietary energy concentration is below of approximately 9.5 MJ/kg of DE, and by a chemostatic mechanism that reduces the feed intake as a consequence of high levels of glucose in blood when the threshold value is overcome (Xiccato and Trocino, 2008). In this latter condition, a daily ingestion of 900 to 950 kJ DE per day per kg of metabolic weight is expected in growing rabbits.

The energy requirements of the animals vary according to body weight, vital and productive functions (maintenance, growth, lactation and pregnancy) and environmental conditions (temperature, humidity, air speed).

Crude protein (CP) requirement of the rabbit differs according to its physiological state and age. The rabbit needs a dietary CP concentration of 160–170 g/kg as-fed diet during the post-weaning period and 150–160 g/kg in the subsequent fattening period. An average digestibility of CP of 70% to 75% is measured in compound diets and the dietary concentration of digestible protein (DP) should be considered for defining accurately the protein requirement and intake. Indeed, due to the chemostatic regulation of appetite, the nitrogen requirements of rabbits are mainly expressed by the relationship between DP and DE. Several authors have studied the best DP to DE ratio for the different productive categories: an optimal level between 10.5 and 11.0 g/MJ is recommended for growing rabbits, that ensures a daily intake of 19 to 20 g CP or 12 to 13 g of DP and optimizes the growth rate (Xiccato and Trocino, 2008; de Blas and Mateos, 2010; Xiccato and Trocino, 2010).

Fibre is an essential component in rabbit feeding and an inadequate dietary level may be responsible of digestive problems especially during the post-weaning period (Gidenne, 2003; Gidenne *et al.*, 2010; Trocino *et al.*, 2013). The requirement of crude fibre (CF) in rabbits ranges from 17–18% to 15–16% from post-weaning to growing period. However it is necessary to evaluate the intake of the different fibrous fractions which are characterized by different effects on digestive physiology and health. Several methods have been developed to estimate the fibre constituents in animal feeds making complex the formulation of balanced diets on the basis of requirements for preventing digestive problems (Maertens, 2013).

Blas and Gidenne (2010) report that the starch content in diets for weaning periods should not overcome 120 g/kg as-fed. However it can reach approximately 200 g/kg during post-weaning (25–39 d of age) in presence of optimal dietary levels of neutral detergent fibre (NDF), i.e. between 300 g/kg to 360 g/kg (Gidenne and García, 2006). According to de Blas (2012), the starch is quickly fermentable at caecum and can be responsible of pathogenic intestinal microbiota proliferation when supplied at high amounts in young rabbits.

In the past, all categories of animals (young and reproducing does, weaning and growing rabbits, males) in the rabbit farm were fed a unique diet. In the current closed-cycle intensive farms the feeds are differentiated according to anatomical and physiological status, age and productive sector (Xiccato and Trocino, 2008). In particular, the feeding of growing rabbits is divided in two phases related to the two rearing periods (post weaning and fattening). In the first rearing period (from weaning at 30 to 35 d of age until 55–60 d), known as post-weaning, the feed

is poor in DE (9.2–9.6 MJ/kg as–fed) and starch (<120 g/kg) and rich in fibre (ADF>180–190 g/kg, ADL>45 g/kg) in order to limit digestive diseases and prevent mortality at this age. During this period the voluntary feed intake ranges from 80–100 g/d in the first week to 120–140 g/d in the third week. Frequently, the weaning diet is medicated with antibiotics and coccidiostatic drugs to prevent digestive diseases and epizootic rabbit enteropathy (ERE) in particular.

In the second rearing period, known as fattening period (from 50–60 d until slaughter age, i.e. 75–90 d), the feed is always antibiotic free and is characterized by a higher energy content (10.0–10.5 MJ/kg) and lower protein (150–155 g/kg of CP) and fibre (ADF>160–170 g/kg; ADL>40 g/kg) levels in order to improve feed conversion (FC) and allow a sufficient fat deposition in carcasses. In this period feed intake ranges from 150 to 180 g/d (Xiccato and Trocino, 2008).

Digestive disorders in growing rabbits

A brief overview

Digestive diseases are the main causes of morbidity and mortality in commercial rabbits farms especially during weaning and post–weaning periods (from 28 to 49 d of age). However, these pathologies may affect fattening rabbits up to slaughter age (65–80 d of age). Therefore digestive diseases are responsible of relevant economic losses for the farmers not only due to the increase of mortality rate but also because of an impairment of growth performance and feed conversion. The immaturity of rabbit gastrointestinal tract at weaning as well as poor hygienic and environmental conditions, stress events or the use of unbalanced diets may promote the pathogens development and spread out (Peeters, 1992).

Until 1997 the main pathogenic agents associated with digestive diseases in growing rabbits had been primarily of parasitic origin such as *Eimeria* spp., or bacterial origin such as enteropathogenic *Escherichia coli* (EPEC), *Clostridium spiroforme*, *Klebsiella* and *Clostridium piliforme* (Licois *et al.*, 2005). Other digestive diseases without a well–defined etiology had been recognized as non–specific enteritis (Bennegadi *et al.*, 2000).

In 1997 a new and severe gastrointestinal syndrome called epizootic rabbit enteropathy (ERE) appeared in French rabbit farms and rapidly spread all over the Europe. The disease mainly affected young rabbits during post–weaning period (from 42 to 60 d of age), but it has also been observed in older rabbits and sometimes in kits or adults. The rabbits affected by this intestinal pathology show a strong reduction of feed and water consumption, distension of whole gastrointestinal tract, filling of stomach with gas and fluid, caecal paresis, emission of liquid diarrhoea and sometimes fluid mucus. Moreover, Pérez de Rozas *et al.* (2005) reported strong decrease in the pH value of stomach and urine. The main features of ERE cecal microbiota are represented by dysbiosis and reduced taxonomic diversity (Bäuerl *et al.*, 2014).

Because of the high mortality (30–70%) and morbidity (up to 100%) rates, ERE is still considered the most serious disease in commercial rabbit farms (Pérez de Rozas *et al.*, 2005). Le Bouquin *et al.* (2009) sustain that late weaning (age at weaning ≥ 35 d), transfer of weaned rabbits, high volume of the fattening room (air volume/rabbit weight in fattening room at weaning ≥ 0.14 m³/kg) and a high mortality rate in rabbits before weaning are the main risk factors for an acute expression of ERE.

The ERE was experimentally reproduced by inoculating intestinal contents of ill or dead rabbits, the contact among animals or with contaminated breeding material (Licois *et al.*, 2000), confirming a contagious feature of this disease. Generally, non-specific lesions are detected throughout the intestinal tract (atrophy and fusion of the villus and loss of epithelial cells both in the ileum and jejunum) in presence of this pathology (Licois *et al.*, 2005; Dewrée *et al.*, 2007). However, in several animals affected by ERE no intestinal lesion are observed (Licois *et al.*, 2004). Therefore it is difficult to identify accurately the pathogenic agent implicated in the ERE development which is commonly ascribed to multifactorial features.

Initially, it has been supposed a role of feed in the disease appearance, such as the nature of raw materials, the presence of mycotoxins, pesticides or other chemical and microbiological contaminants. However, all these assumptions have been excluded (Licois *et al.*, 2005), but a role of feed as passive vector has been proved (Duperray *et al.*, 2000).

Several aspects of the disease, i.e. its transmissibility, the presence of lung lesions and the inefficacy of many antibiotics commonly used in rabbit farms, have suggested the implication of a virus in the ERE occurrence. Nevertheless, mainly rotavirus (Céré *et al.*, 2000; Marlier *et al.*, 2006) have been found on field samples, but strains of this virus, isolated from animals affected by ERE, have not developed the pathology in animals experimentally inoculated (Licois, 2004).

The use of prolonged antibiotic treatments, which results the most effective method for preventing the emergency of ERE, demonstrates a bacterial implication in the pathogenesis of disease. Enteropathogenic *Escherichia coli* have been sometimes found in ERE rabbits but the serotypes of the isolated strains have not been constant. Among potentially pathogenic coliforms in rabbits affected by ERE, enterotoxigenic *E. coli* strains have been also identified (Bäuerl *et al.*, 2014). However, the presence of *E. coli* has not been detected in most of the field samples obtained from ill rabbits (Licois, 2004). On the other hand, the implication of *Clostridium perfringens* in the occurrence of ERE has been found (Pérez de Rozas *et al.*, 2005; Marlier *et al.*, 2006; Szalo *et al.*, 2007; Carabaño *et al.*, 2008; Romero *et al.*, 2009) even if the experimental reproduction of this disease has not been achieved by inoculating this bacterium in healthy rabbits (Licois, 2004; Marlier *et al.*, 2006). Moreover *Clostridium perfringens* and its main toxin were absent in

numerous experimental samples obtained from acute phase of ERE reproduction. Other opportunistic and pathogenic bacteria, such as *Akkermansia muciniphila*, that contribute to the worsening of disease, have been found in ERE rabbits (Bäuerl *et al.*, 2014). Indeed, in presence of ERE, the development of secondary infections (including bacteria or coccidia), that are often associated with higher mortality rate in growing rabbits, are frequent in field conditions (Licois, 2004).

Therefore the use of antibiotics still remains the most effective solution to prevent the appearance of ERE and to control mortality and morbidity rates in growing rabbits. However, because of the limits imposed by European rules on the use of antibiotics in prophylactic treatments, the limited number of molecules currently available for the rabbit and their costs, the acquisition of antibiotic resistances and also the reserve of the consumers on the use of drugs in animal production, alternative strategies to antibiotic therapy should be used (Licois, 2004; Maertens, 2011). In this regard, any improvement in hygiene, environmental conditions and management of the rabbit farms may contribute to minimize drug utilization (Maertens, 2011, 2013). Furthermore, nutritional approaches play an important role in preserving animal health by reducing the incidence of digestive diseases (Gidenne, 2003; Carabaño *et al.*, 2008; Gidenne *et al.*, 2012a).

The barrier function

The intestinal saprophyte microflora and the animal defence mechanisms limit or reduce the growth of pathogens and the development of digestive troubles. Indeed, some animals survive to infections experimentally reproduced with pathogenic strains, despite the presence of disease symptoms. As regards competitive exclusion, non-pathogenic species predominate over pathogenic ones. Competitive exclusion can be achieved with different mechanisms, including different growth on a specific substrate, efficiency of mucosa colonization and production of substances with inhibitory activity against pathogens development (short chain fatty acids, sulphuric acid, non-conjugated biliary acids and bactericides) (Hampson *et al.*, 2001).

When the pathogen agent enters in the gut, the intestinal barrier limits the mucosa colonization and avoids the transport of bacteria and toxins through different mechanisms. The acidification of intestinal lumen and the protection of epithelium with a mucosal layer, secreted by the Goblet cells, are the first steps of the barrier effect. In particular, the mucosal layer protects the epithelium from mechanical, chemical and enzymatic damages and also from the adhesion of bacteria. Indeed, the development of main gastrointestinal pathogens, such as the virulent strains of *E. coli*, starts from an initial contact with the mucosa. On the contrary, other bacteria and in particular clostridia

(*C. spiroforme*, *C. perfringens*, *C. difficile*, *C. sporogenes*, etc.) do not adhere to the mucosa, but produce toxins. However, these toxins must overcome the mucosal layer and reach internal organs.

In the intestinal lumen, the immune system of mucosa produces a biological film of IgA immunoglobulin. In addition, antimicrobial peptides are generated by specific cells (known as Paneth cells) in the crypts (Kelly *et al.*, 2005). The main role of IgA is to preserve the mucosa integrity, permitting the survival of saprophyte bacteria and limiting the development of pathogens, while the antimicrobial peptides inhibit the growth of pathogen as well as saprophyte bacteria.

When the lumen barriers are overcome, bacteria and toxins enter in contact with the intestinal mucosa thus activating the local immune system, which acts by modulating the inflammatory response and developing tolerance mechanisms.

The structure integrity and the functionality of the intestinal mucosa are essential in order to prevent the mucosa colonization by pathogenic agents and also to ensure the correct work of the digestive tract for the processes of digestion, absorption, secretion and metabolism of nutrients. In fact, digestive disease are more frequent in rabbits during weaning and post-weaning periods because of the immaturity of intestinal mucosa and the damages on its structure due to the feeding change (from milk to solid feed). Therefore nutritional management can play a key role in avoiding structural damages of the intestinal mucosa and promoting mechanisms of renewal by supplying the required nutrients correctly (Carabaño *et al.*, 2008).

According to the diet composition

Feeding management is becoming a priority in rabbit rearing, especially in the prevention of digestive diseases. Under optimal breeding conditions, a nutritional imbalance should be responsible only for a decrease of performance without compromising animal health. However, dietary imbalances could induce a higher susceptibility of rabbits to digestive troubles. Furthermore, the excess of some minerals or vitamins can be toxic also in the presence of a good sanitary status of animals and optimal environmental conditions (Gidenne *et al.*, 2010).

The level and type of fibre and protein are the most important nutritional factors related to the occurrence of digestive troubles (Carabaño *et al.*, 2008; Gidenne *et al.*, 2010; Trocino *et al.*, 2013; Gidenne, 2015).

In healthy growing rabbits, when a fibre deficiency occurs (intake of ADF lower than 8–11 g/kg live weight/day), the retention time of digesta in the caecum increases, the level of fibre decreases while the starch concentration remains stable and there are irrelevant changes in ammonia and VFAs concentrations as well as in caecal pH. However, VFAs proportions are modified by rising significantly the proportion of butyrate and decreasing acetate. The role of these

changes on favouring digestive problems through low-fibre diets is still unclear (Gidenne *et al.*, 2010). Fibre, which modifies intestinal transit time and acts as a substrate for bacterial growth, can control the gut microbiota thus preserving digestive health (Carabaño *et al.*, 2008). Reducing dietary NDF (from 300 g/kg to 250 g/kg) in weaned rabbits (from 25 to 55 d of age), the microbiota biodiversity in the ileum increases, whereas in the caecum it decreases and a higher mortality rate is detected (Nicodemus *et al.*, 2004; Gidenne *et al.*, 2010). On the other hand, an excess in NDF content (>400 g/kg) should be avoided for preserving good feed efficiency and guaranteeing rabbit health (de Blas *et al.*, 2012). In fact, a reduction of dietary NDF (from 360–380 to 300–320 g/kg) decreases mortality, improves growth performance and feed efficiency (Gutiérrez *et al.*, 2002; Feugier *et al.*, 2006), and enhances gut mucosal structure. In the same way a significant decrease of *C. perfringens* concentration in caecal contents has been observed by reducing dietary NDF concentration from 420 to 330 g/kg (Romero *et al.*, 2009).

Moreover, the favourable effect of a high ADF intake on rabbit digestive health was also proved by means of experimental colibacillosis or ERE infections (Gidenne and Licois, 2005).

Therefore, minimal contents of NDF (300 g/kg), ADL (50 g/kg) and large particles (>0.3 mm, 210 g/kg) are required in the diets used during post-weaning period (Table 1) in order to accelerate the transit time of digesta through intestinal tract, dilute dietary and ileal starch and protein contents and reduce total microbial growth (Carabano *et al.*, 2008, de Blas, *et al.*, 2012), thus contrasting the occurrence of dysbiosis (Gidenne, 1996; Bennegadi *et al.*, 2000).

Table 1 Fibre and starch requirements (as fed corrected to a dry matter content of 900 g/kg) for the young rabbit after weaning to prevent digestive troubles (adapted from Gidenne *et al.*, 2010).

	INRA ¹		UPM ²	
	Post weaning (28–42 days old)	Growing (42–70 days old)	Post weaning (25–39 days old)	Growing (39–70 days old)
Neutral detergent fibre (NDF)	≥310	≥270	300≤NDF<360	320≤NDF<350
Lignocellulose (ADF)	≥190	≥170	–	160≤NDF<185
Lignin (ADL)	≥55	≥50	–	≥55
Cellulose (ADF–ADL)	≥130	≥110	–	–
Lignin/cellulose	>0.40	>0.40	–	–
Hemicelluloses (NDF–ADF)	>120	>100	–	–
DgF/ADF	≤1.3	≤1.3	–	–
Neutral detergent soluble fibre (NDSF ³)	–	–	12.0	–
Particles >0.3 mm	–	–	–	> 21.0
Starch	–	–	<200	145<starch<175

ADF, acid detergent fibre; ADL, acid detergent lignin; DgF, Digestible fibre fraction = [hemicelluloses (NDF – ADF) + water-insoluble pectins]. ¹INRA, Institut National Recherche Agronomique. ²UPM, Technical University of Madrid. ³According to Hall *et al.*, 1997.

Not only fibre intake is important for the digestive health, but also the type of dietary fibre might interfere with the incidence of intestinal troubles in growing rabbits. From a qualitative point

of view fibre could be important since it promotes the growth of beneficial microbiota, improves the competitive exclusion and affects the mucosal structure and the intestinal barrier (Carabaño *et al.*, 2008). Several studies have demonstrated the positive effect of ADF on the incidence of digestive disorders and mortality in fattening rabbits, but within lignocellulose components, has also evidenced the important effect of lignin (ADL) in reducing sanitary risk (morbidity + mortality) (Nicodemus *et al.*, 1999; Gidenne *et al.*, 2001a). Also the increase of cellulose concentration in the diet improves digestive health, but its role seem to be less important compared to that of ADL (Gidenne, 2003). In fact, increasing lignin to cellulose ratio the incidence of digestive pathologies decreases and lower sanitary risk is observed (Gidenne *et al.*, 2001a).

The occurrence of digestive troubles in growing rabbits varies greatly for the same ADF content because the level of hemicelluloses and water-insoluble fibre (DgF, as described by Gidenne, 2003) could also vary independently of lignin and cellulose contents. The DgF fraction could play an important role on the digestive health of rabbits because it is rapidly fermented more than ADF and compatible with the caecal retention time. By maintaining the same levels of ADF in the diet, the frequency of digestive problems decreases when DgF content increases at the expenses of starch or protein (Gidenne *et al.*, 2001a). However, the DgF to ADF ratio should remain <1.3 (with dietary ADF level >150 g/kg) in order to reduce sanitary risk during fattening (Gidenne, 2003) (Table 1).

The addition of soluble fibre (SF), mostly calculated as difference between total dietary fibre (TDF) and neutral detergent fibre (TDF – NDF) (Trocino *et al.*, 2013), in the diet could be useful in promoting the growth of saprophyte bacteria in order to increase the competitive exclusion of pathogenic flora. Indeed the caecal microbiota of young rabbits is limited with respect to that of adult animals and it is specialized in the fermentation of soluble fibrous carbohydrates such as fructans, galactans, β -glucans and pectins (Lavrenčič, 2007).

The increase of SF also leads to an improvement of the caecal environment with a reduction of pH and an increase in VFAs concentration (Jehl and Gidenne, 1996; García *et al.*, 2002; Trocino, *et al.*, 2011; Tazzoli *et al.*, 2013, 2015) and might contribute to contrast the growth of *E. coli* (de Blas *et al.*, 2012). Furthermore, the inclusion of moderate levels of SF in the fattening diet can decrease the incidence of ERE, the microbial load of *C. perfringens* and opportunistic pathogens (e.g. *Campylobacter*) in the digestive content, improving the immune response and reducing mortality (Gómez-Conde *et al.*, 2007, 2009). In some studies, the dietary inclusion of SF has also been related to an improvement of the mucosa morphology and functionality, favouring the growth of intestinal villi and the activity of the enterocytes, whereas the addition of lignified fibre is responsible of structural atrophy, a lower activity of intestinal cells and the proliferation of

C. perfringens (Álvarez *et al.*, 2007; Gómez–Conde *et al.*, 2007). However, the positive effect of the SF inclusion in the diet on gut mucosa has not been observed in other studies (Xiccato *et al.* 2008; Trocino, *et al.*, 2010, 2011; Tazzoli, *et al.*, 2015). Probably the changes in mucosa morphology are age–dependent and also vary according to the sampling site, time after weaning and the health status of animals (Trocino *et al.*, 2013).

In summary, a minimum level of NDF (300–320 g/kg), ADF (180 g/kg) and SF (120 g/kg) should be guaranteed in diets during post–weaning and fattening periods in order to reduce ERE incidence and to maintain mortality below 5% (Carabaño *et al.*, 2008; de Blas *et al.*, 2012; Trocino *et al.*, 2013; Gidenne, 2015).

As in general the dietary level of fibre decreases with increasing starch content, also the starch concentration could play an important role on affecting the digestive health of the rabbits. Indeed, high starch concentrations in the diet are related to increased incidence of diarrhoea (Blas and Gidenne, 2010), particularly in weaning rabbits because the gastrointestinal tract is immature and gut amylase activity is still scarce. According to Cheeke (1987), high starch and low fibre diets could involve an overload of indigested starch at caecum, favouring a rapid increase of amylolytic bacteria with high concentration of VFAs and low values of caecal pH (5.0–5.5). These conditions should lead to an over–proliferation of *Clostridium spiroforme* and a consequent production of an iota–toxin which is responsible of enteritis and mortality (Xiccato and Trocino, 2008).

Nevertheless, the relationship between dietary level of starch and mortality is not fully demonstrated and appears questionable. In fact, an overload of starch seems to be very unlike since its digestion is efficient already in weaning rabbits (35 days of age) (Blas and Gidenne, 2010; Gidenne, 2015). The relationship between starch and insoluble fibre contents seems to be more important for controlling digestive health and growth performance (Blas and Gidenne, 2010), but often the evaluation of the effect of each dietary component is difficult because of the simultaneous variation of the main nutrients (starch and fibre fractions) in the experimental diets.

The increase of starch (from 120 to 180 g/kg) and starch to ADF ratio (from 0.71 to 1.16) was related to a higher mortality rate in growing rabbits in the presence of ERE (Carraro, *et al.*, 2007), but no significant effect on health status was observed in the absence of ERE (Xiccato *et al.*, 2002; Trocino *et al.*, 2011).

On the other hand, by comparing four diets obtained by the combination of two levels of starch (193 vs. 123 g/kg) and two levels of ADF (191 vs. 155 g/kg), a significant reduction in morbidity and mortality has been observed only depending on the ADF content (Gidenne *et al.*, 2004). In addition, also varying the source of starch (maize vs. wheat vs. barley) in iso–fibre diets for weaned rabbits, no relevant effect has been detected in the occurrence of diarrhoea (Gidenne *et al.*, 2005).

So the influence of starch on the digestive health of growing rabbits seems to be limited when fibre requirements are fully covered (Gidenne *et al.*, 2010).

In young rabbits, protein and amino acids (AAs) requirements are relatively high for body growth as well as for intestinal mucosa development, maintenance and renewal (Carabaño *et al.*, 2008; de Blas *et al.*, 2012; Gidenne *et al.*, 2010).

The use of unbalanced diets, particularly with an excessive protein content, could modify the caecal fermentative activity, altering the microflora composition and favouring the appearance of digestive diseases with an increase of mortality (Carabaño *et al.*, 2008, 2009; Gidenne *et al.*, 2010; de Blas *et al.*, 2012). In fact, a surplus of dietary protein may promote the proliferation of *Clostridia* and rise the incidence of *E. coli* (Gidenne *et al.*, 2010). In this way the population of *Clostridium perfringens* and mortality significantly decreased when dietary CP level were reduced from 180 to 160 g/kg (Xiccato *et al.*, 2006; Chamorro *et al.*, 2007). Martínez–Vallespín *et al.*, (2011) observed a relevant decrease of mortality (-19.9 percentage points) when dietary protein was reduced from 175 to 145 g/kg. Also with a slightly decrease of dietary protein (from 150 to 140 g/kg) a significant reduction of mortality in growing rabbits has been detected (Tazzoli *et al.*, 2013). In young rabbits, the increase of protein ileal flow is also related to a lower caecal pH (Gutiérrez *et al.*, 2003; Nicodemus *et al.*, 2004) and, consequently, the commensal microbiota may be altered. Therefore, it is assumed that with high–protein diets the availability of substrates for microbial growth is greater and the prevalence of pathogenic species increases (Gidenne *et al.*, 2010). Moreover, excesses in protein supply should be avoided also in order to contain feeding costs and to reduce nitrogen excretion (Maertens, 1999; Maertens *et al.*, 2005).

Around weaning the protein requirements for gut maintenance are very high and the relative needs for some essential and non–essential AAs can increase substantially. Therefore, several AAs such as threonine (the major component of mucin proteins) or glutamine (used by enterocytes as energy source) can play an important role in preventing intestinal mucosa degradation and supporting immunological response (Carabaño *et al.*, 2008; de Blas *et al.*, 2012). The dietary supplementation of 10 g/kg of glutamine modified ileal microbiota, by limiting the intestinal spread of several bacteria (*Helicobacter* spp. and *Clostridium* spp.) and *Eimeria* spp., therefore decreasing mortality due to ERE (Chamorro *et al.*, 2010).

Weaning also implies a change in the protein sources of feed, since the easily hydrolysable milk proteins are replaced with the less digestible vegetable proteins. In addition, raw materials may contain anti–nutritional factors such as lectins, antitrypsin or antigenic compounds (Gidenne *et al.*, 2010; Villamide *et al.*, 2010). Consequently, the apparent ileal digestion could impair and the morphology of gut mucosa may be altered. The importance of the level and source of vegetable

protein in affecting the digestive health has been stated in several study. Indeed, limiting the flow of protein to the gross intestine, by decreasing dietary protein content or by increasing ileal protein digestibility, has significantly reduced mortality due to ERE (Gutiérrez *et al.*, 2000, 2003; García *et al.*, 2006; Chamorro *et al.*, 2007). Moreover, a rise of the nitrogen flow reaching the terminal ileum has been related with a higher load of total anaerobic bacteria (García *et al.*, 2006), *E. coli*, *C. spiroforme* and *C. perfringens* (Chamorro *et al.*, 2007; Carabaño *et al.*, 2008).

Also the nitrogen of endogenous origin (digestive enzymes, mucoproteins, desquamation cells, urea) is an important source of protein for the gut microorganisms (Carabaño *et al.* 2009; de Blas *et al.*, 2012). In rabbit the endogenous nitrogen can contribute for about 40–60% of the total ileal protein flow (García–Ruiz *et al.*, 2004, 2005). This wide variability is firstly related to the dry matter intake and secondly to the diet composition, in particular the level and type of dietary fibre or the presence of anti–nutritional factors that could damage gut mucosa and increase the flow of nitrogen towards the caecum (Carabaño *et al.*, 2008, 2009; de Blas *et al.*, 2012). In fact, the replacement of soybean meal with protein sources characterized by lower anti–nutritional factors (e.g. sunflower or meat meal) improved the mucosal integrity in weaning rabbits (Gutiérrez *et al.*, 2000) and decreased mortality during fattening (Scheele and Bolder, 1987; Gutiérrez *et al.*, 2003; García *et al.*, 2006).

In presence of ERE, a reduction of fattening mortality has been observed with the addition of hydrolysable tannins (Maertens and Štruklec, 2006), probably because of the formation of non–degradable complexes of tannins with dietary protein (reducing protein substrate for microbial growth) and/or by the direct inhibition of microbial growth (Carabaño *et al.*, 2008; de Blas *et al.*, 2012).

Feed restriction programs in growing rabbits

General motivations and goals

Usually, in the rabbit farms, animals are fed *ad libitum* (AL) to meet nutrient requirements, optimize productive performance and obtain final live weights in accordance with market demands (Xiccato and Trocino, 2008; Maertens, 2009). The implementation of feed restriction programs is diffused in European rabbit farms (especially in France) in order to reduce digestive diseases, achieve standardized growth curves and improve global farm feed efficiency. In fact, feed restriction could be more advantageous from the point of view of feed efficiency than *ad libitum* feeding by increasing diet digestibility, modifying animal body composition with an enhanced deposition of protein rather than fat (Gidenne *et al.*, 2012a). In the past, feed restriction programs were introduced with the aim of increasing feed efficiency and standardizing the growth curve of growing rabbits (Ouhayoun *et al.*, 1986; Cavani *et al.*, 1991).

Since 1997, the interest in feed restriction as a strategy to reduce animal losses during post-weaning and growing periods has increased because of the appearance and spread out of ERE (Licois, 2004; Licois *et al.*, 2005). Moreover, since 2006, in European Union, the use of antibiotics as growth promoters has been banned in animal farms and restricted in prophylaxis and metaphylaxis, so limiting the possibility of defence against digestive diseases, especially ERE that could be responsible for mortality up to 80% in the post-weaning period (Dewrée *et al.*, 2007).

Therefore the research of effective nutritional strategies and feeding plans alternative to the use of traditional antibiotics is growing. In this regard feed restriction programs could improve not only the health status in commercial rabbit farms by increasing the resistance of animals against digestive diseases (Boisot *et al.*, 2003), but also feed efficiency and could reduce the farm costs (Martignon, 2010; Gidenne *et al.*, 2012a; Knudsen *et al.*, 2014).

Methods of application

In the last decades several feed restriction programs have been tested in post-weaning rabbits for evaluating the effects of different feeding levels (from 90% to 40% of *ad libitum*) and finding the best strategy in terms of duration of restriction period and method of application. Furthermore, a qualitative feed restriction could be applied by modifying the chemical composition of the diets supplied to animals (reducing dietary energy level).

The quantitative feed restriction can be obtained, directly, by reducing the amount of feed administered to rabbits or limiting the access time to the feeders or, indirectly, by reducing the amount of drinking water. In the latter strategy, the feed intake is associated to water consumption with a feed intake to water consumption ratio equal to 1:1.68 (Boisot *et al.*, 2005). Several studies have been carried out in order to develop a feed restriction program based on limiting the access of animals to water and evaluating the indirect effects on the reduction of feed intake. In details, a feed restriction level of 82% of *ad libitum* has been observed by limiting water access to 120 minutes daily (Boisot *et al.*, 2004), 78% with 90 minutes drinking (Verdelhan *et al.*, 2004), 77% with 60 minutes drinking (Boisot *et al.*, 2005). Recently, an averaged feed restriction level of 82% of *ad libitum* has been achieved in a trial carried out under field conditions by gradually limiting the access to water from 2 to 4 hours daily during the first period of post-weaning (from 35 to 60 days of age) (Bovera *et al.* 2013). However these relevant restrictions are debatable in terms of animal welfare especially in conditions of high environmental temperatures (Ben Rayana *et al.*, 2008). In addition, the effects of feed restriction applied by limiting the access to water on productive performance and the health status of rabbits are more variable than those usually obtained with a direct reduction of feed supplied (Gidenne *et al.*, 2012a).

A desired restriction level could be achieved by offering daily a defined amount of feed that may be supplied all at once or as several meals per day (Gidenne *et al.*, 2009b; Martignon, 2010). However, this technique is difficult to be applied in commercial farms because it takes a lot of time and labour when an automatic feeding system is not available (Foubert *et al.*, 2008b). Moreover the optimum control on the feeding level could be impaired using collective pens where the amount of diet supplied should be adjusted according to the mortality rate within each pen. In addition, hierarchies and competitions for access to feeders could take place when a severe feed restriction is applied (Tudela and Lebas, 2006). Therefore, in cases of large groups of rabbits and severe feed restriction programs, the heavier and more aggressive animals may consume more feed than lighter ones which are subjected consequently to an even more severe feed restriction.

Another simple and alternative way to restrict feed intake is a time-limited access to the feeder for some hours daily (Foubert *et al.*, 2007; Romero *et al.*, 2010; Salaun *et al.*, 2011; Duperray and Guyonvarch, 2013) or some days weekly (Lebas and Laplace, 1982). In particular, a feed restriction level equal to 80% and 90% of *ad libitum* has been achieved by limiting the access to feed respectively to the light period (from h08:00 to h18:00) and the dark period (feeding during the night) (Jérôme *et al.*, 1998). A 20% reduction of feed intake has been also achieved with a temporary feed restriction program characterized by a weekly administration of feed as follows: two days of *ad libitum* feeding and one day of fasting; three days of *ad libitum* feeding and one day of fasting (Lebas and Laplace, 1982).

Interaction with health status

Several studies have been conducted under experimental and field conditions and with different feed restriction techniques in terms of feeding levels, period and methods of application, in order to evaluate the efficacy of feed restriction in enhancing health status of growing rabbits. The amount of feed ingested daily by rabbits subjected to *ad libitum* feeding increases quickly during the weaning and post-weaning period, with great variations from day to day. This feeding behaviour that is affected also by the environmental conditions (e.g. temperature and humidity), would compromise the intestinal functionality of animals by exposing the gut to pathogens development. Although the physiological mechanisms of feed restriction as a protective system against the digestive diseases of weaned rabbits have not been clarified yet, it is usually accepted that a limitation of feed intake in this period could prevent caecal imbalances and enteric troubles of multifactorial origin (e.g. ERE).

In this regard, Boisot *et al.* (2003) reported that a feeding level equal to 65% of *ad libitum* applied directly on feed or indirectly by water restriction significantly decreased the mortality rate

in rabbits experimentally infected with EEC in comparison to the control group characterized by free access to water and feed.

Foubert *et al.* (2008b) compared the effect of a restriction (only in feed or in combination with a water rationing) with that of an *ad libitum* administration of water and feed on the health status of rabbits experimentally infected with ERE. Both restriction techniques, that achieved a feeding level of 70% for the first three weeks of post-weaning, were unable to significantly decrease the mortality rate of animals in the whole period of trial, even if the feed restriction with free access to the water tended to be more efficient in reducing mortality. However, during the first rearing period associated to the spread of enteric disease, the restriction applied directly on feed improved the health status of animals compared to other experimental groups.

On the contrary, a feed restriction level equal to 80% of *ad libitum* did not improve the health status of animals that were naturally affected by colibacillosis or were experimentally infected with enteropathogenic strains of *E. coli* (0128:C6) (Martignon, 2010).

In a large scale trial, Gidenne *et al.* (2009a) showed a decrease in the mortality due to ERE or other digestive diseases in rabbits restricted at different levels during the first three weeks of post-weaning. The mortality rates of restricted rabbits (80%, 70%, 60% of *ad libitum*) were significantly ($P < 0.001$) lower (5.5%, 5.4% and 2.8%, respectively) compared to those of rabbits fed *ad libitum* (10.2%) or restricted at 90% (14.2%) during the post-weaning period (from 35 to 54 days of age). However, during the fattening period (from 54 to 71 days of age) characterized by *ad libitum* feeding, all experimental groups were affected by the same digestive diseases, which diminished the advantages obtained in the previous feed restriction period. Nonetheless, taking into account the whole trial period (from 35 to 70 days of age), the mortality rate was significantly decreased with a 20% or more reduction of feed intake ($P < 0.05$; probability for the contrast *ad libitum*+90% vs. 80%+70%+60%). In addition, only severe feed restriction programs (70% and 60% of *ad libitum*) were effective in controlling morbidity.

Another study carried out under field conditions confirmed the positive effect of feed restriction on digestive health of growing rabbits (Gidenne *et al.*, 2009b). In details, the reduction of feed intake (-25%) during the first four weeks of post-weaning (from 35 to 63 days of age) led to a significant decrease in mortality (11.9% vs. 21.6%) and morbidity (14.0% vs. 18.7%) with respect to an *ad libitum* feeding during the entire period of trial (from 35 to 70 days of age). Similar results were achieved with a feed restriction level equal to 80% of voluntary intake by limiting the access to water for the first growing period (from 35 to 60 days of age) (Bovera *et al.*, 2013).

Some authors studied the effects of the reduction of the ingested DE, keeping stable the intake levels of other nutrients (Szendrő *et al.*, 2008; Metzger *et al.*, 2009). In details, a 20% reduction in

the voluntary intake of DE resulted effective in the control of the digestive health of rabbits by significantly decreasing mortality compared to rabbits subjected to a voluntary intake of DE (4.8% vs. 29.5%; $P < 0.05$). Other studies were focused on studying the effects of different DE contents in the diets combined with different feeding levels. Generally, rising dietary content of DE, without important changes in fibre level, impaired animal health status especially when rabbits were in good sanitary conditions (Knudsen *et al.*, 2014). On the contrary, decreasing feed intake (at about -20/-25% of the voluntary intake) improved health, while no significant interaction was observed among feeding level and dietary DE content (Gidenne *et al.* 2009c; Knudsen *et al.*, 2014).

A lot of studies based on the application of feed restriction programs directly on feed or indirectly on water access have not shown positive effects of feed restriction on the digestive health of animals, however (Bovera *et al.*, 2008; Matics *et al.*, 2008; Birolo *et al.*, 2013). The divergent results obtained from various trials could be related to differences in terms of application method and duration of feed restriction programs, feeding levels, sanitary and environmental conditions of rearing as well as genotypes used.

It is possible to affirm that the mortality associated to the digestive diseases during the post-weaning period could be significantly reduced by applying feed restriction programs based on a 20% reduction of voluntary intake in rabbits while the morbidity could be controlled with stricter feed restriction levels ($\leq 70\%$ of *ad libitum*). However the positive effects of feed restriction on the health status of growing rabbits are limited to its application phase (Gidenne *et al.*, 2009a). Indeed, the re-feeding phase, characterized by the transition from a restricted to voluntary intake, is critical for animals that tend to consume an excessive amount of feed for compensating the feed restriction period (over-eating). This compromise the digestive health of rabbits which are more exposed to dismetabolies and intestinal troubles especially when the transition from restricted to voluntary feeding occurs quickly (Birolo *et al.*, 2013).

Implications on growth performance, slaughter results and meat quality

Before the spread of ERE in rabbit farms, the interest in the strategies of feed restriction was addressed mainly to improve the feed efficiency, standardize the productive performance of growing rabbits and also evaluate the effects of feed restriction on carcass traits and meat quality (Ouhayoun *et al.*, 1986; Ouhayoun, 1989; Cavani *et al.*, 1991; Perrier e Ouhayoun, 1996).

As reviewed by Gidenne *et al.* (2012a), a severe feed intake reduction (higher than 20% of voluntary intake) leads to an important decrease in growth rate, decreasing live weight of the animals at the end of restriction phase (Figure 1a). In addition, despite the compensatory growth rate observed during the re-feeding phase, restricted rabbits could not achieve the same live weight at slaughter of the rabbits always fed *ad libitum* (Figure 1b). The variability among studies can be

explained by differences on the length of restriction and re-feeding periods, health status, diets composition, environmental conditions and genetic types used.

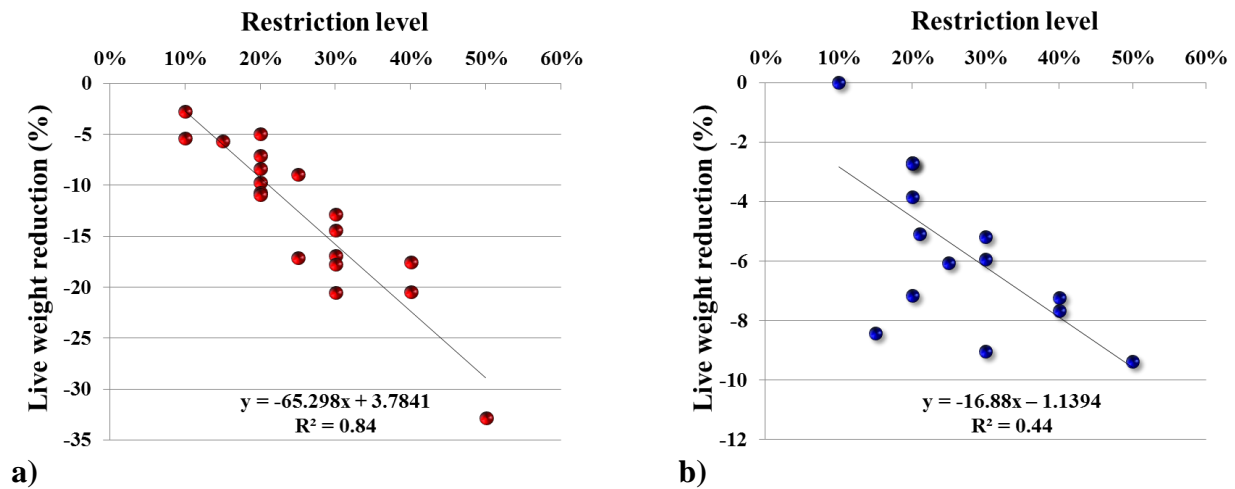


Figure 1 Live weight reduction at the end of restriction period (Figure 1a) and at slaughter (Figure 1b) according to the restriction level. *Sources:* Lebas and Laplace, 1982; Jérôme et al., 1998; Perrier, 1998; Boisot et al., 2003; Foubert et al. 2008a; Bergaoui et al., 2008; Gidenne et al., 2009a, 2009b, 2009c; Martignon, 2010; Romero et al., 2010; Gidenne et al., 2012b; Birolo et al., 2013; Knudsen et al., 2014).

Generally speaking, the reduction in terms of productive performance in restricted rabbits occurs mostly during the first period when the feed restriction is applied (Martignon, 2010).

However, as mentioned above, the results obtained from different studies concerning the productive performance of restricted rabbits are variable according to the health status of animals and the composition of diets supplied (e.g. DE content). For example, the administration of diets rich in DE (10.13 MJ/kg) throughout the rearing period can act synergistically with feed restriction, contributing to a further decrease of feed conversion in restricted rabbits (Knudsen *et al.*, 2014).

During the transition from feed restriction period to *ad libitum* feeding it is usually observed the occurrence of compensatory growth whose intensity is related to the level of feed restriction applied in the previous period (Perrier, 1998; Matics *et al.*, 2008; Gidenne *et al.*, 2009a). Nevertheless, the slaughter weights of rabbits subjected to severe restriction ($\leq 80\%$ of voluntary intake) are lower with respect to those of rabbits fed *ad libitum* during the whole rearing period (Gidenne *et al.*, 2012a) also after 2–3 weeks of re-feeding (Perrier, 1998; Boisot *et al.*, 2003). In details, the application of restriction levels equal to 75% or 80% of *ad libitum* during the first 3–4 weeks after weaning led to a reduction of final live weight around 4–5% (Gidenne *et al.*, 2009c; Knudsen *et al.*, 2014) also after 24 d of re-feeding (Bovera *et al.*, 2013).

In a trial carried out under field conditions, Gidenne *et al.* (2009a) applied a linear decrease of the feeding level (90%, 80%, 70% e 60% of *ad libitum*) from weaning (34–38 d of age) to 54 d of age. At the end of this period, the live weight of animals, the growth rate and the feed intake were significantly lower in all restricted groups. In the following re-feeding period until

commercial slaughter (about two weeks), all restricted rabbits improved their growth rate and feed conversion compared to the control group, but only the slighter feed restriction (90% of the voluntary intake) permitted to reach slaughter weights similar to those of rabbits always fed *ad libitum*.

One of the most interesting points of feed restriction regards the possibility to improve feed efficiency (Tudela, 2008). A recent study, where the feeding levels were equal to 90% and 80% of *ad libitum* for the first two or three weeks of post-weaning, showed a relevant decrease of feed conversion in restricted rabbits in the whole trial period (-0.08 units with respect to AL group) (Birolo *et al.*, 2013). These results were confirmed also by applying restriction programs characterized by a time-limited access to water in the first growing period and average levels of feed intake equal to 82% of *ad libitum* (Bovera *et al.*, 2013).

The amount of feed ingested during the re-feeding phase by the rabbits previously restricted represents a key point regarding the effect of restriction programs on the productive performance of animals. In fact, after the feed restriction period, the over-feeding behaviour generally observed in restricted rabbits is proportionally lower with respect to the increase in growth rate (Gidenne *et al.*, 2012a). Therefore, during the re-feeding phase the feed conversion is significantly reduced, with a relevant improvement also considering the whole rearing period (Table 2). On the other hand, several studies report a relevant decrease of feed conversion also in the restriction period (Table 2). This later result could be partially explain by an enhancement of the digestive efficiency in rabbits submitted to feed restriction (Gidenne *et al.*, 2012a; Knudsen *et al.*, 2014), probably due to an increase in the transit time of particles through the digestive tract (Gidenne and Feugier, 2009).

Feed restriction is responsible of reduced growth rates in post-weaning as well as of the change in the body composition of animals by modifying the tissue deposition and the development of internal organs (Dalle Zotte e Ouhayoun, 1998; Xiccato, 1999). As reported by Parigi Bini and Xiccato (1986, 1998), rabbits under fasting lose 12.6 g/day of empty body weight (EBW) per kg of metabolic live weight ($LW^{0.75}$), while maintain their weight with an intake of 273 kJ DE/day/kg $LW^{0.75}$. At this intake level, the body water content increases at the expenses of body fat and a loss of energy is observed (-62 kJ/day/kg $LW^{0.75}$).

The energy balance is achieved when the DE intake required for maintenance is around 425 kJ/day/kg $LW^{0.75}$ because the loss of energy as body fat is equivalent to the gain of energy as retained protein. On the contrary, in presence of *ad libitum* feeding the rabbit can reach the voluntary intake of DE (about 1000 kJ/day/kg $LW^{0.75}$) and the retained body energy is maximized (Xiccato, 1999). In practice, a feeding level equal to 90% of the voluntary intake applied during

the whole growing period implies lower daily gain (-12%), a delay of slaughter (+5 days) and a decrease in body fat concentration (-15%) compared to rabbits always fed *ad libitum* (Table 3).

Table 2 Feed conversion index according to the feeding level in post-weaning rabbits (adapted from Gidenne et al., 2012a).

Intake level (IL), %	Restriction period (R)		Whole period (R+AL)		Reference
	FC	FC reduction, %	FC	FC reduction, %	
100	–	–	3.22 ^a	–	Birolo <i>et al.</i> , 2013
90	–	–	3.14 ^b	–2.5	
80	–	–	3.14 ^b	–2.5	
100	2.36 ^a	–	3.13 ^a	–	Boisot <i>et al.</i> , 2003
80	2.26 ^{ab}	–4.2	2.70 ^b	–13.7	
60	2.18 ^b	–7.6	2.57 ^b	–17.9	
100	2.29 ^a	–	2.88 ^a	–	Foubert <i>et al.</i> , 2007
80	2.30 ^a	–	2.81 ^b	–2.43	
75	2.20 ^b	–3.9	2.74 ^c	–4.9	
65	2.14 ^c	–6.6	2.68 ^d	–6.9	
100	2.19	–	2.75 ^a	–	Foubert <i>et al.</i> , 2008a
70	2.17	–0.9	2.49 ^b	–9.5	
100	2.49	–	2.69 ^a	–	Gidenne <i>et al.</i> , 2009a
80	2.49	0.0	2.54 ^b	–5.6	
70	2.43	–2.4	2.46 ^{bc}	–8.6	
60	2.48	–0.4	2.38 ^c	–11.5	
100	2.99 ^a	–	3.31 ^a	–	Gidenne <i>et al.</i> , 2009b
75	2.65 ^b	–11.4	3.04 ^b	–8.2	
100	2.83 ^a	–	2.85 ^a	–	Gidenne <i>et al.</i> , 2009c
80	2.66 ^b	–6.0	2.55 ^b	–10.5	
100	2.93 ^a	–	3.20 ^a	–	Knudsen <i>et al.</i> , 2014
75	2.58 ^b	–11.9	2.91 ^b	–9.1	

^{a,b,c}Significant differences among means within study.

Moreover, during the feed restriction period, the development of internal organs, especially the digestive tract, is slowed. However, as a consequence of the compensatory growth that occurs during the re-feeding phase, the size of digestive organs rapidly increases. Therefore a greater size of the digestive organs could occur in restricted rabbits compared to rabbits always fed *ad libitum* (Gidenne *et al.*, 2012a).

Table 3 Effect of feeding level from 0.7 to 2.5 kg on daily weight gain and final empty body (EB) composition (adapted from Xiccato *et al.*, 1999).

Feeding level (%)	DEI (kJ/d/kg LW ^{0.75})	DEI (kJ/d)	Growing period (d)	Weight gain (g/d)	Final EB composition ¹				
					Water (%)	Protein (%)	Fat (%)	Ash (%)	Energy (MJ/kg)
80	800	1136	52.6	34.2	66.2	21.0	9.4	3.4	8.29
85	850	1207	48.5	37.1	65.2	20.9	10.6	3.3	8.70
90	900	1278	45.1	39.9	64.2	20.9	11.7	3.3	9.08
95	950	1349	42.2	42.6	63.2	20.8	12.7	3.2	9.42
100	1000	1420	39.8	45.3	62.3	20.8	13.8	3.1	9.81

DEI: digestible energy intake. LW: live weight. ¹Calculation is based on the following initial body composition: EBW=0.87 live weight; water 71.0 %; protein 19.7%; fat 5.8%; ash 3.5%; energy 6.70 MJ/kg.

The development of the digestive tract is influenced by the level of weight gain during compensatory growth phase and the restriction strategy applied (Gidenne *et al.*, 2012a). Gidenne *et al.* (2009d) reported that the increasing weight of full digestive tract highly affects the compensatory growth of animals during re-feeding phase by decreasing consequently the slaughter yield (almost 2 percentage points) in rabbits restricted at 75% of voluntary intake compared to rabbits fed *ad libitum*. However, if a 30% reduction of feed intake is applied for short periods (1 or 2 weeks) and followed by a re-feeding phase of 2 weeks, this should not influence significantly rabbit dressing percentage (Tůmová *et al.*, 2006).

Although the re-feeding phase is mainly applied in the second rearing period, in which the animals are mostly subjected to adipose tissue deposition, the carcass fattening of restricted rabbits is usually diminished. The weights of perirenal and interscapular fat in the carcass of rabbits subjected to a restriction period during 3–4 weeks of post-weaning and a re-feeding phase until slaughter (from 1 to 3 weeks) are significantly lowered in comparison with those of control groups fed *ad libitum* (Bergaoui *et al.*, 2008; Bovera *et al.*, 2008, 2013; Gidenne *et al.*, 2009d). On the contrary, the application of feed restriction programs does not significantly affect either carcass traits, muscle to bone ratio and the type of muscle fibre or meat quality (pH, colour, cooking losses) (Perrier, 1998; Dalle Zotte *et al.*, 2005; Tůmová *et al.*, 2006).

In conclusion, the final live weights and slaughter yields of rabbits subjected to feed restriction programs based on restriction levels $\leq 80\%$ of *ad libitum* are lower than those of rabbits fed *ad libitum*. However, the decrease in live weight can be limited thanks to the compensatory growth of rabbits during the re-feeding phase. Besides the application of feed restriction during post-weaning improves FC (from 10% to 20%) and consequently reduces farm costs (Gidenne *et al.*, 2012a; Gidenne *et al.*, 2013c). Therefore the rearing cycle should be prolonged by 2–3 days for achieving final live weights in accordance to market requirements and avoiding dressing out reduction. As a consequence, the economic benefits, associated to the FC improvement, seem

limited. In addition, the economic benefits obtained by applying restriction programs are related also to market and slaughter weights. In this regard, in Spain, where the market requires small rabbits (2.0-2.2 kg of live weight at slaughter), the application of feed restriction techniques is debatable because it negatively affects the growth rates, final live weights and FC in rabbits restricted during the first weeks of post-weaning (from 35 to 49 days of age) (Romero *et al.*, 2010). On the other hand, in Italy and Hungary production systems, where the market requires high slaughter weights, the extension of fattening period of 2-3 d or more is not sustainable because of a too long rearing cycle.

Effects on feeding behaviour, digestion, digestive physiology and welfare

In conditions of free access to feed, the rabbit takes feed in several small meals throughout the day. However, the amount of feed ingested during the day ranges from 5% to 13% of the total feed intake depending on the photoperiod. In details, in rabbits fed *ad libitum* and subjected to a daily light:dark schedule equal to 10:14 h, the phase of the lowest feed intake is recognised 2–3 h after the starts of light period (Martignon *et al.*, 2009) according to the caecotrophy activity (Gidenne and Lebas, 2006). The feed intake of AL rabbits remains low in the middle of the light period and then progressively increases up to reach the maximum level after about 2 h starting from the dark period (Figure 2). Moreover, the most of daily feed intake (up to 65–70% of the total) in rabbits fed *ad libitum* occurs in the dark period (Tudela, 2008; Martignon *et al.*, 2009).

The application of feed restriction programs modifies the normal feeding behaviour of animals (considering rabbits reared in cage with free access to water and feed) that take most of feed supplied during the first 6–10 hours of meal administration (Martignon, 2010). Anyway, the rabbits are quickly adapted to the feed restriction strategies, even in conditions of strict restriction levels (75% of *ad libitum*) (Martignon *et al.*, 2009).

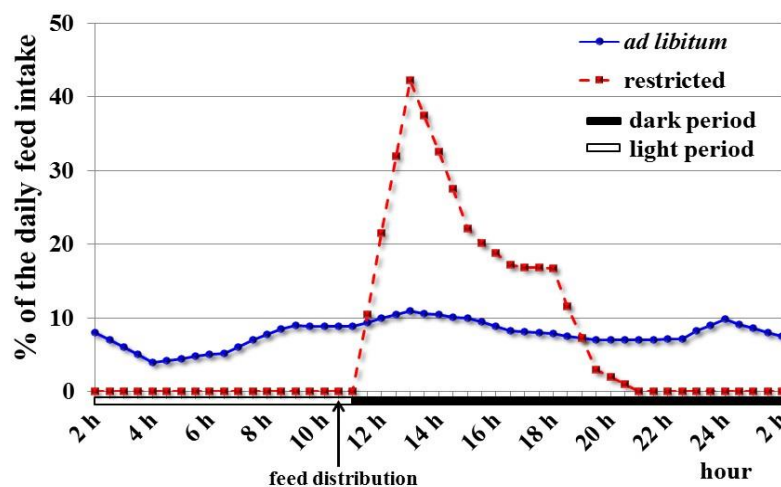


Figure 2 Feed intake patterns of rabbits at 36 days of age, according to the feeding level: *ad libitum* vs. restricted (75% of the voluntary intake) since weaning at 28 days (adapted from Martignon *et al.*, 2009).

The feed restriction affects the feeding behaviour of animals also in terms of meal numbers and the time spent on feeding during the day. In this regard, the restricted rabbits consume the daily feed ration distributed into 27 meals in 2 h 30 min. Conversely, the rabbits fed *ad libitum* spend 3 h 17 min on 24 h feeding that is distributed into 41 daily meals.

The number of access to water decreases as a consequence of feed restriction, although the total amount of water ingested by restricted rabbits is higher than that ingested by rabbits fed *ad libitum* (Boisot *et al.*, 2005). After switching to re-feeding phase, the circadian feeding and drinking activity of restricted rabbits is greater than that of rabbits fed *ad libitum*, even if the normal feeding behaviour is restored within a few days after coming back to *ad libitum* feeding (Martignon, 2010).

Moreover, the number of feeders per cage could also influence the feeding behaviour of animals. In particular, the use of two feeders per cage rather than one allows to rabbits restricted to 80% of *ad libitum* to complete the daily feed intake much more slowly (12 h *vs.* 8 h) thanks to the decreased competition for the access to feed (Tudela e Lebas, 2006). Although a high competition for the access to feeders could be responsible of hostile or even aggressive behaviours in restricted rabbits, any increase in aggressiveness or lesions related to the application of feed restriction programs was observed (Martignon, 2010).

As a consequence of the changes in feeding behaviour, the faecal excretion pattern results strongly modified (Figure 3). These variations do not regard only hard faeces excretion, but also caecotrophy (Martignon, 2010). In case of *ad libitum* feeding, rabbits exhibit a circadian rhythm of faecal excretion, reaching a peak at the end of light period and maintaining high excretion during the first six hours of the dark period, in accordance with the phase of greater feed ingestion.

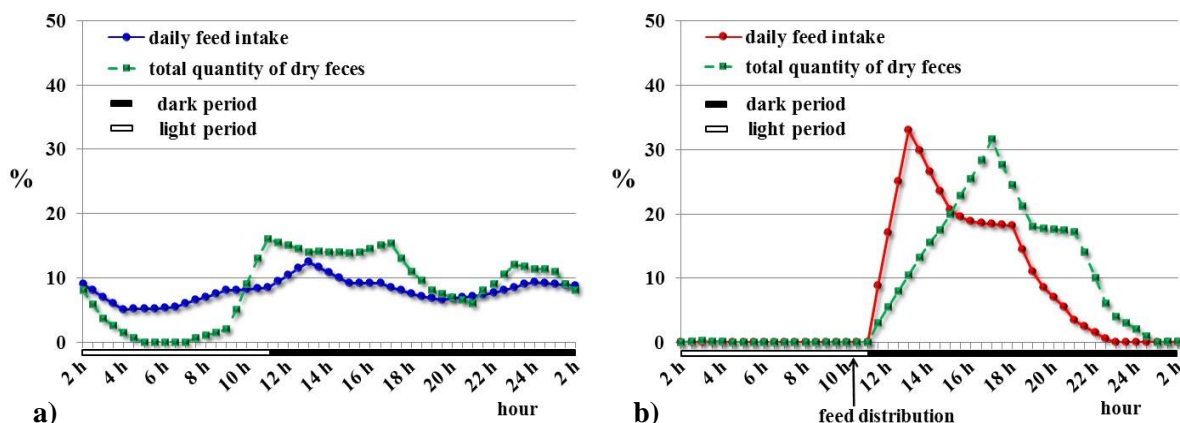


Figure 3 Feed intake (46 days of age) and hard faeces excretion (47 days of age) patterns in rabbits according to the feeding level: *ad libitum* (Figure 3a) or restricted (75% of *ad libitum*) (Figure 3b) (adapted from Martignon *et al.*, 2009).

On the contrary, no excretion of hard faeces is observed for about two hours in the middle of light period (Figure 3a). In restricted rabbits the peak of hard faeces excretion occurs between 5

and 8 hours after feed distribution (Figure 3b) and caecotrophy is shifted to about 8 to 10 h after feed administration (Gidenne *et al.*, 2012a). However, any significant change is detected in hard to soft faeces ratio (Martignon, 2010).

Finally, the changes in feeding behaviour also vary depending on the restriction strategy applied. For example, when water was restricted by limiting drinking at only 2 hours per day, the rabbits achieved 40% of total intake 4 hours after accessing to the water source (Ben Rayana *et al.*, 2008).

The changes in feeding behaviour during the restriction phase could affect digestive physiology, by modifying stomach pH, gut mucosa maturation and altering caecal fermentation processes and microbiota composition. Nonetheless, neither villi height and area nor crypt depth were significantly modified in rabbits restricted at 75% of the voluntary intake (Martignon *et al.*, 2010). On the contrary, the stomach pH values of restricted animals were higher than those of *ad libitum* ones, probably due to the great flow of digesta in the stomach of restricted rabbits just after feed distribution. At about 5–6 hours after the access to the feed, the caecal pH decreased as VFAs concentration increased (Gidenne and Feugier, 2009). However these changes are transitory and quite dependent to the time of sampling (hours after feed distribution) (Gidenne *et al.*, 2012a). The fermentation pattern, ammonia concentration, caecal bacterial activity and microbiota composition generally remain unaltered (Gidenne and Feugier, 2009; Martignon, 2010).

On the other hand, the positive effect of a feed restriction programme on FC is probably related to an increase in nutrient digestibility during restriction period and possibly also in the re-feeding phase (Gidenne *et al.*, 2012a). Indeed, many studies have detected a better DM faecal digestibility (Xiccato *et al.*, 1992; Di Méo *et al.*, 2007; Gidenne *et al.*, 2009d; Tazzoli, 2011; Knudsen *et al.*, 2014) and protein digestibility (Xiccato *et al.*, 1992; Tumova *et al.*, 2007; Gidenne *et al.*, 2009d; Knudsen *et al.*, 2014) in restricted rabbits compared to *ad libitum* ones. Differently, Gidenne and Feugier (2009) did not observe significant effect on faecal digestibility in growing rabbits by applying feeding level equal to 80%, 70% and 60% of the voluntary intake. Furthermore, an adaptation period of at least 8–10 days from the beginning of feed restriction seems to be required for achieving a relevant improvement in digestive efficiency of growing rabbits (Gidenne *et al.*, 2012a). In addition, diet composition can play an important role in feed digestibility and partially explains the different results among studies.

The improvement of digestive efficiency could be associated to an increase in the mean retention time of digesta in the intestine, particularly in the caeco–colic segment (Gidenne *et al.*, 2012a). In this way, Gidenne and Feugier (2009) have observed that the mean retention time of particles raise up to 65% in rabbits restricted at 60% of the voluntary intake compared to the *ad*

libitum rabbits, while the retention time of liquids moderately increased with feed restriction (+7.5% and +20% for rabbits restricted at 80% and 60% respectively) (Figure 4).

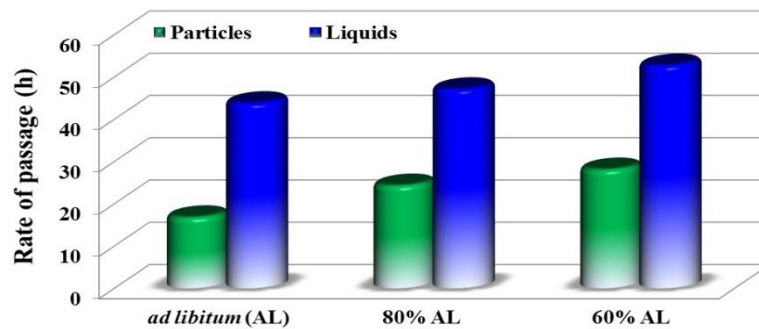


Figure 4 Rate of passage through the whole gut according to the feeding level of growing rabbits (adapted from Gidenne and Feugier, 2009).

The feed restriction program could be ethically questionable and in contrast with some animal welfare standards in rabbit farms according to the first of the five rules of the Farm Animal Welfare Committee (FAWC, 2011):

1. freedom from hunger and thirst – by ready access to fresh water and a diet to maintain full health and vigour;
2. freedom from discomfort – , by providing an appropriate environment including shelter and a comfortable resting area;
3. freedom from pain, injury or disease – by prevention or rapid diagnosis and treatment;
4. freedom of having normal behaviour – by providing sufficient space, proper facilities and company of the animal’s own kind;
5. freedom from fear and distress – by ensuring conditions and treatment which avoid mental suffering.

In details, the first rule is not respected when a feed restriction period is applied. However, during the whole day, the rabbits are subjected only to a transitory phase of fasting which is also a normal condition in nature. Moreover, several studies have showed an improved digestive health of rabbits and a consequent reduction in the incidence of enteric diseases associated with high rates of mortality and morbidity after the application of feed restriction programs. In this regard, the use of feed restriction agrees with the third rule of FAWC (Gidenne *et al.*, 2012a).

Objectives

Meat rabbit production is characterized by high rearing costs related to high feed conversion ratios and low slaughter yields. Moreover, high mortality rates due to digestive troubles occur, mainly during weaning and post-weaning periods. Over the last decades, several studies have highlighted that the application of feed restriction strategies in growing rabbits can contribute to reduce the incidence of digestive diseases, to increase diet digestibility, and to improve feed efficiency. For these reasons, the use of feed restriction has been widely spread in rabbit farms, especially in France, where it represents a current rearing practice. However, some negative impact on growth performance and slaughter traits often occur with restriction and the optimum strategy for balancing negative effects and benefits on gut health has not been defined yet. In Italy, few studies have dealt the effect of feed restriction programs in growing rabbits, using genetic types with slower growth rates compared to the current commercial hybrids. Moreover, the studies regarding the impact of feed restriction on productive and qualitative performances, and digestive health of growing rabbits produce variable and sometime contrasting results.

Hence, the main objective of the present PhD thesis was to define an efficacious restriction program for enhancing feed efficiency and gut health in growing rabbits, without impairing growth performance, carcass yield and meat quality. The general goal was pursued by focusing on three specific objectives; *i*) to evaluate the effect of quantitative feed restriction systems; *ii*) to develop an adequate time-based feed restriction program; *iii*) to optimize the management of the re-feeding phase. These points were addressed in four experimental contributions organized as follows:

First contribution. Two theoretical feeding curves were developed to obtain a feeding level varying from 80% to 100% of *ad libitum* during the first three week after weaning, which were characterized by small daily increments (+4 g/d: day-by-day restriction) or by large weekly increments (+23 g/week on average: week-by-week restriction) of the diet supply. The effect of the feed restriction system was evaluated on nutrient digestibility, sanitary status, growth performance, slaughter traits and nitrogen balance in growing rabbits weaned at 37 d of age, reared in individual cages and slaughtered at two ages (73 d or 80 d of age).

Second contribution. The effect of a time-based feed restriction, applied by increasing step-by-step the access time to the feeders from 10 to 16 h/d during the first three weeks after weaning and then progressively (+1 h/d) until reaching the free access, was evaluated on growth performance, feeding behaviour, slaughter traits and carcass and meat quality of growing rabbits housed collectively from weaning (32 d of age) to slaughter (74 d). Moreover the effect of the group composition for gender (only female vs. only males vs. mixed sexes) was tested.

Third contribution. The effect of a progressive and variable access time to the feeders (decrease from 14 to 8 h/d in the first week; stable phase of 8 h/d in the second week; progressive increase of +1 h/d during the third and fourth weeks) was evaluated on growth performance, health status, carcass traits and meat quality in growing reared from weaning (31 d of age) to slaughter (73 d) in groups of different size (groups of 8, 16 and 32 rabbits kept at the same stocking density).

Fourth contribution. The effect of the restriction method (diurnal *vs.* nocturnal access to feed), the re-feeding speed (fast: +4 h/d *vs.* slow: +1/+2 h/d of access to feed) and the re-feeding level (complete: up to 24 h/d *vs.* incomplete: up to 12 h/d of access to feed) was evaluated on growth performance, digestive physiology, health status, slaughter traits and carcass and meat quality of growing rabbits housed in collective pens from weaning (28 d of age) to slaughter (70 d).

First contribution:

Effects of feeding system and slaughter age in growing rabbits

Introduction

Over the last decades, the use of drugs in commercial rabbit farms has been the main solution against the rapid spread of enteric diseases such as Epizootic Rabbit Enteropathy (ERE). However, prophylactic antibiotic supplementation involves increased production costs and represents a critical point for food safety (Romero *et al.*, 2012). For these reasons, to look at alternative solutions to control digestive disease is becoming a priority. The application of feed restriction techniques in growing rabbits may reduce the economic impact of digestive troubles by decreasing mortality and morbidity rates (Gidenne *et al.*, 2009a; Romero *et al.*, 2010; Gidenne *et al.*, 2012a;). This way, the development of feed restriction programs could reduce the use of antibiotics in rabbit farms (Maertens, 2011). In addition, several authors reported that feed restriction programs can be used to improve feed conversion (Gidenne *et al.*, 2012a; Bovera *et al.*, 2013; Knudsen *et al.*, 2014), increase diet digestibility (Ledin, 1984a, 1984b; Xiccato *et al.*, 1992; Di Méo *et al.*, 2007; Knudsen *et al.*, 2014) and reduce nitrogen emission of rabbit farms (Gidenne *et al.*, 2013c).

In Italy, considering the high slaughter weights required by market and consequently the long fattening period, the real efficacy of feed restriction in reducing farm costs has not been completely proved since even a light rationing can depress growth rate of rabbits, mainly in the restriction phase (Gidenne *et al.*, 2012a). On the other hand, the lower growth performance can be partially recovered during re-feeding period thanks to the occurrence of compensatory growth (Matics *et al.*, 2008; Gidenne *et al.*, 2009a). However, a strong feed restriction ($\leq 80\%$ of *ad libitum*) is required for obtaining a significant enhancement of animal health status (Gidenne *et al.*, 2009a, 2012a) and this severe feed limitation may compromise the overall productive performance of rabbits by decreasing final live weight, dressing percentage and impairing carcass traits (Xiccato, 1999; Gondret *et al.*, 2000; Gidenne *et al.*, 2012a). In addition, during re-feeding the rabbits tend to an over-consumption for compensating the feed restriction period (Gidenne *et al.*, 2012b; Knudsen *et al.*, 2014) that could compromise the digestive health increasing morbidity and mortality during fattening period especially when the transition from restricted to voluntary feeding occurs rapidly (Birolo *et al.*, 2013). Furthermore, the worst growth performance are not always counterbalanced by the improvement of feed conversion (Romero *et al.*, 2010).

Considering the wide variability of the results obtained in several studies, the problems related to the correct development of feed restriction strategies in terms of intensity and application methods still appear unsolved.

On the basis of these considerations, the present study aimed at evaluating the effects of two feed restriction systems (day-by-day restriction vs. week-by-week restriction) in comparison with *ad libitum* feeding on nutrient digestibility, health status, growth performance, slaughter traits and nitrogen balance of growing rabbits reared in individual cages. Moreover, the effect of slaughter age (73 vs. 80 d of age) was assessed.

Materials and methods

Animals and experimental conditions

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

The trial was realized at the rabbit farm of the University of Padova, in a brick shed during the months of September and October under a natural photoperiod. Extraction fans and automatic heating system were used to maintain the temperature in a range of 17–25°C and to control air circulation and relative humidity.

A total of 282 rabbits (Hypharm, Groupe Grimaud, Roussay, France) of both sexes were selected in a commercial farm from healthy litters of multiparous does (≥ 3 kindling) and moved to the rabbit farm at 36 d of age. The rabbits were put in individual cages (285 mm x 410 mm x 285 mm) and monitored from 37 d to slaughter age (75 or 80 d of age).

At 37 d, 6 rabbits were slaughtered in order to measure initial empty body weight (EBW) and composition according to the comparative slaughter technique (Xiccato *et al.*, 2003). The other 276 rabbits were randomly allocated to six experimental groups (49 animals each one), homogeneous by live weight (LW) and variability, according to a bi-factorial arrangement with three feeding systems (AL: *ad libitum*, D: day-by-day restriction, W: week-by-week restriction) and two slaughter ages (73 or 80 d of age).

Diets and restriction programs

Two experimental diets were formulated and pelleted with 3.5 mm of diameter and 10–11 mm of length; their ingredients and chemical composition are listed in Table 4. The diets were formulated to satisfy the requirements of rabbits during post-weaning (diet P) and fattening (diet F) according to de Blas and Mateos (2010) and were supplemented with synthetic lysine and methionine, vitamins, macro- and micro-minerals.

Table 4 Ingredients composition (g/kg) of the experimental diets.

	Diets	
	P	F
<i>Ingredients</i>		
Dehydrated alfalfa meal 160 g/kg CP	340	253
Wheat bran	240	240
Barley	120	160
Dried beet pulp	150	160
Soybean meal 49%	40	40
Sunflower meal 30%	70	100
Sunflower oil	10	15
Molasses	15	15
Calcium carbonate	1.0	4.0
Dicalcium phosphate	3.5	2.5
Sodium chloride	4.0	4.0
L-lysine HCl	1.0	1.0
DL-methionine	1.0	1.0
Vitamin-mineral premix*	4.0	4.0
Coccidiostat (Diclazuril)	0.5	0.5
<i>Chemical composition</i>		
Dry matter	902	899
Crude protein	157	155
Ether extract	41.5	42.6
Ash	73.7	67.7
aNDF	349	343
ADF	197	188
Lignin (sa)	49.7	45.7
Starch	123	142
Gross energy, MJ/kg	16.7	16.8

*Supplementation per kg of feed: vit. A, 12000 UI; vit. D3, 1000 UI; vit. E acetate, 50 mg; vit. K3, 2 mg; Biotin, 0.1 mg; Thiamine, 2 mg; Riboflavin, 4 mg; vit. B6, 2 mg; vit. 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.

The diets were not supplemented with antibiotics, but with coccidiostatic (Diclazuril, 1 mg/kg). The rabbits fed the diet P from the beginning of trial to the end of the third week (58 d of age), thereafter they received the diet F.

The feed restriction programs were based on the administration of a restricted amount of the diet varying from 80% of *ad libitum* intake (AL) at the beginning of trial (37 d of age) and 100% of AL at the beginning of fourth week (58 d of age). The average restriction level around 90% was obtained by following two feeding curves calculated on the basis of the theoretical curve of AL rabbits. The day-by-day system (D) was characterized by small daily increments (+4 g/d starting from 76 g distributed on the first day of trial), while the week-by-week system (W) was characterized by large weekly increments (+33, +20 and +17 g/week respectively for the first, second and third week starting from 90 g/d distributed on the first week). From 58 d of age onwards, when the restricted rabbits

achieved the feeding level of the AL group, the amount of feed supplied for both restricted group was increased by 4 g/d up to 67 d of age in order to prevent large picks in food ingestion and realize a gradual re-feeding phase. Then all rabbits were fed *ad libitum* until the end of trial.

Growth performance and health status

During the trial the individual live weight was recorded two times a week. Individual feed intake was recorded daily. The live weight at 72 and 79 d of age was considered as the final live weight and used to calculate growth performance in the second and in the whole rearing period. The feeders were weighed every day at h09.00. Health status was controlled daily to detect the possible occurrence of digestive diseases. Rabbits were considered ill in the presence of diarrhoea and mucus or in case of a clear reduction of feed intake (at last -20% in comparison to the previous day) and of growth (weight loss during 2–3 consecutive d). According to Bennegadi *et al.* (2000), ill rabbits were considered only once for calculating morbidity while dead animals were taken into account only for determining mortality rate; the sanitary risk was expressed as the sum of morbidity and mortality. During the trial 35 animals died: in details 19 from group AL, 6 from group D and 10 from group W. All of the died animals were analysed in a specialized laboratory (Istituto Zooprofilattico Sperimentale delle Venezie – Viale dell'Università, 10 Legnaro, Padova, Italy) where autopsy, parasitological and bacteriological exams were performed.

Digestibility trials

Coefficients of total tract apparent digestibility (CTTAD) of dry matter (DM) and nutrients were measured during two *in vivo* digestibility assays performed according to the European standardised method (Perez *et al.*, 1995). The first trial was carried out on 36 rabbits (12 per feeding system) from 49 to 53 d of age when rabbits were fed diet P. The second trial was performed on 36 rabbits (12 per feeding system) from 64 to 68 d of age when rabbits were given access to diet F *ad libitum*.

Comparative slaughters and nitrogen balance

At 37 d, 6 rabbits representative of all other rabbits in terms of average live weight and variability were submitted to an initial comparative slaughter for measuring empty body weight (EBW) and the empty body chemical composition (Xiccato *et al.*, 2003). At the end of the trial, the day before the commercial slaughter (i.e. at 72 or 79 d of age), 18 rabbits (six rabbits per group), representative of the corresponding experimental group in terms of average live weight and variability, were slaughtered to determine their EB weight and EB composition. The rabbits were weighed immediately before slaughter and killed by cervical dislocation from h08:00 to h12:00 h. Stomach, gut and bladder were removed, emptied and weighed. Then, the fresh empty organs were joined to the whole body and weighed to determine the EBW.

To calculate the nitrogen balance, the body N content of all animals was estimated at the beginning (37 d of age) and at the end of trial (73 or 80 d of age) on the basis of the body protein content of rabbits sacrificed in the comparative slaughters. The ingested N was calculated on the basis of the individual feed intake and the diet nitrogen content ($N = \text{crude protein} / 6.25$). The retained N was calculated as the difference between the N body content at the end of trial and the body N content at the beginning of trial. According to ERM (1999), the excreted N was calculated as the difference between the ingested N and the retained N.

Commercial slaughters and carcass and meat quality recordings

At 73 d of age, all alive rabbits of S73 group (99 animals: 28 from group AL, 37 from group D and 34 from group W) were weighed at the experimental farm and then transported to a commercial slaughterhouse in an authorized truck (about one hour of transport). Two rabbits (one from group D and one from group W) were excluded from the commercial slaughter because of inadequate live weight (<2.2 kg). At slaughterhouse, the rabbits were individually weighed, stunned by electro-anaesthesia and killed by jugulating. The fur and distal legs were separated and the full gastro-intestinal tract of each animal was individually collected and weighed. After 2-h chilling, the commercial carcasses of all animals were weighed and transported to the laboratory and stored at 3–4°C for 24 h. The chilled carcasses were then dissected according to harmonised methods (Blasco *et al.*, 1993).

At 80 d of age, all remaining rabbits of S80 group (104 animals: 33 from group AL, 36 from group D and 35 from group W) were slaughtered and the carcasses were dissected and analysed as described for the first commercial slaughter.

Chemical analyses

Diets and faeces were analysed to determine the concentrations of dry matter (934.01), ash (967.05), crude protein (2001.11), and starch (amyloglucosidase α amylase method, 996.11) using AOAC (2000) methods following harmonised procedures (Gidenne *et al.*, 2001b). The ether extract was analysed after acid hydrolysis (EC, 1998). The fibre fractions, aNDF (neutral detergent fibre without sodium sulphite), ADF (acid detergent fibre expressed inclusive of residual ash), and lignin (sa), 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). The gross energy was measured with an adiabatic bomb calorimeter.

The empty bodies of the rabbits subjected to comparative slaughters were frozen at –18°C, ground, and freeze-dried. Finally the freeze-dried samples were ground and analysed to determine their moisture and protein contents by AOAC methods (2000).

Statistical analyses

The individual data of CCTAD, feed intake, growth rate, slaughter traits and nitrogen balance were analysed by a two-way ANOVA using the PROC GLM of SAS (Statistical Analysis System, 2013). The model included the feeding system (F: AL, D and W), the slaughter age (S: S73 and S80) and their interaction as fixed effects. Mortality, morbidity and sanitary risk were analysed by the PROC GENMODE of SAS (Statistical Analysis System, 2013) and the feeding system, the slaughter age and their interaction were included in the model. Least-square means were chosen for presenting all data and compared using the Bonferroni test. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences. Differences among means with $0.05 < P < 0.10$ were accepted as approaching statistical significance.

Results

Digestibility and nutritive value of experimental diets

The nutrient digestibility of diet P was not significantly affected by the feeding system and only the CTTDA of crude protein was higher ($P < 0.001$) in the restricted rabbits compared to rabbits fed *ad libitum* (Table 5).

The digestibility energy (DE) content of diet P averaged 10.26 MJ/kg, while the digestible protein (DP) content ranged from 11.8 g/kg to 12.1 g/kg according to the increase on the CTTAD of crud protein in restricted rabbits. Therefore the DP to DE ratio varied from 11.5 g/MJ in AL group to 11.8 g/MJ in restricted groups, irrespective by the restriction system applied.

Table 5 Coefficients of total tract apparent digestibility (CTTAD) and nutritive value (as fed) of the diet P. Data measured from 49 to 53 d of age.

	Feeding System			Prob.	RMSE ¹
	AL	D	W		
Number of rabbits	12	12	12		
Dry matter	0.619	0.615	0.620		1.3
Crude protein	0.751 ^a	0.765 ^b	0.773 ^b	***	1.1
Ether extract	0.800	0.793	0.802		1.6
aNDF	0.309	0.302	0.307		2.5
ADF	0.232	0.226	0.233		3.2
Gross energy	0.614	0.610	0.614		1.3
Digestible energy (DE), MJ/kg	10.28	10.21	10.28	–	–
Digestible protein (DP), g/kg	11.8	12.0	12.1	–	–
DP to DE ratio, g/MJ	11.5	11.8	11.8	–	–

AL: rabbits fed *ad libitum*; D: rabbits fed according to a day-by-day restriction curve; W: rabbits fed according to a week-by-week restriction curve.

¹Root mean square error.

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

*** $P < 0.001$.

The digestibility of diet F was not affected by the previous restriction treatment (Table 6). The DE content averaged 10.10 MJ/kg, the DP was equal to 11.0 g/d on average and the DP to DE ratio averaged 10.9 g/MJ without differences according to the feeding system.

Table 6 Coefficients of total tract apparent digestibility (CTTAD) and nutritive value (as fed) of diet F. Data measured from 64 to 68 d of age.

	Feeding System			Prob.	RMSE ¹
	AL	D	W		
Number of rabbits	12	12	12		
Dry matter	0.612	0.601	0.597		2.11
Crude protein	0.712	0.706	0.707		1.62
Ether extract	0.801	0.787	0.787		1.82
aNDF	0.315	0.298	0.285		3.91
ADF	0.234	0.206	0.194		4.84
Gross energy	0.602	0.605	0.598		1.97
Digestible energy (DE), MJ/kg	10.11	10.16	10.04	–	–
Digestible protein (DP), g/kg	11.0	10.9	11.0	–	–
DP to DE ratio, g/MJ	10.9	10.7	11.0	–	–

AL: rabbits fed *ad libitum*; D: rabbits fed according to a day-by-day restriction curve; W: rabbits fed according to a week-by-week restriction curve.

¹Root mean square error.

Effect on health status

Severe digestive troubles appeared soon from the first d of trial in *ad libitum* rabbits (Figure 5a). At the beginning of the second week (43–44 d of age), also the rabbits subjected to feed restriction were affected by enteric disease; after that (50–52 d of age), the morbidity rate achieved about 10–12% in all experimental group. During the same period (from 40 to 50 d of age) the mortality rate was rising (Figure 5b).

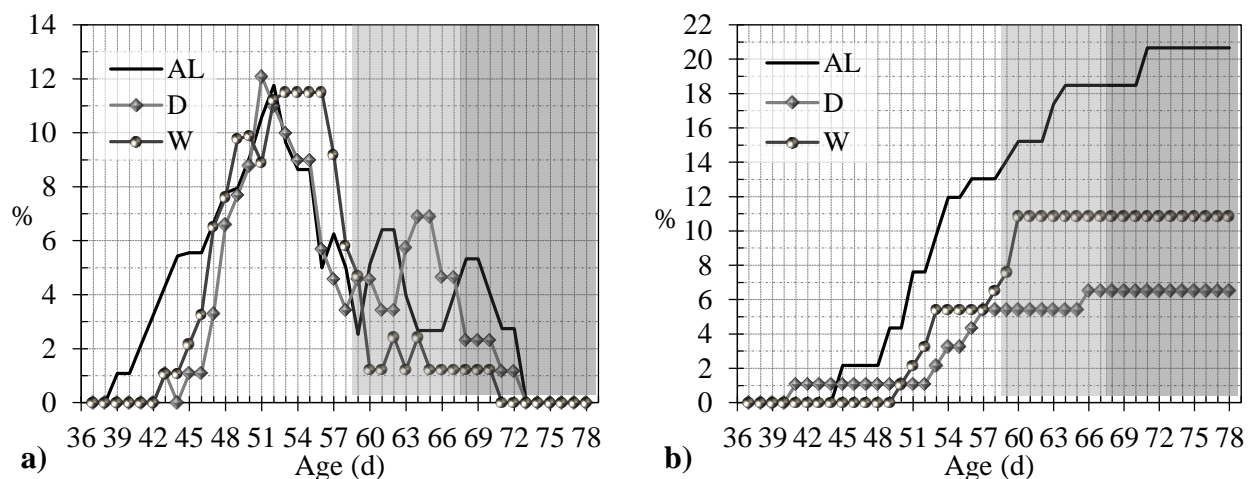


Figure 5 (a) Daily change of ill rabbits calculated as proportion of ill rabbits on the present animals of each group. (b) Cumulative mortality rate calculated on the initial number of 92 animals for each group. AL: rabbits fed *ad libitum*; D: rabbits fed according to a day-by-day restriction curve; W: rabbits fed according to a week-by-week restriction curve. White, light grey and dark grey boxes correspond to data detected during feed restriction, gradual re-feeding and *ad libitum* phases respectively.

In the AL group, the mortality increased by about 1% per day from 44 to 64 d of age, therefore reaching 21% at 71 d of age. Differently, in the D group only 5% losses were detected from 53 to 57 d and thereafter, the number of dead rabbits remained almost stable up to the end of trial. Finally, in the W group the mortality rate was very low during the first two weeks and rapidly increased until 11% from 50 to 60 d of age, concurrently with the reaching of *ad libitum* feeding level.

All cases of illness and death were caused by digestive diseases characterized by the typical symptoms of ERE. Distension of gastro-intestinal tract and caecal paresis with presence of mucous were observed during post-mortem examinations. Over-proliferations of *Clostridium perfringens* and *Escherichia coli* were detected by microbiological analysis in the veterinary lab, while parasitic infections were absent.

In the whole trial period, mortality, morbidity and sanitary risk reached respectively 12.7%, 11.3% and 24.0% on average (Table 7). The mortality was significantly ($P<0.05$) higher in AL rabbits (20.7%) compared to rabbits subjected to a daily restriction system (6.5%), whereas the averaged mortality of W group (10.9%) did not differ from the other groups.

Morbidity was not influenced by the feeding system, while the sanitary risk (mortality + morbidity) was higher ($P<0.05$) in AL group compared to D group (32.7% vs. 18.5% respectively).

Table 7 Sanitary status of rabbits from weaning (37 d age) to slaughter (73 or 80 d age).

	Feeding System (F)			Slaughter age (S)		Prob.		
	AL	D	W	S73	S80	F	S	F×S
Initial number of rabbits	92	92	92	138	138			
Mortality (%)	20.7 ^b	6.5 ^a	10.9 ^{ab}	13.8	11.6	*		
Morbidity (%)	12.0	12.0	9.8	8.1	14.5			
Sanitary risk (%)	32.7 ^b	18.5 ^a	20.7 ^{ab}	21.9	26.1	*		

AL: rabbits fed *ad libitum*; D: rabbits fed according to a day-by-day restriction curve; W: rabbits fed according to a week-by-week restriction curve.

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

* $P<0.05$.

Effect on growth performance and carcass traits

Regarding the effects on growth performance during the first period of trial, feed intake and daily growth rate were higher ($P<0.01$) in AL rabbits (Table 8). Therefore restricted groups, especially W group, were less heavy than AL ones at the end of restriction period (-3% and -4% for D and W group, respectively). In the second phase of the trial, only rabbits subjected to a weekly restriction system showed a relevant ($P<0.05$) increase of feed consumption with a significant ($P<0.001$) recovery in daily growth with respect to *ad libitum* rabbits (42.7 g/d vs. 48.9 g/d for AL and W groups, respectively). At the same time, the feed conversion ratio was lower in W group compare to AL and D groups (-9.8%; $P<0.01$). However, considering the whole trial, weight gain and feed conversion were similar among groups. At slaughter, the live weight of the animals averaged 2748 g (mean at

the two slaughter ages) without significant differences among groups. As expected, the effect of slaughter age on growth performance was more evident (Table 8). In fact, extending the fattening period of one week the final live weight increased (2647 g vs. 2847 g; $P<0.001$) as well as daily feed intake (138 g/d vs. 142 g/d; $P<0.05$).

In contrast, daily weight gain decreased (47.3 g/d vs. 44.3 g/d; $P<0.001$) and feed conversion worsened (3.77 vs. 4.09; $P<0.001$).

Table 8 Rabbit performance from weaning (37 d age) to the day before slaughter (72 or 79 d age).

	Feeding system (F)			Slaughter age (S)		Prob.			RMSE ¹
	AL	D	W	S73	S80	F	S	F×S	
Number of rabbits	73	85	80	116	122				
Live weight (g)									
At 37 d	985	989	992	989	987				72
At 58 d	2011 ^b	1951 ^{ab}	1930 ^a	—	—	*	—	—	185
At 72 or 79 d	2755	2710	2780	2650	2848		***		260
Feed restriction period (37 to 58 d)									
Weight gain (g/d)	48.9 ^b	45.8 ^a	44.7 ^a	—	—	**	—	—	7.87
Feed intake (g/d)	120 ^b	111 ^a	112 ^a	—	—	**	—	—	14.4
Feed conversion	2.50	2.47	2.55	—	—		—	—	0.33
Re-feeding period (58 d to 72 or 79 d)									
Weight gain (g/d)	42.7 ^a	43.9 ^a	48.9 ^b	46.8	43.5	***	*		10.7
Feed intake (g/d)	168 ^a	167 ^a	175 ^b	170	171	*			22.4
Feed conversion	4.09 ^b	4.00 ^b	3.69 ^a	3.77	4.09	**	**		0.78
Whole period (37 d to 72 or 79 d)									
Weight gain (g/d)	46.1	44.9	46.6	47.4	44.3		***		6.04
Feed intake (g/d)	142 ^b	136 ^a	141 ^{ab}	138	142	*	*		14.2
Feed conversion	3.10	3.06	3.04	2.91	3.22		***		0.22

AL: rabbits fed *ad libitum*; D: rabbits fed according to a day-by-day restriction curve; W: rabbits fed according to a week-by-week restriction curve.

¹Root mean square error.

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

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

As observed for final live weights, slaughter results and carcass traits were not affected by the feeding system (Table 9). Cold carcass weight were similar among groups (1634 g on average), as well as cold dressing (59.8% on average) and reference carcass weight (1359 g on average). In the same way, the incidence of dissectible fat and the muscle to bone ratio of hind leg were not modified by the feeding system.

On the contrary, delaying slaughtering of one week slaughter results and carcass quality changed. In particular, the incidence of full gut was lower in older rabbits, while cold dressing percentage (59.3% vs. 60.5%), cold carcass (1563 g vs. 1705 g) and reference carcass weight (1293 g vs. 1425

g) were higher ($P<0.001$) at 80 d than at 73 d. Similarly, carcass fatness and muscularity increased with slaughter age (+15.9% and +10.8% respectively; $P<0.001$) (Table 9).

Table 9 Slaughter traits and main characteristics of 24-h chilled carcasses.

	Feeding system (F)			Slaughter age (S)		Prob.			RMSE ¹
	AL	D	W	S73	S80	F	S	F×S	
Number of rabbits	61	73	69	99	104				
Slaughter weight (SW), g	2735	2698	2762	2641	2822		***		253
Full gut incidence, % SW	18.0	18.3	18.4	18.6	17.9		**		1.70
Cold carcass, g	1638	1611	1654	1563	1705		***		158
Cold dressing, % SW	59.8	59.9	59.8	59.3	60.5		***		1.67
Reference carcass (RC), g	1366	1338	1373	1293	1425		***		134
Dissectible fat, % RC	2.96	2.83	3.16	2.76	3.20		***		0.83
Muscle/bone ratio	6.36	6.41	6.31	6.04	6.69		***		0.49

AL: rabbits fed *ad libitum*; D: rabbits fed according to a day-by-day restriction curve; W: rabbits fed according to a week-by-week restriction curve.

¹Root mean square error.

** $P<0.01$; *** $P<0.001$.

Nitrogen balance

The nitrogen balance from weaning (37 d of age) to slaughter (73 or 80 d of age) was significantly modified by the feeding system (Table 10). The nitrogen intake was higher in rabbits fed *ad libitum* or subjected to a weekly restriction curve compared to those of D group (137 g vs. 131 g vs. 135 g for AL, D and W group, respectively; $P<0.05$). At the same time, the retained nitrogen of D group was lower than AL one. The nitrogen output was reduced by the daily feed restriction system (2.14 g/d vs. 2.06 g/d) with respect to AL rabbits, whereas the nitrogen excretion of weekly restricted rabbits (2.13 g/d) was similar to that of AL and D animals. Therefore, the total N output reached 83.4 g, 79.9 g and 82.3 g for AL, D and W groups respectively.

Table 10 Nitrogen balance expressed per rabbit from weaning (37 d of age) to the day before slaughter (72 or 79 d of age).

	Feeding system (F)			Slaughter age (S)		Prob.			RMSE ²
	AL	D	W	S73	S80	F	S	F×S	
Rabbits, n.	75	85	80	116	122				
Body N ¹ at 37 d, g	23.0	23.1	23.2	–	–		–	–	1.68
Body N ¹ at slaughter, g	76.6	74.2	75.9	72.9	78.3	‡	***		7.14
Total ingested N, g	137 ^b	131 ^a	135 ^b	120	148	*	***		14.0
Total retained N, g	53.6 ^b	51.1 ^a	52.7 ^{ab}	49.8	55.2	*	***		6.59
Total excreted N, g	83.4 ^b	79.9 ^a	82.3 ^{ab}	70.2	92.8	*	***		8.87
Daily excreted N, g/d	2.14 ^b	2.06 ^a	2.13 ^{ab}	2.01	2.21	*	***		0.23

AL: rabbits fed *ad libitum*; D: rabbits fed according to a day-by-day restriction curve; W: rabbits fed according to a week-by-week restriction curve. ¹Data calculated on the basis of the body protein content of rabbits sacrificed in the comparative slaughters. ²Root mean square error. ^{a, b}Means within each row and with different superscript letters are statistically different ($P<0.05$). ‡ $P<0.10$; * $P<0.05$; *** $P<0.001$.

The increase of the slaughter age from 73 to 80 d led to a significant ($P < 0.001$) increase of the total ingested (+28 g) and retained (+5.4 g) nitrogen and also of the daily nitrogen excretion (+0.21 g/d per rabbit). Therefore also the total excreted nitrogen (70.2 g vs. 92.8 g per rabbit) increased in older rabbits (Table 10).

Discussion

Effect of the restriction system

It is generally assumed that the application of a feed restriction leads to the improvement in the nutrient digestibility and feed conversion ratio either during the restriction phase or during the re-feeding period (Gidenne *et al.*, 2012a). Unlikely, in our study only the digestibility of crude protein increased when rabbits were subjected to a feed restriction plane. Gidenne and Feugier (2009) observed an improved protein digestion when rabbits were rationed with a feed amount equal to 70% or 60% of the voluntary intake, while digestibility coefficients of the other nutrients remained unchanged.

Several authors (Xiccato *et al.*, 1992; Xiccato and Cinetto, 1988; Gidenne *et al.*, 2009b, Knudsen *et al.*, 2014) reported a higher digestive efficiency in rabbits under feed restriction, whereas no residual effects of the previous restriction treatments were observed during the re-feeding (Tumova *et al.*, 2003, 2007; Knudsen *et al.*, 2014). The different results obtained by various studies may depend on the length of the adaptation period to the feeding system before the digestibility assay and also by the diet composition (Xiccato *et al.*, 1992; Gidenne and Feugier, 2009). In the present trial, the slight restriction level (92% of AL on average for both restricted groups during the four days of digestibility assay) explains the absence of significant differences among groups on digestive efficiency also in the rationing phase.

Weaning and post-weaning are very stressful periods for young rabbits that are subjected to important changes in feeding and digestive physiology. As a consequence, the effect of nutrition including feed restriction strategies on digestive health of growing rabbits have been widely investigated (de Blas *et al.*, 2012). Most of studies agree about the protective role of feed restriction against digestive diseases during post-weaning. In particular, the mortality associated to enteric troubles of multifactorial origin in growing rabbits could be significantly reduced through a feed restriction level at least equal to 80% of *ad libitum* intake. On the other hand, feed restriction levels lower than 70% of *ad libitum* may be necessary for controlling the incidence of morbidity (Gidenne *et al.*, 2012a). Likewise, in the present trial, daily and weekly restriction systems achieved positive effects on the animal health status by reducing mortality (-12% on average, $P < 0.01$ for the contrast AL vs. D+W, data not shown) and sanitary risk (-13% on average, $P < 0.05$ for the contrast AL vs. D+W, data not shown) with respect to *ad libitum* group. In particular, a relevant improvement in the

sanitary status was observed by applying a daily restriction curve. Since D and W rabbits were submitted to a slight restriction level during the first three weeks of trial (91% and 93% of AL, respectively) morbidity was not affected by the feeding system, in agreement with the literature (Gidenne *et al.*, 2009a, 2012a).

The daily feeding curves of AL, D and W groups and their respective feeding levels are reported in Figure 6. Considering the first trial period (from 37 to 57 d of age) the intake level of both restricted groups was reduced in comparison with the *ad libitum* group (by 91% and 93% for D and W groups, respectively). Daily increments of diet (+4 g/d, starting from 76 g) corresponded to a progressive feed restriction curve, while weekly increments led to a step-by-step trend (Figure 6a). Therefore, the feeding level of W group reached the *ad libitum* intake after several fluctuations (Figure 6b).

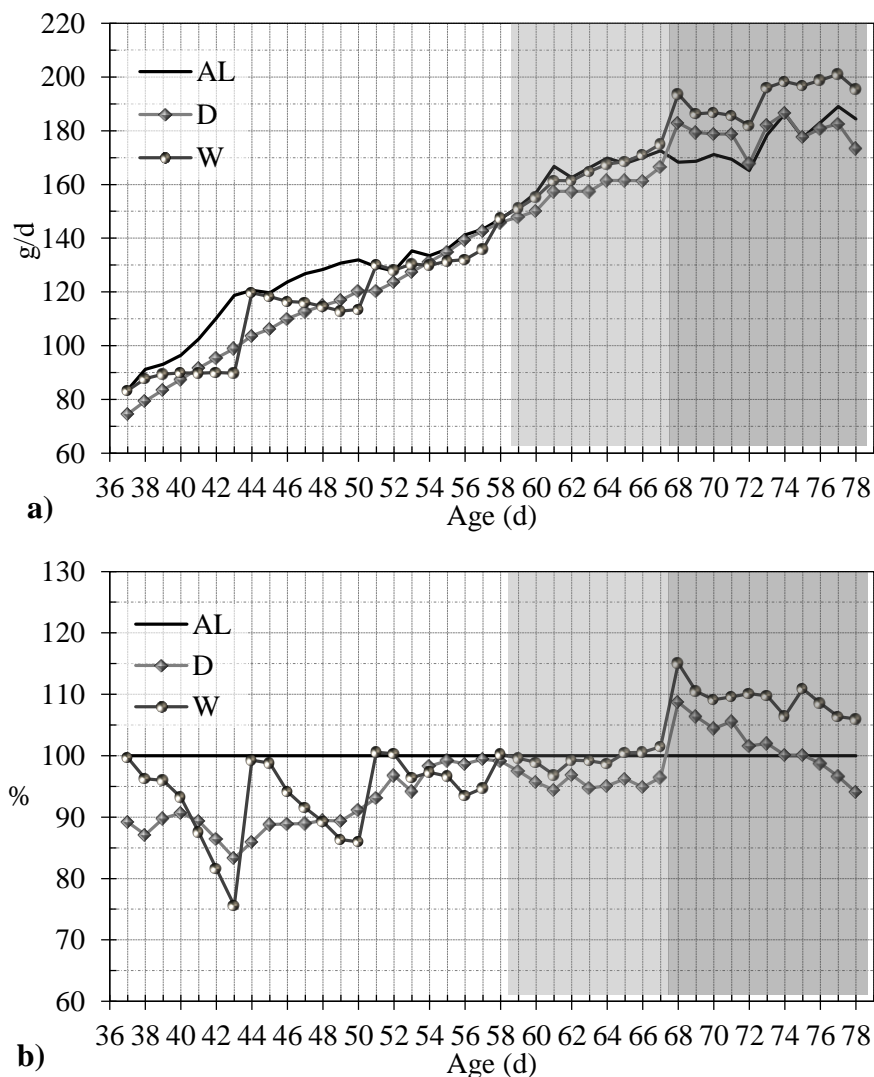


Figure 6 (a) Daily feed intake according to the feeding systems. (b) Feed restriction levels of restricted groups considering a feeding level equal to 100% for *ad libitum* rabbits. AL: rabbits fed *ad libitum*; D: rabbits fed according to a day-by-day restriction curve; W: rabbits fed according to a week-by-week restriction curve. White, light grey and dark gray boxes correspond to data detected during feed restriction, gradual re-feeding and *ad libitum* phases respectively.

From 58 d to 67 d of age, the feed distribution increased by 4 g/d for both restricted groups in order to realise a gradual transition from the restriction to the *ad libitum* phase. In this period, W rabbits achieved a feeding level equal to that of AL rabbits, whereas the D group preserved a slight feed restriction (96% of AL). From 59 d to slaughter, the true re-feeding phase was applied and an over-feeding behaviour was observed in the previously restricted rabbits (+2% and +9% of AL intake for D and W groups, respectively).

Both theoretical feed restriction curves (D and W systems) led to a reduction of daily intake in the first trial period, decreasing significantly the growth performance of restricted rabbits. Therefore, the feed conversion ratio was not modified by the feeding system since lower feed intake (-7.1% on average) corresponded to lower daily growth (-7.5% on average) in restricted groups compared to AL ones. Similarly, other studies reported that feed efficiency of the rabbit was scarcely affected during restriction phase (Foubert *et al.*, 2008b; Romero *et al.*, 2010; Bovera *et al.*, 2013), regardless of the rationing level applied (from 90% to 60% of *ad libitum*) (Gidenne *et al.*, 2009a). In opposite, several authors observed an important reduction of feed conversion during restriction phase when the intake level was limited at 75% or 70% of *ad libitum* (Bergaoui *et al.*, 2008; Knudsen *et al.*, 2014).

In the present trial, during the second period, when feed availability for restricted rabbits was increasing, higher feed intake (+4.8%) and daily growth (+14.5%) were observed only for W group compared to AL group. The fluctuations of feed intake in the weekly restriction curve could explain the capability of the W rabbits to rapidly increase food ingestion. In fact, this type of feeding system possibly enhanced the stomach elasticity permitting a quick enlargement of the organ when rabbits were fed freely. On the contrary, the progressive feature of daily restriction curve probably slowed the adaptation of D rabbits to ingest great amount of food during the *ad libitum* period. Indeed, according to Gidenne and Feugier (2009) the stomach of restricted rabbits is relatively small and animals need to at least 10 d for increasing their feed intake by 20%. On the other hand, the higher intake of W rabbits in comparison with AL and D animals during the re-feeding phase maybe partially depended on the lower incidence of digestive troubles in this group. Other authors (Knudsen *et al.*, 2014) observed that when rabbits were fed freely after a restriction period they showed a sharp increase in feed intake. Therefore the present trial aimed at controlling the problems due to the over-feeding of rabbits in the re-alimentation period in both restricted groups thanks to the gradual increment (+4 g/d) of the supplied feed up to reach the voluntary intake. Nevertheless, a sudden increase of intake was observed in restricted groups during the fifth week of trial when the free re-alimentation started (Figure 6).

On the other hand, as reviewed by (Gidenne *et al.*, 2012a) most studies stated that rabbits subjected to feed restriction did not show higher intake levels than *ad libitum* rabbits during re-

feeding period irrespective by the rationing level previously applied. This result could explain the best feed efficiency in restricted animals. In our study, daily growth and feed conversion were similar among groups in the whole rearing period, although the feed intake was higher in AL and W rabbits compared to D ones.

On the basis of our results, it is possible to assert that in our conditions the feed restriction can improve the animal health status without compromising the growth performance in the whole trial period. Moreover, the presence of digestive troubles which have modified feeding and growth curves in all animals by leading to slight feed restriction levels (91–93% for daily and weekly system) can explain the absence of significant differences in daily growth and feed conversion according to the results obtained by Gidenne *et al.* (2009a).

According to the weak effect on growth performance, feeding systems used in our trial scarcely affected slaughter yield and carcass traits. Similar results were observed by Bovera *et al.* (2013) applying hydric restriction. Other studies showed greater differences in carcass traits due to the application of feed restriction strategies (Perrier, 1998; Metzger *et al.*, 2009). In these later studies, the different slaughter weight achieved by restricted and AL rabbits was mainly associated to a stricter feed restriction applied for longer periods with respect to our trial.

As regard the nitrogen balance, the daily N excretion reached 2.11 g/d per rabbit on average in the whole trial period resulting 9.9% higher than the daily output reported by Gidenne *et al.* (2013b) for growing rabbits weaned at 34 d of age (884 g of live weight) and slaughtered at 70 d (2451 g of live weight), but 17.3% lower with respect to the N output previously estimated in another study of ours with rabbits weaned at 37 d (1007 g of live weight) and slaughtered at 79 d (2953 g of live weight) (Tazzoli *et al.*, 2013) where all animals were fed *ad libitum*. In our trial the nitrogen excretion per kg of rabbit produced averaged 46.4 g (data not shown). In shorter fattening periods (33 d), Calvet *et al.* (2008) measured nitrogen excretions around 10% lower (40.1 g and 42.4 g of N per kg produced rabbit) with respect to our results. A similar value (38 g) was estimated by Maertens *et al.* (2005) for rabbits reared from 0.8 kg to 2.5 kg of live weight. Discrepancies in the N output measured or estimated in various studies may depend on different live weights at slaughter (Calvet *et al.*, 2008) or N contents in feed (Xiccato *et al.*, 2005; Gidenne *et al.*, 2013b; Tazzoli *et al.*, 2013). In our conditions, total and daily nitrogen excretion significantly ($P < 0.05$) decreased in rabbits subjected to a daily restriction system compared to those fed *ad libitum* or fed according to weekly restriction curve. The lower N output of D group was due to the lower N intake which mainly depended on the reduction of feed consumption. On the other hand, a better protein digestion was measured during the second week of trial in both the restricted groups, but this effect disappeared in the re-feeding period. Accordingly, when the N intake was reduced also the retained N decreased.

Effect of slaughter age

As expected, delaying slaughtering of one week (from 73 d to 80 d of age) growth performance and carcass traits changed significantly. In particular, the final live weight increased by 7.6%, whereas the daily weight gain decreased by 6.3% and feed conversion impaired (+8.5%). Similar findings were previously obtained in our trials for growing rabbits reared in colony (Trocino *et al.*, 2014). Furthermore, the increment of final body weight with age was widely described in literature (Dalle Zotte, 2002; Tumova *et al.*, 2006; Metzger *et al.*, 2011).

When slaughtering was delayed, carcass weight significantly increased according to the final live weight (Dalle Zotte, 2002; Trocino *et al.*, 2014). At the same time, dressing out improved in older rabbits (Rao *et al.*, 1978; Milisits *et al.*, 2000; Metzger *et al.*, 2011), the incidence of fat increased as well as the carcass muscularity (Tumova *et al.*, 2006). Besides, the incidence of full gut was lower in older rabbits. As reviewed by Dalle Zotte, (2002) these results can be explained by the reduction of the allometric coefficients of tissues and organs with growth, excluding adipose tissue and the skin.

The extension of the fattening period was not advantageous from the environmental impact point of view. In fact, total and daily nitrogen excretion per rabbit significantly increased with age. Gidenne *et al.* (2013a) reported that N faecal digestibility decreased significantly from 7 to 9 week of age as well as the body nitrogen retention (Xiccato and Cinetto, 1988). In our study, the greater impact in terms of nitrogen output obtained for the older rabbits was probably related either to a decreased on protein digestibility or to higher N intake (+23%).

Conclusions

In the present study, even a slight feed restriction (about 90%) improved rabbit health status without compromising growth performance, slaughter results or carcass traits. The progressive day-by-day restriction curve avoided sharp fluctuations in food consumption, allowed to obtain a restriction level closer to that expected and reflected better the feeding behaviour of growing rabbits compared to the week-by-week restriction system. Moreover the daily feeding program decreased nitrogen excretion. The increase of slaughter age was much more effective in modifying growth and slaughter performance by increasing final live weight, slaughter yield, dressing out and carcass fatness and muscularity. Nonetheless, a longer fattening period involved higher nitrogen output and environmental pollution.

Second contribution:

Time–based feed restriction and group composition for gender: effects on growth performance, feeding behaviour and meat quality of growing rabbits

Introduction

Currently, feed restriction programs in growing rabbits are widely applied for improving the global farm efficiency by increasing the diet digestibility (Xiccato *et al.*, 1992; Knudsen *et al.*, 2014), decreasing the feed conversion (Gidenne *et al.*, 2012a; Bovera *et al.*, 2013) and reducing the mortality due to digestive diseases (Boisot *et al.*, 2003; Romero *et al.*, 2010; Gidenne *et al.*, 2009a). However, the enhancement of health status is not always achieved in restricted rabbits (Matics *et al.*, 2008) even when severe feed restriction strategies (<80% of *ad libitum*) are used (Martignon *et al.*, 2009). Moreover, a lower slaughter yield in restricted rabbits compared to rabbits fed *ad libitum* can partially compromise the advantage due to the improved feed conversion (Xiccato, 1999). In fact, when feed restriction programs are used in growing rabbits, a longer fattening period is often required for reaching the same slaughter weight of rabbits fed *ad libitum* (Perrier, 1998). On the other hand, a slight feed restriction (around 90% of *ad libitum*) can be applied in the post–weaning period in order to reduce feed conversion without impairing daily growth, slaughter traits and carcass and meat quality (Gidenne *et al.*, 2009a), but only few studies deal with this feeding level (Gidenne *et al.*, 2012a).

In field conditions, a feed restriction can be easily applied through the reduction of the feeding time (Szendrő *et al.*, 1988; Foubert *et al.*, 2007; Romero *et al.*, 2010). Nevertheless, there is a lack of information regarding the effect on the feeding behaviour of growing rabbits and the daily changes of feed intake according to a limited access time to the feeders.

Over the last years, the housing system of growing rabbits has been a topic of great importance especially from the point of view of animal welfare (EFSA, 2005; Trocino and Xiccato, 2006; Szendrő and Dalle Zotte, 2011; Trocino *et al.*, 2014). The group–housed system allowed rabbits to express better their species–specific behaviour, but also led to increased aggressiveness among animals especially nearby the period of sexual maturity (Verga *et al.*, 2006; Lambertini *et al.*, 2005). The increase in animal distress and injuries can cause a greater risk of illness impairing animal welfare and carcass and meat quality. The studies concerning the effect of sex composition in groups of growing rabbits are scarce (Di Méo *et al.*, 2003; Szendrő *et al.*, 2012) and results still appear inconsistent. Moreover, the competition for feeding could increase when a feed restriction strategy is applied (Tudela and Lebas, 2006) and different group compositions for gender could favour the occurrence of aggressiveness among animals.

Therefore, the present study aimed at evaluating the effect of the feeding system (AL: *ad libitum* vs. R: feed restriction) and the group composition for gender (X: only female vs. Y: only males vs. Z: mixed sexes) on growth performance, feeding behaviour, slaughter traits and carcass and meat quality of growing rabbits housed in collective pens.

Materials and methods

Animals and experimental conditions

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

The trial was realized at the rabbit farm of the University of Padova, in a close shield during the months of January and March under a natural photoperiod. Extraction fans and automatic heating system were used to maintain the temperature in a range of 18–24 °C and to control air circulation and relative humidity.

A total of 288 rabbits (Hypharm, Groupe Grimaud, Roussay, France), half females and half males, were selected in a commercial farm from multiparous does (≥ 3 kindling) by healthy litters homogenous in size and moved to the experimental rabbit farm. The rabbits were housed in 18 open-top pens (1.28×0.78 m, i.e. 1 m^2) with 16 animals per pen (16 animals/m^2) and monitored from weaning (33 d) to commercial slaughter (75 d of age).

Rabbits were randomly allocated to six homogeneous experimental groups (48 animals per group) by live weight and variability, according to a bi-factorial arrangement with two feeding systems (AL: *ad libitum*, R: time-based feed restriction) and three group compositions for gender (X: only females; Y: only males; Z: mixed sexes). In details, the groups were organized as follows:

- AL–X fed *ad libitum* for the whole trial period, housed in groups of 16 females;
- AL–Y fed *ad libitum* for the whole trial period, housed in groups of 16 males;
- AL–Z fed *ad libitum* for the whole trial period, housed in groups of 8 females and 8 males;
- R–X fed according to time-based feed restriction during the first three weeks, fed *ad libitum* thereafter, housed in groups of 16 females;
- R–Y fed according to time-based feed restriction during the first three weeks, housed in groups of 16 males;
- R–Z fed according to time-based feed restriction during the first three weeks, fed *ad libitum* thereafter, housed in groups of 8 females and 8 males.

Diets and feeding plans

All animals had *ad libitum* access to fresh water through nipple drinkers. During the first period (from weaning until 54 d of age), all rabbits were fed with a post-weaning diet supplemented with an antibiotic (Oxitetracyclin, 1,450 mg a.p./kg) and a coccidiostatic (Diclazuril, 1 mg a.p./kg). In the second period (from 54 d until slaughter), a fattening diet without antibiotics and coccidiostatics was provided. The ingredients and chemical composition of experimental diets are listed in Table 11.

Table 11 Ingredients and chemical composition (g/kg) of the experimental diets.

	Diets	
	P	F
<i>Ingredients</i>		
Dehydrated alfalfa meal (160 g/kg CP)	340	254
Wheat bran	190	240
Barley	120	160
Dried beet pulp	190	160
Soybean meal (490 g/kg CP)	50	40
Sunflower meal (300 g/kg CP)	70	100
Sunflower oil	10	15
Molasses	15	15
Calcium carbonate	1.0	4.0
Dicalcium phosphate	3.5	2.5
Sodium chloride	4.0	4.0
L-lysine HCl	1.0	1.0
DL-methionine	1.0	1.0
Vitamin-mineral premix*	4.0	4.0
Coccidiostat (Diclazuril)	0.5	–
<i>Chemical composition</i>		
Dry matter	890	876
Crude protein	158	151
Ether extract	35	42
Crude fibre	159	148
Ash	68	65
aNDF	354	348
ADF	197	190
Lignin (sa)	48	46
Starch	93	117
Digestible energy (MJ/kg – estimated)	10.12	10.49

*Supplementation per kg of feed: vit. A, 12000 UI; vit. D₃, 1000 UI; vit. E acetate, 50 mg; vit. K₃, 2 mg; Biotin, 0.1 mg; Thiamine, 2 mg; Riboflavin, 4 mg; vit. B₆, 2 mg; vit. 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.

Both experimental diets were pelleted with 3.5 mm of diameter and 10–11 mm of length and supplemented with synthetic lysine and methionine, vitamins, macro- and micro-minerals. The diets were formulated to satisfy the requirements of rabbits during weaning and post-weaning according to de Blas and Mateos (2010).

The feed restriction was realized varying the access time to the feeders from 10 h/d at the beginning of trial until 16 h/d at the end of the third week (53 d of age), as shown in Figure 7. After that, feeding time was increased 1 h per day up to reach the free access to the feeders (24 h/d) at the

end of the fourth week (60 d of age) in order to avoid abrupt changes of the intake level during the re-feeding phase. Then all rabbits had free access to the feed until the end of the trial.

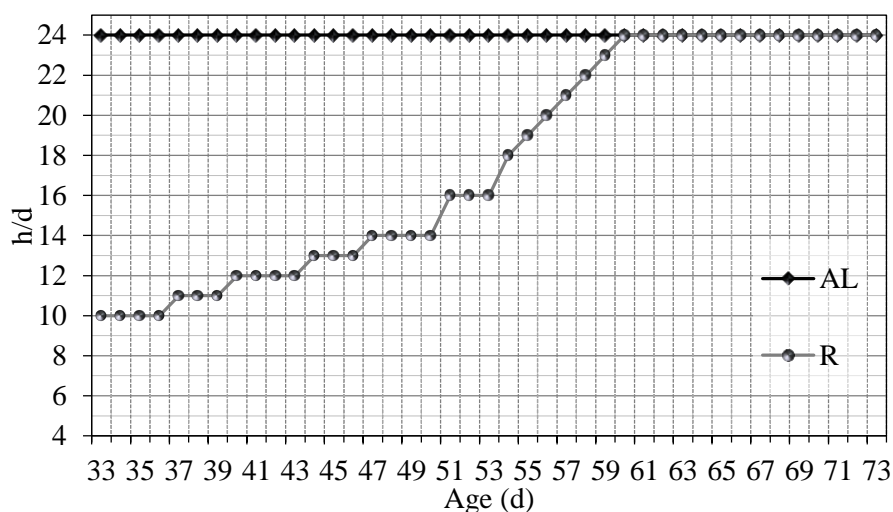


Figure 7 Daily access time to the feeders of *ad libitum* (AL) and restricted rabbits (R) from weaning to the day before slaughter.

This program was developed to achieve a feed restriction around 90% of the voluntary intake ranging from 80% to 100% in the first rearing period. The access to the feeders was permitted mainly during the hours of darkness. The feed restriction was carried out by manually closing the feeders always at the same time (h09:00) and by anticipating gradually the time of their opening (from h23:00 to h09:00).

Growth performance and feeding behaviour

During the trial the individual live weight was measured two times a week. Cage feed intake was recorded daily from the arrival of rabbits until the commercial slaughter. Health status was monitored daily to detect any clinical sign of digestive diseases. During the trial, four animals died; in details two from group AL-Z, one from group AL-Y and one from group R-Z.

The feeding behaviours of *ad libitum* and restricted rabbits were monitored at different ages (39 d, 46 d, 53 d and 60 d old) by weighing the feeders five times a day i.e. every 4–5 hours.

Before the final commercial slaughter, all animals were individually examined for the presence of injuries due to bites and grazes on the ears, body, and genitals.

Commercial slaughter and carcass and meat quality recordings

At 75 d of age, all rabbits (284 animals: 48 from group AL-X; 47 from group AL-Y, 46 from group AL-Z; 48 from group R-X; 48 from group R-Y and 47 from group R-Z) were weighed at the experimental farm and then transported to a commercial slaughterhouse in an authorized truck (about one hour of transport). Nine rabbits (one from group AL-X, two from group AL-Y, three from group AL-Z; one from group R-X; one from group R-Y and one from group R-Z) were excluded from the

commercial slaughter because of inadequate live weight (<2.2 kg). The slaughtering took place after about 6 h of fasting. At the slaughterhouse, the rabbits were individually weighed, stunned by electro-anaesthesia and killed by jugulating. After 2.5-h chilling, the commercial carcasses were weighed to measure the individual dressing out percentages. The carcasses of 144 rabbits (24 per experimental group that were representative in terms of average live weight and variability) were transported to the departmental laboratory and stored at 3–4°C for 24 h. The chilled carcasses were then dissected according to harmonised methods (Blasco *et al.*, 1993).

The pH of the *longissimus lumborum* muscles were measured in duplicate with a pH meter (Basic 20; Crison Instruments Sa, Carpi, Italy) equipped with a specific electrode (cat. 5232; Crison Instruments Sa). The L*a*b* colour indexes (Commission International de l'Eclairage, 1976) were measured in duplicate in the same muscles using a Minolta CM-508 C spectrophotometer (Minolta Corp., Ramsey, NJ, USA). The hind legs and *l. lumborum* muscles were dissected, and the meat of the right hind leg was separated from the bones to measure the meat-to-bone ratio (Blasco and Ouhayoun, 1996). The weight, length, and minimum and maximum diameters of the femur were recorded. The resistance to fracture was tested at the middle of the femur (i.e. the point corresponding to the minimum diameter) with a TA.HDI dynamometer (Stabel Micro System Ltd, Godalming, Surrey, UK) and a structure analyser with a 100-kg load cell (resolution 2 g) and an attached three-points-Bend-Rig (HDP/2PB) with a 40-mm distance between the points and speed of 2 mm/s. The *l. lumborum* muscles were stored under vacuum in plastic bags at -18°C until meat analyses. Thawing and cooking losses were measured. After thawing, the *l. lumborum* were kept in plastic bags and cooked in a water bath for 1.0 h, until an internal temperature of 80°C was achieved. After a 1-h cooling period, the *l. lumborum* were cut into three parts (length: 4 cm; thickness: 1 to 3 cm). On these sections, the maximum shear force was measured with the TA.HDI dynamometer using the Allo-Kramer (10 blades) probe (load cell: 100 kg; distance between the blades: 5 mm; thickness: 2 mm; cutting speed: 500 mm/min) according to the method of Bianchi *et al.* (2007).

Statistical analyses

The individual data of live weight, daily growth, slaughter traits and carcass and meat quality traits were analysed by a two-way ANOVA using the PROC MIXED of SAS (Statistical Analysis System, 2013). The model included feeding system (F: AL and R), group composition for gender (G: X, Y and Z) and their interaction as fixed effects with the pen as a random effect. The cage data of feed intake and feed conversion were analysed by a two-way ANOVA using the PROC GLM of SAS. The model included feeding system, group composition for gender and their interaction as fixed effects. The frequency of injuries was analysed with the GENMOD SAS procedure according to feeding system, group composition and their interactions. Least-square means were chosen for

presenting all data and they were compared using Bonferroni test. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences. Differences among means with $0.05 < P < 0.10$ were accepted as representative of tendencies close to statistical significance.

Results

Growth performance and feeding behaviour

The daily feeding curves of *ad libitum* and restricted rabbits and the feed restriction level obtained during the trial were reported in Figure 8.

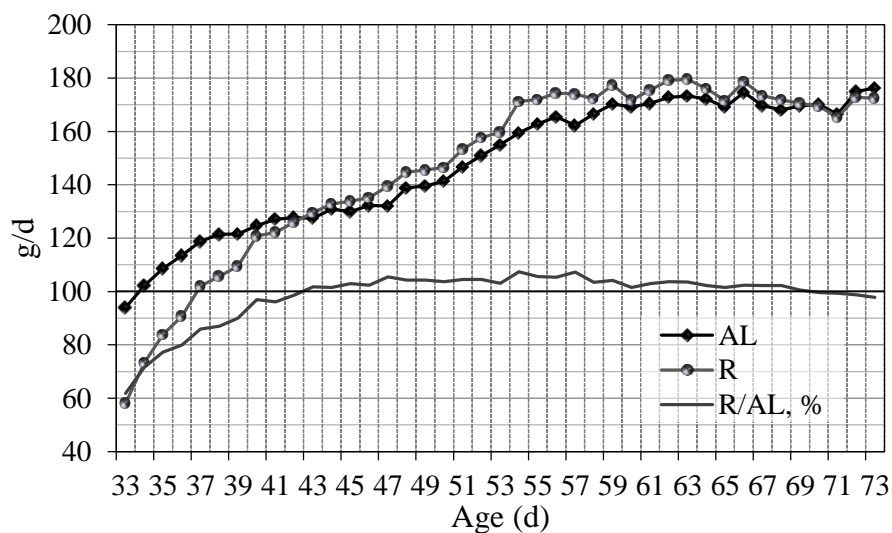


Figure 8 Daily feeding curve of *ad libitum* (AL) and restricted (R) rabbits and feed restriction level from weaning to the day before commercial slaughter.

The feeding level of restricted rabbits averaged 80% of *ad libitum* (111 g/d vs. 89 g/d for AL and R group respectively) during the first week, ranging from 62% at the beginning of trial (32 d of age) to 90% at the seventh day (39 d of age). In this period the access time to the feeders was maintained at 10–11 h/d. In the second week (from 40 d to 46 d of age) with 12–13 h/d of access to the feed, R rabbits rapidly reached the same intake level of AL rabbits (129 g/d on average). During the third week (from 47 to 53 d of age), when the feeding time increased from 14 h/d to 16 h/d, R rabbits showed a higher feed consumption (+4%) compared to AL group.

Therefore, considering the first rearing period, the average feed restriction level of R rabbits reached 95% of *ad libitum*. In the fourth week (from 54 to 60 d of age), the access time to the feeders increased from 16 to 24 h/d and the feed intake of R group was 5% higher than AL group (165 g/d vs. 173 g/d respectively). The overfeeding behaviour of R rabbits continued for another week (from 61 to 67 d of age) and disappeared during the last week of trial.

Since feeding system did not significantly affect the daily growth of rabbits in the first period (52.6 g/d vs. 53.7 g/d for AL and R group respectively), no difference was observed in terms of live weight between *ad libitum* and restricted rabbits at 54 d of age (Table 12).

Table 12 Effect of the feeding system and the group composition for gender on performance from weaning (32 d age) to the day before slaughter (74 d age) and occurrence of lesions in growing rabbits housed in collective pens (16 animals/m²).

	Feeding program (F)		Group composition (G)			Significance			RMSE ¹
	AL	R	Female	Male	Mixed	F	G	F×G	
Number of rabbits	137	138	94	92	89				
Live weight (g)									
At 33 d	831	832	830	831	833				73.9
At 54 d	1937	1959	1941	1948	1954				147
At 74 d	2852	2868	2881	2824	2875				225
Post-weaning period (33 to 54 d)									
Weight gain (g/d)	52.6	53.7	52.9	53.2	53.4				5.1
Feed intake ² (g/d)	128	122	126	125	125	**			4.4
Feed conversion ²	2.42	2.29	2.38	2.34	2.34	***			0.04
Fattening period (54 to 74 d)									
Weight gain (g/d)	45.8	45.5	47.0 ^b	43.8 ^a	46.1 ^b		**		6.7
Feed intake ² (g/d)	169	173	172	171	171				9.4
Feed conversion ²	3.70	3.83	3.66 ^a	3.91 ^b	3.72 ^a	*	**		0.10
Whole period (33 to 74 d)									
Weight gain (g/d)	49.3	49.7	50.0	48.6	49.8				4.7
Feed intake ² (g/d)	148	147	148	147	147				6.7
Feed conversion ²	3.00	2.97	2.96	3.03	2.96				0.05
Occurrence of lesions at slaughter (%)	11.7	10.3	5.2 ^a	18.3 ^b	13.3 ^{ab}		*		

AL: *ad libitum* rabbits; R: restricted rabbits.

¹Root mean square error. ²Pen data.

^{a, b}Means within each row and with different superscript letters are statistically different (P<0.05).

*P<0.05; **P<0.01; ***P<0.001.

Likewise, AL and R rabbits showed similar daily growth rates (46.3 g/d on average) during fattening and reached high live weights at 74 d (2860 g on average). On the other hand, significant effects of the feeding system were observed on feed intake and feed conversion in the first period of trial. In details, AL rabbits showed higher daily intake (128 g/d vs. 122 g/d; P<0.01) and feed conversion (2.42 vs. 2.29; P<0.001) compared to restricted ones. However, in the second phase the daily feed intake of AL and R groups was similar, while feed conversion of previously restricted rabbits significantly impaired (3.70 vs. 3.83; P<0.05). In the whole trial analogous daily feed intakes (148 g/d on average) and feed conversion ratios (2.99 on average) were observed for rabbits always fed *ad libitum* or subjected to the restriction program.

The sex significantly (P<0.01) affected the growth performance of animals in the second trial period (from 54 d to 74 d of age) when the pens with only females or with mixed sexes showed higher daily growths compared to those of only males (47.0 g/d and 43.8 g/d vs. 46.1 g/d for X, Y and Z groups, respectively). At the same time, the feed conversion ratio decreased in pens X and Z in comparison to Y ones (3.66 vs. 3.91 vs. 3.72; P<0.01) as a consequence of the same daily feed intake

observed among groups. Considering the whole rearing period, daily weight gain, feed intake and feed conversion did not change according to the group composition and the animals reached similar live weights and feed efficiency at the end of trial.

At the end of trial the frequency of injuries was significantly higher in rabbits housed in pens with only males compared to those kept in pens with only females (5.2% vs. 18.3% for X and Y groups, respectively; $P < 0.05$). On the other hand, Z group showed an intermediate degree of injuries (Table 12).

The feeding behaviours of AL and R rabbits (measured at 39, 46, 53 and 60 d of age) are reported in Figure 9. The feeding rhythm (hourly feed intake) of AL rabbits was characterized by a progressive increment during afternoon (from h13:00 to h18:00) and a peak of intake (around 7–10 g/h) during the evening (from h19:00 to h21:00); then the hourly feed consumption gradually decreased during the night and early morning until reaching the minimum level (about 2 g/h) from h09:00 to h11:00. This phase of low feed intake was usually preceded by a slight recovery of the feeding rhythm between h04:00 and h06:00. The circadian feeding curve of AL rabbits was scarcely modified during the trial, whereas the total quantity of feed ingestion obviously increased with the age.

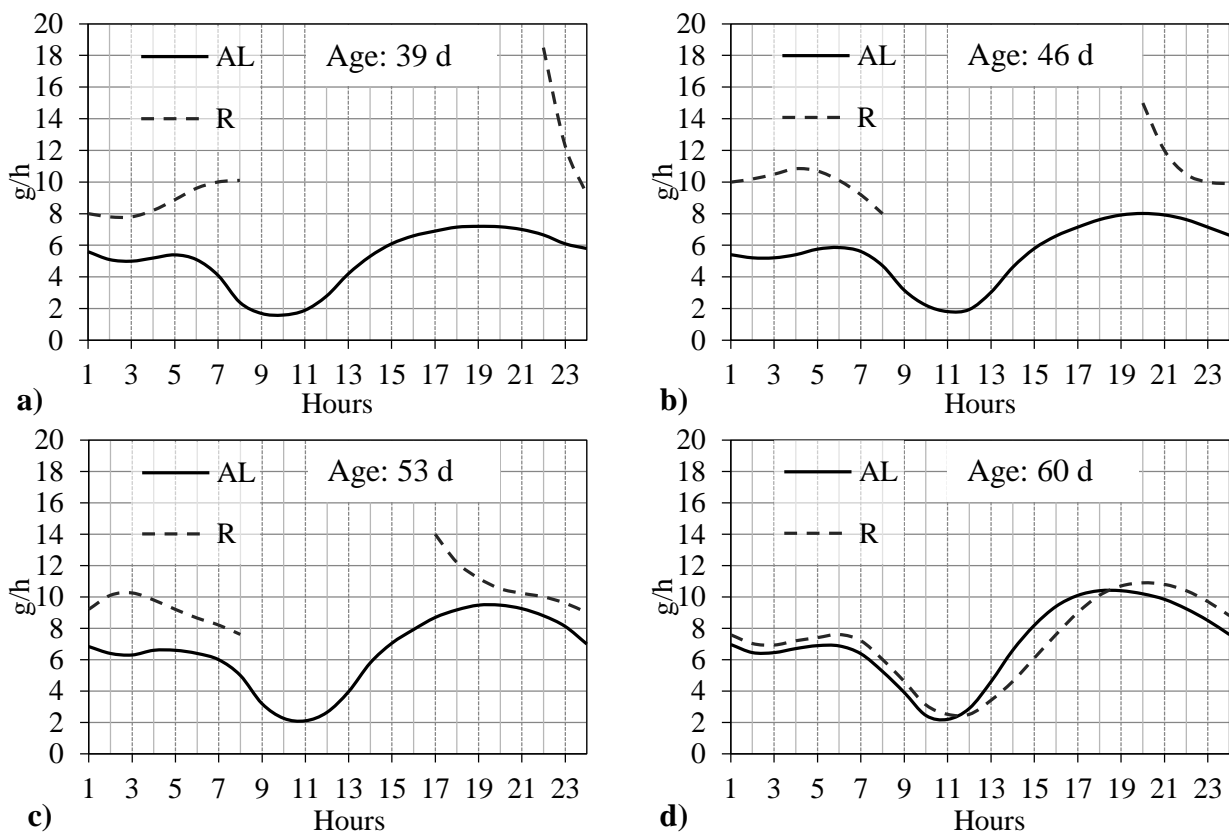


Figure 9 Feeding behaviour of rabbits fed *ad libitum* (AL) or subjected to a limited access time to the feeders of 11 h/d (Figure 9a), 13 h/d (Figure 9b), 16 h/d (Figure 9c) and 24 h/d (Figure 9d).

As expected, the feed restriction led to important changes in the rabbits feeding behaviour. At 39 d of age, with 11 h/d of access to feed (from h22:00 to h09:00), R rabbits showed high feed intake

(>18 g/h) in the first hour after the opening of the feeders. Subsequently, the feeding rhythm of R group decreased rapidly for reaching a minimum level (about 8 g/h) after 4–5 h. However, the hourly intake of restricted rabbits remained higher than that of AL group (Figure 9a). At 46 and 53 d of age, the restricted rabbits had an access to the feeders of 13 h/d and 16 h/d, respectively. In these conditions the R group overcame the feeding level of the AL one. Nonetheless, peaks of feed ingestion (14–15 g/h) were detected in R animals at the time of access to the feeders. These peaks were followed by a quick reduction of the intake rhythm (Figure 9b, Figure 9c). As observed in the previous week (39 d of age), the hourly feed consumption were higher in restricted rabbits compared to AL ones and characterized by relevant daily changes. Finally, when the R animals were allowed to have free access to feed, they achieved a “normal” feeding behaviour within two d, showing an intake curve comparable to that of AL rabbits (Figure 9d).

Slaughter results, carcass traits and meat quality

Slaughter traits, carcass characteristics and meat quality were not affected by the feeding system. The slaughter weight of AL and R rabbits was similar (2795 g on average) as well as the cold carcass weight (1702 g on average), the dressing percentage (61.0%), the incidence of dissectible fat (2.77%) and the meat to bone ratio of hind leg (5.68) (Table 13).

In the same way, meat pH (5.58), lightness (50.7) and colour indexes (-1.92 and 1.47 for redness and yellowness respectively) of *longissimus lumborum* did not differ according to the feeding system (Table 14). Also the values of shear force, thawing and cooking losses measured in the same muscles were similar among groups.

As regards the femur characteristics, only the maximum diameter was significantly affected by the feeding system and exhibited higher values in AL group than restricted one (9.11 mm vs. 8.79 mm; $P < 0.01$) (Table 15).

The effect of group composition was relevant for some slaughter results (Table 13). In particular lower transport losses were observed in Y rabbits compared to X and Z ones (3.00% vs. 2.45% vs. 2.80%; $P < 0.01$). Moreover, the animals housed in groups of only males achieved the best performance in terms of dressing percentage (61.8%; $P < 0.01$); the slaughter yield of X group was the lowest (60.2%), while Z group reached an intermediate value (61.0%). On the other hand, the carcasses of Y and Z rabbits showed a higher head proportion with respect to groups of only females (7.41% vs. 7.65% vs. 7.60% for X, Y and Z groups respectively; $P < 0.05$), whereas the incidence of liver and thoracic organs did not differ among groups. Accordingly, all groups exhibited analogous reference carcass weights (1438 g on average) and similar indexes of carcass fatness and muscularity.

Meat pH was significantly higher in Y and Z rabbits compared to X ones (5.55 vs. 5.60 vs. 5.58 for X, Y and Z groups respectively; $P < 0.01$), while neither meat lightness nor colour indexes differed

among groups. Furthermore, rabbits housed in groups with only females showed higher thawing losses and higher meat shear force in comparison to groups of rabbits composed by only males (Table 14). The femur characteristics were not affected by the group composition for gender (Table 15).

Table 13 Effect of the feeding system and the group composition for gender on slaughter traits and main characteristics of 24–h chilled carcasses of growing rabbits housed in collective pens (16 animals/m²).

	Feeding program (F)		Group composition (G)			Significance			RMSE ¹
	AL	R	Female	Male	Mixed	F	G	F×G	
Number of rabbits	137	138	94	92	89				
Weight at farm, g	2871	2869	2902	2828	2879				224
Slaughter weight (SW), g	2796	2793	2818	2762	2802				212
Transport losses, %	2.73	2.77	3.00 ^b	2.45 ^a	2.80 ^b		**		1.12
Cold carcass weight (CW), g	1699	1705	1696	1703	1707				145
Dressing percentage, % SW	60.8	61.1	60.2 ^a	61.8 ^c	61.0 ^b		***		1.73
Head, % CW	7.55	7.56	7.41 ^a	7.65 ^b	7.60 ^b		*		0.45
Liver, % CW	4.48	4.55	4.56	4.42	4.56				0.57
Thoracic organs, % CW	3.00	2.98	3.02	3.01	2.94				0.32
Reference carcass (RC), g	1437	1439	1438	1436	1440				106
Dissectible fat, % RC	2.70	2.84	2.93	2.65	2.73				0.92
Hind legs, % RC	32.8	32.5	32.7	32.8	32.4				0.90
Hind leg muscle/bone ratio	5.75	5.61	5.81	5.61	5.63				0.38

AL: *ad libitum* rabbits; R: restricted rabbits.

¹Root mean square error.

^{a, b}Means within each row and with different superscript letters are statistically different (P<0.05).

*P<0.05; **P<0.01; ***P<0.001.

Table 14 Effect of the feeding system and the group composition for gender on meat traits of *longissimus lumborum* muscle of growing rabbits housed in collective pens (16 animals/m²).

	Feeding program (F)		Group composition (G)			Significance			RMSE ¹
	AL	R	Female	Male	Mixed	F	G	F×G	
Number of rabbits	72	72	48	48	48				
<i>Longissimus lumborum</i>									
pH	5.57	5.58	5.55 ^a	5.60 ^b	5.58 ^b		**		0.08
<i>L</i> [*]	50.7	50.6	51.0	50.5	50.5				2.42
<i>a</i> [*]	-2.00	-1.83	-1.91	-1.97	-1.87				0.62
<i>b</i> [*]	1.36	1.57	1.50	1.42	1.49				1.92
Thawing losses, %	6.27	6.25	7.23 ^b	5.88 ^a	5.67 ^a		*		1.93
Chocking losses, %	32.7	32.6	32.5	32.4	33.0				1.98
Shear force, kg/g	2.13	2.26	2.36 ^b	2.03 ^a	2.18 ^{ab}		*		0.42

AL: *ad libitum* rabbits; R: restricted rabbits; *L*^{*}: lightness; *a*^{*}: redness; *b*^{*}: yellowness.

¹Root mean square error.

^{a, b}Means within each row and with different superscript letters are statistically different (P<0.05).

*P<0.05; **P<0.01; ***P<0.001.

Table 15 Effect of the feeding system and the group composition for gender on femur characteristics of growing rabbits housed in collective pens (16 animals/m²).

	Feeding program (F)		Group composition (G)			Significance			RMSE ¹
	AL	R	Female	Male	Mixed	F	G	F×G	
Number of rabbits	36	36	24	24	24				
Femur weight, g	14.4	14.2	14.2	14.6	14.1				1.10
Femur length, mm	91.5	91.3	91.1	91.7	91.4				1.94
Maximum diameter, mm	9.11	8.79	9.01	8.85	9.00		**		0.53
Minimum diameter, mm	7.00	6.89	6.97	6.93	6.93				0.34
Femur fracture force, kg	40.1	41.4	39.7	41.4	41.2				5.35

AL: *ad libitum* rabbits; R: restricted rabbits.

¹Root mean square error.

**P<0.01.

Discussion

Effect of feeding system

As previously described, the feed restriction program proposed in the present study was developed in order to obtain an average feeding level around to 90% of *ad libitum*, increasing gradually from 80% to 85–90% to 95–100% through an access time to the feeders of 10–11 h/d, 12–13 h/d and 14–16 h/d during the first, the second and the third week of rearing respectively. The expected restriction level was fully achieved in the first week, but this result depended mainly on the severe feed limitation observed on the first day of trial (62% of *ad libitum*). This was probably due to an initial “surprise” effect, i.e. R rabbits were not accustomed to have a limited access time for feeding and they ate with a normal rhythm till the first withdrawal of feeders. After that, the ingestion capacity of restricted rabbits increased very quickly reaching the intake of the *ad libitum* group already during the second week of trial (42 d of age). Therefore, in the three-week post-weaning period the feeding access time for R rabbits was in fact too long, leading to an average restriction level lower than that expected (96% vs. 90% of *ad libitum*). In a previous study (Foubert *et al.*, 2007), the intake level of growing rabbits was limited to 62.8%, 73.9% and 80.4% of *ad libitum* in the first three weeks after weaning (31 d of age) by reducing the access time to feeders to 6, 8 and 10 h/d respectively. Romero *et al.* (2010) reported that rabbits fed for 8 h/d in the first rearing period achieved a feeding level around to 80% of the voluntary intake. In these studies the stricter feed restriction with respect to our results was related mainly to the shorter feeding time. However, also the daily phase of feed availability (during the day or during the night) could play an important role. Indeed, the previous authors allowed the food access during daylight in order to obtain more severe rationing levels, as suggested by Jerome *et al.* (1988). On the other hand, Wissman *et al.* (2009) have not observed significant differences in the feed intake of growing rabbits fed during dark or light periods.

The first studies on feed restriction strategies in growing rabbits aimed at evaluating the effects on growth performance, carcass traits and meat quality. Moreover, rationing programs were applied to improve feed efficiency and standardize the growth curves of the animals (Ouhayoun, 1989). However, at slaughter, rabbits previously subjected to a strict feed restriction ($\leq 80\%$ of *ad libitum*) did not catch up live weights similar to those of rabbits always fed *ad libitum*, despite of prolonged re-feeding phases and the occurrence of compensatory growths (Bovera *et al.*, 2013). These findings were confirmed in our previous study (Birolo *et al.*, 2013), where rabbits fed at 90% of the voluntary intake in the first rearing period completely recovered growth performance during re-feeding phase, while rabbits subjected to a restriction equal to 80% of *ad libitum* showed daily growth rates and final live weights significantly lower compared to those of rabbits fed

always freely. In the present work, the growth performance was not significantly affected by the feeding system, therefore restricted and *ad libitum* rabbits showed similar final live weights. In fact, the effects of the feed restriction on daily intake and feed conversion were limited to the first period.

Martignon (2010) stated that the weight gain of restricted rabbits impaired in the first phase of feed restriction according to the rationing level applied. Afterwards, during re-feeding phase, the feed conversion of restricted rabbits could be greatly improved thanks to the occurrence of compensatory growths. Furthermore, as widely reviewed by Gidenne *et al.* (2012a), the feed conversion of rabbits subjected to feed restriction programs could be significantly enhanced in the whole rearing period. However, in our conditions, the feed efficiency was not affected by the feeding system because the improvement achieved by restricted rabbits in the first trial period was lost during fattening phase.

Previous studies also detected relevant effects of the feed restriction strategies on the carcass traits. In particular, restricted animals showed higher incidences of the full digestive tract and liver compared to *ad libitum* ones. Moreover, the carcass fatness could be strongly reduced in rabbits subjected to rationing programs (from 50 to 80% of *ad libitum* intake) (Xiccato, 1999; Gidenne *et al.*, 2012a; Knudsen *et al.*, 2014). In our study, the feeding system did not modify neither carcass characteristics nor meat quality, in accordance with the slight effects observed on growth performance. However it is necessary to underline that a very weak feed restriction (around to 95% of the voluntary intake) in the first three weeks associated to long re-feeding phase (three weeks) did not improve feed efficiency.

The feeding system significantly ($P < 0.01$) affected the femur characteristics, in details the maximum diameter of the bone was higher in *ad libitum* rabbits compared to restricted ones, while the fracture force did not differ between groups. This result may depend on a lower rate of skeletal development in restricted rabbits related to different nutrients availability for the growth of tissues in the post-weaning period.

The intake pattern of rabbits fed *ad libitum* is characterised by several meals during the day, reaching the maximum intake level (10% of the total daily feed consumption) 2–4 hours after the end of light period and a very low intake 2–4 hours after the end of dark period, in concomitance with the beginning of caecotrophy (Gidenne and Lebas, 2006; Martignon *et al.*, 2009). In our trial, the feeding behaviour was greatly modified by feed restriction also when a slight rationing level was applied. In particular, restricted rabbits showed a higher feeding activity compared to *ad libitum* ones in the whole period of access to feeders with the most important peak of food ingestion during the first hour after the fasting phase. Thanks to this intake pattern, rabbits fed for 13 h/d

were capable of overcoming the daily feed intake of rabbits fed freely. Therefore, a fasting phase applied during daylight appeared less stressful from the point of view of animal welfare, as it partially reflected the normal feeding behaviour of rabbits fed freely. Moreover, during the re-feeding phase, with the achievement of the free access to the feeder, R rabbits adopted the regular intake pattern of the AL group within few days. In accordance with our findings, Martignon *et al.* (2009) reported that rabbits subjected to a feed restriction equal to 75% of *ad libitum* ingested 42% of their daily ration within 2 h at 36 d of age, while after 10 d (46 d of age) under the same restriction level (75%) the peak of ingestion were considerably lower (33% of the total daily ration within 2 h). Once fed freely, the rabbits previously restricted showed very soon an intake pattern similar to that of the *ad libitum* group (Martignon, 2010). On the other hand, the number of feeders could also play an important role on the feeding behaviour of restricted rabbits by affecting the competition among animals (Tudela and Lebas, 2006). However, in our conditions all rabbits accessed at the same time to feed. Moreover, in our trial the feed restriction level applied was not so severe and limited at the first 2–3 weeks after weaning when any aggressive behaviour was usually observed among rabbits. In fact, at the end of trial no significant difference was observed on the occurrence of injuries according to the feeding system.

Effect of the group composition for gender

Usually, growing rabbits are housed in groups of mixed sexes because sexual dimorphism is scarce in this species. However, at the end of the rearing period, approaching the sexual maturity, the occurrence of aggressive behaviour among animals can sharply increase. Housing rabbits in groups of only females and only males rather than groups with animals of both sexes could represent an important topic from the point of view of productive performance as well as animal welfare.

Di Méo *et al.* (2003) observed significant effects of the group composition for gender: in particular the daily weigh gain was higher in females than in males from 49 to 70 d of age. Conversely, from 70 to 90 d of age, pens with only males achieved higher daily growth rates compared to those with only females or with mixed sexes. Furthermore, pens with mixed sexes showed a greater frequency of injuries and a lower final live weight with respect to groups of only females and only males. Therefore the authors suggested the housing of growing rabbits in groups of animals of the same sex. By contrast, Szendrő *et al.* (2012) reported that housing rabbits in groups of only females or only males led to a higher incidence of injuries originated from aggression without any significant advantage on growth performance.

In the present study, the effect of the group composition on growth performance was significant in the second period, when pens with only females or with mixed sexes showed higher

daily growth and better feed conversion in comparison to pens of only males. Nevertheless, considering the whole trial period, no difference on productive performance among groups was measured. On the contrary, an important effect was detected on the frequency of injuries. In particular, the higher incidence of wounded animals in pens with only males compared to those with only females could be related to a major aggressiveness of males who approached before to the sexual maturity.

In literature, the effect of sex on slaughter traits of growing rabbits is treated mainly by comparing males and females kept together in bi-cellular cages or in collective pens. According to our findings, several studies reported that females exhibited a lower slaughter yield than males due to a higher incidence of the digestive tract as found by other authors (Parigi Bini *et al.*, 1992; Petracci *et al.*, 1999; Trocino *et al.*, 2003; Trocino *et al.*, 2015). Higher transport losses measured in females were also probably related to greater intestinal contents with respect to males. Contrarily to the results of the present study, some authors described significant variations on meat colour indexes according to the sex of the animals (Trocino *et al.*, 2003; Carrilho *et al.*, 2009).

As other carcass traits are concerned, similar thawing and cooking losses are usually detected in males and females reared in bi-cellular cages (Petracci *et al.*, 1999). In our trial, the differences observed in meat pH, thawing losses, and tenderness could be explained by different stress conditions of animals kept in pens with only females, only males or with mixed sexes. In particular, the increased aggressiveness in only-male groups, which could require more time for establishing hierarchy than only-female groups, could explain the increased pH values in the meat as a consequence of a reduced glycogen in the muscles of animals subjected to extended stress periods (Dalle Zotte, 2002). Nevertheless, this hypothesis has to be validated on the basis of further physiological and behavioural observations.

Conclusions

Feed restriction can be easily applied by limiting the access time to the feeders. However, the precise control of the feeding level is not simply to be reached, because rabbits adjust very quickly their voluntary ingestion capacity. The progressive increase in the access time to the feeders from 10 to 16 h/d in the first three weeks of trial leads to a feed restriction level around to 95% of *ad libitum* without compromising growth performance, slaughter results, carcass traits and meat quality of growing rabbits. On the meanwhile, in our conditions, feeding restriction does not improve either feed efficiency or health status and therefore the economic meaning of feed restriction technique should be carefully evaluated.

In fact, a gradual decrease of the feeding time in the first week after weaning could avoid abrupt changes on the feed intake related to a surprise effect. Moreover, an access time to the

feeders lower than 10 h/d during the second week followed by a progressive increase in the third and fourth week might allow a better control on the feeding level, also improving the feed efficiency. From the point of view of animal welfare, feeding the rabbits during the dark period, with the fasting phase during daylight, could be a less stressful strategy because it partially reflects the normal intake pattern of animals fed freely.

As what regards the effects of the group composition, our findings did not justify the housing of rabbits in separated groups of males and females, also considering the complications on rearing management and the risks of aggressiveness in only-male groups.

Third contribution:
Effect of the feeding program and group size on growth performance, health status and meat quality in growing rabbits

Introduction

Several studies have been carried out on the application of rationing programs in growing rabbits during the post-weaning period (2–4 weeks after weaning) followed by a re-feeding phase with free access to feeders until commercial slaughter. In particular, a reduction of feeding level by at least 20% in the first period after weaning could improve animal health status and feed conversion, while the losses on weight gain could be partially recovered thanks to the compensatory growth during re-feeding (Gidenne *et al.*, 2012a). A quantitative feed restriction can be obtained directly by administering daily a restricted amount of diet (Gidenne *et al.*, 2009a; Knudsen *et al.*, 2014), or indirectly by reducing the time of access to feed or water (Boisot *et al.*, 2005; Bovera *et al.*, 2013). The latter technique is of easy application also in field conditions, but it results debatable from the animal welfare viewpoint because a long daily period of thirst (22–23 h/d) is required to achieve a considerable intake reduction (Boisot *et al.*, 2004). Moreover, a hydric restriction leads to a further suffering for the animals because of thirst and hungry especially in conditions of high room temperatures (Ben Rayana *et al.*, 2008). On the other hand, a direct reduction of the feed administered daily is effective in controlling the feeding level, but it is a labour- and time-consuming technique especially in the absence of automatic feeders. Differently, feed intake can be easily reduced by limiting the access time to the feeders without producing additional stress to the animals. Several authors report that a feed restriction at about 75–80% of *ad libitum* intake can be achieved through an access time to feed of 8 h/d during the first three weeks after weaning (Foubert *et al.*, 2007; Romero *et al.*, 2010), with positive effects on the health status and/or feed efficiency. However, the sanitary conditions often impair when a free access to feeders is permitted in the successive re-feeding period. Furthermore, restricted rabbits cannot always reach the same slaughter weight and dressing percentage of *ad libitum* ones.

In the second contribute of this thesis we increased progressively the feeding time from 10 to 16 h/d during the first three weeks in order to obtain a feeding level around 90% of *ad libitum* on average. However, it was difficult to achieve an accurate control on the feeding level due to the “surprise” effect at the beginning of rationing and the capability of rabbits of increasing rapidly their feed intake during the subsequent days.

The control of feed intake could be even more difficult when rabbits are reared in groups and could be affected by the group size. According to EFSA (2005), growing rabbits should be housed

in groups of at least 7–8 animals, with a stocking density not higher than 16 animals/m² and a maximum load of live weight of 40 kg/m². Several studies confirmed the possibility to improve rabbits welfare through the pen–housing system (Dal Bosco *et al.*, 2002), even if an increase of aggressiveness can occur in large groups (Rommers and Meijerhof, 1998; Verga *et al.*, 2006) and negative effects could be observed on growth performance (Trocino *et al.*, 2014) and sanitary status (Dal Bosco *et al.*, 2002).

The present study aimed at evaluating productive performance, health status, carcass traits and meat quality in rabbits subjected to two feeding systems (*ad libitum* vs. restricted) and reared in groups of different size (groups of 8, 16 and 32 rabbits/pen). The restriction program was developed to obtain a restriction level around 85% of *ad libitum* in the first three weeks through a progressive and variable management on the feeding time.

Materials and methods

Animals and experimental conditions

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

The trial was realized at the experimental farm of the University of Padova, in a close shield during the months of February and April under a natural photoperiod. Extraction fans and automatic heating system were used to control air circulation and maintain the temperature in a range of 20–23 °C.

A total of 320 cross–bred rabbits (Hypharm, Groupe Grimaud, Roussay, France), including 160 females and 160 males, were selected in a commercial farm from multiparous does (≥ 3 kindling) by healthy litters homogenous in size and moved to the experimental farm. The animals were housed in 20 open–top pens composed by one, two or four modules (0.64 × 0.78 m, i.e. 0.5 m²) communicating each other through an opening (0.2 m × 0.3 m) on the side wall in order to obtain pens of different sizes in which to house different number of rabbits (8, 16 or 32 animals/pen, respectively) at the same stocking density (16 animals/m²). Each group consisted of females and males with a ratio 1:1. The animals were monitored from weaning (31 d of age) to commercial slaughter (73 d).

The rabbits were randomly allocated to six homogeneous experimental groups by live weight and variability, according to a bi–factorial design with two feeding systems (AL: *ad libitum*, R: time–based feed restriction) and three group sizes (8, 16 or 32 animals/pen). In details the following groups were organized:

- AL-8 fed *ad libitum* for the whole trial period, housed in pens composed by one module with 8 animals (4 replicates);
- AL-16 fed *ad libitum* for the whole trial period, housed in pens composed by two modules with 16 animals (4 replicates);
- AL-32 fed *ad libitum* for the whole trial period, housed in pens composed by four modules with 32 animals (2 replicates);
- R-8 fed according to time-based feed restriction during the first three weeks, housed in pens composed by one module with 8 animals (4 replicates);
- R-16 fed according to time-based feed restriction during the first three weeks, housed in pens composed by two modules with 16 animals (4 replicates);
- R-32 fed according to time-based feed restriction during the first three weeks, housed in pens composed by four modules with 32 animals (2 replicates).

Diets and feeding plans

All animals had *ad libitum* access to fresh water through nipple drinkers. During the first three-week period (from weaning until 52 d of age), all rabbits were fed with a post-weaning diet supplemented with antibiotics (Oxitetracilin, 1,450 mg a.p./kg) and with coccidiostatic (Diclazuril, 1 mg a.p./kg). In the final three-week period (from 53 d until slaughter), a fattening diet without antibiotics and coccidiostatics was provided.

Both experimental diets were pelleted with 3.5 mm of diameter and 10–11 mm of length and their ingredients and chemical composition are listed in Table 16. The diets were formulated to satisfy the requirements of rabbits during post-weaning and fattening according to de Blas and Mateos (2010) and were supplemented with synthetic lysine and methionine, vitamins, macro- and micro-minerals.

Table 16 Ingredients and chemical composition (g/kg) of the experimental diets.

	Diets	
	P	F
<i>Ingredients</i>		
Dehydrated alfalfa meal (160 g/kg CP)	340	253
Wheat bran	190	240
Barley	120	160
Dried beet pulp	190	160
Soybean meal (490 g/kg CP)	50	40
Sunflower meal (300 g/kg CP)	70	100
Sunflower oil	10	15
Molasses	15	15
Calcium carbonate	1.0	4.0
Dicalcium phosphate	3.5	2.5
Sodium chloride	4.0	4.0
L-lysine HCl	1.0	1.0
DL-methionine	1.0	1.0
Vitamin-mineral premix*	4.0	4.0
Coccidiostat (Diclazuril)	0.5	–
<i>Chemical composition</i>		
Dry matter	879	880
Crude protein	153	152
Ether extract	31	37
Crude fibre	166	151
Ash	68	66
aNDF	357	348
ADF	197	188
Lignin (sa)	50	50
Starch	112	131
Digestible energy (MJ/kg – estimated)	10.12	10.49

*Supplementation per kg of feed: vit. A, 12000 UI; vit. D3, 1000 UI; vit. E acetate, 50 mg; vit. K₃, 2 mg; Biotin, 0.1 mg; Thiamine, 2 mg; Riboflavin, 4 mg; vit. B₆, 2 mg; vit. 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.

Feed restriction was realized varying progressively the access time to the feeders as shown in Figure 10. In details the feeding time for restricted rabbits decreased (from 14 to 8 h/d) during the

first week of trial (from 31 to 37 d of age), remained stable (8 h/d) during the second week (from 38 to 44 d of age) and increased gradually (+1 h/d) from the beginning of the third week (45 d of age) up to the full free access to the feeders (24 h/d) at the beginning of the fifth week (60 d of age). Then all rabbits had free access to the feeders until the end of the trial.

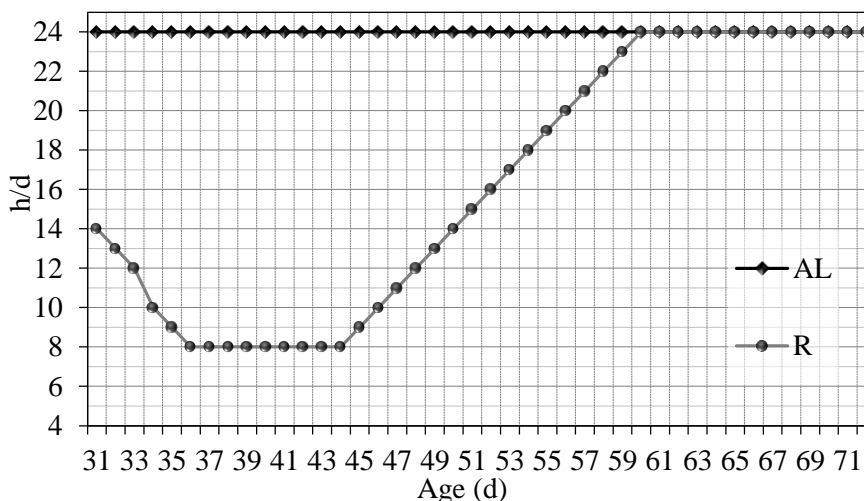


Figure 10 Feeding restriction program with the variation of daily access time (h/d) to the feeders of *ad libitum* (AL) and restricted rabbits (R) from weaning to the day before slaughter.

The access to the feeders was permitted mainly during the hours of darkness and the feeding program was implemented initially by extending and then by shortening the time of fast in the daylight period. The feed restriction was carried out by manually closing the feeders in the morning and by re-opening them in the evening. This feeding program was developed to achieve an average feed restriction around 85% of the voluntary intake, ranging from 75% to 100% in the first rearing period.

Growth performance and health status

During the trial live weight was recorded individually three times a week. Cage feed intake was recorded daily from the arrival of rabbits until slaughter. The live weight at 72 d of age was considered as the final live weight and used to calculate growth performance in the second and in the whole rearing period. Health status was controlled daily to detect any clinical signs of digestive diseases. Rabbits were considered ill in the presence of diarrhoea and mucus or with a clear reduction of growth (weight loss during 2–3 consecutive d). According to Bennegadi *et al.* (2000) ill rabbits were counted only once for calculating morbidity while death animals were taken into account only for determining mortality rate. The sanitary risk was expressed as the sum of morbidity and mortality. During the trial 29 animals died; in details 1 from group AL–8, 4 from group AL–16, 5 from group AL–32; 2 from group R–8, 9 from group R–16 and 8 from group R–32.

Commercial slaughter and carcass and meat quality recordings

At 73 d of age, all alive rabbits (289 animals: 31 from group AL-8; 59 from group AL-16, 59 from group AL-32; 30 from group R-8; 55 from group R-16 and 55 from group R-32) were weighed at the experimental farm and then transported to a commercial slaughterhouse in an authorized truck (about one hour of transport). Two rabbits (1 from group AL-16 and 1 from group R-32) were excluded from the commercial slaughter because of their insufficient live weight (<2.2 kg). The slaughtering took place after 6 hours of fasting. At slaughterhouse, the rabbits were individually weighed, stunned by electro-anaesthesia and killed by jugulating. After 2.5-h chilling, the commercial carcasses were weighed to measure the individual dressing out percentages. The carcasses of 144 rabbits (24 per experimental group that were representative in terms of average weight and variability) were transported to the departmental laboratory and stored at 3–4°C for 24 h. The chilled carcasses were then dissected according to harmonised methods (Blasco *et al.*, 1993).

The pH of the *longissimus lumborum* and *biceps femoris* muscles were measured in duplicate with a pH meter (Basic 20; Crison Instruments Sa, Carpi, Italy) equipped with a specific electrode (cat. 5232; Crison Instruments Sa). The L*a*b* colour indexes (Commission International de l'Eclairage, 1976) were measured in duplicate on the same muscles using a Minolta CM-508 C spectrophotometer (Minolta Corp., Ramsey, NJ, USA). The hind legs and *l. lumborum* muscles were separated, and the meat of the right hind leg was separated from the bones to measure the meat-to-bone ratio (Blasco and Ouhayoun, 1996). The weight, length, and minimum and maximum diameters of the femur were recorded. The resistance to fracture was tested at the middle of the femur (i.e. the point corresponding to the minimum diameter) with a TA.HDI dynamometer (LS5, Lloyd Instruments Ltd, Bognor Regis, UK) and a structure analyser with a 500-kg load cell (resolution 1 g) and an attached three-points-Bend-Fixture (3PBF) with a 40-mm distance between the points and speed of 2 mm/s. The *l. lumborum* muscles and the left hind legs were stored under *vacuum* in plastic bags at -18°C until the meat analyses were performed. Thawing and cooking losses were measured. After thawing, the *l. lumborum* were kept in plastic bags and cooked in a water bath for 1.0 h until an internal temperature of 80°C was achieved. After a 1-h cooling period, the *l. lumborum* was cut and the middle part selected (length: 70 mm). On this section, the maximum shear force was measured with the dynamometer using the Allo-Kramer (10 blades) probe (load cell: 500 kg; distance between the blades: 5 mm; thickness: 2 mm; cutting speed: 500 mm/min) according to the methods of Bianchi *et al.* (2007). The left hind legs were kept in plastic bags and cooked in a water bath for 2.5 h, until reaching an internal temperature of 80°C. After 1-h cooling period, the hind legs were sectioned into 3 portions by separating the

regions of the tibia and the hip from that of the femur. From the femur region (middle part), the bone was extracted without damaging the muscles of the thigh. On this section, the maximum shear force was measured according to the method described for the *l. lumborum* muscle.

Chemical analyses

The diets were analysed to determine the concentrations of dry matter (934.01), ash (967.05) and crude protein (2001.11), and starch (amyloglucosidase α amylase method, 996.11) using AOAC (2000) methods following harmonised procedures (Gidenne *et al.*, 2001b). Ether extract was analysed after acid hydrolysis (EC, 1998). The fibre fractions; i.e. aNDF (neutral detergent fibre without sodium sulphite), ADF (acid detergent fibre expressed inclusive of residual ash), and lignin (sa), 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 of live weight, daily growth, slaughter traits and carcass and meat quality were analysed by a two-way ANOVA using the PROC MIXED of SAS (Statistical Analysis System, 2013). The model included feeding system (F: AL and R), group size (G: 8, 16 and 32) and their interaction as the fixed effects with the pen as a random effect. The cage data of feed intake and feed conversion were analysed by a two-way ANOVA using the PROC GLM of SAS. The model included feeding system (F: AL and R), group size (G: 8, 16 and 32) and their interaction as fixed effects. Mortality, morbidity and sanitary risk were analysed by the PROC GENMODE of SAS (Statistical Analysis System, 2013). The feeding system, the group size and their interaction were included in the model as fix effects. Least-square means were chosen for presenting all data and they were compared by using Bonferroni test. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences. Differences among means with $0.05 < P < 0.10$ were accepted as representative of tendencies close to statistical significance.

Results

Growth performance

The expected feed restriction level for R rabbits (85% of *ad libitum*) was perfectly achieved during the first three weeks of trial (from 31 to 51 d of age). In fact, the intake level of restricted animals decreased progressively, from 80% to 65% of *ad libitum*, during the first week (from 31 to 36 d of age) according to the gradual reduction in the access time to the feeders (from 14 to 8 h/d) (Figure 11).

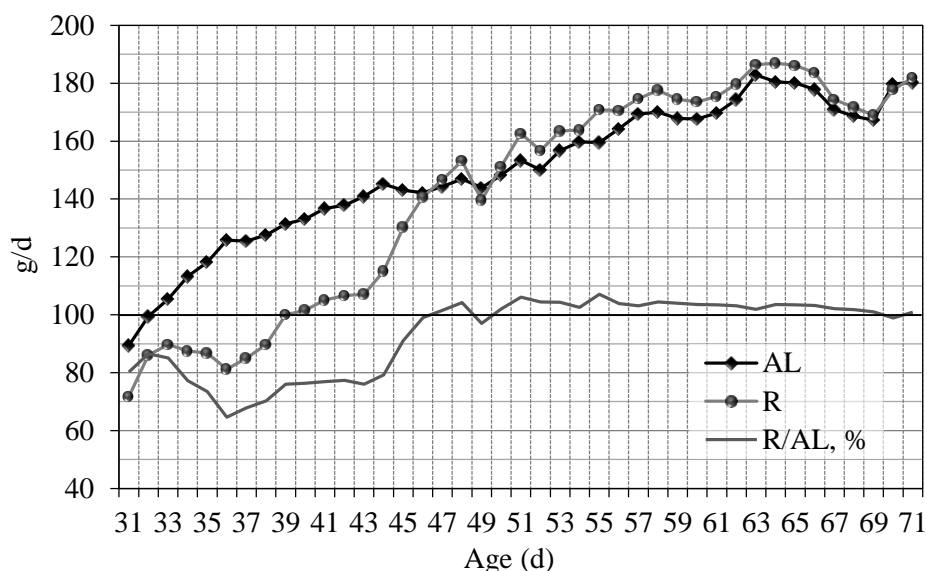


Figure 11 Daily feed intake curve of *ad libitum* (AL) and restricted (R) rabbits and feed restriction level (%) from weaning to the day before slaughter.

In the second week, the feeding level increased (from 71% to 81%), although the access time to feed was maintained constant at 8 h/d, while in the third week (45 to 51 d of age) the feeding level increased rapidly from 90 to 108% of *ad libitum* at increasing feeding times (+1 h/d of access to the feeders). During the fourth week (from 52 to 58 d of age), the R rabbits continued to show a higher feed intake compared to the AL ones (+5% on average) although their access time to the feeders (16–22 h/d) was still lower than that of the control group. The overfeeding behaviour of R rabbits slightly decreased in the fifth week (59 to 65 d of age) with the achievement of the free access to the feed and then disappeared during the last week of trial (66 to 72 d of age).

In the first rearing period, daily weight gain was only slightly lower in R groups compared to AL ones (51.9 g/d vs. 50.2 g/d; $P < 0.10$), whereas feed intake was considerably ($P < 0.001$) reduced (130 g/d vs. 111 g/d for AL and R groups, respectively). Therefore, feed conversion was significantly improved in R rabbits during the restriction phase (-11.6%; $P < 0.001$) that reached live weights similar to AL ones at 52 d of age (Table 17).

In the fattening period, when the re-feeding was completed, R group showed higher daily growth rate compared to AL one (47.2 g/d vs. 48.8 g/d), while feed intake and feed conversion did not differ among groups. Considering the whole trial, no differences in terms of daily weight gain (49.5 g/d on average) and final live weight (2842 g on average) were detected according to the feeding system, while the feed intake was lower for rabbits subjected to the feed restriction program (151 g/d vs. 144 g/d for AL and R groups, respectively; $P < 0.01$). Accordingly, the feed conversion index significantly decreased in restricted rabbits (3.05 vs. 2.93; $P < 0.001$ for groups AL and R, respectively).

Group size did not significantly affect growth performance of rabbits either in the first or in the second rearing period. Only during the final phase, the feed intake tended to be higher in rabbits housed in groups of 8 individuals compared to those kept in pens with 16 or 32 animals ($P<0.10$) (Table 17).

Table 17 Effect of the feeding system and the group size on growth performance from weaning (31 d of age) to the day before slaughter (72 d of age).

	Feeding program (F)		Group size (G)			Probability			RMSE ¹
	AL	R	8	16	32	F	G	F×G	
Number of rabbits	149	140	61	114	114				
Live weight (g)									
At 31 d	814	811	816	810	812				50.6
At 52 d	1904	1865	1900	1880	1874				171
At 72 d	2845	2838	2848	2842	2835				211
Post-weaning period (31 to 52 d)									
Weight gain (g/d)	51.9	50.2	51.7	51.0	50.6	‡			7.49
Feed intake ² (g/d)	130	111	122	120	120	***	‡		2.38
Feed conversion ²	2.51	2.22	2.35	2.36	2.38	***			0.08
Fattening period (53 to 72 d)									
Weight gain (g/d)	47.2	48.8	47.5	48.3	48.2	*			6.30
Feed intake ² (g/d)	172	178	173	174	178				6.45
Feed conversion ²	3.64	3.65	3.64	3.64	3.70				0.13
Whole period (31 to 72 d)									
Weight gain (g/d)	49.5	49.4	49.6	49.6	49.4				4.85
Feed intake ² (g/d)	151	144	147	147	149	**			3.84
Feed conversion ²	3.05	2.93	2.97	2.98	3.02	***			0.06

AL: *ad libitum* rabbits; R: restricted rabbits.

¹Root mean square error.

‡ $P<0.10$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Health status

During the trial a total of 29 animals died, leading to an average mortality rate of 9.1% despite the use of a medicated feed during the first period. However, no additional antibiotic treatment was carried out. All cases of illness and death were caused by digestive diseases characterized by the typical symptoms of epizootic rabbit enteropathy (ERE). Distension of gastro-intestinal tract and caecal paresis with the presence of mucous were observed at the post-mortem examination.

Enteric troubles appeared before in AL groups, starting from 40 d of age, and the peak of morbidity (about 7.5% of ill rabbits) was detected 8–10 d later (Figure 12a). After that, the cases of illness decreased considerably in this group and disappeared 14–15 d later, around 64 d of age. The morbidity evolution was similar in R rabbits, but the disease appeared about one week later than L group and stopped within 20 d (from 47 to 67 d of age). The first cases of death were detected after one week from the occurrence of the disease in L group, when morbidity rate was

reaching its peak (Figure 12b). After few d, some animals died also in R group and the mortality rate increased rapidly during the re-feeding phase up to reach the maximum (11.9%) at 67 d of age. On the other hand, the number of losses in L group slowed around to 56 d of age, achieving a mortality rate equal to 6.3%.

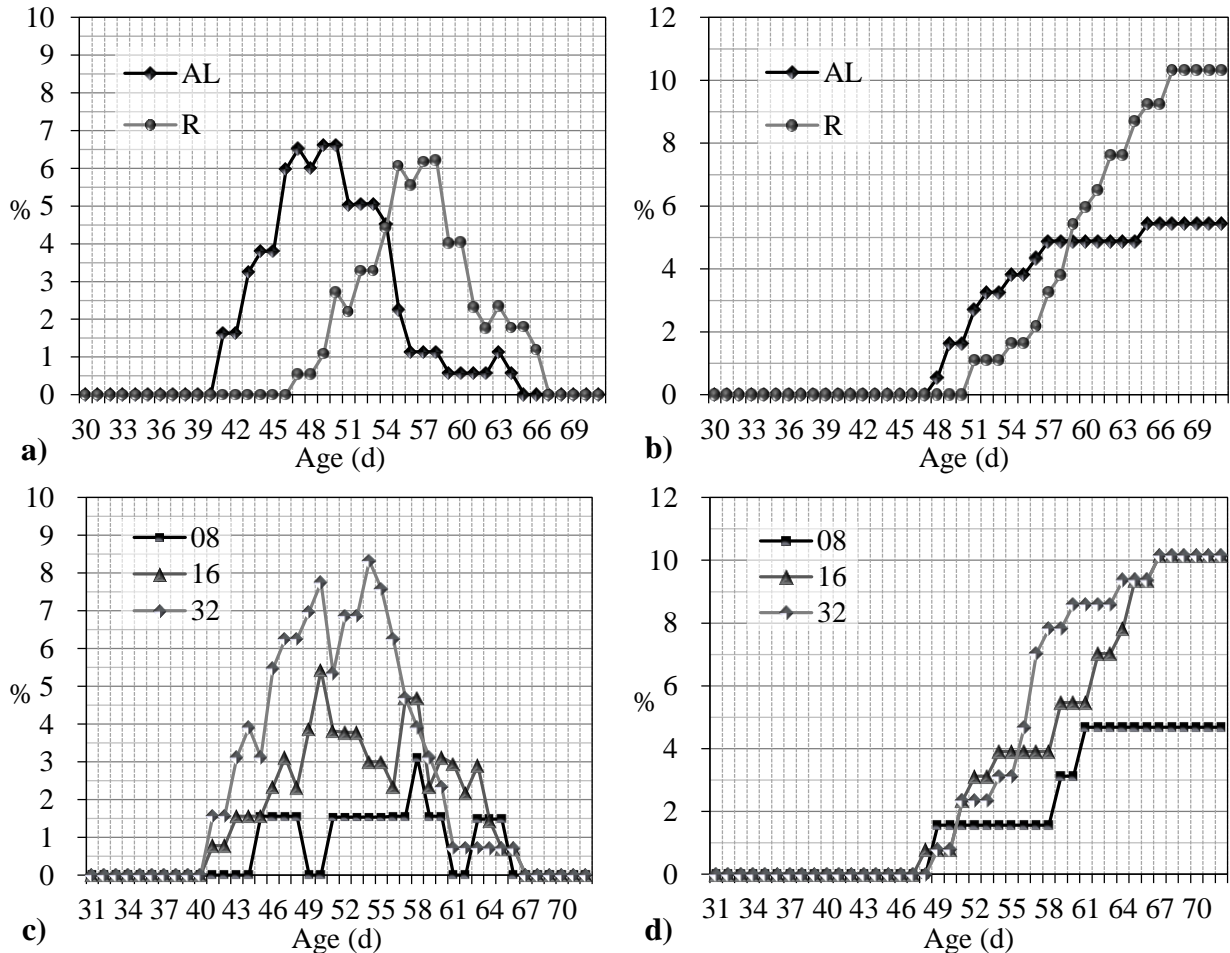


Figure 12 (a) Daily change of morbidity calculated as the proportion of ill rabbits on the reared animals according to the feeding system (AL: *ad libitum* rabbits vs. R: restricted rabbits). (b) Cumulative mortality rate calculated on the initial number of 160 animals for each feeding group. (c) Daily change of morbidity according to the group size (8 vs. 16 vs. 32 animals/pen). (d) Cumulative mortality rate calculated on the initial number of 64 animals for group 8 and 128 animals for groups 16 and groups 32.

The evolutions of morbidity and mortality according to the group size are reported in Figure 12c and Figure 12d respectively. The morbidity reached around 8.5% in large groups (32 rabbits/pen) and 3% for animals kept in small groups (8 rabbits/pen). Similarly, the mortality trend quickly increased in groups composed by 16 or 32 rabbits up to about 10% at the end of trial, while stopped around 5% at 61 d of age in the smallest groups.

Considering the whole trial, mortality tended to be higher in restricted rabbits compared to *ad libitum* ones ($P < 0.10$), whereas no significant differences were detected on morbidity and sanitary risk according to the feeding system (Table 18).

The sanitary risk was significantly ($P < 0.05$) lower in the smaller groups than in the greater ones (6.3% vs. 14.8% and 18.0% for groups with 8, 16 and 32 rabbits/pen, respectively), while the differences on morbidity and mortality rates did not reach the statistical significance.

Table 18 Effect of the feeding system and the group size on the sanitary status from weaning (31 d of age) to slaughter (73 d of age).

	Feeding program (F)		Group size (G)			Probability		
	AL	R	8	16	32	F	G	F×G
Initial number of rabbits	160	160	64	128	128			
Mortality (%)	6.3	11.9	4.7	10.2	10.2	‡		
Morbidity (%)	6.9	3.8	1.6	4.7	7.8			
Sanitary risk (%)	13.1	15.6	6.3	14.8	18.0		*	

AL: *ad libitum* rabbits; R: restricted rabbits.

¹Root mean square error.

[‡] $P < 0.10$; * $P < 0.05$ for the contrast “8 vs. (16+32)”.

Slaughter results, carcass traits and meat quality

Slaughter results were in accordance with the genetic type used and the final live weight achieved by the animals. The cold carcass weight averaged 1676 g, with a slaughter yield of 61.3% and the incidence of *longissimus lumborum* muscles and hind legs on reference carcass (RC) was equal to 12.5% and 33.6% respectively. The incidence of dissectible fat reached 2.56%, while the muscle to bone ratio of hind leg was equal to 5.76 (Table 19).

The *l. lumborum* muscles showed an average pH of 5.60, with a lightness (L^*) of 52.8 and both redness (a^*) and yellowness (b^*) indexes close to zero (-2.01 and 1.27 respectively). The hind legs showed the average pH of 5.81 and colour indexes equal to 49.3, 4.06, and -2.56 for lightness, yellowness and redness, respectively (Table 20). Thawing losses, cooking losses and shear force were of 6.80%, 34.6% and 4.79 g/kg, respectively, in *l. lumborum* muscles, and 2.09%, 28.7% and 2.62 g/kg in the hind legs (Table 20).

Table 19 Effect of the feeding system and the group size on slaughter traits and main characteristics of 24-h chilled carcasses.

	Feeding program (F)		Group size (G)			Probability			RMSE ¹
	AL	R	8	16	32	F	G	F×G	
Number of rabbits	149	140	61	114	114				
Weight at farm, g	2814	2795	2812	2802	2799				207
Slaughter weight (SW), g	2746	2722	2741	2732	2729				199
Transport losses, %	2.42	2.57	2.50	2.48	2.50				1.20
Cold carcass weight (CW), g	1688	1664	1677	1677	1673				134
Dressing percentage, % SW	61.4	61.1	61.1	61.4	61.3				1.70
Head, % CW	7.72	7.64	7.71	7.59	7.75				0.54
Liver, % CW	4.24	4.42	4.15	4.54	4.29				0.68
Thoracic organs, % CW	3.17	3.30	3.18	3.28	3.24	‡			0.39
Reference carcass (RC), g	1446	1431	1436	1442	1437				107
Dissectible fat, % RC	2.47	2.64	2.48	2.52	2.66				0.70
Muscle <i>l. lumborum</i> , % RC	12.6	12.3	12.3	12.6	12.3	*			0.77
Hind legs, % RC	33.7	33.4	33.6	33.3	33.7				0.86
Hind leg muscle/bone ratio	5.79	5.73	5.81	5.69	5.78				0.48

AL: *ad libitum* rabbits; R: restricted rabbits.¹Root mean square error.

‡P<0.10; *P<0.05.

Table 20 Effect of the feeding system and the group size on meat traits of *longissimus lumborum* and hind leg.

	Feeding program (F)		Group size (G)			Probability			RMSE ¹
	AL	R	8	16	32	F	G	F×G	
Number of rabbits	149	140	61	114	114				
<i>Longissimus lumborum</i>									
pH	5.59	5.60	5.58	5.59	5.61				0.09
<i>L</i> [*]	53.1	52.5	52.1	53.4	53.0				2.24
<i>a</i> [*]	-1.97	-2.04	-2.06	-1.98	-1.98				0.57
<i>b</i> [*]	1.27	1.27	1.60	1.35	0.84				1.76
Thawing losses, %	7.02	6.57	6.48	6.68	7.21				2.16
Cooking losses, %	34.4	34.8	34.3	34.6	34.9				1.89
Shear force, kg/g	4.86	4.72	4.94	4.86	4.57				0.90
Hind leg									
pH	5.80	5.82	5.80	5.80	5.83				0.11
<i>L</i> [*]	49.4	49.2	49.3	49.6	49.0				1.66
<i>a</i> [*]	-2.52	-2.59	-2.53	-2.57	-2.56				0.44
<i>b</i> [*]	4.11	4.02	3.94	4.00	4.24				1.36
Thawing losses, %	2.13	2.04	2.23	2.06	1.97				0.78
Cooking losses, %	28.7	28.7	28.6	28.7	28.8				1.29
Shear force, kg/g	2.63	2.60	2.55	2.62	2.68				0.44

AL: *ad libitum* rabbits; R: restricted rabbits; *L*^{*}: lightness; *a*^{*}: redness; *b*^{*}: yellowness.¹Root mean square error.

The feeding system did not produce relevant effects on slaughter traits and meat quality of growing rabbits. Indeed, animals of both experimental groups showed similar slaughter weights, transport losses, carcass weights and dressing percentages. The incidence of thoracic organs tended to be higher in R rabbits compared to AL ones ($P < 0.10$), whereas the incidence of head and liver did not differ among groups. The proportion of *I. lumbrorum* on the reference carcass (RC) was significantly ($P < 0.05$) higher in AL group than R one (12.6% vs. 12.3% RC) (Table 20). Meat pH values and colour indexes of *I. lumbrorum* and hind leg were similar among groups, as well as thawing and cooking losses and shear force (Table 20). The minimum diameter of femur tended to be lower in restricted rabbits compared to those fed *ad libitum* (7.09 vs. 6.95 mm; $P < 0.10$), while other femur characteristics (weight, length, maximum diameter and fracture force) did not differ between AL and R groups (Table 21).

Table 21 Effect of the feeding system and the group size on femur characteristics.

	Feeding program (F)		Group size (G)			Probability			RMSE ¹
	AL	R	8	16	32	F	G	F×G	
Number of rabbits	48	48	32	32	32				
Femur weight, g	15.0	14.7	14.6	15.1	14.8				1.17
Femur length, mm	91.1	90.5	90.8	91.1	90.6				1.91
Maximum diameter, mm	8.87	8.71	8.67	8.76	8.95				0.77
Minimum diameter, mm	7.09	6.95	7.05	7.01	7.02	‡			
Femur fracture force, kg	41.5	42.6	40.6	42.8	42.8				6.13

AL: *ad libitum* rabbits; R: restricted rabbits.

¹Root mean square error. ‡ $P < 0.10$.

Also the group size scarcely affected slaughter results and meat quality. In fact, the only significant effect of the group size concerned the lightness index that resulted lower in small groups (52.1 vs. 53.4 and 53.0 for groups 8, 16 and 32 respectively; $P < 0.05$).

Discussion

Effect of the feeding system

The feed restriction level achieved during the first rearing period (85% of *ad libitum*) was in accordance with what expected, confirming that the progressive feed restriction curve permitted to control the feed intake of restricted rabbits. In particular, the gradual reduction in the access time to the feeders in the first week was effective in avoiding to surprise animals at the beginning of rationing program and led to a progressive reduction of the intake level. During the second week, the feeding level raised by about 1.3% per day, confirming the capability of rabbits to quickly adapt their feeding behaviour to the time-based feed restriction, especially when the diet was supplied during the dark period. In fact, the increase in the intake level during this phase was

the consequence of the acceleration of feeding rhythm (i.e. to ingest a great amount of feed in a short time) of rabbits subjected to a feed restriction, as described in previous studies (Martignon *et al.*, 2009; second contribute of this thesis). Accordingly, at 46 d of age (third week of trial) with only 10 h/d of access to the feeders, the restricted rabbits reached the same feed intake of the *ad libitum* ones and after that, they showed a slight over-feeding behaviour (+4% on average) until the last week of trial. Foubert *et al.* (2007) observed even more important increments of feed intake (about 8–10%) with respect to our results when rabbits changed from a limited access time to feed (6, 8 or 10 h/d) in the first three weeks after weaning to a regime of free feeding in the last two weeks of fattening. Knudsen *et al.* (2014) reported similar results by applying a feed restriction equal to 75% of *ad libitum* in post-weaning period. However, the over-eating behaviour of previously restricted rabbits during re-feeding phase could negatively affect the digestive health of the animals with a consequent higher sanitary risk during fattening. In our trial the gradual increase (+1 h/d) of the access time to feed was programmed in order to avoid abrupt changes on the intake level. However from 48 to 53 d of age the intake curve of R group showed several fluctuation, therefore suggesting that the extension of 1 h/d could be excessive for realizing a safe progressive re-feeding phase.

The losses in terms of daily weight gain was partially recovered during the re-feeding phase when rabbits previously restricted showed higher feed intake and daily growth rates compared to those of AL rabbits, improving significantly the feed efficiency and decreasing the negative impact on final live weight (Tudela *et al.*, 2008; Gidenne *et al.*, 2009a). Nevertheless, this latter result depended also on the level of feed restriction and on the duration of rationing and re-feeding phases (Birolo *et al.*, 2013). In the present trial, reducing the intake level by 15% in the first three weeks of trial the daily weight gain decreased by only 3.3% (-0.11 g/d per each percentage point of feed intake reduction), therefore the feed restriction program led to a relevant improvement of feed conversion (-12%) during restriction phase, in agreement with the findings by other authors (Szendrő *et al.*, 2008; Knudsen *et al.*, 2014). During the re-feeding phase, R animals achieved higher daily growth rate in comparison to AL ones (+3.4%; $P < 0.05$) without affecting feed conversion. In fact, the positive effect on digestive efficiency observed during the restriction period usually disappeared when rabbits returned to an *ad libitum* intake (Tumova *et al.*, 2007; Knudsen *et al.*, 2014; first contribute of this thesis) and the compensatory growth was mainly related to the increase of feed intake and the enlargement of internal organs (Gidenne *et al.*, 2012a; Knudsen *et al.*, 2014). However, in our study, considering the whole trial, the feed restriction program led to a significantly decrease of feed intake (-4.6%; $P < 0.01$) and feed conversion (-4%; $P < 0.001$) as reviewed by Gidenne *et al.* (2012a), without affecting the final live weight of the animals. In this

way, the margin of feed costs was clearly improved (Gidenne *et al.*, 2012a). On the other hand, Romero *et al.* (2010) stated that feed restriction was not an advantageous technique from the economic viewpoint when rabbits were slaughtered at lighter live weights as required by the Spanish market.

In our trial, a preventive antibiotic treatment was administered in the diet during post-weaning period because the primary objective of this work was to test the effect of feed restriction on growth performance and slaughter traits. Nonetheless, the drug was not effective in preserving animal gut health and digestive troubles appeared just after 9–10 d from the beginning of the trial in the AL group. By contrast, the restricted rabbits were not affected by the enteric disease until the end of restriction phase. However the morbidity rate rapidly increased in this group immediately after the re-feeding started. In this sense our findings were in agreement with the literature since the protective role of feed rationing (<80% of *ad libitum*) on the digestive health was observed during the restriction phase, but it disappeared when rabbits returned to a free intake regime (Gidenne *et al.*, 2012a). Moreover it is necessary to underline that mortality tended to be higher in restricted groups and the worsening of sanitary conditions during fattening could reduce the global farm efficiency by increasing the economic impact of animal losses.

Reducing feed intake by 15 to 40% of *ad libitum* in the post-weaning period, a decrease of about 2 percentage points in slaughter yield was generally expected (Xiccato, 1999; Gidenne *et al.*, 2012a). This result was mainly related to the higher incidence of the digestive tract in rabbits subjected to a feed restriction due to the sharp development and repletion of gut that occurs during re-feeding phase. Moreover, also the carcass fatness was often reduced by applying feed restriction strategies (Gidenne *et al.*, 2012a). In the present study, the restricted rabbits reached the same slaughter weight of the AL ones. Accordingly, cold carcass weight, slaughter yield and the main carcass characteristics did not differ between R and AL groups, except for the lower loin proportion observed in the restricted rabbits. Similar results were reported by Tumova *et al.* (2006) by limiting the feed intake of the animals from 56 to 63 d of age or during three weeks after weaning. The progressive feature of our feed restriction program and the moderate rationing level can explain the absence of significant effects on slaughter results. Furthermore, the scarce effect of feed rationing on carcass and meat quality of growing rabbits was confirmed (Xiccato, 1999; Dalle Zotte *et al.*, 2005; Tumova *et al.*, 2006).

The minimum diameter of femur tended to decrease in the restricted rabbits compared to the AL ones, in accordance with our previous findings (second contribute of this thesis). Indeed, the skeletal development of recently weaned rabbits could be slowed when a feed restriction is applied

because the reduction in nutrients availability could impaired mainly the tissues characterised by early deposition (Gidenne *et al.*, 2012a; second contribute of this thesis).

Effect of the group size

In literature, group–housing systems for growing rabbits were compared to the classical rearing conditions (small groups of 2–6 animals per cage) in order to evaluate the animal response in terms of behavioural repertoire, growth performance, slaughter traits and meat quality (Rommers and Meijerhof, 1998; Dal Bosco *et al.*, 2002). In the present trial, three group sizes within a pen–rearing system were tested. The increase of animal group size was obtained by joining pens with the same dimensions. Increasing the number of rabbits from 8 (as the group size representing a single litter) to 16 or 32 animals per pen, and keeping stable the stocking density (16 animals/m²), growth performance, slaughter results and meat quality were scarcely modified. Similarly, Rommers and Meijerhof (1998), increasing the group size from 6 to 54 animals per cage, with a stocking density of 17 animals/m², did not observe significant effects on growth performance. Szendrő *et al.* (2009) obtained final live weights and daily growth rates similar among groups in the presence of group sizes ranging from 2 to 26 animals per cage/pen at two stocking densities (12 vs. 16 animals/m²). On the other hand, the same authors observed lower perirenal fat in rabbits housed in large groups, while meat quality was not affected. Martens and Van Herck (2000) reported that daily weight gain and final live weight decreased (-8% and -4% respectively; P<0.001) in pen–housed rabbits (30 animals per pen, stocking density of 15.8 rabbits/m²) in comparison with animals kept in standard fattening cages (4 animals per cage, stocking density of 15.4 rabbits/m²), while feed efficiency did not change (the reduction of daily growth corresponded to a proportional reduction in feed intake). Different results among studies can be partially explained by different types of pen and cage used.

In our study the increase of group size from 8 to 16 or 32 animals led to a higher sanitary risk due to the occurrence of digestive disorders. This could be related to the contagious enteric diseases that can be more easily spread with increasing group size (Martens and Van Herck, 2000; Dal Bosco *et al.*, 2002).

Conclusions

In our study, the application of a feed restriction program characterized by three main steps on the feeding time management (*i*) progressive reduction during the first week (14 to 8 h/d); *ii*) constant phase in the second week (8 h/d); *iii*) progressive increase (+1 h/d) during the third and fourth week) allowed to obtain the expected restriction level in the first trial period (85% of *ad libitum*), limiting the surprise effect in the rabbits at the beginning of feed restriction and the abrupt

changes on feed intake during re-feeding period. The rationing program improved feed efficiency, without reducing final live weight and slaughter yield and without impairing carcass and meat quality. However, no improvement on gut health was achieved since the protective role of feed rationing was limited to the restriction phase and digestive troubles rapidly spread during re-feeding phase, therefore increasing mortality rate. On the basis of these considerations, the management of re-feeding phase deserves further investigations.

The increase in group size from 8 to 16 or 32 animals per pen, by maintaining the same stocking density (16 animals/m²), did not affect growth performance, slaughter results, and meat quality but impaired sanitary conditions.

Fourth contribution:
Effect of different rationing and re-feeding programs on growth performance, digestive physiology, health status, slaughter traits and meat quality in group-housed rabbits

Introduction

Over the last decades the feed conversion of intensive rabbit farms has been greatly enhanced through progresses in genetics, sanitary conditions, management and feeding techniques but further improvements are required in view of guaranteeing an economic sustainability to farmers (Gidenne *et al.*, 2013c; Cartuche *et al.*, 2014). Recently, the main topics of researches in rabbit nutrition focused on the role of feeding strategies to preserve gut health (Gidenne and Garcia, 2006; Gidenne *et al.*, 2010) and enhance farm feed efficiency (Maertens, 2009). However, an effective protection against the emergence and spread of enteric diseases such as Epizootic Rabbit Enteropathy (ERE), which is the most common cause of death in growing rabbits (Licois *et al.*, 2005; Dewrée *et al.*, 2007), still appears difficult to be achieved without the use of antibiotics. Indeed, during weaning and post-weaning young rabbits are subjected to important changes of feeding and digestive physiology including variations on caecal microflora, fermentation patterns, and gut enzymatic activity (Gidenne and Fortun-Lamothe, 2002; Gallois *et al.*, 2008). In this context of precarious balance, the nutritional strategy obviously plays a key role. Among feeding strategies, the application of quantitative rationing programs during the post-weaning period has been widespread over the last years, since that a feed restriction of about 80% of *ad libitum* can protect young rabbits by digestive disorders and improve feed efficiency (Gidenne *et al.*, 2012a). However, during the re-feeding phase, with the return to the voluntary intake, the animals health status could impair due to the occurrence digestive troubles, therefore increasing the mortality during fattening period (Birolo *et al.*, 2013). Furthermore, an extension of the fattening period may be required to allow the complete recover of the animals in terms of live weight and avoid a substantial decrease of slaughter yield (Perrier, 1998; Xiccato, 1999). In this way, the economic margin obtained with the enhancement of feed conversion can be reduced (Knudsen *et al.*, 2014). In addition, several aspects concerning the most efficient method to apply feed restriction and to realize the re-feeding phase need to be already defined.

The findings described in the previous contributes of this thesis confirm that the management of the re-feeding phase is probably the most critical point of rationing programs, not only for the

possible worsening of sanitary conditions, but also for the impairment of feed efficiency with respect to the restriction phase.

Therefore, the present trial aimed at evaluating the effect of feeding system (*ad libitum* vs. feed restriction) and restriction method (diurnal vs. nocturnal access to feed) on growth performance, digestive physiology, health status, slaughter traits and carcass and meat quality of 400 growing rabbits housed from weaning (28 d of age) to slaughter (70 d of age) in collective pens. Moreover, the effect of re-feeding speed (fast vs. slow) and level (complete vs. incomplete) was also assessed.

Materials and methods

Animals and experimental conditions

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

The trial was realized at the rabbit farm of the University of Padova, in a close shield during the months of February and April under a natural photoperiod. Extraction fans and automatic heating system were used to control air circulation and to maintain the temperature in a range of 20–23 °C.

A total of 400 rabbits (Hypharm, Groupe Grimaud, Roussay, France), including both sexes, were selected in a commercial farm from multiparous does (≥ 3 kindling) by healthy litters homogenous in size and moved to the rabbit farm of the University. The animals were housed in 40 open-top pens (0.64×0.78 m, i.e. 0.5 m²). The stocking density ranged from 20 animals/m² at the beginning of trial to 16 animals/m² in the last week. The animals were monitored from weaning (28 d of age) to commercial slaughter (70 d).

Rabbits were randomly allocated to five experimental groups balanced by live weight and variability. In details the following groups were organized:

- AL fed *ad libitum* for the whole trial period (8 replicates);
- DF diurnal access to feed with fast and complete re-feeding (8 replicates);
- NF nocturnal access to feed with fast and complete re-feeding (8 replicates);
- NS nocturnal access to feed with slow and complete re-feeding (8 replicates);
- NI nocturnal access to feed with very slow and incomplete re-feeding (8 replicates).

Diets and feeding programs

All animals had *ad libitum* access to fresh water through nipple drinkers. During the first period (from weaning until 49 d of age), all rabbits were fed with a post-weaning diet supplemented with antibiotics (Oxitetraclín, 1,450 mg a.p./kg) and coccidiostatic (Diclazuril, 1 mg a.p./kg). In the second period (from 53 d until slaughter), a fattening diet without antibiotics and coccidiostatics was provided.

Both experimental diets were pelleted with 3.5 mm of diameter and 10–11 mm of length and their ingredients and chemical composition are listed in Table 22.

Table 22 Ingredients and chemical composition (g/kg) of the experimental diets.

	Diets	
	P	F
<i>Ingredients</i>		
Dehydrated alfalfa meal 160 g/kg CP	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	2.5
Sodium chloride	4.0	4.0
L-lysine HCl	1.0	1.0
DL-methionine	1.0	1.0
Vitamin-mineral premix*	4.0	4.0
Coccidiostat (Diclazuril)	0.5	–
<i>Chemical composition</i>		
Dry matter	902	902
Crude protein	159	160
Ether extract	32.4	38.2
Crude fibre	169	162
Ash	78.3	72.6
TDF	427	412
aNDF	376	366
ADF	210	196
Lignin (sa)	59.3	53.0
Soluble fibre (TDF–aNDF corrected for protein and ash)	101	90
Starch	107	132
Gross energy, MJ/kg	16.5	16.6

*Supplementation per kg of feed: vit. A, 12000 UI; vit. D3, 1000 UI; vit. E acetate, 50 mg; vit. K₃, 2 mg; Biotin, 0.1 mg; Thiamine, 2 mg; Riboflavin, 4 mg; vit. B₆, 2 mg; vit. 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.

The diets were formulated to satisfy the requirements of rabbits during weaning and post-weaning according to de Blas and Mateos (2010) and were supplemented with synthetic lysine and methionine, vitamins, macro- and micro-minerals.

Feed restriction was realized varying progressively the access time to the feeders according to the programs shown in Figure 13.

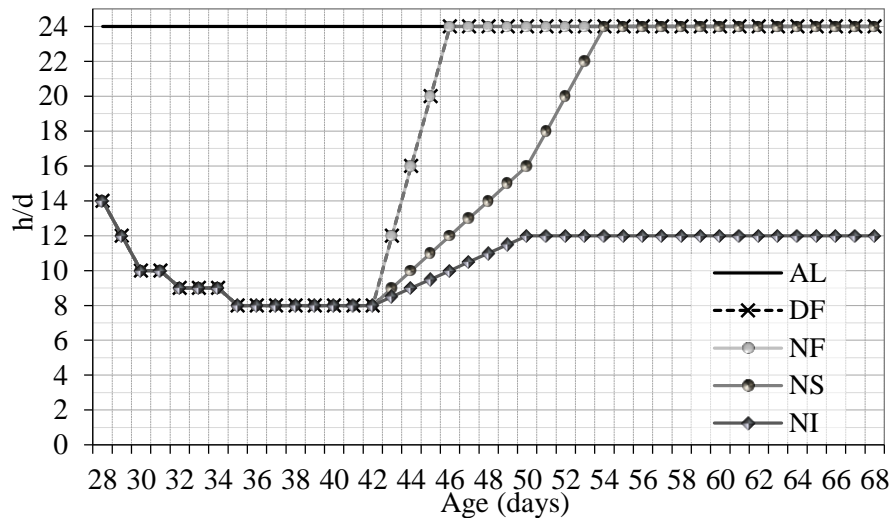


Figure 13 Daily access time to the feeders according to the feeding program (AL: *ad libitum*; DF: diurnal access to feed followed by fast and complete re-feeding; NF: nocturnal access to feed followed by fast and complete re-feeding; NS: nocturnal access to feed followed by slow and complete re-feeding; NI: nocturnal access to feed followed by incomplete re-feeding) in rabbits housed in collective pens.

In details, a diurnal rationing program (DF group with access to the feeders allowed during daylight period) was compared with a nocturnal rationing program (NF, NS and NI groups, with access to the feeders during dark period); within nocturnal rationing programs, different re-feeding strategies were compared among them i.e. fast (NF group *vs.* slow (NS group) *vs.* incomplete (NI group)). In details the feeding time for restricted rabbits decreased (from 14 to 9 h/day) during the first week of trial (from 28 to 34 d of age), remained stable (8 h/d) during the second week (from 35 to 41 d of age) and increased from the beginning of the third week (43 d of age) according to three re-feeding curves: the first one was a fast (+4 h/d) and complete (until 24 h/d of access to feed) re-feeding curve applied to both diurnal and nocturnal groups (DF and NF groups), the second one was a slow (+1/+2 h/d) and complete (until 24 h/d of access to feed) re-feeding curve (NS group) and the third one was a very slow (+30 min/d) and incomplete (until 12 h/d of access to the feeders) re-feeding curve (NI group). In this way DF and NF groups reached the free access to feed at 46 d of age, the NS group at 54 d, while the NI group achieved the 12 h/d of access to feed at 50 d of age onward. The feeding programs were implemented before by extending and then by shortening the time of fasting in the daylight period (nocturnal programs) or reversely in the dark period (diurnal program). The feed restriction was carried out by manually closing the feeders at the two times.

Growth performance and health status

During the trial individual live weight was recorded three times a week. Cage feed intake was recorded daily from the housing of rabbits until the commercial slaughter. The live weight at 69 d of age was considered as the final live weight and used to calculate growth performance in the second and in the whole rearing period. The feeders were weighed every day at h09:00. Health status was controlled daily to detect any clinical signs of digestive diseases. Rabbits were considered ill in the presence of diarrhoea and mucus or a clear reduction of growth (weight loss during 2–3 consecutive weighing). Ill rabbits were considered only once for calculating morbidity while death animals were taken into account only for determining mortality rate. The sanitary risk was expressed as the sum of morbidity and mortality (Bennegadi *et al.*, 2000).

The health status was good during the main part of the trial, but strongly worsened during the last week, during which 24 animals died; in details 1 from group AL, 2 from group DF, 8 from group NF; 7 from group NS and 6 from group NI. Moreover, 90 animals were discarded from the commercial slaughter because of inadequate live weight (<2.2 kg) or severely ill; in details 17 from group AL, 22 from group DF, 14 from group NF, 20 from group NS and 17 from group NI. A large part of the discarded animals did not reach the expected commercial weight due to the need to anticipate the slaughter because of the worsening of health conditions and the impossibility to make any antibiotic treatment close to slaughtering.

Digestibility trials

Faecal apparent digestibility coefficients of dry matter (DM) and other nutrients were measured during two *in vivo* digestibility assays performed in rabbits housed in collective pens adapting the European standardised method (Perez *et al.*, 1995). The first trial was carried out on 12 pens (4 from the AL group, 4 from the DF group and 4 from the NF group) from 36 to 40 d of age when rabbits fed diet P. The second trial was performed on 16 pens (4 from the AL group, 4 from the NF group, 4 from the NS group and 4 from the group NI) from 60 to 64 d of age when rabbits fed diet F. The pens were equipped with a wire net (2 mm mesh) under the floor in order to collect the entire daily quantity of hard faeces excreted by rabbits.

Sampling of caecal content

At 43 d, when the restricted groups had 8 h/d of access to the feeders, 24 rabbits (8 from group AL, 8 from group DF and 8 from group NF, 1 rabbit per pen), representative of the corresponding experimental groups in terms of average live weight and variability, were slaughtered to sample caecal content. The animals were slaughtered in two moments: 8 rabbits belonging to the group NF and 4 rabbits belonging to the group AL in the morning (between h07:00 and h08:00); and 8

rabbits belonging to the group DF and 4 rabbits belonging to the group AL in the evening (between h19:00 and h20:00 pm). In this way the caecal content was sampled one hour after the closure of feeders for both restricted groups. AL rabbits were divided in two groups and slaughtered just after the restricted animals. The rabbits were weighed immediately before slaughter and killed by cervical dislocation. Stomach and gut were removed and weighed. The caecum was removed and weighed and the pH of the caecal content was immediately measured. The caecal content was then diluted with a 15% HPO₃ solution (25% wt/wt), and stored at -20°C until chemical analyses (Trocino *et al.*, 2011). In the groups not subjected to the sampling, one rabbit per pen was removed and excluded from the trial in order to maintain the same number (9 rabbits/pen) and stocking density in all experimental groups.

At 51 d of age, when NF, NS and NI groups reached respectively 24, 18 and 12 h/d of access to the feeders, other 32 rabbits (8 per group and for groups AL, NF, NS and NI), representative of the corresponding experimental groups in terms of average live weight and variability, were slaughtered to sample caecal content according to the procedure described above. The rabbits of NI group were slaughtered between h08:00 and h08:30 (one hour after the closure of the feeders); AL and NF rabbits were slaughtered between h08:30 and h09:30 am; NS rabbits were slaughtered between h10:00 and h10:30 am (one hour after the closure of the feeders). In the groups not subjected to sampling, one rabbit per pen was removed and excluded from the trial in order to reach the same final number (8 rabbits/pen) and stocking density (16 animals/m²) in all experimental groups.

Commercial slaughters and carcass and meat quality recordings

At 70 d of age, all healthy rabbits were weighed at the experimental farm and the rabbits heavier than 2.2 kg (206 animals: 46 from group AL; 40 from group DF, 42 from group NF; 37 from group NS and 41 from group NI) were then transported to a commercial slaughterhouse in an authorized truck (about one hour of transport). The slaughtering took place after 8 hours of fasting. At the slaughterhouse, the rabbits were individually weighed, stunned by electro-anaesthesia and killed by jugulating. After 2.5-h chilling, the commercial carcasses were weighed to measure the individual dressing out percentages. The carcasses of 160 rabbits (32 per experimental group that were representative in terms of average weight and variability) were transported to the departmental laboratory and stored at 3–4°C for 24 h. The chilled carcasses were then dissected according to harmonised methods (Blasco *et al.*, 1993).

The pH of the *longissimus lumborum* and *biceps femoris* 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). The L*a*b* colour indexes (Commission International de

l'Eclairage, 1976) were measured in duplicate in the same muscles using a Minolta CM-508 C spectrophotometer (Minolta Corp., Ramsey, NJ, USA). The hind legs and *l. lumborum* muscles were separated, and the meat of the right hind leg was dissected from the bones to measure the meat-to-bone ratio (Blasco and Ouhayoun, 1996). The *l. lumborum* muscles and the left hind legs were stored under vacuum in plastic bags at -18°C until the meat analyses. At this moment, thawing and cooking losses were measured. After thawing, the *l. lumborum* were kept in plastic bags and cooked in a water bath for 1.0 h until an internal temperature of 80°C was achieved. After a 1-h cooling period, the *l. lumborum* was cut to separate the middle part (length: 70 mm). On this section, the maximum shear force was measured with a TA.HDI dynamometer (LS5, Lloyd Instruments Ltd, Bognor Regis, UK) using the Allo-Kramer (10 blades) probe (load cell: 500 kg; distance between the blades: 5 mm; thickness: 2 mm; cutting speed: 500 mm/min) according to the methods of Bianchi *et al.* (2007). Also the left hind legs were thawed, kept in plastic bags and cooked in a water bath for 2.5 h, until reaching an internal temperature of 80°C . After 1-h cooling period, the hind legs were sectioned into three portions by separating the regions of the tibia and the hip from that of the femur. From the femur region (middle part) the bone was extracted without damaging the muscles of the thigh. On this section, the maximum shear force was measured with the same method described for the *l. lumborum* muscles.

Chemical analyses

Diets and faeces were analysed to determine the concentrations of dry matter (934.01), ash (967.05), crude protein (2001.11), and starch (amyloglucosidase α amylase method, 996.11) using AOAC (2000) methods following harmonised procedures (Gidenne *et al.*, 2001b). The ether extract was analysed after acid hydrolysis (EC, 1998). The fibre fractions, i.e. aNDF (neutral detergent fibre without sodium sulphite), ADF (acid detergent fibre), and lignin (sa), 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). The total dietary fibre (TDF) was determined with a gravimetric enzymatic procedure with α -amylase, protease, and amyloglucosidase treatments (Megazyme Int. Ireland Ltd., Wicklow, Ireland) (Method AOAC 991.43). The soluble fibre was calculated by subtracting aNDF from TDF (Van Soest *et al.*, 1991; Xiccato *et al.*, 2012). The gross energy was measured with an adiabatic bomb calorimeter.

The thawed samples of caecal content were centrifuged at 9000 rpm for 10 min. Caecal N ammonia was determined on the supernatant with a pH meter (Crison GLP 22) equipped with ammonia specific electrode (mod. 9663 combined with the reference electrode mod. 5044) (Crison Instruments S.A., Riera Principal 34, Barcelona, Spain). Volatile fatty acids (VFAs) contents was

measured on the supernatant with gas chromatography (Agilent 7820A, equipped with flame ionization detector FID, Split–splitless injection system, programmable oven) on a cross bond capillary column DB–FFAP (30 m x 0.25 mm I.D., 0.25 µm film thickness) (Agilent J&W, USA). The injector and the detector temperatures were set at 250°C. The program provided an initial oven temperature of 100°C, maintained for 3 min, increased of 10°C per min until 200°C, maintained for 0 min. Hydrogen was used as carrier with a flow of 1.20 ml/min and linear speed of 38.47 cm/s at 180 °C.

Statistical analyses

The individual data of live weight, daily growth, caecal content, slaughter traits and carcass and meat quality were analysed by a two–way ANOVA using the PROC MIXED of SAS (Statistical Analysis System, 2013). The model included the experimental group as fixed effect with the pen as a random effect. The cage data of feed intake, feed conversion and digestibility coefficients were analysed by a two–way ANOVA using the PROC GLM of SAS. The model included the experimental group as fixed effect. Mortality, morbidity, sanitary risk were analysed by the PROC GENMODE of SAS (Statistical Analysis System, 2013). Least–square means were chosen for presenting all data. To test the effect of: *i*) feeding system (*ad libitum* vs. restricted); *ii*) restriction method (diurnal vs. nocturnal); *iii*) re–feeding speed (fast vs. slow); *iv*) re–feeding level (complete vs. incomplete) different contrasts were computed as follows:

$$i) \text{ AL vs. Restricted feeding} = \text{AL} - \left[\frac{\text{DF} + \text{NF} + \text{NS} + \text{NI}}{4} \right]$$

$$ii) \text{ Diurnal vs. Nocturnal access to feed} = \text{DF} - \text{NF}$$

$$iii) \text{ Fast vs. Slow re– feeding} = \text{NF} - \text{NS}$$

$$iv) \text{ Complete vs. Incomplete re– feeding} = \frac{[\text{NF} + \text{NS}]}{2} - \text{NI}$$

Differences among means with $P < 0.05$ were accepted as representing statistically significant differences. Differences among means with $0.05 < P < 0.10$ were accepted as approaching statistical significance.

Results

Growth performance and health status

The daily feeding curves of the experimental groups and the corresponding feed restriction levels (AL = 100%) are reported in Figure 14a and Figure 14b. During the first two weeks of trial (from 28 to 42 d of age), when the access time to the feeders was firstly decreased from 14 to 9 h/d (first week) and then kept stable at 8 h/d (second week), the feeding level of nocturnal rationing programs was reduced at 79% of AL, while a stricter feed restriction was achieved with a diurnal

rationing (74% of AL). At 43 d of age, that is the first day of re-feeding, DF and NF rabbits showed a great peak of feed intake (142 and 137 g/d respectively) overcoming the feeding level of AL group (+11% and +7% respectively) although the access time to the feeders was increased from 8 to 12 h/d. In the following two d, both groups subjected to the fast re-feeding (+4 h/d of access to feed) exhibited other relevant fluctuations of feed intake, firstly with a reduction to intake levels equal to 100% (DF group) and 89% (NF group) of AL and then with new peaks on feed ingestion (+14% and +10% of AL). After that, an overfeeding behaviour was detected in these groups (+8% on average) until the end of the trial.

By contrast, the application of a slow re-feeding curve (+1/d on 4th week and +2 h/d on 5th week) allowed a more progressive transition from the restriction to the free feeding phases. Indeed, NS rabbits reached the AL feeding level at 47 day of age with an access time to the feeders of only 13 h/d. The gradual increase of feed intake was evident also in NI rabbits, that were submitted to a very slow (+30 min/d) and incomplete re-feeding (12 h/d as a maximum access time). Furthermore, this rationing program allowed to avoid high intake peaks and minimize the typical overfeeding behaviour of restricted rabbits when return to the voluntary 24-h intake (Figure 14).

Growth performances were significantly ($P<0.001$) affected by the feeding system in the first three weeks of trial. In particular, AL rabbits exhibited higher daily growth (52.8 g/d vs. 49.3 g/d) and feed intake (112 g/d vs. 95 g/d) compared to restricted animals (Table 23). Accordingly, the AL rabbits reached greater live weight at 49 d of age in comparison to the restricted ones (1761 vs. 1672 g). Nevertheless, the reduction on daily growth (-6.6%) was not proportional to the decrease on feed intake (-14.2%), therefore feed conversion improved in restricted rabbits (2.11 vs. 1.95). In the same period, among the restricted groups, NF rabbits achieved a higher daily weight gain compared to DF animals and a better feed conversion ($P<0.05$). The lowering of re-feeding speed led to lower feed intake (98 g/d vs. 93 g/d for NF and NS groups, respectively) without significant differences on daily growth and feed efficiency. Furthermore, rabbits subjected to an incomplete re-feeding exhibited lower feed intake (-3.7%) and feed conversion (-3.9%) compared to rabbits subjected to a complete re-feeding (NF+NS vs. NI groups).

In the second rearing period, daily weight gain (47.1 g/d vs. 51.1 g/d) and feed intake (157 g/d vs. 165 g/d) were lower for AL group in comparison to restricted ones ($P<0.001$). In this period the restriction method did not affect growth performance, while the effect of the re-feeding technique was relevant. In details, NF rabbits showed higher growth rate and feed intake compared to NS rabbits ($0.05<P<0.01$). Furthermore, the incomplete re-feeding determined lower feed intake (167 g/d vs. 159 g/d; $P<0.001$) and feed conversion ratio (3.26 vs. 3.14; $P<0.01$) with respect to the complete re-feeding curves.

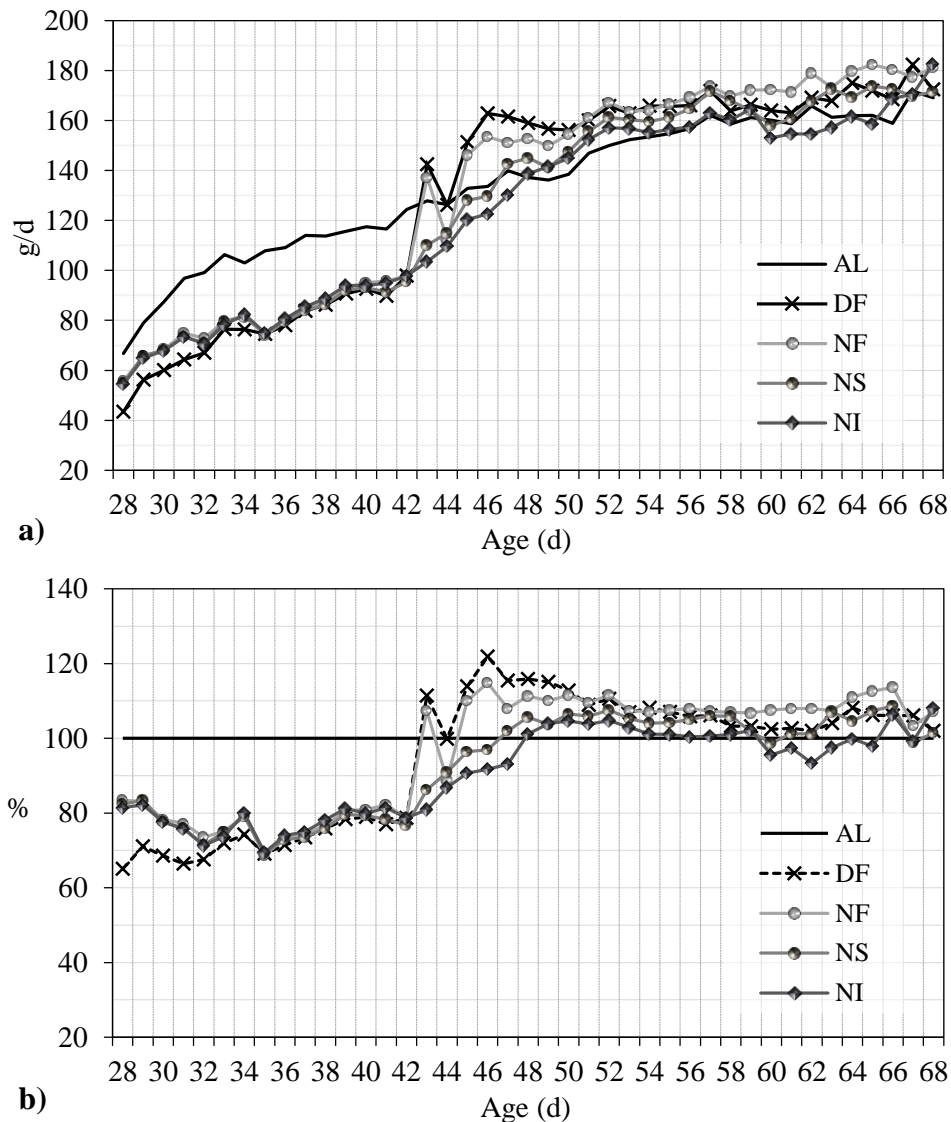


Figure 14 Daily feeding curves (a) and feed restriction levels (b) detected during the trial according to the feeding program (AL: *ad libitum*; DF: diurnal access to feed followed by fast and complete re-feeding; NF: nocturnal access to feed followed by fast and complete re-feeding; NS: nocturnal access to feed followed by slow and complete re-feeding; NI: nocturnal access to feed followed by incomplete re-feeding) in rabbits housed in collective pens.

Considering the whole trial period, the rationing programs significantly reduced feed intake (134 g/d vs. 129 g/d; $P < 0.001$) and feed conversion (2.67 vs. 2.59; $P < 0.05$) compared to the *ad libitum* system, without affecting daily growth (50.1 g/d on average) and final live weight (2696 g on average). Productive performance did not change according to the restriction method. A fast re-feeding curve increased feed intake (+5 g/d) and daily growth (+2.1 g/d) rates in comparison to a slow curve, leading to higher final live weights (+3%; $P < 0.10$). The NI rabbits exhibited the lowest intake and the best feed conversion, reaching final live weights similar to those of rabbits subjected to complete re-feeding (Table 23).

During the first four weeks of trial a good health status was detected in all experimental groups. However, in the last two weeks a severe enteric disease, characterized by the typical

symptoms of epizootic rabbit enteropathy (ERE), appeared and rapidly spread out in all pens. The illness led to an average mortality equal to 7.5%, whereas morbidity and sanitary risk reached 27.8% and 35.3% respectively (Table 24).

The administration of antibiotics in the water was not possible because of the proximity of slaughtering. For this reason, the rate of total animal losses (dead + discarded by the commercial slaughter) averaged 35.6%. Mortality was significantly lower in AL group compared to R ones (1.6% *vs.* 9.0%; $P < 0.05$). Among the R groups, the nocturnal feeding showed a higher mortality with respect to the diurnal feeding (3.1% *vs.* 12.5% for DF and NF groups, respectively; $P < 0.05$), while the re-feeding technique did not modify the incidence of dead animals. Morbidity and sanitary risk did not differ among groups (Table 24).

Table 23 Effect of different feeding programs on performance from weaning (28 d of age) to the day before slaughter (69 d of age) in growing rabbits housed in collective pens.

Feeding system [†]	AL	Diurnal R	Nocturnal R			Probability of the contrasts				RMSE ¹
			Fast	Fast	Slow	Incomplete	AL vs. R	DF vs. NF	NF vs. NS	
Re-feeding technique [§]	–	Fast	Fast	Slow	Incomplete					
Group	AL	DF	NF	NS	NI	AL vs. R	DF vs. NF	NF vs. NS	(NF+NS) vs. NI	
Number of rabbits	46	40	42	37	41					
Live weight (g)										
At 28 d	650	633	634	638	638					66.2
At 49 d	1761	1647	1696	1667	1677	***				140
At 69 d	2704	2699	2736	2654	2685			‡		185
Post-weaning period (28 to 49 d)										
Weight gain (g/d)	52.8	48.2	50.5	48.9	49.4	***	*			4.67
Feed intake [£] (g/d)	112	97	98	93	92	***		**	**	2.80
Feed conversion [£]	2.11	2.04	1.93	1.96	1.87	***	*		‡	0.10
Fattening period (50 to 69 d)										
Weight gain (g/d)	47.1	52.6	52.0	49.3	50.4	***		*		5.21
Feed intake [£] (g/d)	157	167	170	164	159	***		**	***	4.61
Feed conversion [£]	3.34	3.20	3.28	3.23	3.14				*	0.17
Whole period (28 to 69 d)										
Weight gain (g/d)	50.1	50.3	51.2	49.1	49.9			*		3.78
Feed intake [£] (g/d)	134	131	133	128	124	***		***	***	3.02
Feed conversion [£]	2.67	2.63	2.60	2.63	2.49	*			**	0.09

[†]AL: *ad libitum* feeding; Diurnal R: diurnal access to feed; Nocturnal R: nocturnal access to feed.

[§]All re-feeding techniques started from the beginning of the 3th week; Fast: +4 h/d of access to feed up to 24 h/d; Slow: +1/+2 h/d of access to feed up to 24 h/d; Incomplete: +30 min/d of access to feed up to 12 h/d.

¹Root mean square error.

[£]Pen data.

[‡]P<0.10; *P<0.05; **P<0.01; ***P<0.001.

Table 24 Effect of different feeding program on the sanitary status of growing rabbits housed in collective pens from weaning (28 d of age) to the day before slaughter (69 d of age).

Feeding system [†]	AL	Diurnal R	Nocturnal R			Probability of the contrasts			
			Fast	Slow	Incomplete	AL vs. R	DF vs. NF	NF vs. NS	(NF+NS) vs. NI
Re-feeding technique [§]	–	Fast	Fast	Slow	Incomplete				
Group	AL	DF	NF	NS	NI				
Initial number of rabbits	64	64	64	64	64				
Mortality (%)	1.6	3.1	12.5	10.9	9.3	*	*		
Morbidity (%)	26.6	34.4	21.9	29.7	26.6				
Sanitary risk [‡] (%)	28.2	37.5	34.4	40.6	35.9				

[†]AL: *ad libitum* feeding; Diurnal R: diurnal access to feed; Nocturnal R: nocturnal access to feed.

[§]All re-feeding techniques started from the beginning of the 3th week; Fast: +4 h/d of access to feed up to 24 h/d; Slow: +1/+2 h/d of access to feed up to 24 h/d; Incomplete: +30 min/d of access to feed up to 12 h/d.

[‡]Sanitary risk = Mortality + Morbidity.

*P<0.05.

Digestibility and nutritive value of experimental diets

Eight days after the beginning of trial, when all restricted groups were maintained with an access time to feed of 8 h/d, the nutritive value of diet P resulted significantly affected by the feeding system (*ad libitum* vs. feed restriction), while the period of restriction (feeding during the day or during the night) did not produce any relevant effect on the coefficients of total tract apparent digestibility (CTTDA) of the main nutrients (Table 25).

In details, both feed restriction programs improved the digestibility of DM on average (+3.5%; $P<0.01$), gross energy (+3.4%; $P<0.01$), crude protein (+4.6%; $P<0.001$), ether extract (+2.7%; $P<0.001$), crude fibre (+15.9%; $P<0.05$), insoluble fibre fractions (aNDF, ADF and ADL; $0.05<P<0.001$) and total dietary fibre ($P<0.05$) with respect to the AL system. The post-weaning diet showed a high nutritive value, similar in the AL, D and N groups (DE = 10.9 MJ/kg on average). The digestible protein (DP) content ranged from 122 g/d and 128 g/kg according to the variations in protein digestibility coefficients. The DP/DE ratio averaged 11.6 g/MJ.

At the moment of the second digestibility assay (60–64 d), all rabbits fed the fattening diet. The rabbits of NF and NS groups had reached the free access to the feeders since 17 and 5 d respectively, while NI animals maintained an access time to feed of 12 h/d. No significant difference was observed according to the feeding system, whereas the fast re-feeding decreased significantly ($P<0.05$) the digestibility coefficient of DM (-1.6%), GE (-1.7%) and TDF (-4.8%) compared to the slow re-feeding and at a lower extent ($P<0.10$) reduced CP and soluble fibre digestibility. On the other hand, the incomplete re-feeding led to higher CTTAD of DM (+1.8%), GE (+2.0%) and CP (+2.9%) than the complete re-alimentations (Table 26). Accordingly, diet F achieved the greatest nutritive value in the NI group (Table 26).

Caecal content and caecal fermentative activity

At 43 d of age, two weeks after the beginning of trial, the live weight was higher in AL rabbits compared to restricted ones (1446 g vs. 1324 g; $P<0.01$), while the incidence of full gut, full stomach and full caecum was higher in the restricted groups ($P<0.001$), independently by the rationing method (Table 27). The N-ammonia content was higher in AL animals compared to R ones (+29%; $P<0.05$), as well as the caecal pH value (5.46 vs. 5.34; $P<0.01$). Accordingly, the total content of volatile fatty acids (VFAs) at caecum increased ($P<0.05$) with feed restriction (67.7 mmol/L vs. 76.6 mmol/L, for AL and R groups, respectively). The molar proportion of propionic and valeric acids decreased in R rabbits compared to AL ones ($P<0.05$). By contrast, the rationing method slightly affected the caecal fermentative activity of the animals. Indeed, only the

proportion of valerianic acid changed significantly ($P<0.01$), resulting lower in rabbits subjected to a nocturnal rationing program rather than a diurnal program (0.56% vs. 0.36%, respectively).

The second sampling of caecal content was carried out at 51 d of age, in the middle of the re-feeding phase, when rabbits belonging to groups NF, NS and NI had an access time to the feeders of 24, 18 and 12 h/d respectively. Live weight did not differ among groups, whereas the proportions of full gut, full stomach and full caecum were still higher in the R groups (Table 28). Decreasing the speed of re-feeding increased the proportion of full gut (22.7% vs. 24.0% for NF and NS groups respectively; $P<0.10$) and full stomach (7.20% vs. 8.75% for NS and NF groups respectively; $P<0.01$). The greatest incidence of full caecum was detected in the NI group (Table 28).

The pH value of caecal content did not vary among groups, while the N-ammonia content tended to be higher ($P<0.10$) in R groups compared to AL one. Total VFAs concentration was significantly higher in R groups than AL one (76.5 mmol/L vs. 91.4 mmol/L; $P<0.001$) and increased with the decrease on the re-feeding speed ($P<0.01$). The different re-feeding techniques significantly affected the proportions of VFAs, in details the percentage of acetic acid was higher in NF and NS rabbits compared to NI rabbits (84.0% and 83.1% vs. 82.0%, respectively; $P<0.05$). On the opposite, the percentage of butyric acid increased in NI group ($P<0.05$), while the proportion of propionic acid did not change. Therefore, the C3 to C4 ratio tended to decrease in NI rabbits compared to NF and NS rabbits (Table 28).

Table 25 Effect of different feeding programs on the coefficients of total tract apparent digestibility (CTTAD) and nutritive value of the post-weaning diet. Data measured from 36 to 42 d of age in growing rabbits housed in collective pens.

	Feeding system [†]			Probability of the contrasts		RMSE ¹
	AL	D	N	AL vs. (D+N)	D vs. N	
Number of replicates	4	4	4			
Dry matter	0.611	0.633	0.632	**		0.76
Crude protein	0.769	0.807	0.801	***		0.66
Ether extract	0.814	0.838	0.834	***		0.54
Crude fibre	0.201	0.235	0.230	*		2.24
TDF	0.424	0.440	0.439	*		1.11
aNDF	0.311	0.337	0.334	***		0.64
ADF	0.201	0.224	0.221	*		1.33
Soluble fibre	0.889	0.878	0.880			3.56
Gross energy	0.618	0.640	0.638	**		0.73
Digestible protein (DP), g/kg	122	128	127			
Digestible energy (DE), MJ/kg	10.79	10.93	10.89			
DP to DE ratio, g/MJ	11.33	11.74	11.69			

[†]AL: *ad libitum* feeding; D: diurnal access to feed; N: nocturnal access to feed.

¹Root mean square error.

*P<0.05; **P<0.01; ***P<0.001.

Table 26 Effect of different feeding programs on the coefficients of total tract apparent digestibility (CTTAD) and nutritive value of the fattening diet. Data measured from 60 to 64 d of age in growing rabbits housed in collective pens.

Feeding system [†]	AL	Nocturnal R			Probability of the contrasts			
		–	Fast	Slow	Incomplete	AL vs. R	NF vs. NS	(NF+NS) vs. NI
Re-feeding technique [§]	AL	NF	NS	NI				
Group	AL	NF	NS	NI				
Number of replicates	4	4	4	4				
Dry matter	0.606	0.597	0.607	0.613		*	**	0.55
Crude protein	0.729	0.715	0.731	0.744		‡	*	1.02
Ether extract	0.808	0.811	0.812	0.825				1.30
Crude fibre	0.223	0.218	0.230	0.215				1.34
TDF	0.413	0.401	0.421	0.419		*		0.90
aNDF	0.308	0.308	0.319	0.317				0.86
ADF	0.201	0.193	0.201	0.199				1.50
Soluble fibre	0.830	0.774	0.846	0.841		‡		4.33
Gross energy	0.615	0.605	0.615	0.622		*	**	0.47
Digestible protein (DP), g/kg	116	114	117	119				
Digestible energy (DE), MJ/kg	10.31	10.29	10.35	10.41				
DP to DE ratio	11.29	11.10	11.30	11.41				

[†]AL: *ad libitum* feeding; Nocturnal R: nocturnal access to feed.

[§]All re-feeding programs started from the beginning of the 3th week; Fast: +4 h/d of access to feed up to 24 h/d; Slow: +1/+2 h/d of access to feed up to 24 h/d; Incomplete: +30 min/d of access to feed up to 12 h/d.

¹Root mean square error.

‡P<0.10; *P<0.05; **P<0.01.

Table 27 Effect of different feeding programs on the caecal fermentative activity in growing rabbits at 43 d of age.

	Feeding system [†]			Probability of the contrasts		RMSE ¹
	AL	D	N	AL vs. (D+N)	D vs. N	
Number of rabbits	8	8	8			
Live weight (LW), g	1446	1310	1337	**		106
Full gut, % LW	22.1	26.4	26.3	***		1.24
Full stomach, % LW	6.08	9.36	9.05	***		1.03
Full caecum, % LW	6.98	8.41	8.32	***		0.74
Caecal content						
pH	5.46	5.36	5.32	**		0.08
N-NH ₃ , mmol/L	7.24	4.23	6.08	*		2.22
Total VFAs, mmol/L	67.7	75.4	79.7	*		8.63
C2, %	83.8	84.9	85.1			2.30
C3, %	5.32	4.56	4.55	*		0.84
C4, %	10.3	10.0	10.0			1.68
C5, %	0.62	0.56	0.36	**	**	0.12
C3/C4	0.52	0.46	0.46			0.08

[†]AL: *ad libitum* feeding; D: diurnal access to feed; N: nocturnal access to feed.

¹Root mean square error.

*P<0.05; **P<0.01; ***P<0.001.

Table 28 Effect of different feeding programs on the caecal fermentative activity in growing rabbits at 51 d of age.

Feeding system [†]	AL	Nocturnal R			Probability of the contrasts			RMSE ¹
		Fast	Slow	Incomplete	AL vs. R	NF vs. NS	(NF+NS) vs. NI	
Re-feeding technique [§]	–							
Group	AL	NF	NS	NI				
Number of rabbits	8	8	8	8				
Live weight (LW), g	1844	1778	1761	1779				159
Full gut, % LW	20.6	22.7	24.0	24.7	***	‡	*	1.46
Full stomach, % LW	6.31	7.20	8.75	8.47	***	**		0.92
Full caecum, % LW	6.57	7.50	7.03	7.65	**			0.71
Caecal content								
pH	5.85	5.79	5.64	5.56				0.33
N-NH ₃ , mmol/L	6.42	8.29	10.7	8.22		‡		3.50
Total VFAs, mmol/L	76.5	83.4	96.2	94.6	***	**		7.21
C ₂ , %	83.8	84.0	83.1	82.0			*	1.62
C ₃ , %	4.86	4.60	4.58	4.41				0.71
C ₄ , %	10.8	10.9	11.8	13.1			*	1.63
C ₅ , %	0.54	0.50	0.52	0.49				0.08
C ₃ /C ₄	0.45	0.42	0.39	0.34			‡	0.10

[†]AL: *ad libitum* feeding; Nocturnal R: nocturnal access to feed.

[§]All re-feeding techniques started from the beginning of the 3th week; Fast: +4 h/d of access to feed up to 24 h/d; Slow: +1/+2 h/d of access to feed up to 24 h/d; Incomplete: +30 min/d of access to feed up to 12 h/d.

¹Root mean square error.

‡P<0.10; *P<0.05; **P<0.01; ***P<0.001

Slaughter results, carcass traits and meat quality

Slaughter results were in accordance with the genetic type used and the final live weight achieved by the animals. The slaughter weight was similar among *ad libitum* and restricted groups (2607 g on average), but tended to be lower in rabbits submitted to a slow re-feeding compared to the animals of NF group (Table 29). R rabbits showed higher transport losses with respect to AL ones (1.64% vs. 2.00%; $P < 0.05$). The cold carcass weight averaged 1568 g, with a slaughter yield of 60.2% without significant differences among groups. The incidence of liver was greater in R groups compared to AL one (4.10% vs. 4.52%; $P < 0.01$).

Feeding system did not affect other carcass traits, while the re-feeding method had some significant effects. In particular, the proportion of liver increased ($P < 0.01$) in rabbits submitted to an incomplete refeeding (NI group). Furthermore in this group the reference carcass weight and the incidence of dissectible fat were slightly reduced with respect to groups subjected to a complete re-feeding ($P < 0.10$). By contrast, the incidence of *l. lumborum* muscles was higher in NI group compared to groups completely re-fed (13.3% vs. 13.9%; $P < 0.05$), while the proportion of hind legs on reference carcass did not change among groups (33.5% on average). The muscle to bone ratio of hind leg averaged 5.55 (Table 29).

In accordance with the slight effect on slaughter traits, different feeding systems, restriction methods and re-feeding techniques did not modify the meat quality (Table 30).

In details *l. lumborum* muscle showed an average pH of 5.64, with a lightness (L^*) of 51.8 and both redness (a^*) and yellowness (b^*) indexes close to zero (-1.86 and 1.50, respectively). The average meat pH detected in the hind legs was equal to 5.85, while L^* , a^* and b^* indexes averaged respectively 50.0, -2.42 and 3.86.

Thawing losses, cooking losses and shear force were of 8.45%, 36.4% and 6.30 g/kg, respectively, in *l. lumborum*, and 2.76%, 29.0% and 2.64 g/kg in the hind legs (Table 30).

Table 29 Effect of different feeding programs on the slaughter traits and main characteristics of 24-h chilled carcasses in growing rabbits at 70 d of age.

Feeding system [†]	AL	Diurnal R	Nocturnal R			Probability of the contrast				RMSE ¹
			Fast	Slow	Incomplete	AL vs. R	DF vs. NF	NF vs. NS	(NF+NS) vs. NI	
Re-feeding technique [§]	–	Fast	Fast	Slow	Incomplete					
Group	AL	DF	NF	NS	NI	AL vs. R	DF vs. NF	NF vs. NS	(NF+NS) vs. NI	
Number of rabbits	46	40	42	37	41					
Live weight at farm [§] , g	2680	2684	2705	2629	2637			‡		179
Slaughter weight (SW), g	2628	2621	2643	2572	2570			‡		174
Transport losses, %	1.64	2.02	1.92	1.88	2.19	*				1.01
Cold carcass weight (CW), g	1591	1557	1597	1557	1540					113
Dressing percentage, % SW	60.4	60.0	60.6	60.2	60.0					1.85
Head, % CW	7.87	7.87	7.79	7.84	7.86					0.43
Liver, % CW	4.10	4.38	4.51	4.31	4.86	**		**		0.66
Thoracic organs, % CW	3.07	3.12	3.24	3.23	3.28					0.33
Reference carcass (RC), g	1351	1316	1346	1316	1292			‡		101
Dissectible fat, % RC	2.08	2.19	2.11	2.10	1.71			‡		0.68
<i>L. lumborum</i> muscle, % RC	13.4	13.5	13.2	13.3	13.9			*		0.85
Hind legs, % RC	33.4	33.4	33.4	33.4	33.7					0.80
Hind leg muscle/bone ratio	5.65	5.44	5.57	5.44	5.64					0.47

[†]AL: *ad libitum* feeding; Diurnal R: diurnal access to feed; Nocturnal R: nocturnal access to feed.

[§]All re-feeding techniques started from the beginning of the 3th week; Fast: +4 h/d of access to feed up to 24 h/d; Slow: +1/+2 h/d of access to feed up to 24 h/d; Incomplete: +30 min/d of access to feed up to 12 h/d.

¹Root mean square error.

[§]Live weight after 5 hours of fasting.

‡P<0.10; *P<0.05; **P<0.01.

Table 30 Effect of different feeding programs on the meat traits of *longissimus lumborum* and hind in growing rabbits at 70 d of age.

Feeding system [†]	AL	Diurnal R	Nocturnal R			Probability of the contrast				RMSE ¹
			Fast	Slow	Incomplete	AL vs. R	DF vs. NF	NF vs. NS	(NF+NS) vs. NI	
Re-feeding technique [§]	–	Fast	Fast	Slow	Incomplete					
Group	AL	DF	NF	NS	NI					
Number of rabbits	32	32	32	32	32					
<i>Longissimus lumborum</i>										
pH	5.67	5.66	5.62	5.63	5.64					0.09
<i>L</i> [*]	51.7	51.7	52.3	51.6	51.8					2.38
<i>a</i> [*]	–1.64	–1.81	–1.86	–2.05	–1.92					0.55
<i>b</i> [*]	1.25	1.94	1.23	1.31	1.77					1.94
Thawing losses, %	8.12	8.29	9.06	8.51	8.26					1.98
Cooking losses, %	36.6	36.6	36.1	36.2	36.4					1.52
Shear force, kg/g	6.38	6.15	6.07	6.36	6.55					1.22
Hind leg										
pH	5.86	5.85	5.82	5.84	5.87					0.10
<i>L</i> [*]	49.9	49.9	49.6	50.1	50.3					2.27
<i>a</i> [*]	–2.30	–2.35	–2.47	–2.50	–2.48					0.48
<i>b</i> [*]	3.91	3.98	3.75	3.83	3.83					1.33
Thawing losses, %	3.05	2.80	2.51	2.77	2.68					0.82
Cooking losses, %	29.2	29.2	28.9	28.9	29.0					1.25
Shear force, kg/g	2.64	2.69	2.56	2.71	2.60					0.38

[†]AL: *ad libitum* feeding; Diurnal R: diurnal access to feed; Nocturnal R: nocturnal access to feed.

[§]All re-feeding techniques started from the beginning of the 3th week; Fast: +4 h/d of access to feed up to 24 h/d; Slow: +1/+2 h/d of access to feed up to 24 h/d; Incomplete: +30 min/d of access to feed up to 12 h/d.

¹Root mean square error.

L^{*}: lightness; *a*^{*}: redness; *b*^{*}: yellowness.

Discussion

During the first two weeks of trial daily feeding curves and feed restriction levels were consistent to those expected for the nocturnal feeding programs (77% on average), while diurnal rationing method led to a more severe feed restriction in the first week, therefore achieving in the restriction phase a feeding level lower than that planned (72% of *ad libitum*). This result could be related to the lower activity of the rabbit during daylight especially in the morning which corresponds to the caecotrophy period (Gidenne and Lebas, 2005), while the higher feed consumption happens during evening and night (Lebas *et al.*, 1996; Fortun-Lamothe and Gidenne 2006). Hence, the diurnal program involves a deeper change on the feeding behaviour of rabbits compared to the nocturnal method. This might explain the period of adaptation (about one week) required in DF rabbits to reach the intake level of animals fed in the dark period. Our results agree with the findings of Jerome *et al.* (1998) which reported that a stricter feed restriction could be achieved by feeding rabbits during daylight rather than during the night. On the other hand, in the whole first rearing period (first three weeks) no differences were observed in terms of feed intake (87% of *ad libitum* on average) between DF and NF groups because with the start of the re-feeding phase (from 43 d of age) DF rabbits overcame the feeding level of NF ones (+5%), therefore recovering the delay of the first week. Accordingly, several authors did not observe significant changes on feed intake by rationing rabbits during daylight or during darkness (Szendrő *et al.*, 1988; Weissman *et al.*, 2009). Moreover, in this study, it was confirmed the rabbit adaptability to feed-limited conditions. This result probably depended on the rising of feeding rhythm (i.e. the capability to ingest a great quantity of feed in a short time) of restricted rabbits (Martignon *et al.*, 2009). This behaviour was clear also during the second week, when the feeding level was increasing (from 70% to 80% of *ad libitum*) although the access time to the feeders was kept stable at 8 h/d, similarly to that observed in the previous trial of this thesis. Accordingly, other authors detected a remarkable over-eating behaviour during re-feeding especially in the absence of a gradual transition from the restriction phase to the *ad libitum* phase (Foubert *et al.*, 2007; Gidenne *et al.*, 2012b; Knudsen *et al.*, 2014).

As expected the fast curve (+4 h/d of access to feed) involved a sharp increase on feed intake at the first day of re-feeding with several fluctuations in the following d, while the slow curve (+1 or +2 h/d) led to a gradual increase on the feeding level also avoiding abrupt changes on the intake level. On the other hand, the restricted rabbits reached the intake level of the control groups also applying the very slow (+30 min/d of access to feed) and incomplete curve confirming that an increase until 12 h/d could be sufficient to realize and adequate re-feeding.

In the first trial period, weight gain, feed intake and feed conversion decreased in the restricted rabbits. In particular, feed conversion improved by 8% on average since feed intake decreased by about 15% and weight gain only by 7%. A significant improvement on feed conversion in restricted rabbits compared to *ad libitum* ones during the restriction phase was previously reported by other authors (Foubert *et al.*, 2007; Salaun *et al.*, 2011; Gidenne *et al.*, 2012b; Knudsen *et al.*, 2014). This result could be partially explained by the enhancement on the CTTAD of most nutrients observed in rabbits subjected to rationing programs during the second week of trial when the feeding level was around 75% of *ad libitum*. Our results are in agreement with several studies that reported a significant increase of diet digestibility by applying similar restriction levels (Gidenne *et al.*, 2009d; Knudsen *et al.*, 2014). According to Gidenne and Feugier (2009), the mean retention time of digesta raised at 44.6% when the feed intake was reduced by 20% of *ad libitum* which could explained the increase on nutrient digestibility, together with the increase of the enzyme/substrate ratio.

Applying different restriction strategies, several studies did not detect a significant enhancement of feed efficiency during the restriction phase irrespective of the feeding level, stating that the feed conversion improved only during re-feeding as a consequence of the compensatory growth (Tumova, 2008; Gidenne *et al.*, 2009a). Knudsen *et al.* (2014) assumed that the enhancement of feed efficiency in restricted rabbits during re-feeding could also be of metabolic origin. Indeed, the basal metabolism of animals submitted to feed restriction decreased and it remained relatively low in the initial phase of re-feeding as a consequence of the lower live weight of restricted animals (Hornick *et al.*, 2000). Therefore greater proportions of energy and protein could be addressed to cover growth requirements (Hornick *et al.*, 2000; Sahraei, 2012).

In our trial the compensatory growth in previously restricted rabbits happened during the re-feeding phase, but it was not associated to a better feed conversion because the increase on daily growth (+8.4% on average) was rather proportional to the increase on feed intake (+5.1%). Only the incomplete re-feeding technique allowed to maintain a high feed efficiency in the second rearing period. The diet digestibility did not differ among *ad libitum* and restricted (or previously restricted) groups during fattening, confirming that the improvement on nutrient utilization due to feed restriction was not maintained during re-feeding period as previously observed by other authors (Tumova *et al.*, 2003, 2007; Knudsen *et al.*, 2014). However, the CTTAD of several nutrients was higher in rabbits submitted to an incomplete re-feeding compared to those completely re-fed especially with respect to the fast re-feeding curve, therefore explaining, at least partially, the lowest feed conversion achieved in the NI group.

Considering the whole trial period, the feed efficiency was significantly ($P < 0.05$) improved in restricted groups compared to *ad libitum* one (-3%) in agreement with the findings of most studies as reviewed by Gidenne *et al.* (2012a). The best feed conversion was obtained in NI group (2.49) as a consequence of the lower feed intake but the similar weight gain with respect to the other groups completely re-fed and also to those of *ad libitum* ones. This result was also associated to satisfying carcass yield and meat quality as for not restricted rabbits. Accordingly, Salaun *et al.* (2011) reported that keeping stable the access time to the feeders at 12 h/d from weaning (35 d of age) to 66 d of age led to a significant reduction of feed conversion (-4.4%) in the whole rearing period (from 35 to 70 d of age) with respect to an *ad libitum* feeding, without impairing the live weight of rabbits at 70 d of age.

As regards the animals health status, it was not possible to evaluate accurately the effect of different feeding programs because the administration of a preventive antibiotic treatment in the diet during the first three weeks guaranteed optimal health conditions in all groups until the end of the fourth week. The reason for the inclusion of an antibiotic in the diet was the presence in the origin commercial farm of some digestive problem and the study aimed at evaluating growth performance, intake variation and carcass quality rather than sanitary status. By contrast, after the change of the diet to a fattening diet without antibiotic, the appearance of an acute form of ERE in the last two weeks impaired very quickly the health conditions of rabbits leading to a higher mortality in the restricted groups with respect to *ad libitum* one (+7.4% on average). However this result was not so relevant considering that the sanitary risk, that is the proportion of rabbits dead or not suitable for slaughtering (35.6% on average) did not differ among groups. Indeed, a strict feed rationing ($\leq 80\%$ of *ad libitum*) applied during the first three/four weeks after weaning was generally required to decrease significantly the mortality rate also in the whole rearing period, whereas morbidity rate could be reduced only through a feeding level equal to 70% of the voluntary intake (Gidenne *et al.*, 2012a). On the other hand, allowing an access time to the feeders of 12 h/d throughout the rearing period a lower mortality rate with respect to a free access to feed was previously observed by Salaun *et al.* (2011).

At 43 d of age, the restricted rabbits exhibited a lower live weight compared to AL ones ($P < 0.01$), irrespective of the restriction method. On the contrary, the incidences of full gut, full stomach and full caecum, measured one hour after the feeder were closed, were higher in rabbits submitted to rationing programs ($P < 0.001$). Several authors reported that a feed restriction (from 80% to 60% of *ad libitum*) during the first three weeks after weaning did not imply morphological changes of the digestive tract (Lebas and Laplace, 1982; Gidenne and Feugier, 2009), therefore the higher incidence of full tract observed here in restricted rabbits depended mainly on an increase

of organ contents. This result could be related either by an increased on feeding rhythm (Martignon *et al.*, 2009) or by a longer retention time of particles in rabbits subjected to feed restriction (Gidenne and Feugier, 2009). Moreover, a higher flow of digesta in the caecum explained the increase on the total VFAs production in D and N rabbits compared to AL ones (+14.5%; $P < 0.05$). Caecal pH and N-ammonia values decreased as VFAs production increased. However, it is known that these variables are scarcely correlated with the VFAs production in the caecum (Garcia *et al.*, 2002). Sampling caecal contents after 5 hours from meal distribution, Gidenne and Feugier (2009) observed a total VFAs production significantly higher in rabbits submitted to a severe feed restriction (60% of *ad libitum*) compared to the *ad libitum* group, while pH values decreased also with slightly restriction level (80%). The same authors did not detect significant variations on VFAs proportions according to the restriction level.

At 51 d of age there were no differences on live weight among AL and restricted rabbits. However, the incidence of full digestive tract including stomach and caecum was still higher in rabbits subjected to rationing programs. Also in this case it was possible to assume that the higher incidence of full organs in restricted rabbits was related to the great amount of diet ingested in a short time (Gidenne and Feugier, 2009). Accordingly the total VFAs production was higher in restricted groups compared to AL one and it significantly increased with the decrease of re-feeding speed. Nevertheless, this latter result probably depended on the total access time to feed reached by NS and NF groups (18 h/d and 24 h/d, respectively) rather than on the re-feeding speed *per se*.

The total VFAs production increased at the second sampling probably because in the second period of trial an antibiotic-free diet was administered, whereas the VFAs proportions were quite similar between two samplings. In general the VFAs profile detected in our trial agreed with those of healthy rabbits (Gidenne *et al.*, 2010).

As the slaughter weight was not affected, also dressing percentage and main carcass traits did not differ among groups. Indeed, considering the whole trial period, the feeding level in restricted rabbits was equal to 96% of *ad libitum* on average, varying from 93% (NI group) to 99% (NF group). Moreover the period of severe feed restriction (until 76% on average) was limited at the first two weeks of trial and therefore, the absence of significant differences on slaughter results and carcass traits according to the feeding program was not surprising. Our findings agree with those of Tumova *et al.* (2006) which did not observe a significant decrease on slaughter yield by applying different feed restriction strategies during one or two weeks in the post-weaning period followed by an *ad libitum* feeding until 84 d of age. Moreover Salaun *et al.* (2011) reported that a feeding time of 12 h/d from 35 to 66 d of age did not impair the dressing percentage of rabbits slaughtered at 71 d of age with respect to those always fed *ad libitum*.

The higher proportion of liver in restricted groups compared to AL one could have been originated by an increased demands of glycogen storage during the re-feeding phase (Knudsen *et al.*, 2014). Indeed, liver is an organ with a very high metabolic activity, therefore resulting greatly affected by changes on the feeding level (Hornick *et al.*, 2000).

Furthermore, *ad libitum* or restricted feeding programs did not modify meat quality of growing rabbits in terms of meat pH and colour as previously observed by other authors by applying different feed restriction strategy (Combes *et al.*, 2003; Dalle Zotte *et al.*, 2005; Tumova *et al.*, 2006).

Conclusions

The diurnal access to the feeders led to an initial restriction level more severe than that obtained with the nocturnal access rationing programs, the former requiring more important changes on the normal feeding behaviour of growing rabbits. However after one week of adaptation there were no differences in terms of feed intake between diurnal and nocturnal rationing method. The feeding time reduction (until 8 h/d) in the first two weeks decreased weight gain of restricted rabbits, but improved diet digestibility and feed efficiency compared to the *ad libitum* system. In the second rearing period, with the starts of re-feeding, compensatory growth occurred in previously restricted rabbits especially when a fast re-feeding curve was applied. Moreover, the incomplete re-feeding technique allowed to maintain the highest diet digestibility and feed efficiency. At the end of the trial, all restricted groups reached live weights similar to those of the *ad libitum* group with a significant improvement on feed conversion ratio. Slaughter results, carcass traits and meat quality were not significantly affected by the feeding program.

From the economic viewpoint, the best results were obtained by applying a nocturnal rationing program in the first two weeks of post-weaning followed by a very slow and incomplete re-feeding (up to 12 h/d) until slaughter, which optimized feed efficiency throughout the rearing period without impairing growth performance, slaughter results and carcass quality. On the other hand, in our conditions, the occurrence of a severe digestive disease in the last two weeks of the trial compromised the health status of rabbits irrespective by the previously restriction method and by the re-feeding technique (fast, slow or incomplete).

General discussion

The present thesis aimed to define an efficacious feed restriction strategy for improving gut health and feed efficiency in growing rabbits guaranteeing, at the same time, optimum growth performance and slaughter yield and high carcass and meat quality. For this purpose, four experimental trials were performed at the rabbit farm of the University of Padova using 288–400 rabbits (Hypharm, Groupe Grimaud, Roussay, France) per trial, coming from commercial farms and reared from weaning (28–37 d of age) to commercial slaughter (70–80 d of age) in individual cages or in collective pens.

The first contribution focused on the effect of quantitative feed restriction systems on nutrient digestibility, health status, growth performance, slaughter results and nitrogen balance in growing rabbits kept individually. A feed restriction level varying from 80% to 100% of *ad libitum* (AL) was obtained following two feeding curves characterized by small daily increments (day-by-day: +4 g/d) or by large weekly increments (week-by-week: +23 g/week on average) of the diet supplied during the first three weeks after weaning. From the beginning of the 4th week the amount of diet supplied was increased by 4 g/d for 9 consecutive days in both restricted groups in order to avoid peaks of intakes. Then all rabbits were fed *ad libitum*.

The other three contributions were dedicated to develop an adequate time-based feed restriction program in group-housed rabbits. In details, in the second contribution, the access time to the feeders (allowed mainly during the hours of darkness) increased step-by-step (+1 h every 3–4 d) from 10 h/d to 16 h/d in the first three weeks in order to obtain a feeding level around 90% on average. After that, feeding time increased gradually (+1 h/d) up to reach the free access to the feeders. The effect of the feeding program was evaluated on growth performance, feeding behaviour, slaughter traits and meat quality. The third contribution aimed to obtain a restriction level around 85% of *ad libitum* in the first three weeks through a progressive and variable management of the feeding time. In details, the access time to the feeders decreased from 14 to 8 h/d in the first week, remained stable at 8 h/d in the second week and increased of +1 h/d during the third and fourth week until reaching the free access to feed. This program was developed to support the rabbit adaptation to the feeding program either during restriction or during re-feeding phase. The effect of the feeding program was evaluated on growth performance, health status, slaughter traits and meat quality. Finally, in the fourth contribution, the effect of feeding system (*ad libitum* vs. feed restriction), restriction method (diurnal vs. nocturnal access to feed), re-feeding speed (fast: +4 h/d vs. slow: +1/+2 h/d of access to feed) and re-feeding level (complete: up to 24 h/d vs. incomplete: up to 12 h/d of access to feed) was evaluated on growth performance,

digestive physiology, health status, slaughter traits and carcass and meat quality of growing rabbits.

Effect on health status

One of the most interesting points of feed intake limitation strategies regards the possibility of reducing the incidence of digestive troubles in growing rabbits. In this way the use of antibiotic treatments can be substantially decreased as required by the European rules. Several studies have dealt the relationship between feed intake reduction and digestive health, often showing encouraging results in terms of reduced mortality and morbidity rates (Boisot *et al.*, 2005; Gidenne *et al.*, 2009a; Romero *et al.*, 2010; Bovera *et al.*, 2013; Knudsen *et al.*, 2014). However the mechanisms through which a limitation of the feed intake can control gut health still remain almost unexplained (Knudsen *et al.*, 2015). Furthermore the enhancement of health status is not always achieved (Bovera *et al.*, 2008; Matics *et al.*, 2008; Martignon *et al.*, 2009). Different experimental conditions, feed restriction levels and methods of application could partially explain the variability among studies.

The study of the relationship between feed restriction and digestive health was a primary objective in the first contribution of the thesis. Indeed, any antibiotic treatment was administered during the whole trial period. In the other three contributions the effect of feed restriction programs on health status was also evaluated, but it was a secondary objective and antibiotics were included in the diets provided during the first three weeks after weaning. In the conditions of the first trial, despite the slight restriction level applied in the first period (92% on average), both restriction systems (small daily increments or large weekly increments of the diet supplied) significantly reduced mortality (-12 percentage points on average with respect to AL group; $P < 0.01$) due to ERE in the whole rearing period. On the contrary, morbidity was not affected by the feeding system. Indeed, the daily trend of ill animals during the trial was quite similar among groups excepted for the time of appearance of the disease. In fact, digestive troubles appeared before in the AL group, whereas the first cases of illness were detected after 5–6 d in restricted rabbits.

Similarly, in the third contribution, cases of ERE appeared after 9–10 d from the beginning of the trial in the rabbits fed *ad libitum*, while in the restricted animals no symptoms of enteric disease were observed until the start of re-feeding phase (3th week). On the other hand, the number of ill and dead animals rapidly increased in the R group during the second period. Therefore, considering the whole trial there were no differences in terms of morbidity among AL and R rabbits, while mortality tended to be higher in the R group (+5.6 percentage points; $P < 0.10$).

In the fourth trial, after the change of the post-weaning diet with a fattening diet without antibiotic, the appearance of an acute form of ERE in the last two weeks rapidly impaired animal

health causing a high proportion of rabbits dead or not suitable for slaughtering (35.6% on average) without significant differences among groups. Indeed, the application of a very slow (+30 min/d) and incomplete re-feeding (up to 12 h/d) was not effective in preserving the gut health of the animals. This latter result was in contrast with the findings of other authors, which stated that an access time to the feeders of 12 h/d throughout the rearing period reduced the mortality rate with respect to an *ad libitum* feeding (Salaun *et al.*, 2011). On the other hand, also the decrease of mortality rate detected in R rabbits in the first trial was quite unexpected, since a restriction level lower than 80% of *ad libitum* during post-weaning is generally required to reduce significantly the proportion of dead animals (Gidenne *et al.*, 2009a; Gidenne *et al.*, 2012a). The housing conditions could play a key role on the spread of the disease. Indeed, in the first trial the rabbits were kept individually, therefore the contacts among individuals were limited. By contrast, when rabbits are housed collectively, a quicker infection transmission could be hypothesized (Martens and Van Herck, 2000). Nonetheless, our findings highlight that the positive effect of a strict feed restriction (<80%) on gut health occurs during the restriction phase, but it disappears when rabbits return to a free intake regime. This mechanism leads to a relevant increase of mortality during the last weeks of fattening in previously restricted rabbits, therefore increasing the economic impact of animal losses.

Effect on growth, feed efficiency, slaughter traits and meat quality

According to the study of Tudela (2008), daily growth of restricted rabbits should be reduced by 0.5 g/d per each percentage point of feed intake reduction during the rationing period. Once re-fed freely, the previously restricted rabbits show compensatory growth rates, recovering about 0.3 g/d per each percentage point of feed intake reduction with respect to the animals always fed *ad libitum*. Therefore, in the whole rearing period the losses of weight gain in R rabbits are limited (-0.13 g/d per each percentage point of feed intake reduction) and feed conversion linearly improves (-0.008 units per each percentage point of feed intake reduction). Hence, the final live weights of R rabbits should not be greatly reduced compared to those of AL rabbits. However, this result depends on the level of feed restriction and the extension of rationing and re-feeding phases (Birolo *et al.*, 2013). Gidenne *et al.* (2009a) reported that feed efficiency did not enhance during the restriction phase (from 34 to 54 d of age) irrespective of the feeding level applied (60%, 70%, 80%, and 90% of *ad libitum*) stating that the feed conversion improves only during re-feeding (from 54 d to 70 d of age) as a consequence of the compensatory growth. By contrast, other studies reported a relevant decrease of feed conversion either during restriction or during re-feeding phase by applying a feed restriction around 75% *ad libitum* in the first four weeks after weaning followed by a re-feeding period of one week (Gidenne *et al.*, 2012b; Knudsen *et al.*, 2014). The improvement

of feed conversion may be related to an increased diet digestibility during the restriction phase (Gidenne *et al.*, 2012a), while the changes in the basal metabolism, which remains relatively low in restricted rabbits during the initial phase of re-feeding, might lead to greater proportions of energy and protein available for growth (Hornick *et al.*, 2000; Sahraei, 2012). Moreover, the decrease in digestible energy (DE) intake during the restriction phase modifies the EB composition. In details, the feed restriction affects the body energy retention by promoting the body deposition of protein rather than fat (Xiccato and Trocino, 2010). This could partially explain the compensatory growth during the re-feeding period.

Summarizing our results, in the first trial, a feeding level equal to 91% of AL was obtained through a day-by-day restriction curves during the first three weeks, while the feeding level reached 93% by applying a week-by-week restriction. Since the reduction of feed intake was proportional to the reduction of daily growth, no improvement on feed conversion was observed in R rabbits during post weaning period, in agreement with Gidenne *et al.* (2009a). During the re-feeding phase, feed intake and daily growth raised in rabbits subjected to the week-by-week system, which showed the best feed conversion (-10% with respect to AL). However, in the whole rearing period growth performance were scarcely affected by the feeding system and feed conversion did not differ among groups.

In the second trial, the progressive increase of the access time to feed from 10 to 16 h/d in the first three weeks led to a restriction level equal to 95% of *ad libitum* on average ranging from 62% to 107%. This way, weight gain was not affected by the feeding system and feed conversion decreased in R rabbits during post-weaning period (-5%). However, also in the second trial, there were no differences on feed efficiency between AL and R groups.

In the third trial, when a feeding level around 85% of *ad libitum* was achieved during the first three weeks, feed conversion significantly decreased in R rabbits (-12% with respect to AL; $P < 0.001$). Considering the whole trial, feed conversion decreased by 0.12 points in the restricted rabbits (-4% with respect to *ad libitum* group) according to the equation proposed by the French authors (Tudela, 2008; Gidenne *et al.*, 2009a). Moreover, the feed restriction program did not affect the final live weight of the animals.

Also in the fourth contribution, feed conversion decreased in all restricted groups (-8% on average) during the first three weeks, but a diurnal access to the feeders was less effective compared to the nocturnal feeding program on enhancing feed conversion. Furthermore, only the application of an incomplete re-feeding allowed to increase feed efficiency also in the second period by reducing the over-eating behaviour of the previously restricted rabbits. The incomplete re-feeding led to an intake level around 93% on average in the whole rearing period and allowed

to achieve the best feed conversion (-7% with respect to AL group), without reducing the final live weight. In agreement with our results, Salaun *et al.* (2011) reported that an access time to feed of 12 h/d during darkness throughout the entire rearing period led to a feeding level equal to 95% of *ad libitum* and improved feed conversion (-4% with respect to AL group).

The reduction of feed conversion plays a key role not only for increasing the profitability of rabbit farm, but also for decreasing environmental pollution (Gidenne *et al.*, 2013c). In the first contribution, the daily nitrogen excretion significantly decreased in rabbits subjected to a day-by-day restriction system with respect to those fed *ad libitum* (-4%; $P < 0.05$). This decreased N output was associated to a reduced N intake and enhanced protein digestion during restriction phase.

According to the slight restriction levels and the length of re-feeding phases applied in our trials, no difference in terms of slaughter weight, dressing percentage, main carcass traits and meat quality was detected among restricted and *ad libitum* groups. Indeed, Gidenne *et al.* (2009a) observed that slaughter yield significantly decreased only when a severe feed restriction ($\leq 80\%$) was applied for three weeks after weaning and followed by two weeks of re-feeding. The same authors stated that other carcass traits (fatness, muscularity, etc.) as well as meat quality parameters were not or scarcely affected by the feeding system. Similarly, other studies did not detect significant variations on meat pH and colour by applying different restriction strategies (Combes *et al.*, 2003; Dalle Zotte *et al.*, 2005; Tumova *et al.*, 2006). On the other hand, when the digestible energy intake was reduced by 20% throughout the entire trial period, slaughter weights, carcass weights and carcass fatness significantly decreased and relevant changes on meat quality parameters were observed (Metzger *et al.* 2009).

Effect on feeding behaviour, digestive physiology and nutrient digestion

Growing rabbits fed *ad libitum* split their total intake in several meals over the day (around 40 meals). This feeding behaviour does not vary with age, but the amount of food ingested increases from 2.6 g per meal at 42 d of age to 4.9 g per meal at 84 d of age (Tudela, 2008). However, the daily intake pattern changes during the day according to photoperiod. In details, more than 60% of the total daily intake is consumed during the dark period when rabbits are submitted to a light:dark schedule of 12:12 h/d (Tudela, 2008). Indeed, Martignon *et al.* (2009) observed that the lowest feed consumption of animals fed *ad libitum* occurred 2–4 hours after the beginning of the light period, when rabbits are dedicated to caecotrophy (Gidenne and Lebas, 2006). The application of feed restriction strategies obviously alters the “normal” rabbits feeding behaviour. For example, Martignon *et al.* (2009) reported that rabbits subjected to a quantitative feed restriction (75% of *ad libitum*) ingested high quantity of food in the first hours after the meal

distribution, consuming about 40% of the daily ration within the first two hours, while the total amount of diet supplied was consumed within 10 hours.

The day-by-day feed intake variation of rabbits was measured in all our trials. Moreover, in the second trial the feeding behaviour through the day was measured at different ages in rabbits always fed *ad libitum* or submitted to a progressive increase of the access time to the feeders. Our results agree with the literature, indicating that the feeding behaviour of growing rabbits is strongly modified when feed restriction is applied. Indeed, limiting the feeding time at 11 h/d, the restricted rabbits showed peaks of intake up to 18 g/h during the first hour of access to feed, that was about three times higher than the maximum intake of rabbits fed *ad libitum*. Moreover, the restricted rabbits showed higher feeding activity with respect to AL ones throughout the access time to the feeders. Relevant peaks in feed consumption (up to 14–15 g/h) were observed also with access times to the feeders of 13 or 16 h/d.

The changes in feeding behaviour, could modify the digestive physiology of the rabbit. Applying different feed restriction levels (80%, 70% and 60% of *ad libitum*), Gidenne and Feugier (2009) observed a significant decrease of caecal pH (-0.3 units on average) after 5–6 h from the meal distribution, probably associated with the increase of the volatile fatty acids (VFAs) production (+24% with respect to AL group), while the VFAs proportions did not changed according to the intake level. In the fourth trial of ours, caecal contents were sampled one hour after closing the feeders and higher VFAs contents (+15% on average) and lower pH values (-0.12 units on average) were found in R rabbits (8 h/d of access time to feed) compared to AL ones, irrespective by the restriction method (diurnal or nocturnal access to the feed). Moreover, the French authors observed that the mean retention time of particles in the digestive tract significantly raised with the decrease of feed intake.

The changes in digestive physiology could partially explain the enhancement on diet digestibility observed in R rabbits during restriction phase (fourth contribution of this thesis). In agreement, the findings of several studies highlight the positive effect of feed restriction (varying from 50% to 80% of *ad libitum*) on nutrient utilization in the post-weaning period (Gidenne *et al.*, 2012a; Knudsen *et al.*, 2014). However, this result seems to be limited at the restriction phase since no residual effect is observed during the re-feeding period (Tumova *et al.*, 2003; 2007; Knudsen *et al.*, 2014). On the other hand, the application of an incomplete re-feeding (up to 12 h/d of access to feed) allowed to maintain higher diet digestibility compared to complete re-feeding techniques (fourth contribution of the thesis).

From the welfare viewpoint, the results of the third contribution confirm that the rabbits feeding behaviour changes greatly when the animals are subjected to limited access time to the

feeders. However, in our conditions, when rabbits reached the free access to feed, they adopted a feeding behaviour similar to that of rabbits always fed *ad libitum* within few days. Martignon (2010) reported similar results by applying a quantitative feed restriction (75% of *ad libitum*) during post-weaning followed by free feeding in the last two weeks. According to the findings of the fourth contribution, a diurnal access to feed involves a deeper change in the feeding behaviour of rabbits compared to a nocturnal feeding. However, in our trials, no increase of aggressiveness among animals was observed by applying feed restriction programs during the first weeks after weaning, in agreement with the findings of other studies (Gidenne *et al.*, 2012a).

Main conclusions

The main objective of the present PhD thesis was to define an efficacious feed restriction program for enhancing feed efficiency and gut health in growing rabbits, without impairing carcass and meat quality. The goal was pursued by focusing on three specific objectives (*i*) to evaluate the effect of quantitative feed restriction systems (First contribution); *ii*) to develop an adequate time-based feed restriction program (Second contribution and third contribution); *iii*) to optimize the management of the re-feeding phase (Fourth contribution).

The results obtained in the four contributions highlight that:

- I. a theoretical progressive feeding curve characterized by small daily increments of the supplied diet allows to control accurately the feed intake of growing rabbits, but its application under field conditions would require expensive technology innovations;
- II. feed restriction can be easily applied in rabbit farms by limiting feeding time, but the daily program of access time to the feeders should take into account that rabbits can adjust very quickly their ingestion capacity to the new distribution time;
- III. a feeding time shorter than 10 h/d is required to maintain feed intake around 80% of *ad libitum*;
- IV. feed restriction does not always produce the expected enhancement of health status;
- V. re-feeding phase is a critical period because health condition can worsen in previously restricted rabbits, therefore increasing the economic impact of animal losses;
- VI. feeding the rabbit during the dark period, with the fasting phase during daylight, could be a less stressful strategy because it partially reflects the normal intake pattern of animals fed freely;
- VII. a gradual decrease of the access time to the feeders (up to 8 h/d) during the first two weeks after weaning, followed by a gradual and incomplete re-feeding (until 12 h/d) could represent the best choice in order to reduce feeding costs without compromising growth performance and slaughter results;
- VIII. feed restriction levels around 80% of *ad libitum* in the first three weeks after weaning followed by three weeks of re-feeding does not affect slaughter traits and carcass and meat quality;
- IX. the application of feed restriction strategies could contribute to lowering nitrogen excretions and thus the environmental pollutions.

In the first three contributions, the effects of slaughter age (73 or 82 d of age), group composition for gender (only-females, only-males or mixed sexes) and group size (8, 16 or 32

rabbits per pen with a stocking density equal to 16 animals/m²) were also assessed. None of these factors showed significant interactions with the feeding program, but the following conclusions can be drawn:

- i. a slaughter age around 72–74 d of age is recommended in order to reduce the feed conversion and to optimize the rearing cycle;
- ii. housing rabbits in separated groups of only males and only females is not justified considering the complications on rearing management and the higher risks of aggressiveness in only–male groups;
- iii. the increase in group size from 8 to 32 rabbits per pen by maintaining the same stocking density does not affect growth performance, slaughter results and meat quality but it could impair sanitary conditions.

Future perspectives

The present thesis evidenced the possibility to successfully apply feed restriction programs to growing rabbits in order to enhance feed efficiency without impairing growth performance, slaughter traits and meat quality. On the other hand, the variability of the results concerning the effects of this feeding technique on health status highlights that the control of digestive diseases is difficult to be achieved. Therefore, the relationship between feed restriction and gut health needs to be further clarified through multidisciplinary approaches.

Further investigations may be also carried out to prove a possible synergistic effect by combining feed restriction programs with different diet compositions. Moreover, the diet formulation should be reviewed to improve the expression of the genetic potential in the current commercial hybrids also in conditions of feed restriction.

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