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CICLO XXIV

FEEDING STRATEGIES TO IMPROVE HEALTH STATUS AND FEED EFFICIENCY IN GROWING RABBITS

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Riassunto

Introduzione e obiettivi

L’Enteropatia Epizootica del Coniglio (*Epizootic Rabbit Entropathy*, ERE) è diventata, negli ultimi decenni, la principale causa di perdite a livello economico negli allevamenti cunicoli europei, con aumento della morbilità e della mortalità traducibili in un importante peggioramento della conversione alimentare a livello aziendale. È una patologia caratteristica del periodo di post-svezzamento e può essere descritta come una grave forma di enteropatia mucoide, complicata da affezioni batteriche secondarie che, spesso, ne rendono difficile l’interpretazione dei sintomi e l’attribuzione sicura della malattia (Marlier e coll., 2003; Rosell, 2003).

Non essendo stato ancora identificato l’agente eziologico della malattia cui piuttosto si riconosce un carattere multifattoriale, le principali soluzioni adottate fino ad oggi hanno riguardato l’utilizzo di antibiotici in profilassi e meta filassi orientati al controllo di clostridi e coli, ma la vigente normativa comunitaria ne limita l’utilizzo a fini preventivi. Inoltre, a causa della rapida evoluzione della malattia, una volta che l’ERE è stata diagnosticata, l'intervento con antibiotici a scopo terapeutico non sempre è sufficientemente tempestivo da limitare le perdite economiche (Xiccato e coll., 2008). A ciò si aggiunga il costo della terapia antibiotica, che risulta avere un considerevole impatto sui costi di produzione degli allevamenti cunicoli e l’elevata frequenza di scarsa efficacia del trattamento antibiotico dovuta a ceppi di clostridi e coli resistenti.

A tal proposito, gli studi effettuati negli ultimi decenni hanno puntato a definire al meglio i fabbisogni nutrizionali degli animali e a sviluppare nuove strategie alimentari soprattutto nelle fasi di svezzamento e post-svezzamento in relazione alla possibilità di ridurre le perdite causate dall’ERE. Infatti, la nutrizione gioca un ruolo fondamentale nello sviluppo delle popolazioni microbiche intestinali e nella possibilità che batteri enteropatogeni possano prevalere sulle popolazioni simbionti.

In particolare, parlando dei nutrienti della dieta è risaputo come il contenuto ottimale di amido in mangimi per conigli nella fase di svezzamento si attesti attorno al 10-15%, mentre può superare il 15% nelle ultime fasi di ingrasso e finissaggio (De Blas e Mateos, 2010). L'utilizzo di diete ad alto tenore in amido viene spesso associato alla comparsa di disturbi digestivi durante le fasi di svezzamento e post-svezzamento (Blas e Gidenne, 2010). Quanto detto trova riscontro nella teoria di Cheeke (1987), secondo la quale diete ricche in amido e povere di fibra aumenterebbero il flusso di amido a livello ciecale, soprattutto negli animali giovani che presentano una scarsa attività delle amilasi intestinali. A livello ciecale, l’amido viene fermentato dai batteri amilolitici, aumenta la produzione di acidi grassi volatili (AGV) provocando un importante riduzione del pH. Queste condizioni risulterebbero favorevoli alla proliferazione di *Clostridium spiroforme* che, utilizzando il glucosio in eccesso, produrrebbe una tossina iota-simile, principale causa di enteriti e diarree.

I fabbisogni proteici si attestano attorno al 15-16% di proteina grezza (PG) o al corrispondente 10,5-11% di proteina digeribile (PD). Per quanto riguarda le relazioni intercorrenti tra il livello proteico della dieta e la salute degli animali, la carenza (<12%) o l'eccesso (>18%) di PG possono favorire la presenza di problemi digestivi e quindi aumentare la mortalità modificando l'attività fermentativa ciecale e la composizione della microflora intestinale (Maertens e De Groote, 1988; Lebas, 1989; Carabaño e coll., 2008, 2009). Tutte le popolazioni microbiche beneficiano della disponibilità proteica a livello ciecale per il loro sviluppo e la loro proliferazione, ma alcune specie (in particolare *E. coli* e *Clostridia*) sembrerebbero maggiormente avvantaggiate da squilibri nell'apporto proteico.

Per quanto concerne i fabbisogni delle diverse frazioni fibrose, vengono consigliati quantitativi minimi pari al 5% di ADL e 16-17% di ADF (Gidenne e García, 2006). Bassi livelli di fibra insolubile diminuirebbero la velocità di transito, prolungando la permanenza dell'alimento nel tratto intestinale; questo aumenterebbe la fermentazione delle proteine, con conseguente aumento dell'azoto ammoniacale e del pH ciecale favorendo le disbiosi intestinali (Gidenne, 1996;. Bennegadi e coll., 2000). Anche un eccesso di fibra insolubile, tuttavia, associato ad una carenza di sostanze fermentabili a livello ciecale, promuove lo sviluppo di una microflora patogena nel cieco, in particolare *Escherichia coli.*, come descritto dalla nota teoria di Morisse e coll. (1985).

D'altra parte è stato evidenziato un effetto positivo della fibra solubile sullo stato di salute dei conigli soprattutto quando il suo incremento avviene a spese dell'amido in diete con un contenuto di ADF costante (Jehl e Gidenne, 1996; Perez e coll., 2000; Soler e coll., 2004; Xiccato e coll., 2008). In questo caso risultano migliorate le condizioni a livello ciecale con pH più bassi, conseguenza di una maggior produzione di AGV e di una minore concentrazione di azoto ammoniacale. Inoltre, non è modificata la digeribilità della sostanza secca e il contenuto energetico delle diete, pur migliorando considerevolmente la digeribilità delle frazioni fibrose delle diete stesse (Gidenne e Jehl, 1996; Gidenne e Bellier, 2000; Gidenne e Perez, 2000; Xiccato e coll., 2008, 2010; Trocino e coll., 2011).

Al fine di controllare i disturbi digestivi si possono utilizzare anche programmi di restrizione alimentare. In particolare essi possono essere utilizzati al fine di migliorare l'efficienza globale dell'allevamento, aumentando l'utilizzazione digestiva della dieta fornita, modificare la composizione corporea in termini energetici, favorendo la deposizione di proteina a scapito di quella di grasso, ed infine ridurre la mortalità e la morbilità in allevamento causate da disturbi digestivi. Mediante l'applicazione di programmi di restrizione alimentare nella prima fase di allevamento, l'accrescimento viene controllato e quindi limitato durante la fase di restrizione ma, l'accrescimento compensativo durante la successiva fase di re-alimentazione permette agli animali di recuperare le performance produttive durante la fase finale di allevamento.

I primi studi effettuati sui piani di restrizione alimentare nel coniglio valutavano l'effetto di tali piani sulle performance produttive degli animali, sulle caratteristiche della carcassa e sulla qualità della carne. In seguito, il razionamento è stato utilizzato al fine di migliorare l'efficienza alimentare e standardizzare le curve di crescita negli animali con differenti capacità di ingestione. (Ouhayoun e coll., 1986; Ouhayoun, 1989; Cavani e coll., 1991). Dalla fine degli anni '90, la restrizione alimentare è stata utilizzata soprattutto nel periodo di post-svezzamento al fine di controllare i disturbi digestivi. Gli effetti positivi di queste strategie alimentari sono risultati evidenti sull'insorgenza e la diffusione dell'ERE, in quanto hanno un'azione positiva in termini di contenimento della patologia e di migliore indice di conversione globale (Maertens, 1992; Gidenne e coll., 2003; Tudela, 2009).

Fatte queste premesse, obiettivo generale della presente Tesi di dottorato è stato lo sviluppo e la messa a punto di strategie nutrizionali per il miglioramento dell'efficienza alimentare e dello stato di salute di conigli in accrescimento. Tale obiettivo è stato perseguito mediante quattro attività sperimentali, in cui si è articolata la presente Tesi di Dottorato, orientate ai seguenti specifici obiettivi: i) definizione dei fabbisogni di fibra solubile in rapporto al contenuto di fibra insolubile, di amido e di proteina in conigli in post-svezzamento e ingrasso (contributi sperimentali 1 e 2); ii) valutazione dell'effetto del livello e della durata della restrizione alimentare rispetto ad un'alimentazione *ad libitum* nell'ottica della massimizzazione delle prestazioni produttive e qualitative e del controllo dei problemi digestivi (contributi sperimentali 3 e 4).

Contributo sperimentale 1

Effetto dell'aumento della fibra solubile e dell'amido e della riduzione del contenuto di proteina in diete per conigli in accrescimento.

All'età di 36 giorni, 282 conigli (951±31 g) ibridi di entrambi i sessi sono stati suddivisi in sei gruppi sperimentali di 47 animali e alimentati *ad libitum* con sei diete sperimentali formulate secondo un disegno bi-fattoriale 3 x 2, con tre rapporti (amido+fibra solubile)/ADF (0,6, 1,2 e

2,0) e due livelli di proteina grezza (15% e 17%). La digeribilità apparente dei nutrienti della dieta e la concentrazione dell'energia digeribile (ED) sono stati determinati *in vivo* (Perez *e coll.*, 1995). A 45 giorni di età sono stati prelevati campioni di contenuto ciecale e mucosa intestinale al fine di misurarne l'altezza dei villi e la profondità delle cripte. Le feci e i mangimi sono stati analizzati secondo i metodi AOAC (2000) e le procedure armonizzate EGRAN (2001). Il contenuto di fibra totale dietetica (TDF) è stato determinato attraverso una procedura gravimetrico-enzimatica (Megazyme Int. Ireland Ltd., Wicklow, Ireland) (Metodo AOAC 991.43). Il contenuto di fibra solubile è stato calcolato sottraendo il contenuto di aNDF (senza sodio solfito) da quello di TDF (Van Soest e coll., 1991). L'energia lorda è stata misurata mediante bomba calorimetrica adiabatica.

L'azoto ammoniacale è stato determinato su un campione di contenuto ciecale mediante pHmetro munito di elettrodo specifico. La concentrazione di AGV è stata misurata attraverso analisi gas-cromatografica secondo il metodo di Osl (1988).

All'età di 79 giorni, i conigli sono stati avviati alla macellazione presso un macello commerciale e le carcasse degli animali sono state sottoposte a dissezione secondo protocolli internazionali armonizzati (Blasco e coll., 1993). Le misurazioni del pH (Xiccato e coll., 1994) e del colore della carne (CIE, 1976) sono state effettuate sui muscoli *longissimus lumborum* e *biceps femoris*.

I dati raccolti sono stati analizzati mediante ANOVA con tre fattori principali (rapporto amido+fibra solubile/ADF, livello proteico e sesso) e rispettive interazioni, utilizzando la procedura GLM del SAS (SAS, 1991). I contrasti ortogonali sono stati utilizzati per valutare la probabilità della componente lineare e quadratica della varianza in relazione al rapporto amido+fibra solubile/ADF. La mortalità, la morbilità ed il rischio sanitario sono stati analizzati tramite la procedura CATMODE del SAS.

La digeribilità apparente della sostanze secca è aumentata linearmente ($P<0,001$) all'aumentare del rapporto (amido+fibra solubile)/ADF dal 50,8% al 60,5% e 71,5%. Tale rapporto non ha influenzato la digeribilità della proteina grezza né dell'estratto etereo, mentre la digeribilità delle frazioni fibrose è aumentata significativamente in maniera lineare ($P<0,001$). La digeribilità dell'ADF è cambiata in modo quadratico ($P<0,01$), mentre le emicellulose e la fibra solubile mostrano un miglioramento dell'efficienza digestiva all'aumentare del rapporto (amido+fibra solubile)/ADF.

L'effetto del livello proteico è risultato meno importante e limitato alla maggiore digeribilità della sostanza secca, dell'energia lorda e della proteina grezza ($P<0,01$). Anche la digeribilità della TDF e della fibra solubile è risultata maggiore nelle diete contenenti il 17% di proteina rispetto a quelle contenenti il 15% (P<0,05): ciò potrebbe essere dovuto alla minore inclusione di erba medica a favore della farina di estrazione di soia e a quella di girasole.

Durante la prova, la mortalità si è attestata attorno all'8,5% ed è diminuita all'aumentare del rapporto (amido+fibra solubile)/ADF (15,8% *vs.* 4,5% e 4,9%; P=0,02). In maniera simile, è stata osservata una tendenziale riduzione del rischio sanitario (mortalità+morbilità).

Nonostante non si siano stati rilevati effetti dovuti al livello proteico delle diete sullo stato di salute, bisogna evidenziare come la somministrazione di una dieta ad alto contenuto di amido e fibra solubile ed un basso contenuto in ADF ha tendenzialmente aumentato la morbilità (interazione P=0,08) nei conigli alimentati con diete ad alto tenore proteico.

La somministrazione di diete contenenti rapporti crescenti di (amido+fibra solubile)/ADF, ha aumentato linearmente l'incidenza del cieco (P<0,01) e dell'intestino (P<0,001) pieni. La maggior disponibilità di carboidrati fermentabili ha diminuito linearmente il pH ciecale (da 6,02 a 5,75; P=0,06) e aumentato la produzione totale di acidi grassi volatili (AGV) (da 63,0 a 78,5 mmol/L; P=0,02), riducendo la proporzione di acido propionico (da 4,0 a 3,5 mol/100 mol AGV; P=0,04). Anche in questo caso, il livello proteico non ha modificato le fermentazioni ciecali, se non per un tendenziale aumento (P=0,07) del livello ciecale di azoto ammoniacale negli animali alimentati con diete al 17% di PG.

La mucosa dell'ileo non è stata influenzata dal trattamento alimentare, mentre l'altezza dei villi intestinali del digiuno (P=0,06) e il rapporto villi/cripte (P=0,03) sono linearmente diminuiti all'aumentare del rapporto (amido+fibra solubile)/ADF. L'aumento del contenuto proteico della dieta ha indotto un tendenziale aumento (P=0,07) dell'altezza dei villi del digiuno.

Durante l'intero periodo di prova, l'escrezione azotata giornaliera negli animali alimentati con elevati rapporti di (amido+fibra solubile)/ADF (1,2 e 2,0) è risultata pari all'80% e al 60% di quelli misurati nei conigli alimentati con un rapporto pari a 0,6. Tenendo in considerazione il contenuto proteico della dieta, l'escrezione azotata è diminuita del 15% al ridursi del contenuto proteico della dieta dal 17% al 15%.

Contributo sperimentale 2

Effetto dell'ulteriore aumento del rapporto (amido+fibra solubile)/ADF e della diminuzione del livello proteico in diete per conigli in accrescimento.

A 33 giorni, 306 conigli (975±97 g) sono stati selezionati in un allevamento commerciale. Gli animali sono stati divisi in sei gruppi da 51 animali assegnati a sei diete fornite *ad libitum* e formulate secondo un disegno bi-fattoriale con tre livelli di rapporto (amido+fibra solubile)/ADF (1,2, 1,8 e 2,8) e due livelli di proteina (14% e 15%). La digeribilità in vivo, le analisi di feci, mangimi e contenuto ciecale, le analisi morfometriche della mucosa intestinale e l'elaborazione statistica sono state compiute con le metodologie indicate per il precedente contributo sperimentale.

La digeribilità della sostanza secca, dell'energia lorda e di tutti gli altri nutrienti della dieta sono aumentate significativamente con l'aumento del rapporto (amido+fibra solubile)/ADF. La digeribilità delle frazioni insolubili della fibra (ADF e lignina) non è stata modificata, mentre la digeribilità delle emicellulose è diminuita e quella della fibra solubile è aumentata.

I primi sintomi di problemi digestivi riferibili all'insorgenza e alla presenza di enteropatia sono stati osservati una settimana dopo l'inizio della prova. In media la mortalità si è attestata attorno al 22.6% senza variazioni in funzione del rapporto (amido+fibra solubile)/ADF, mentre morbilità e rischio sanitario hanno mostrato un tendenziale aumento con l'aumento del rapporto indicato.

L'aumento del rapporto (amido+fibra solubile)/ADF ha aumentato il valore nutritivo delle diete e diminuito il consumo di alimento (P<0,001), senza effetti su velocità di accrescimento e peso degli animali. Di conseguenza, la conversione alimentare è migliorata linearmente (da 3,65 a 3,22 e 2,76; P<0,001). Alla macellazione comparativa, la somministrazione di diete con un più alto rapporto (amido+fibra solubile)/ADF ha modificato in modo quadratico l'incidenza dello stomaco pieno (P=0,02) e dell'intestino pieno (P=0,07). A livello ciecale, ha aumentato linearmente (P<0,05) la produzione di AGV (da 52,1 a 61,9 mmol/L), ridotto il pH (da 5,90 a 6,67) e la concentrazione di azoto ammoniacale (da 4,85 a 1,93 mmol/L); inoltre ha ridotto in modo non lineare l'altezza dei villi della mucosa del digiuno (839 *vs.* 733 e 751 μm) e come conseguenza il rapporto villi/cripte (4,42 *vs.* 3,81 e 3,95).

L'aumento del livello proteico dal 14% al 15% ha determinato un aumento significativo della mortalità (16,3% *vs.* 28,9%, P=0,01), accompagnata da un moderato e significativo aumento della digeribilità della proteina grezza, mentre non ha modificato le prestazioni nell'intero periodo di allevamento né le caratteristiche del contenuto ciecale alla macellazione comparativa o le condizioni della mucosa intestinale.

I trattamenti alimentari non hanno modificato i risultati di macellazione, fatta eccezione per il rapporto muscolo/ossa dell'arto posteriore (P=0,04) che è risultato più elevato con il rapporto (amido+fibra solubile)/ADF intermedio.

Contributo sperimentale 3

Effetto del livello e della durata del periodo di restrizione alimentare in conigli in accrescimento.

All’età di 32 giorni, 264 conigli (912±99 g) sono stati suddivisi in sei gruppi sperimentali di 47 unità ciascuno, omogenei per peso medio e variabilità, e trattati secondo un disegno bi-fattoriale, con tre livelli di razionamento (*ad libitum*, 90% e 80% dell’*ad libitum*) per due periodi (2 settimane *vs.* 3 settimane) dopo lo svezzamento.

La prova ha avuto una durata di 6 settimane ed è stata suddivisa in 2 periodi in funzione del mangime somministrato:

a) periodo di post-svezzamento durante il quale i conigli sono stati alimentati con una dieta da svezzamento (S) *ad libitum* (R100) o razionati al 90% (R90) oppure all’80% (R80) durante un periodo di 2 settimane (T2) o 3 settimane (T3);

b) periodo di ingrasso e finissaggio, durante il quale i conigli sono stati alimentati con una dieta da ingrasso (I) somministrata *ad libitum* per 4 settimane (nei gruppi T2) o 3 settimane (nei gruppi T3).

La digeribilità in vivo, le analisi di feci, mangimi e contenuto ciecale e le analisi statistiche sono state compiute con le metodologie indicate per il precedente contributo sperimentale. All’inizio (33 d) e alla fine (74 d) della sperimentazione, un numero rappresentativo di animali è stato sottoposto a macellazione comparativa per misurare la composizione chimica dei corpi netti e stimare il bilancio corporeo in tutti gli animali giunti alla fine della prova.

La digeribilità della sostanza secca, dell’energia e dei nutrienti della dieta è risultata diversa a seconda delle diete (dieta S: 9,8 MJ ED/kg; dieta I: 10,6 MJ ED/kg) e del livello di restrizione alimentare adottato. La digeribilità della sostanza secca (56,6% *vs.* 58,6% *vs.* 60,3%, $P<0,001$), dell’energia lorda (56,6% *vs.* 58,4% *vs.* 60,0%, $P<0,001$), della proteina grezza e dell’estratto etereo sono aumentate all’aumentare del livello di razionamento.

Lo stato di salute degli animali è peggiorato durante la seconda e la terza settimana di prova con il manifestarsi di diarrea e muco, sintomi associabili a ERE, negli animali sottoposti ai diversi trattamenti, rendendo necessario un trattamento antibiotico supplementare, oltre a quello incluso nel mangime S. Durante l’intero periodo di prova, la mortalità si è attestata attorno al 9,8%, mentre la morbilità ha raggiunto livelli pari al 41,9%. L’analisi dell’evoluzione dello stato di salute nelle diverse settimane ha evidenziato che i minori problemi osservati durante il primo periodo di restrizione alimentare è stato seguito da un peggioramento dello stato di salute nel periodo di ingrasso e re-alimentazione, e questa tendenza è stata tanto più accentuata quanto più spinto è stato il livello e più lungo il periodo di restrizione alimentare. In tutto il periodo della prova, nel suo complesso, è stato evidenziato un significativo aumento della mortalità (dal 5,7% al 13,8%; P=0,03) nei conigli razionati per un periodo più lungo (2 *vs.* 3 settimane), senza effetti significativi del livello di restrizione alimentare.

Il peso vivo degli animali è risultato significativamente più basso nei conigli razionati più severamente, sia dopo due settimane (a 47 giorni di età, 1646 g e 1592 g *vs.* 1507 g per i gruppi R100, R90 e R80, rispettivamente; P<0,001) sia dopo 3 settimane (a 54 giorni di età, 2002 g e 1979 g *vs.* 1883 g, P<0,001). Alla fine del periodo di sperimentazione, a 75 giorni di età degli animali, dopo la fase di re-alimentazione, le differenze di peso vivo tra i tre gruppi si sono ridotte così da non risultare più significative.

Nei conigli sottoposti ad una restrizione alimentare più severa, il peso del corpo netto alla fine del periodo sperimentale è risultato inferiore (P=0,03) rispetto agli altri gruppi sperimentali, così come il contenuto di acqua, proteine e ceneri. Diversamente, il valore minore (220-240 g) di contenuto lipidico è stato misurato nei conigli alimentati con la dieta S a volontà per 2 settimane (R100-T2) ed in quelli razionati per 3 settimane (R90-T3 e R80-T3) (P<0,001). Questo risultato dipenderebbe principalmente dall’interazione fra il livello di restrizione e la sua durata che penalizzerebbe da un lato i conigli alimentati *ad libitum* con una dieta da svezzamento per sole 2 settimane, e dall’altro gli animali razionati (80% e 90%) per un periodo più lungo (3 settimane) rispetto a tutti gli altri. Inoltre, l’applicazione di un razionamento più severo riduce in modo significativo la quantità di azoto ingerito, ritenuto ed escreto rispetto agli animali non razionati o razionati al 90%. Negli animali sottoposti alla restrizione più severa, la quantità di azoto ingerito diminuisce del 6% (P<0,01), quella ritenuta del 4% (P<0,10) e quella escreta dell’8% (P<0,001). Aumentando da 2 a 3 settimane la durata del razionamento, il bilancio azotato peggiora con un incremento dell’escrezione azotata (+6% nei gruppi T3 confrontati con i gruppi T2, P<0,01).

I trattamenti sperimentali hanno scarsamente influenzato i risultati di macellazione, le caratteristiche della carcassa e la qualità della carne.

Contributo sperimentale 4

Effetto del piano alimentare e del programma di restrizione alimentare in conigli in accrescimento.

A 33 giorni di età, 300 conigli ibridi (953±110 g) sono stati suddivisi in sei gruppi sperimentali ciascuno composto da 50 animali. I gruppi, omogenei per peso medio e variabilità, sono stati sottoposti a un disegno sperimentale bi-fattoriale 3 x 2, con tre piani alimentari combinati a due livelli di razionamento. Sono state preparate due diete (dieta A e dieta B) con differente concentrazione energetica e uguale rapporto proteina digeribile/energia digeribile (rapporto teorico: 11 g PD/MJ ED). I programmi alimentari si sono differenziati in relazione alle diete somministrate durante il primo ed il secondo periodo di accrescimento: per il piano A-A, i conigli sono stati alimentati con la dieta A per tutto il periodo della prova (5 settimane); per il piano B-A, i conigli hanno ricevuto la dieta B durante le prime tre settimane e quella A durante le ultime due settimane di prova; il piano B-B ha previsto la somministrazione della dieta B durante l'intero periodo di prova (5 settimane).

Entro piano alimentare i conigli sono stati alimentati *ad libitum* per tutto il periodo di prova oppure soggetti ad una restrizione alimentare media del 90% durante le prime tre settimane. L'intero periodo di prova, durato 5 settimane, è stato diviso in due periodi:

a) periodo di post-svezzamento (prime tre settimane), nel quale due gruppi sono stati alimentati con la dieta A e i rimanenti quattro con la dieta B. In ciascun trattamento alimentare metà degli animali sono stati razionati e l'altra metà alimentati *ad libitum*;

b) periodo di ingrasso (ultime 2 settimane), nel quale quattro dei sei gruppi sperimentali hanno ricevuto la dieta A e gli altri due la dieta B. Tutti gli animali sono stati alimentati *ad libitum* durante tutto questo periodo.

La digeribilità dei principi nutritivi è stata scarsamente influenzata dal programma di restrizione alimentare, mentre è stata influenzata dalla dieta: la digeribilità della sostanza secca, dell'energia lorda, della proteina e delle frazioni fibrose è stata significativamente superiore per la dieta A rispetto alla dieta B.

Sia il razionamento che il piano alimentare non hanno influenzato la mortalità e il rischio sanitario, mentre la morbilità è risultata significativamente influenzata da entrambi i fattori. In particolare la percentuale di animali malati è risultata superiore nei conigli sottoposti a razionamento alimentare rispetto e quelli alimenti *ad libitum* e nei conigli sottoposti al piano alimentare B-A rispetto ai conigli dei gruppi A-A- e B-B.

Durante la prima settimana, gli accrescimenti sono stati penalizzati dalla restrizione alimentare (53,6 e 46,8 g/d nei conigli alimentati *ad libitum* e razionati, P<0,001). Durante la seconda settimana di prova, la comparsa di disturbi digestivi associabili a ERE ha ridotto l'assunzione di alimento negli animali alimentati *ad libitum* di modo che le differenze di ingestione con i gruppi razionati si sono ridotte (134 *vs.*129 g/d, P=0,05, corrispondente ad una restrizione alimentare reale del 4%). Per questa ragione, durante la seconda settimana gli accrescimenti degli animali sono stati simili tra i diversi gruppi, mentre durante la terza settimana i conigli alimentati *ad libitum* hanno evidenziato ingestioni (151 *vs.* 149 g/d, P>0,10) e accrescimenti (48,3 *vs.* 51,0 g/d, P=0,05) simili a quelli degli animali razionati. In seguito, le prestazioni sono state simili fra i due gruppi, cosicché anche le prestazioni nell'intero periodo sperimentale non sono risultate significativamente diverse. In modo analogo, anche il piano alimentare non ha modificato le prestazioni produttive.

I conigli sottoposti a restrizione hanno mostrato un minor peso del corpo netto a 55 giorni di età (dopo 3 settimane di prova) e la composizione corporea durante il primo periodo è risultata caratterizzata da incrementi inferiori in termini di proteine, lipidi, ceneri e, quindi, energia lorda. Nel secondo periodo, quando tutti gli animali sono passati a un'alimentazione a volontà, l'effetto del razionamento alimentare è risultato ancora evidente, ma a favore dei conigli precedentemente razionati. Tra i 55 e i 69 giorni di età, i conigli precedentemente sottoposti a restrizione alimentare hanno deposto più proteine (121 contro 126 g), lipidi (91 contro 97 g), ceneri (18 contro 19) ed energia lorda (6,7 contro 7,2 MJ) rispetto a quelli alimentati *ad libitum*, confermando il verificarsi dell'accrescimento compensativo nei conigli precedentemente razionati. La restrizione alimentare, inoltre, ha ridotto significativamente l'azoto corporeo a 55 giorni di età, senza mostrare effetti residui a 69 giorni. Durante il primo periodo, la restrizione alimentare ha ridotto la quantità di azoto ingerito, ritenuto ed escreto. Durante il secondo periodo, invece si è registrato un aumento solo dell'azoto ritenuto. Considerando l'intero periodo di prova, le variazioni di azoto ritenuto non sono risultate significative. Il piano alimentare non ha modificato il contenuto di azoto del corpo a 55 o 69 giorni di età, ma l'escrezione azotata è risultata inferiore nei conigli sottoposti al piano B-B.

Conclusioni

Effetto della variazione di alcuni principi nutritivi nelle diete per conigli

I primi due contributi sperimentali della tesi sono stati dedicati alla definizione dei fabbisogni di fibra solubile e amido in rapporto al contenuto di fibra insolubile delle diete in conigli in post-svezzamento e ingrasso. Allo stesso tempo sono stati considerati anche il ruolo della proteina alimentare e le sue interazioni con gli altri nutrienti in considerazione delle relazioni fra il livello di proteina alimentare e l'efficienza aziendale oltre che l'escrezione di azoto. L'uso di diete con livelli crescenti di fibra solubile e amido in sostituzione della fibra insolubile ha migliorato lo stato di salute dei conigli, aumentato l'utilizzazione digestiva delle diete, soprattutto delle frazioni fibrose, e il loro valore nutritivo. Le prestazioni produttive, in termini di peso vivo e accrescimento ponderale, non sono state influenzate, mentre il consumo alimentare è diminuito e la conversione alimentare è aumentata notevolmente. Grazie ai minori livelli di ingestione, l'escrezione di azoto è fortemente diminuita con l'aumento del rapporto (amido+fibra solubile)/ADF. Le fermentazioni ciecali sono state più intense e la digeribilità dell'alimento è migliorata a seguito del contemporaneo aumento di amido e della riduzione di fibra insolubile, come pure della variazione della struttura della fibra, in quanto la fibra è risultata meno lignificata e più disponibile per l'attacco enzimatico e batterico. Alla macellazione commerciale finale, l'aumento del rapporto (amido+fibra solubile)/ADF ha determinato un aumento del peso vivo, ma ha ridotto la resa di macellazione a causa della maggiore incidenza dell'apparato intestinale pieno. Quest'ultimo risultato, tuttavia, è stato bilanciato da un significativo aumento della muscolosità della carcassa come provato dal maggiore rapporto carne/ossa misurato sull'arto posteriore.

Sulla base dei risultati delle due sperimentazioni e della discussione di cui sopra, si evince che è possibile formulare diete per conigli in post-svezzamento e ingrasso in grado di migliorare notevolmente la conversione alimentare rispetto agli attuali standard, mantenendo le prestazioni di crescita e i risultati di macellazione. Possiamo quindi concludere che il contemporaneo aumento del contenuto di amido (fino al 20%) e di fibra solubile (fino al 12%) ha un effetto positivo su fisiologia digestiva, stato di salute ed efficienza alimentare dei conigli, senza effetti negativi su risultati di macellazione e qualità della carcassa e della carne. Tuttavia, in presenza di enteropatia epizootica, la contemporanea riduzione dell'ADF al di sotto del 12% può influenzare negativamente la salute digestiva dei conigli in accrescimento. Variazioni nel livello proteico della dieta hanno effetti meno rilevanti, anche se talvolta significativi, rispetto alle variazioni del contenuto di amido e delle diverse frazioni fibrose. La riduzione del contenuto proteico della dieta dal 17% al 14% ha permesso di contenere l'escrezione di azoto dell'azienda, senza modificare le prestazioni o lo stato di salute. Tuttavia, conigli alimentati con diete a maggior contenuto proteico e basso rapporto (amido+fibra solubile)/ADF hanno mostrato una maggiore suscettibilità ai disturbi digestivi. Questo risultata merita di essere approfondito anche perché è stato ottenuto con livelli proteici delle diete piuttosto bassi rispetto a quelli correntemente utilizzati in campo.

Effetto delle modalità di alimentazione

Il terzo e il quarto contributo sperimentale della tesi sono stati dedicati allo sviluppo di strategie di alimentazione che permettano un controllo adeguato del livello di ingestione nel periodo post-svezzamento, con l'obiettivo generale di aumentare l'efficienza globale dell'azienda. In entrambi i contributi, la restrizione alimentare non ha prodotto gli effetti attesi in termini di miglioramento dell'efficienza aziendale, e nemmeno in termini di salute o di prestazioni produttive. Nel terzo contributo, durante il post-svezzamento, la restrizione alimentare ha consentito di ridurre il numero di animali malati e morti, ma durante il successivo periodo di re-alimentazione le condizioni di salute sono peggiorate nei conigli in precedenza sottoposti a restrizione alimentare, e questo risultato è risultato tanto evidente quanto più severa era stata la restrizione alimentare e, soprattutto, quanto maggiore era stata la durata della restrizione alimentare. In effetti, durante i primi giorni di re-alimentazione sono stati osservati un notevole aumento ed una forte variabilità nel livello di ingestione alimentare che hanno contribuito a destabilizzare l'equilibrio a livello dell'apparato digerente dei conigli. Nel quarto contributo, la restrizione alimentare è stata effettuata sulla base di una curva teorica e progressiva di ingestione alimentare che è risultata relativamente facile da realizzare in pratica Inoltre, il graduale ritorno all'alimentazione *ad libitum* nella terza settimana ha evitato il verificarsi di sbalzi nei livelli di ingestione e le possibile conseguenze negative sullo stato di salute dei conigli. In entrambe le prove, grazie all'accrescimento compensativo nel periodo di re-alimentazione, l'effetto negativo sulle prestazioni osservato nella fase di restrizione è stato diluito nell'intero periodo sperimentale, di modo che alla macellazione le differenze di peso vivo finale fra i gruppi non sono risultate statisticamente significative. Questo risultato deve essere considerato tipico del sistema di produzione italiano, il cui mercato richiede che i conigli siano macellati a età e pesi maggiori rispetto agli altri mercati europei.

Summary

Introduction and objectives

Epizootic Rabbit Enteropathy (ERE) has become the main cause of losses in intensive rabbit farms: it is typical of post-weaning period and can be described as a severe form of mucoid enteropathy, complicated by secondary etiologic agents. The main solution to ERE are antibiotics, but the European rules limit the possibility of using them in preventive treatments. Moreover, due to the fast evolution of the disease, once ERE occurs and is recognized, the therapeutic use of antibiotics may often not be applied on time to control economic losses. In addition, the cost of the therapy has an important impact in rabbit production costs.

In the last decade, the studies intended to specify the nutritional requirements and feeding strategies in rabbits during weaning and post-weaning with special emphasis on the possibility of reducing the damages caused by ERE.

With regards to nutrients, both experimental studies and field results push towards the reduction of the use of high-insoluble fibre diets in growing rabbits, because they greatly limit growth performance, impair feed conversion and increase nitrogen excretion while sometimes do not avoid the need of using prophylactic and therapeutic drugs. The negative role of starch on the occurrence of digestive diseases has been somewhat put under discussion, whereas a positive effect of the most soluble fibre fractions has been outlines on both health and performance of growing rabbits when used in substitution of insoluble fibre or starch. Finally, both lack and excess of crude protein are critically considered because of their negative effects on caecal fermentative activity, microflora composition and lastly on rabbit health.

With regards to feeding management, feed restriction programs may be used also with some advantages on global farm efficiency as they increase diet utilization, modify the partition of body energy retention as protein instead of fat, and may reduce mortality and morbidity due to digestive problems. With these programs, especially in the early period of breeding, growth is controlled and limited during the restriction period but the compensatory growth during the following period of re-alimentation allows animals to recovery productive performance at the end of the rearing period. From the end of the ‘90s, feed restriction has been widely used in some European Countries (especially France) during the post-weaning period to control the mortality due to digestive disorders. Positive effects of this technique have been outlined since the spread-out of ERE in terms both of a better control of the disease and improvement of the global feed conversion.

The general objective of the present thesis was the development of nutritional strategies for improving feed efficiency and health status of growing rabbits. This goal was pursued by means of experimental activities with the specific objectives of: *i*) defining requirements of soluble fibre in relation to dietary insoluble fibre, starch and protein in rabbits during post-weaning and fattening (experiments 1 and 2); *ii*) measuring the effect of the rate and duration of feed restriction compared to an *ad libitum* feeding, in order to define the most effective restriction technique capable of maximizing performance and controlling digestive problems in growing and fattening rabbits (experiments 3 and 4).

The specific objectives have been pursued through the research activities in the four experiments of the doctoral thesis as summarized below:

Experiment 1. *Effect of increasing soluble fibre and starch and reducing protein in diets for growing rabbits.*

The effect increasing the (soluble fibre+starch)/ADF ratio by rising soluble fibre (18%, 20% and 22%) and starch (10%, 14% and 18%) at the expenses of insoluble fibre and of reducing dietary crude protein (17% to 15%) was evaluated on health, performance, caecal fermentative activity, gut mucosa traits, nitrogen excretion and carcass and meat quality of growing rabbits. At 36 d, 282 rabbits (951±31 g LW) of both genders from a hybrid line were divided into six experimental groups of 47 units and fed *ad libitum* with six diets formulated according to a 2 x 3 factorial arrangement, with three (soluble fibre+starch)/ADF ratio (0.6, 1.2 and 2.0) and two protein levels (17% and 15%). An *in vivo* digestibility trial was carried out on 72 animals (12 animals of both sexes per diet) from 49 to 52 days of age to evaluate the nutritive values of diets. At 79 days, the rabbits were slaughtered to evaluate carcass traits and meat quality.

The apparent digestibility of dry matter greatly and linearly increased from 50.8% to 60.5% and 71.5% (significant linear component of variance, $L<0.001$) with the raising of (starch+soluble fibre)/ADF ratio. The increase of (starch+soluble fibre)/ADF ratio did not affect the digestibility of crude protein and ether extract, while the digestibility of fibre and fibre fractions linearly and significantly increased ($L<0.001$). The digestibility of ADF increased quadratically ($Q<0.01$), that is by reducing lignification degree, cellulose is more and more sensible to digestive and fermentative processes, while hemicelluloses and soluble fibre showed a linear improvement in their digestive utilization as (starch+soluble fibre)/ADF ratio increased. The differences in formulation of diets, with the reduction in the inclusion rate of alfalfa meal in favour of energy concentrates containing starch (barley) and high-soluble fibre raw materials (dried beet pulp) accounted for the increase in the digestibility of dry matter and fibrous fractions, even if differences between treatments were larger than what expected.

The effect of the protein level was less important and limited to the higher digestibility of DM, gross energy and crude protein ($P<0.01$) in the diets with a higher protein content. Also digestibility of TDF and soluble fibre was higher in diets at 17% CP compared to diets at 15% ($P<0.05$): this result may be ascribed to the lower inclusion rate of alfalfa meal in favour of soybean and sunflower meal.

During the trial, mortality reached an average value of 8.5% and changed significantly with experimental factors. In fact it was significantly higher (15.8% *vs*. 4.5% and 4.9%; $P=0.02$) when the (starch+soluble fibre)/ADF ratio was 0.6 compared to diets with 1.2 and 2.0 ratios. Similarly, even if at a lower level of significance ($P=0.09$), the increase of (starch+soluble fibre)/ADF ratio reduced the sanitary risk (mortality+morbidity). Even if no significant effect of the protein level was measured, it is worthy to note that the administration of the diet highest in starch and soluble fibre and lowest in ADF content combined with the high level of protein produced the highest morbidity value (probability of the interaction = 0.08) due to digestive disorders of brief duration which occurred especially in the last 10 days of rearing before slaughter.

The administration of diets at increasing ratio (starch+soluble fibre)/ADF significantly and linearly increased the proportion of full (with its content) caecum ($L<0.01$) and of full gut ($L<0.001$) on live weight and, therefore, the rate of filling of the digestive tract. The higher availability of fermentable carbohydrates linearly decreased caecal pH (from 6.02 to 5.75, $L=0.06$) and increased total volatile fatty acids (VFA) content at caecum (from 63.0 to 78.5 mmol/L; $L=0.02$), with a contemporary reduction of the molar proportion of propionic acid (from 4.0% to 3.5% mol VFA; $L=0.04$). No significant effect of the protein level was measured. Only the level of N-ammonia was higher ($P=0.07$) in rabbits fed the diets with the highest protein level, which could be due to a higher ileal flux of protein to caecum. Ileal mucosa did not change with experimental factors, while at jejunum the increase of the (starch+soluble fibre)/ADF ratio reduced villi height ($L=0.06$) and the villi/crypth ratio ($L=0.03$). The increase of dietary protein increased villi height ($P=0.07$).

During the whole trial, daily nitrogen excretion in rabbits fed diets with high (starch+soluble fibre)/ADF ratio (1.2 and 2.0) was 80% and 60% of that measured in rabbits fed with 0.6 ratio. During the first period, nitrogen excretion tended to be lower when rabbits were fed diets with (starch+soluble fibre)/ADF ratio equal to 2.0. Nitrogen excretion diminished by about 15% when decreasing dietary protein from 17% to 15% and without differences between the first and the second period of growth.

Experiment 2. *Effect of a further increase of (starch+soluble fibre)/ADF ratio and decrease of protein level on diets for growing rabbits.*

The effect of the administration of diets with increasing (starch+soluble fibre)/ADF ratio (from 1.2 to 1.8 to 2.8) obtained by rising starch (from 14% to 20%) and soluble fibre (21% to 25%) levels and by decreasing ADF supply (21% to 13%), combined with two protein levels (14% and 15%) was assessed on health, growth performance, caecal fermentative activity, gut mucosa traits, nitrogen excretion and carcass and meat quality in growing rabbits. At 33 d, 306 rabbits (975±97 g LW) of both genders from a hybrid line with homogeneous weight were selected from healthy litters in a commercial farm. The animals were divided into six groups of 51 units each. The six experimental groups were then given six diets formulated according to a bi-factorial arrangement [3 (starch+soluble fibre)/ADF ratios x 2 protein levels] and fed *ad libitum* all over the trial. The *in vivo* digestibility trial was carried out on 72 animals from 54 to 58 days of age to evaluate the nutritive values of diets. The animals were slaughtered at 76 days of age to evaluate carcass traits and meat quality.

The digestibility of dry matter, energy and all other nutrients significantly and linearly increased with the increase of the (starch+soluble fibre)/ADF ratio and as a consequence of the higher inclusion rate of dried beet pulp and barley and the lower presence of alfalfa meal. The digestibility of insoluble fibre fractions (hemicelluloses and ADF) also increased. Only the digestibility of soluble fibre, which was always higher than 80%, was not affected.

The effect of the protein level, even if significant, was less important and resulted in a moderate increase of crude protein digestibility, which could be explained by the higher rate of protein coming from soybean meal rather than alfalfa in the diets with the higher protein content.

First symptoms of digestive disorders appeared one week after the start of the trial (low feed intake, diarrhoea, mucus) which could be clearly referred to ERE. On average, mortality stood around 22.6% without significant variations according to the (starch+soluble fibre)/ADF ratio. On the contrary, mortality was significantly higher (16.3% *vs*. 28.9%, P=0.01) in rabbits fed the diets with the highest protein content. As what concerns the effect of the changes in soluble and insoluble fibre and starch, the trend towards an increased morbidity (P=0.09) and, at similar mortality rates, the increase of the sanitary risk (P=0.10), may be explained by the reduction of ADF below 12% in the diets with the highest ratio (starch+soluble fibre)/ADF rather than with changes in soluble fibre fractions, which content slightly changes from 9-10% to 11-12%.

When the (starch+soluble fibre)/ADF ratio and the nutritive value of diets increased, growth rate and live weight did not change, whereas feed intake significantly and linearly decreased ($P<0.001$) both in the first and second period of the trial. As a consequence, feed conversion linearly improved (from 3.65 to 3.22 and 2.76; $P<0.001$) until values which were particularly favourable, especially for the (starch+soluble fibre)/ADF ratio equal to 2.8. The improvement of feed conversion was rather expected on the base of diets characteristics and the higher level of starch and soluble fibre in replacement of insoluble fibre.

The increase of CP level from 14% to 15% did not affect productive performance on the whole, whereas improved feed conversion during the first period ($P=0.07$) and increased growth and feed intake during the second period. The interaction between the two main experimental factors was never significant and, similarly, no effect of sex was recorded.

The administration of diets with a higher (starch+soluble fibre)/ADF ratio changed in a quadratic mode the incidence of the full stomach (quadratic component of variance, $P=0.02$) and the full gut ($P=0.07$) expressed on the base of live weight without effect on the filling of caecum. At caecum, the increase of (starch+soluble fibre)/ADF ratio significantly and linearly stimulated VFA production (52.1 to 61.9 mmol/L) which went side by side a reduction of pH value (5.90 to 5.67) and a strong decrease in N-ammonia level (4.85 to 1.93 mmol/L). Even if a lower rate of significance ($P<0.10$), the production of acetic acid (rising) and of propionic acid (decreasing) also changed.

The effect of the protein level was less important and represented by a reduction of the full stomach incidence ($P=0.03$) and a trend to an increased full caecum incidence ($P=0.09$), besides a reduction of caecal pH (5.87 to 5.70) when the protein level increased from 14% to 15%.

As what concerns the effect of the feeding treatments on intestinal mucosa traits, the administration of diets with increasing (starch+soluble fibre)/ADF ratio decreased in a non-linear way the villi height at jejunum (839 *vs.* 733 and 751 μm) and, as a consequence, the villi/crypths ratio (4.42 *vs.* 3.81 and 3.95). The effect of the experimental factors was hardly perceivable on growth performance and absent on slaughter results, apart from a significant and quadratic change of the muscle to bone ratio ($P=0.04$), which was higher in rabbits fed diets with high (starch+soluble fibre)/ADF ratio.

Experiment 3. *Effect of the level and duration of feed restriction in growing rabbits.*

The effect of the level and duration of feed restriction was measured on health, growth performance, caecal fermentative activity, body tissue balance and carcass and meat quality of growing rabbits.

At 32 d, 264 rabbits (912±99 g LW) of both genders from a hybrid line were selected from multiparous does (3-6 kindling) with healthy litters and homogeneous kit weight. The rabbits were divided in six experimental groups of 47 units, homogeneous in average weight and variability, and submitted to different feeding restriction levels according to a bi-factorial arrangement, with three restriction levels (*ad libitum*, 90% and 80% of *ad libitum*) during two different periods (2 weeks *vs.* 3 weeks after weaning).

The trial lasted 6 weeks and was divided into two periods:

a) post-weaning period during which rabbits were fed a weaning diet (S) *ad libitum* (R100) or at a restricted level at 90% (R90) or 80% (R80) during 2 weeks (T2) or 3 weeks (T3);

b) fattening period, during which rabbits were fed a fattening diet (I) always *ad libitum* and during 4 weeks (groups T2) or 3 weeks (groups T3).

Two in vivo digestibility trials were carried out on 42 restricted animals (14 per restriction level) from 49 to 53 days of age for diet S and on 36 animals (12 per restriction level) from 56 to 60 days of age for diet I when all rabbits were fed *ad libitum*. The rabbits were slaughtered at 75 days of age to evaluate carcass traits and meat quality.

The digestibility of dry matter, energy and nutrients changed in the different experimental groups according the restriction level and the diet (S or I, 9.8 *vs.* 10.6 MJ/kg respectively). In details, the digestibility of dry matter (56.6% *vs.* 58.6% *vs.* 60.3%, $P<0.001$) and gross energy (56.6% *vs.* 58.4% *vs.* 60.0%, $P<0.001$) significantly increased with the restriction level, in rabbits fed *ad libitum* *vs.* 90% *vs.* 80%. Also digestibility of protein and ether extract changed accordingly. Only the digestibility of starch, even if in very narrow interval, decreased when the restriction level increased ($P<0.001$).

Health of rabbits impaired during the second and third week of trial, with diarrhoea and mucus in several rabbits of all treatments. The antibiotics included in the diet S were therefore considered insufficient to control ERE and an additional antibiotic treatment in water was offered during the third week of trial to maintain the sanitary status under control, with morbidity and mortality within acceptable values. During the entire trial, mortality averaged 9.8% and morbidity 41.9%. The analysis of the effect of main factor (level and duration of feed restriction) on health evidenced a significant increase of mortality (from 5.7% to 13.8%; $P=0.03$) in rabbits restricted for the longer period (2 *vs.* 3 weeks), while the restriction level did not affect results.

Live weight of rabbits, similar at the beginning of the trial, was significantly lower in rabbits submitted to the most severe restriction (R80) both after two weeks of restriction (at 47 days, 1646 and 1592 g *vs.* 1507 g, P<0.001) and after three weeks (at 54 days, 2002 and 1979 g *vs.* 1883 g, P<0.001; in this case the average values of R100, R90 and R80 groups include all animals, both those half still restricted and those half free of feeding). At the end of the experimental period, at 75 days of age, however, differences in live weights among the three groups were reduced and not statistically significant.

In rabbits submitted to the most intensive restriction level, empty body weight gain was lower (P=0.03) than in the others rabbits groups and, as a consequence, the empty body gains in water, protein and ash were lower. The gain of lipid was unexpected: the lowest values (220-240 g) were recorded both in the rabbits fed the diet S *ad libitum* for 2 weeks (R100-T2) and in those restricted for 3 weeks (R90-T3 and R80-T3) (significant interaction P<0.001).

The most severe feed restriction significantly reduced the amount of ingested, retained and excreted nitrogen compared to not restricted rabbits or rabbits restricted at 90%. The amount of ingested nitrogen decreased by 6% (P<0.01), that of retained nitrogen by 4% (P<0.10) and nitrogen excretion diminished by 8% (P<0.001) in most severe feed restriction compared to *ad libitum* rabbits.

As what concerns the effect of the restriction period, an increase from 2 to 3 weeks impaired nitrogen balance with a significant increase of nitrogen ingestion (+4% in rabbits T3 compared to rabbits T2, P=0.02) and nitrogen excretion (+6% in rabbits T3 compared to rabbits T2, P<0.01), in front of a not significant change in the amount of retained nitrogen.

As for performance, also slaughter results were scarcely affected by the level or period of feed restriction.

Experiment 4. *Effect of feeding plan and feed restriction program in growing rabbits.*

The use of feeding plans based on diets at high digestible energy concentration (DE diet A: 11.1 MJ/kg) or moderate digestible energy concentration (DE diet B: 10.7 MJ/kg) during the whole productive cycle (A-A; B-B) or in a sequence (B-A) was tested together with the use or not of a progressive feed restriction program which started from 80% of *ad libitum* and linearly reached the voluntary feed intake level after 3 weeks in order to avoid sudden changes in feed intake level during re-alimentation period. The effect of the above mentioned feeding systems were evaluated on health, growth performance, caecal fermentative activity, body tissue balance and carcass and meat quality of growing rabbits.

At 34 d, 300 rabbits (953±110 g LW) of both genders from a hybrid line were selected from multiparous does (3-6 kindling) with healthy litters and homogeneous kit weight. The rabbits were divided in six experimental groups of 48 units, homogeneous in average weight and variability, and submitted to three feeding plans per two restriction levels. The six rabbits from the same litter were assigned one per group to distribute the maternal effect over the treatments. Two diets (diet A and diet B) at a different energy concentration and with the same digestible protein (DP)/DE ratio (theoretical ratio: 11 g DP/MJ DE) were used. The feeding plans changed according to the diets administered during the first and second period of growth: plan A-A, rabbits were fed diet A during the whole trial (5 weeks); plan B-A, rabbits were fed diet B during the first three weeks and diet A during the last two weeks; plan B-B, rabbits were fed diet B during the whole trial.

Within the three feeding plans, rabbits were fed *ad libitum* during the whole trial; or restricted on average at 90% during the first three weeks of trial.

The trial lasted 5 weeks and was divided into two periods:

a) first post-weaning, period (3 weeks), during which two groups of animals were fed with diets A and the remaining four groups with diet B. Within each feeding treatment, half of animals was restricted (R) and half was fed *ad libitum* (L);

b) second fattening period (2 weeks), during which four out of the six experimental groups received diet A and the remaining two groups were fed diet B. All rabbits were fed *ad libitum* during this period (L).

The in vivo digestibility trial was carried out on 48 animals (12 rabbits fed *ad libitum* with diet A, 12 rabbits restricted fed with diet A, 12 rabbits fed *ad libitum* with diet B and 12 rabbits restricted fed with diet B) from 47 to 51 days of age to evaluate the nutritive values of diets. The rabbits were slaughtered at 70 days of age to assess the carcass traits and meat quality.

Digestibility of nutrients was scarcely affected by feed restriction program, whereas digestibility and nutritive value changed greatly with the diet: digestibility of dry matter and energy, protein and fibre was significantly higher for diet A than diet B.

As what concerns the effect of experimental treatments, mortality and sanitary risk did not change either with the feeding system or with the feeding plan, while morbidity was significantly affected by both factors, with the lowest values for rabbits submitted to the restriction program and the B-B feeding plan.

During the first week, growth rate was significantly impaired by feeding restriction (53.6 and 46.8 g/d in rabbits fed *ad libitum* and restricted, $P<0.001$) which depended on a 13% lower feed intake (107 *vs.* 93 g/d). During the second week of trial, the appearance of digestive disorders reduced feed intake in rabbits fed *ad libitum* so that differences in feed intake with the restricted groups decreased (134 *vs.* 129 g/d, $P=0.05$, which corresponded to a real restriction of 4%). For this reason, during the second week growth rate was similar among the two groups, while during the third week rabbits fed *ad libitum* showed a similar feed intake (151 *vs.* 149 g/d, P>0.10) and even a lower growth rate compared to restricted rabbits (48.3 *vs.* 51.0 g/d, P=0.05). During the fourth and fifth week, nor growth rates nor feed intake differed between the two groups. As a consequence, growth performance in the whole period did not change with the experimental treatments. Neither the feed restriction program, *ad libitum* or restricted, nor the feeding plan affected slaughter results.

At 55 days of age, the protein content of empty body differed significantly according to the feed restriction program and was higher in rabbits fed *ad libitum* compared to those restricted (19.2% *vs.* 18.9%, respectively; P<0.05). At 69 days of age, after two weeks of re-alimentation of previously restricted rabbits, no significant effects of the treatments were observed on empty body composition. Restricted rabbits showed a lower empty body weight at 55 days, and the composition of the empty body gain during the first period was characterized by lower gains of protein, lipid, ash and gross energy. In the same period, the different diets only affected empty body gain of ash. In the second period, when all rabbits were fed *ad libitum*, the effect of the feed restriction program was still evident, but the restricted rabbits were favoured. In details, between 55 and 69 days of age, the EB of previously restricted rabbits gained more protein (121 *vs.* 126 g), lipid (91 *vs.* 97 g), ash (18 *vs.* 19 g) and energy (67 *vs.* 72 MJ) than those fed *ad libitum*, confirming the existence of a compensative growth.

Feed restriction significantly reduced body N at 55 days of age, without residual effects at 69 days of age. During the first period, feed restriction significantly reduced feed intake, ingested N as well as retained and excreted N. During the second period, a residual effect of feed restriction was measured only on retained nitrogen which was higher in previously restricted rabbits. During the whole trial, changes in retained N were not significant. The feeding plan did not affect body N at 55 or 69 days of age, but N excretion was lower in rabbits submitted to the restriction program and the B-B feeding plan.

Conclusions

Modulating nutrients in the diets for growing rabbits

The use of diets with increasing levels of both starch and soluble fibre in replacement of insoluble fibre, tested in the experiments 1 and 2, increased diet utilization, especially of fibrous fractions, and nutritive value. Growth performance, as live weight and weight gain, was not influenced, whereas feed intake decreased and feed conversion greatly improved. Health status was improved only in one of the two experiments and due to the change of several constituents of the diets, further studies will need to confirm the positive effect of the substitution of insoluble fibre by starch and soluble fibre. Thanks to the lower ingestion levels, nitrogen excretion deeply decreased with increasing dietary (starch+soluble fibre)/ADF ratio. Caecal fermentations were more intense and feed digestibility improved both because of the starch increase and the insoluble fibre reduction, and because fibre had a different structure, that is, fibre was less lignified and more available for the enzymatic and bacterial attack. At the final commercial slaughtering, the increase of the above ratio stimulated live weight, but contemporary reduced dressing percentage because of the higher gut incidence. This last result, however, was counterbalanced by a significant increase of carcass muscularity as proved by the higher meat to bone ratio of hind leg.

On the base of the results of the two experiments and the discussion above, it is possible to formulate diets for post-weaning and fattening rabbits capable of greatly improving feed conversion compared to actual standards, maintaining growth and slaughter results. We can conclude that the contemporary increase of starch (until 20%) and soluble fibre (until 12%) has positive effects on digestive physiology, health, and feed efficiency of rabbits without impairing slaughter results and carcass or meat quality. However, in presence of epizootic rabbit enteropathy, the contemporary decrease of ADF below 12% may negatively affect digestive health of growing rabbits.

Changes in protein level had less important effects, even if sometimes significant, compared to changes in dietary starch and fibrous fractions concentration. The reduction of CP from 17% to 14% permitted to reduce farm nitrogen excretion, while did not affect rabbit performance or health. However, rabbits fed with low dietary (starch+soluble fibre)/ADF presented a higher susceptibility to digestive disorders when CP increased from 14% to 15%. This result deserves to be further investigated also because it takes into account rather low levels of dietary protein compared to those currently used in field practice.

Modulating feeding strategies in growing rabbits

In the conditions of both experiments 3 and 4, feed restriction did not produce the expected results on improved farm feed efficiency, in terms either of health or performance of growing rabbits. In the experiment 3, during post-weaning period, feed restriction permitted to reduce the number of ill and dead rabbits, but during re-alimentation phase the health condition worsened in previously restricted rabbits, and this result was more evident as more severe the restriction was and, especially, as longer the period of restriction was. In fact, during the first days of re-alimentation a great increase and variability in feed intake was recorded which contributed to challenge digestive equilibrium of rabbits.

In the experiment 4, feed restriction realised on the base of a theoretical progressive feed intake curve was easy to be applied and the gradual turn to a voluntary intake during the third week of trial prevented the occurrence of great variations in feed intake levels and possible negative consequences on health.

In both trials, thanks to the compensatory growth during the re-alimentation period, the negative effect on performance occurred during the restriction phase was diluted and the end of the rearing period the differences in final live weight and body composition among experimental groups were not significant. This result is to be considered typical of the Italian production system, which market requires that rabbits are slaughtered at age and weights higher than the other European markets.

Introduction

Rabbit physiology and feeding

The rabbit is an herbivore monogastric which selects concentrates; in wildness, its diet consists of those plant parts low in fibre, high in digestible protein and carbohydrates, such as young leaves, shoots and tubers (Xiccato and Trocino, 2008; Carabaño *et al.*, 2010).

Like other herbivores, in its digestive tract, the rabbit holds a microbial population of symbionts bacteria, which ferment the substances indigestible to the animal, and protozoa localized especially at caecum, the main site of fermentation in rabbits. The real composition of this symbiotic population is almost unknown (Gidenne *et al*., 2010).

The digestive physiology of the rabbit is very similar to the other monogastrics, but is characterized by changes in anatomy and by behavioural adaptations which permits the mechanism of "caecotrophy". It consists in the re-ingestion of caecal content, excreted as "soft faeces" or caecotrophes. This mechanism permits to optimize the utilization of food nutrients since less digestible constituents of the diet are quickly excreted through the hard faeces; soft faeces, enriched with vitamins and proteins of microbial origin, are re-ingested and subsequently digested; through caecal mucosa, volatile fatty acids (VFAs) produced by microbial caecal fermentation may be absorbed.

The products of fermentation of the caecal microflora consist of VFAs and ammonia, derived from the fermentation of carbohydrates and proteins, respectively. The composition of the VFAs produced in rabbit caecum is characteristic of the species, with a predominance of acetic acid (C2) (70-85% VFAs), followed by butyric (C4) (8-20%), propionic (C3) (3-10%) and caprylic acids (C5) (0.5-1%). The proportions of the different fatty acids and the total level of VFAs change with animal age and during the day according to caecotrophy occurrence. Moreover, the diet, depending on its composition, may modify the fermentation patterns and the composition of the caecal microflora.

To guarantee the caecotrophy and the normal digestive physiology, the rabbit needs a specific dietary supply of fibre to ensure an adequate food transit and efficient intestinal peristalsis (Gidenne *et al*., 2010).

General criteria for feeding of growing in commercial farms

The energy system, expression of nutritional value of diets and raw materials and energy requirements, adopted in rabbits is based on digestible energy (DE) (Xiccato and Trocino, 2010a). Food ingestion is conditioned by the dietary energy concentration. The threshold below which feed intake is regulated by a physical mechanism, that is the repletion of the digestive tract, is approximately 9.5 MJ DE/kg. Above this threshold, on the contrary, appetite in rabbits is mostly regulated by a chemiostatic mechanism: the level of glucose in blood acts on hypothalamic receptors (satiety centre) and reduces feed intake above certain concentrations. In other words, when increasing dietary energy concentration above 9.5 MJ DE/kg of diet, feed intake of rabbits decreases accordingly to the increased energy intake and consistently with the energy requirements. The energy requirements change with body weight (depending on age, sex, breed), vital and productive functions (maintenance, growth, lactation and pregnancy,) and environment (temperature, humidity, air speed).

In rabbit farms, animals are usually fed *ad libitum* and different diets are fed according to the age and corresponding nutrient requirements of animals. At least two different diets are fed for the post-weaning and the fattening period. The post-weaning period begins with the separation of litter from the mother at about 30-35 days of age and ends around 45-50 days of age, depending on the reproductive rhythm and the organization of the farm. This period is the most critical period for the breeding success and for the health of animals, which are more susceptible to the digestive diseases at this age. The rapid development of the digestive tract, the colonization and development of a new caecal microbial flora, the start of the caecotrophy mechanism and, finally, the stress caused by the separation from the mother are the main causes of this susceptibility.

Diets used during post-weaning are poor in energy (9.2-9.6 MJ DE/kg), high in fibre (ADF>18-19%, ADL>4.5-5%) and contain less starch (<12%) (Table 1). Raw materials rich in soluble and more digestible fibre fractions, such as beet pulp, may be included in post-weaning diets, whereas low inclusion rates of high-starch raw materials, like cereals, are preferably used (Table 1). To ensure an optimal amino acids balance, protein sources like soybean meal and/or sunflower are included at rates of 10-15% of the diet in order to reach a dietary crude protein around 15.0-16.0%. During post-weaning, feed intake ranges from 80 to 140 g/d depending on DE concentration, age of animals, genetic type and environmental conditions.

At about 45-55 days of age, growing and fattening diets may be fed with increased energy content (10-10.5 MJ/kg) and decreased protein content (14.5-15.5%) compared to post-weaning diets (Table 1). These diets are formulated to maintain the feed conversion index at acceptable values and to permit a sufficient deposition of fat in carcasses. The inclusion rates of cereals and wheat bran increases at the expenses of alfalfa and protein concentrates. During fattening, feed intake ranges from 130 to 200 g/d.

Table 1. Formulation and chemical composition of diets for growing rabbits (Xiccato and Trocino, 2008).

	Weaning and post-weaning diets	Growing and fattening diets	Finishing diets
Raw materials, %			
Alfalfa meal (15-16% CP)	33.0	30.0	26.0
Barley meal	10.0	20.0	28.0
Wheat straw	4.0	0.0	0.0
Dried beet pulp	10.0	14.0	10.0
Citrus pulp	5.0	0.0	0.0
Wheat bran	18.0	20.0	22.0
Soybean meal (44% CP)	9.0	6.0	5.0
Sunflower meal (30% CP)	6.0	6.0	5.0
Soybean oil	1.5	1.0	1.0
Molasses	1.5	1.5	1.5
Calcium carbonate	0.0	0.0	0.3
Dicalcium phosphate	1.0	0.5	0.2
Vitamin and mineral premix	1.0	1.0	1.0
Chemical composition			
Dry matter, %	88.5	88.7	88.5
Crude protein, %	16.0	15.5	14.9
Ether extract, %	3.9	3.4	3.4
Crude fibre, %	16.9	15.0	13.5
NDF, %	35.0	33.4	31.7
ADF, %	20.7	18.4	16.6
ADL,%	4.3	3.9	3.6
Starch, %	8.5	14.0	18.5
Lysine, %	0.74	0.7	0.66
Methionine+cystine, %	0.51	0.51	0.50
Calcium, %	0.99	0.76	0.72
Phosphorus, %	0.63	0.57	0.53
Digestible energy (DE), MJ/kg	9.8	10.2	10.5
Digestible protein/DE ratio, g/MJ	11.4	10.6	9.9

During the last week of rearing before slaughter, a third type of diet, a high-energy finishing diet, is often used, which does not contain coccidiostatic drug and presents increased levels of starch and low digestible protein/digestible energy ratio.

Rearing efficiency and feed conversion

Feeding plays an important role in modern and intensive rearing systems because formulation of diets must ensure the correct functioning of digestive physiology; it must fully satisfy nutrients requirements and provide a good quality product at a convenient economic price. In fact, the costs of feeding represent the major part of farm budget, about 60-65% of total costs (Maertens, 2010).

Farm efficiency may be measured by the global feed conversion index (FRC), which is calculated as the ratio between the total quantity of consumed feed and the total weight of produced rabbits in the farm. Feed efficiency depends on the results of both reproduction and fattening sectors. A global FCR around 3 is expected in an efficient commercial intensive farm when rabbits are slaughtered within 2.3-2.5 kg live weight (Maertens, 2009). At higher slaughter live weights (2.5-2.8 kg), FCR are likely around 3.7-3.8 (Xiccato and Trocino, 2007, 2010b; Xiccato *et al*., 2007).

In Europe, the average FCR in French farms, Spanish and Italian is equal to 3.60, 3.63 and 3.82 respectively (Lebas, 2007; Xiccato *et al.*, 2007, Rosell and González, 2007) and the greatest value measured in Italian farms is a consequence of the higher slaughter weights of rabbits compared to the other Countries. In fact, the feed conversion from weaning to slaughter, at the same dietary energy supply, impairs with age with a perfect trend (R^2=0.99) described by a cubic equation and with an increment in FCR that is more pronounced in the last period of fattening (Xiccato and Trocino, 2010b). Therefore, when the rabbits are slaughtered later and at heavier weights, the conversion of the whole fattening period is negatively affected.

The dietary DE concentration directly influences feed intake and, consequently, FCR. In fact, dietary DE explains a great part of the variability of feed intake (R^2=0.75) and feed conversion (R^2=0.74) in growing rabbits from weaning to 75-79 days of age fed with diets ranging in DE from 8.0 to 12.0 MJ/kg (Figure 1) (Xiccato and Trocino, 2010b).

According to equations in Figure 1, increasing DE content by 1 MJ/kg of feed reduces feed consumption by 12 g/d and feed conversion by 0.29 points. These results agree with those of Maertens (2009), according to whom increasing dietary DE by 1 MJ/kg impairs FRC by 0.30-0.40 points. Similar correlations (R^2=0.65) between dietary DE and feed intake were found by Gidenne and Lebas (2005).

To evaluate farm global conversion, mortality and reproduction results must be taken into account, which depend on farm sanitary status and/or management and reproductive performances (e.g., reproductive rate, fertility, reproductive career, etc.) (Xiccato *et al*., 2007; Rebollar *et al*., 2009).

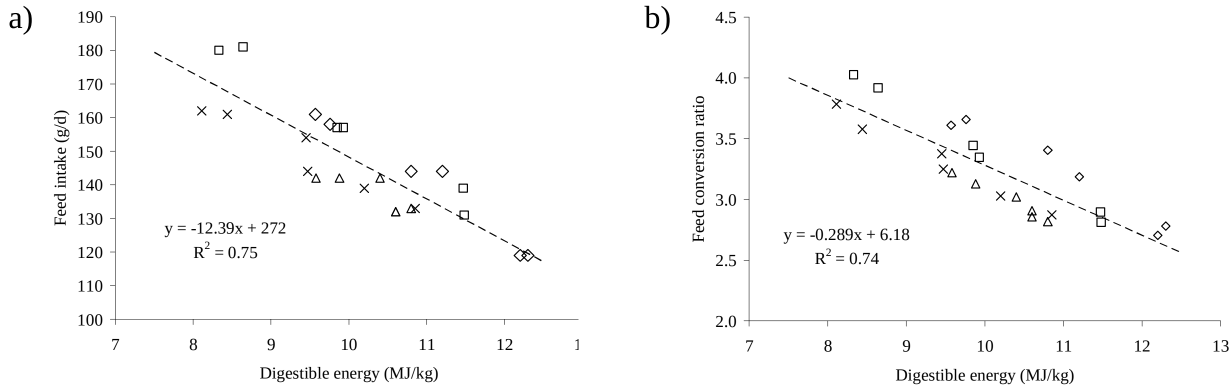


Figure 1. Relationship between DE concentration of diets and feed intake (a) and feed conversion ratio (b) in growing rabbits: data from four studies on rabbits caged individually and reared from weaning (32-34 d) until slaughter (75-79 d) (Xiccato and Trocino, 2010b).

Maertens (2009) also showed that to reduce FCR until about 3.0 in a commercial farm with 10% of mortality in the fattening sector, the number of weaned young/doe/year must reach 62 units (Table 2).

Table 2. Farm feed conversion according to post-weaning mortality and reproduction efficiency (Maertens, 2009).

Mortality (%)	N° of weaned young/doe/year		
fattening period	52	57	62
0	3.31	3.03	2.79
5	3.59	3.27	2.93
10	3.79	3.45	3.09
15	4.01	3.66	3.27

Digestive disorders in commercial farm

Epizootic Rabbit Enteropathy (ERE) and digestive disorders

In the current commercial intensive systems, several sanitary problems which were previously affecting rabbit farms, like salmonellosis, listeriosis, pseudotuberculosis and coccidiosis, have been overcome thanks to the improvement of hygienic conditions and the optimized pharmacological prophylaxis.

The mortality around weaning (20 to 35 days of age) and during post-weaning period (until 40-45 days of age), however, is often high due to digestive disorders, that can be favoured by immaturity of the gastrointestinal tract of the rabbit especially in presence of an unbalanced diet.

Peeters *et al.* (1992) classified the enteric diseases of rabbits in four classes:

- multifactorial enteritis: provoked by agents with moderate pathogenicity (some strains of *Escherichia coli* and *Bacillus piliformi*); they cause 5% to 20% mortality;

- specific enteritis: due to high pathogenic agents (enteropathogenic strains of *E. coli*, *Eimeria piriformis*, *Eimeria intestinalis*, *Eimeria flavescens*). They appear suddenly and may cause more than 30% of mortality;
- iota-enterotoxaemia: induced by an over-proliferation of *Clostridium spiroforme* as a result of caecal dismicrobism with different aetiology;
- sub-clinic enteritis: they begin with a worsening of feed conversion and, occasionally, with diarrhoea-related events.

Stress events during rearing, handling, age and weight of animals at weaning, environmental conditions may predispose rabbits to digestive disorders and enteropathies.

The classification made by Peeters *et al*. (1992) has been overcome by the appearance of Epizootic Rabbit Enteropathy (ERE). This disease has become the main cause of losses in intensive rabbit farms: it is characteristic of post-weaning period and can be described as a severe form of mucoid enteropathy, complicated by secondary etiologic agents that, often, make difficult to interpret the symptoms (Marlier *et al.*, 2003; Rosell, 2003). ERE first appeared in 1997 (Licois, 2004) and spread all over Europe very quickly.

In acute cases, mortality occurs within the first two days, but diarrhoea and mucus are not present. In this case, animals present caecal impaction and, sometimes, abdominal bloating. When the disease prolongs during more days, usually 1-2 weeks, the animals may experience a remission of symptoms, but remain weak and susceptible to new infections. The first symptoms are a drastic reduction of food and water intake, leading to fast dehydration. Then, very liquid diarrhoea and fluid mucus may appear, flowing and generally transparent. Stomach and caecum appear distended and the *pilorus* blocked. The stomach content may be liquid or compact and its pH, in presence of ERE, very acid (Pérez de Rozas *et al.*, 2005). Generally, at necropsy, typical lesions and inflammation process are not observed and, for this reason, it is better to refer to an enteropathy rather than enteritis or enterocolitis (Licois *et al.*, 2005). Histological observations also show major villus destruction and loss of epithelial cells both at ileum and jejunum (Licois *et al.*, 2005; Dewrée *et al.*, 2007; Chamorro *et al.*, 2010).

No etiological agent responsible for ERE is yet recognized and a multifactorial pattern is generally considered the cause for its occurrence. The agents identified as concurrent to the disease are recognized among infectious causes, unbalanced diets, improper antibiotics treatments and environmental conditions. The importance of *Clostridium perfringens* has been demonstrated in the manifestation of ERE (Licois *et al.*, 2003; Pérez de Rozas *et al.*, 2005; Marlier *et al.*, 2006; Szalo *et al.*, 2007; Carabaño *et al.*, 2008, 2009; Romero *et al.*, 2009), even though the ERE occurrence and expression are difficult to be explained only by the presence of this pathogen. Basically, when ERE occurs, a dysbiosis is present and pathogens over-proliferate. Over-proliferation of *C. perfringens* results in the production of nefro- and epato-toxins that could explain the characteristic symptoms of ERE (Peréz de Rozas *et al.*, 2005).

The main solution to ERE is the use of antibiotics, but the European rules limit the possibility of using them in preventive treatments. Moreover, due to the fast evolution of the disease, once ERE occurs and is recognized, the therapeutic use of antibiotics may often not be applied on time to control economic losses (Xiccato *et al.*, 2008). In addition, the cost of the therapy has an important impact in rabbit production costs.

Rearing conditions may favour digestive diseases, affecting the stress level of the animal for management (e.g., early weaning, intensive rhythms of breeding, high rearing density) and environmental conditions (e.g., temperature, humidity, gas concentration, air speed). On the other hand, also nutrition plays an important role in the development and activity of microbial flora, offering a different substrate to bacteria in the terminal ileum and caecum (Gidenne *et al.*, 2003).

The intestinal barrier

The saprophyte intestinal bacteria and the defensive mechanisms of the animal may prevent or reduce the development of pathogens and the appearance of digestive diseases. Such defensive mechanisms are present in the rabbit (Carabaño *et al.*, 2005, 2006) like in other species (swine and poultry) including humans. Feeding strategies may favour mechanisms of competitive exclusion among the bacteria as well as promote the development of intestinal barrier mechanisms.

The competitive exclusion permits a non pathogen species to predominate over pathogens and may be achieved by different ways. In fact, bacteria develop differently according to the type of substrate; they have a different capacity of colonizing the mucosa; some substances of the bacteria metabolism (short chain fatty acids, sulphuric acid, non-conjugated biliary acids and bacteriostatics) may inhibit the development of other pathogenic species (Hampson *et al.*, 2001).

The intestinal barrier plays a fundamental role when the pathogen is already present at a gut level. The defensive mechanism of the barrier relies on limiting the colonization of the mucosa and the translocation of bacteria and their toxins through the mucosa, whereas most pathogens of gut must come into contact with the mucosa to express their activity.

The intestinal barrier starts to work at level of the lumen by means of acidification and protecting the epithelium thanks to a mucus layer produced by the Goblet cells.

Once the protection of mucus is overhauled by pathogens and the pathogens reach the intestinal mucosa, then the gut associated lymphoid tissue (GALT) is activated. Firstly, a non-specific activation provokes transitory inflammatory processes; then, specific immunoglobulin is synthesized.

The conditions of the intestinal mucosa bias both the pathogen bacteria attacks both the digestive functions that is nutrient digestion, absorbance, and metabolism. In young animals, including rabbits, the immaturity of the intestinal mucosa and the damages to mucosa provoked by the change of diet (from liquid to solid one) are the reasons for which animals are more sensible to digestive diseases. Presently, feeding strategies are recognized to play an important role in preventing damages to mucosa as well as favouring mechanisms to repair the mucosa, by supplying the necessary nutrients.

How diet composition may control digestive disorders in rabbit

Role of starch

Starch is largely used in rabbit feeding to provide energy to both growing and reproducing rabbits. The optimal content of starch in diets for weaning rabbits ranges from 10% to 15% while may overpass 15% in the last phases of fattening (De Blas and Mateos, 2010).

The use of high-starch diets is associated with the appearance of digestive disorders during weaning and post-weaning since long time (Blas and Gidenne, 2010). This opinion is based on the theory of Cheeke (1987), according to which high-starch low-fibre diets may provoke an excessive flux of starch at caecum. In young animals, the low gut amylase activity favours this trend. At caecum, starch is fermented by amilolytic bacteria, VFAs production quickly increases and pH (5.0-5.5) decreases. These conditions should favour *Clostridium spiroforme* proliferation and the excess of glucose is used to produce an iota-like toxin, which causes enteritis and diarrhoea.

In the last decades, the spread of epizootic rabbit enteropathy (ERE) and the remarkable impact on mortality have highlighted the negative role of starch on the health status of rabbits, not only in the post-weaning, but also in the period of fattening and finishing (Gidenne and Licois, 2005). As consequence of the reduction of starch level in feed and the decreased of energy concentration in diets, the total efficiency index of production in rabbit farms impaired (Maertens, 2009).

There is a wide bibliography relative to the effects of starch on the performance of young rabbits and their health status even if the relationship between starch level and mortality is not fully demonstrated. Rather it was highlighted the key role of the relationship between the starch and insoluble fibre level (Blas and Gidenne, 2010). On the same time, the study of this relationship is often complex due to the difficulty to separate the effect of main nutrients (starch, soluble and insoluble fibre fractions) that change simultaneously in the experimental diets.

According to Gutiérrez *et al.* (2002), performance improved and mortality decreased in young rabbits weaned at 25 days feed with diets with 22.6% DM of starch and 33.6% DM of NDF compared to rabbits fed diets with 16.8% DM of starch and 40.6% DM of NDF. Debray *et al*. (2002) observed higher feed intake (+13%) and litter weight at weaning (+6%) and lower mortality (0.8% *vs.* 5.2%) in young rabbits from 25 to 32 days fed a diet with 17% starch compared to rabbits fed a diet with 14% starch.

In presence of ERE, an increasing of mortality of growing rabbits was observed when starch levels (12%, 15% and 18%) and starch to ADF ratio (0.71, 0.88 and 1.16) increased (Carraro *et al.,* 2007). On the contrary, in the absence of ERE and with similar diets, the health status was not influenced (Xiccato *et al.*, 2002). Also Trocino *et al.* (2011) did not find significant effects on health of animals fed with diets characterized by a starch to ADF ratio varying from 0.26 to 0.76.

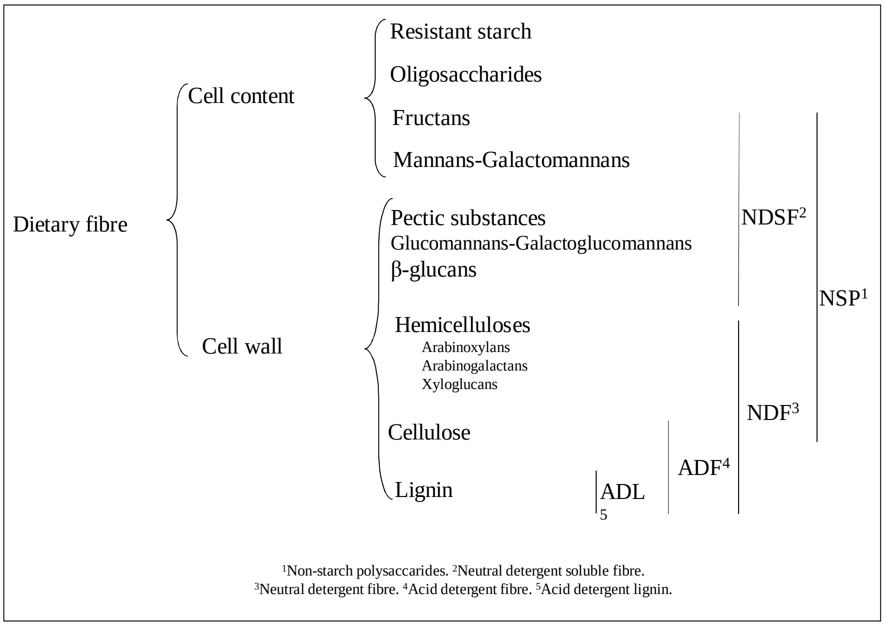
At caecum, high-starch low-fibre diets increased butyrate and valerate production rather than acetate (Parigi Bini *et al.*, 1990; Gidenne and Bellier, 2000; Nicodemus *et al.*, 2003 e 2004; Blas and Gidenne, 2010). The studies about the effects of starch and its ratio with other fibre fractions on gut mucosa traits are limited and not consistent among them (Gutiérrez *et al.*, 2002; Carraro *et al.*, 2007; Álvarez *et al.*, 2007).

Role of fibre

Definition of fibre and analytical methods for its determination

The term “dietary fibre” derived from human nutrition and extended to all mammals. Initially the definition of dietary fibre only referred to hemicelluloses, cellulose and lignin; later it was extended to the structural framework of the plant cell walls that resists to hydrolysis by human digestive enzymes (Trowell, 1972, 1978). Fibre is indigestible in all animals, because their enzymes are not able to break the bonds of polysaccharides of cell wall. Only the intestinal microorganisms can degrade the fibre to a different extent depending on the chemical structure of fibre fractions and make it available to the organism.

Chemically, *fibre* is the structural part of vegetable cells (cell walls) and is formed of cellulose micro fibrils (the backbone) embedded in a matrix composed of a lignin network (phenyl propane units) which cements other matrix polysaccharides (plus some glycoprotein), such as hemicelluloses (arabinoxylans, xyloglucans) and pectins (Gidenne, 2003). Therefore, fibre includes many different chemical constituents of vegetal origin which have different effects and roles on digestive physiology and different nutritive values (Figure 2).



[1]Non-starch polysaccarides. [2]Neutral detergent soluble fibre. [3]Neutral detergent fibre. [4]Acid detergent fibre. [5]Acid detergent lignin.

Figure 2. Major constituents of dietary fibre (Hall, 2003; García *et al.*, 2009).

Lignin is the only non-saccharidic polymer of the cell wall and is made up of three monolignol monomers, methoxylated to various degrees: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Lignin networks tend to fix the other polymers in place, exclude water and make the cell wall more rigid and resistant against various agents, such as bacterial enzymes. *Cellulose* is the most abundant structural polysaccharide of the plant cell walls and is a homopolymer formed from linear chains of β[1-4] linked D-glucose units. Cellulose is soluble in strong acid solutions (i.e., 72% sulphuric acid). *Hemicelluloses* are a group of several polysaccharides with a lower degree of polymerization than cellulose. They have a β[1-4] linked backbone of xylose, mannose or glucose residues that can form extensive hydrogen bonds with cellulose. *Pectic substances* correspond to several classes of polymers, including pectins (rhamnogalacturonan backbone and side chains of arabinose and galactose) and neutral polysaccharides (arabinans, galactans, arabinogalactans). Pectins of the middle lamellae serve as an adhesive in plant tissue, cementing plant cells together. Beet pulp and citrus pulp are among the richest sources of pectins (until 25% in beet pulps).

Several methods are available for analyzing the concentration of fibre and fibre fractions, but the chemicals effectively separated by each method may include constituents with different roles in digestive physiology (Mertens, 2003; Udén, 2005).

The Weende method (AOAC, 1980) measures the “crude fibre” gravimetrically by means of acid and basic sequential alkaline hydrolyses and hemicelluloses and pectins are not included. It measures a variable quote of structural compounds of plant cells (30-100% cellulose, 16-90% lignin and 14-20% pentosanes, depending on the raw materials).

The Van Soest method (Robertson and Van Soest, 1981) separates different insoluble fibrous fractions. The samples are firstly treated with a solution at neutral pH that solubilises the cell contents, thus resulting in a residue named neutral detergent fiber (NDF). The NDF residue consists of hemicelluloses, cellulose, lignin and insoluble ash, but it includes also pectins insoluble in water. The sample or the NDF residue (in the sequential method) is treated with an acid solution. The ADF residue includes cellulose, lignin and insoluble ash. The ADF residue thus obtained is treated with a strong acid solution (sulphuric acid 72%), which dissolves the cellulose and leaves the residue which is represented mainly by lignin (acid detergent lignin, ADL, and insoluble ash, AIA). The hemicelluloses content is calculated by the difference between NDF and ADF, whereas the cellulose content is obtained by the difference between ADF and ADL.

The enzymatic-gravimetric analysis developed by Carré and Brillouet (1989) allows extracting the water-insoluble cell walls (WICW), which includes NDF and insoluble pectins.

On the base of their role in physiology of ruminants and monogastric herbivores, the different fibre fractions may be measured in a different way and classified as insoluble or soluble dietary fibre (Mertens, 2003; Hall, 2003). According to Mertens (2003) “the appropriate physiological definition for selecting insoluble dietary fibre methods may be as follows: the organic fraction of the diet that is indigestible or slowly digesting and occupies space in the gastrointestinal tract”. The indigestible part of fibre is constituted mainly by lignin and slowly digesting organic matter (especially cellulose and hemicelluloses), whereas rapidly fermenting polysaccharides of plant cell walls (such as pectins) and soluble polysaccharides (such as fructans and gums) are excluded.

There is a wide agreement that insoluble fibre is measured by NDF residue (Mertens, 2003), whereas there is still some uncertainty about the best method to measure soluble dietary fibre (Hall, 2003), especially when speaking about rabbits (Gidenne *et al*., 2010).

In the last years, new procedures have been proposed to determinate the total dietary fibre (TDF) and the insoluble dietary fibre (IDF), by treatment with α-amylase, protease and aminoglucosidase (Methods AOAC 991.43, 32-07, 32-21, 985.29, 32-05). The TDF corresponds to the sum of soluble and insoluble non-starch polysaccharides (pectins and β-glucans included) and lignin. The soluble dietary fibre (SDF), composed of soluble pectins, pentosans, β-glucans and other hydrocolloids, is obtained as the difference between TDF and IDF. On the other hand, soluble dietary fibre may be measured as the difference between total dietary fibre and NDF after correction for protein and ash residues (Van Soest *et al.*, 1991). This soluble fibre residue includes fructans, galactans, pectins, and β-glucans.

Fibre in rabbit nutrition

With specific reference to the rabbit, this animal has a specific requirement for dietary fibre which regulates its digestive physiology rather than supplying nutrients or energy (Gidenne *et al.*, 2010). A well balanced fibre supply should stimulate intestinal peristalsis, maintain digestive transit, ensure caecotrophy and regulate intestinal microflora composition with the final effect of reducing the incidence of digestive troubles and diseases (Lebas, 1989; Blas and Gidenne, 1998; De Blas and Mateos, 1998).

During the post-weaning period, minimum supplies of 5% ADL and 16-17% ADF are recommended (Gidenne and García, 2006) (Table 3). Lower levels of insoluble fibre would increase the transit time of digesta and the feed permanence in the intestinal tract, thus favouring the protein fermentation, increasing caecal NH_3 and pH and favouring dysbiosis (Gidenne, 1996; Bennegadi *et al.*, 2000).

On the other hand, already Morisse *et al.* (1985) evidenced that an excess of fibre associated with a lack of fermentable substances would promote the development of a pathogenic microflora in caecum, especially *Escherichia coli*. In these conditions, the utilization of protein as an energy source increases caecal NH_3 levels and pH (>7), thus favouring the proliferation of enteropathogenic *Escherichia coli*. Moreover, high levels of insoluble fibre over-stimulate peristalsis and digesta transit, therefore reducing the time available for digestion and thus the apparent faecal digestibility.

More recent studies outlined that, young rabbits around weaning have a limited capacity of digesting high-fibrous diets rich in insoluble fractions (Gidenne *et al.*, 2004a, 2004b; Gidenne and Licois, 2005). In rabbits weaned at 25 days of age, mortality due to ERE was lower with an intermediate NDF supply (30%) rather than high (38%) or low (25%) levels (Nicodemus *et al.*, 2004). Besides improving health, the reduction of dietary NDF (36-38% to 30-32%) improved performance and feed efficiency (Gutiérrez *et al.*, 2002; Feugier *et al.*, 2006). In fact, soluble fibrous carbohydrates (fructans, galactans, β-glucans, pectic substances) are more easily fermentable (Marounek *et al.*, 1995; Lavrencic, 2007) and could favour the intestinal health even in young rabbits by promoting the proliferation of beneficial microbiota and improving the competitive exclusion with pathogens (Carabaño *et al.*, 2008).

Table 3. Fibre and starch requirements (as fed corrected to a dry matter content of 90%) for the young rabbit after weaning to prevent digestive troubles (Gidenne and García, 2006).

Unit[1]	**INRA[3]**		**UPM[4]**	
	Post weaning (28-42 d old)	Growing (42-70 d old)	Post weaning (25-39 d old)	Growing (39-70 d old)
NDF	≥31.0	≥27.0	30.0≤NDF<36.0	32.0≤NDF<35.0
ADF	≥19.0	≥17.0	–	16.0≤NDF<18.5
ADL	≥5.5	≥5.0	–	≥55
ADF-ADL (cellulose)	≥13.0	≥11.0	–	–
Lignin/cellulose	>0.40	>0.40	–	–
NDF-ADF (hemicelluloses)	>12.0	>10.0	–	–
DF[1]/ADF	≤1.3	≤1.3	–	–
Soluble fibre (NDSF[2])	–	–	12.0	–
Particles > 0.3 mm	–	–	–	> 21.0
Starch	–	–	<20.0	14.5<starch<17.5

[1] Digestible fibre fraction = [hemicelluloses (NDF-ADF)+water-insoluble pectins]. [2] According to Hall *et al.* (1997). [3] INRA, Institut National Recherche Agronomique. [4] UPM, Universidad Politécnica de Madrid.

The relationships between the levels of soluble fibre and other nutrients (insoluble fibre and starch) are not yet clear and the effects on health and performance are not fully elucidated.

Gidenne (2003) used the concept of digestible fibre fraction (DF) that corresponds to the sums of hemicelluloses (NDF-ADF) and water insoluble pectins. This author stated that a DF to ADF ratio higher than 1.3 with ADF>15% should be avoided in order to reduce risks of digestive pathologies and mortality in the fattening period (Table 3). However later studies showed that mortality and morbidity, for ERE or other digestive diseases, may be reduced with a decrease of insoluble fibre content and the increase of soluble fibrous fractions (Gómez-Conde *et al.*, 2007; Xiccato *et al.*, 2007). Gomez-Conde *et al.* (2007) found also a reduction in caecal *C. perfringens* and other opportunistic pathogens as well as a decrease in mortality by ERE when increasing the level of soluble fibre in starter diets. The increase of soluble fibre at the expenses of insoluble fibre in diets for growing rabbits also improved caecal environment, increasing VFA production and decreasing caecal pH (García *et al.*, 2002; Trocino *et al.*, 2011). Some authors also found a positive effect of soluble fibre on gut mucosa (Álvarez *et al.*, 2007; Gómez-Conde *et al.*, 2007), which however was not confirmed in other studies (Xiccato *et al.*, 2008; Trocino *et al.*, 2010 and 2011; Xiccato *et al.*, 2010).

Increasing soluble fibre in replacement of insoluble fibre is associated with higher diet digestibility and energy value (Trocino *et al.*, 1999, 2010, 2011; Falcão-e-Cunha *et al.*, 2004), both depending on the higher amount of more digestible/fermentable fibre fractions, as well as on the increased digestibility of all fibre fractions (Carabaño *et al.*, 1997). In terms of performance, daily weight gain during the post-weaning period may be stimulated (Trocino *et al.*, 2011), due to the high digestive utilization of fibre fractions in the young rabbits (Marounek *et al.*, 1995).

The positive effect of soluble fibre on health of rabbits is even more pronounced when its increase is obtained at the expenses of starch and in iso-fibrous diets (Jehl and Gidenne, 1996; Perez *et al.*, 2000; Soler *et al.*, 2004; Xiccato *et al.*, 2008). In this case, also caecal environment improves with lower pH, higher VFA and lower N-ammonia production (Jehl and Gidenne, 1996; Gidenne and Bellier, 2000; Xiccato *et al.*, 2008).

In iso-ADF diets, the substitution of starch with soluble fibre does not usually change DM and energy digestibility of diets, despite the fact that it often improves the digestive utilization of fibre fractions (Gidenne and Jehl, 1996; Gidenne and Bellier, 2000; Gidenne and Perez, 2000; Xiccato *et al.*, 2010; Trocino *et al.*, 2011). As a consequence, diets have similar nutritive values and insoluble fibre (NDF and/or ADF) concentrations which explains the absence of effects of the soluble fibre to starch ratio on feed intake and growth rate (Xiccato and Trocino, 2010b).

At slaughtering, rabbits fed with a higher soluble fibre level display heavier slaughter and therefore carcass weights. Rabbits also have a higher gut proportion if they are fed with diets containing high levels of soluble fibre and with wide variations in levels of insoluble fibre (NDF, ADF) and starch (García *et al.*, 1993; Carabaño *et al.*, 1997; Falcão-e-Cunha *et al.*, 2004).

Role of protein

Proteins are essential quaternary constituents of all animal and plant cells. They are made up of amino acids linked together by peptide bonds to form polypeptide chains and carry out various functions in the organism, mainly as muscular tissue, but also as intracellular and circulating bioactive molecules, e.g., biological catalysts, transporters (intracellular, extracellular and membrane), receptors, regulators of gene expression as well as polypeptide hormones.

Unlike microorganism and plants, where the synthesis of amino acids occurs from inorganic nitrogen and carbohydrates, animals are not able to synthesize all amino acids and therefore necessarily obtain them with diet.

In rabbit the dietary requirements in proteins and amino acids change with the age and physiological status of animal; they are higher in the first period of growth and lower in later periods, and they are higher in reproducing than not-reproducing adults. In addition, the protein level necessary to satisfy the needs of the rabbit changes with the amino acid profile of diet, the digestibility of the protein and the level of ingestion.

Protein and amino acids supply through caecotrophy is only 15-20% of the total need in rabbits reared in intensive systems (De Blas and Mateos, 2010) and the diet must provide the majority of protein and amino acids. Moreover, commercial diets must be supplemented with synthetic amino acids taking into account the differences in digestibility of synthetic amino acids and those provided by raw materials. In general, the digestibility of synthetic amino acids is about 30% higher than the amino acids made from feedstuffs (De Blas and Mateos, 2010).

The protein requirements correspond to about 15-16% of crude protein (CP) or 10.5-11% of digestible protein (DP). In growing rabbits the optimum DP to DE ratio is between 10.5-11.0 g/MJ (De Blas and Mateos, 2010; Xiccato and Trocino, 2010a), which corresponds to 19-20 g of CP intake or 12-14 g of DP per day. This quantity of protein intake per day permits to maximize the growth of animal.

De Blas and Mateos (2010) recommended levels of lysine of 0.76–0.80% total lysine (0.60–0.64% digestible lysine) and a minimum level of 0.54% of total sulphur-containing amino acids (0.40% of digestible aminoacids) to obtain adequate productivity in growing rabbits, feed efficiency and carcass traits.

When the requirements of the main limiting amino acids (lysine, methionine and threonine) are satisfied, the protein level of the diets could decrease until 14-15% without negative effects on animal performance (Trocino *et al.*, 2000 and 2011, García-Palomares *et al.*, 2006a, 2006b). Only when food consumption is less than 13.8%, daily growth is penalized (-9%) for the whole fattening period (Maertens *et al.*, 1997). In the post-weaning period, however, the growth performance of rabbits weaned at 25 days (Feugier *et al.*, 2006) or 35 days (Trocino *et al.*, 2000) is reduced when the dietary protein level decreases to 15.0% and 14.4%, respectively.

The protein that reaches caecum is partially of dietary origin and partially of endogenous origin (digestive enzymes, mucoproteins, desquamation cells, urea). The contribution of the endogenous N at the total ileal flux is variable: it may reach 64%, depending on the intake of dry matter, the content and characteristics of dietary fibre and the presence of anti-nutritional factors that may damage the intestinal mucosa and increase the ileal flux of endogenous protein (García *et al.*, 1995; Gutiérrez *et al.*, 2000, 2003; García-Ruiz *et al.*, 2005; Llorente *et al.*, 2006; Carabaño *et al.*, 2008, 2009). The protein fermented by microflora is converted to ammonia, which represents the major N source for the synthesis of bacterial protein.

As what concerns the relationships between protein level and rabbit health status, the lack (<12%) or excess of CP (>18%) may favour the presence of digestive troubles and increase mortality, by modifying the caecal fermentative activity and the composition of the microflora (Maertens and De Groote, 1988; Lebas, 1989; Carabaño *et al.*, 2008, 2009). All microbial populations benefit from the protein availability at caecal level for their development and proliferation, but some species (*E. coli* and *Clostridia*) seem to be particularly advantaged under conditions of protein disequilibrium, in particular from the excesses. In fact, reducing dietary CP from 18% to 16% significantly decreased the presence of *Clostridium perfringens* and mortality due to ERE (Xiccato *et al.*, 2006; Chamorro *et al.*, 2007), whereas further reduction from 16% to 14% CP was not effective on mortality but reduced total anaerobic bacteria at ileum (García-Palomares *et al.*, 2006a; Carabaño *et al.*, 2009). On the other hand, Feugier *et al.* (2006) did not observe any significant effect of decreasing dietary CP from 21% to 15% on digestive status of early weaned rabbits.

Few data exist on the effect of the protein level and source on intestinal mucosa in rabbit. Neither the level of dietary protein (Chamorro *et al.*, 2007), nor the integration of specific amino acid (arginine and glutammic acid) (Chamorro *et al.*, 2010) were able to promote the gastro-intestinal integrity or influence positively the intestinal mucosa in weaned rabbits. Trocino *et al.* (2011) observed a reduction in the crypts depth in rabbits fed with higher protein levels, which could be related to a reduced regenerative capability of mucosa and, therefore, a higher susceptibility to enteric disease.

Feeding system to control digestive disorders in growing rabbit: *ad libitum vs.* feed restriction

Monogastrics reared for productive purposes, like poultry, pigs and including rabbits, are fed with diets and systems aimed at maximising lean body mass and optimizing feed conversion ratio. Continuous genetic selection and remarkable technological innovations largely contributes to these goals so that animals have been selected for a very fast growth especially in the first period, which however have brought about number of problems, namely increased body fat deposition, high incidence of metabolic disorders and skeletal diseases and high mortality (Tůmová *et al.*, 2002; Daza *et al.*, 2003; Cerolini, 2008).

To control and prevent some of these problems, feed restriction programs are used in different species, especially in the early period of breeding. With these programs, growth is controlled and limited during the restriction period but the compensatory growth during the following period of re-alimentation allow animals to recovery productive performance at the end of the rearing period.

In commercial rabbit farms, feed restriction is largely used for reproducing bucks and not pregnant and not lactating does to avoid an excessive fattening and a consequent impairment of reproductive performance. Differently, pregnant and lactating does and growing rabbits are usually fed *ad libitum*. Growing rabbits for meat production should be fed *ad libitum* from weaning to slaughter in order to maximize live weight gain and final weight (Maertens, 1992, 2009; Villamide and Maertens, 2010). In these conditions, the voluntary DE intake is utilized for maintenance (45%) and growth (55%) and the overall energy efficiency (retained body energy to DE intake ratio) is maximized (Xiccato and Trocino, 2010a). When DE intake decreases, body energy retention and overall energy efficiency decreases, because of the reduction of energy available for growth. However, in field conditions, a moderate feed restriction may be used with some advantages on global farm efficiency because:

- it increases diet utilization;
- it modifies the partition of body energy retention as protein instead of fat;
- it could reduce mortality and morbidity due to digestive problems.

In fact, recently, feed restriction has become a common technique also in growing rabbits. First studies on feed restriction were conducted to evaluate the effect on productive performance, carcass and meat quality. Then, feed restriction was used to improve feed efficiency and standardise growth curve in animals with different voluntary feed intake (Ouhayoun *et al.*, 1986; Ouhayoun, 1989; Cavani *et al.*, 1991). From the end of the ‘90s, feed restriction has been used in the post-weaning period to control the mortality due to digestive disorders. Positive effects were outlined since the occurrence and diffusion of ERE both in terms of a better control of the disease and improvement of the global feed conversion (Maertens, 1992; Gidenne *et al*., 2003; Tudela, 2008).

When restricting feed, however, the lower energy intake modifies the body composition, since both energy and fat retention decreases. Parigi Bini and Xiccato (1986, 1998) calculated that the rabbit that does not eat is losing 12.6 g/day/kg LW$^{0.75}$, whereas an intake of 273 kJ DE/day/kg LW$^{0.75}$ is necessary to maintain its weight. At this energy intake levels, the body loses energy since body fat is replaced by water. When energy intake reaches the maintenance requirements (425 kJ/day/kg LW$^{0.75}$), the body energy balance is in equilibrium since the gain of energy, as retained protein, is equivalent to the loss of energy as body fat (Xiccato, 1999).

In the field, rabbits may be restricted according to different feeding plans and with different systems, directly (by reducing the supply of diets) or indirectly (by reducing the availability of water), depending on the type of diet distribution (manual or mechanical) and on the personnel availability.

Rather complex feed restriction plans were proposed by Perrier and Ouhayoun (1996) which compared three methods to restrict rabbits at 80% (on average) of the voluntary intake and with different feeding plans: 80% during the whole experimental period (35-77 days of age) (group 80-80); restriction at 70% from 35 to 56 days and restriction at 90% thereafter until slaughtering (group 70-90); restriction at 90% from 35 to 56 days and at 70% in the following period (group 90-70). The best daily growth rate and feed efficiency were recorded for the group 70-90. The higher energy availability during the second period reduced slaughter dressing percentage, increased muscle to bone ratio and did not affect the fattening condition of carcasses compared to the 90-70. Also final pH of meat changed with the restriction method, which could imply also changes in technological and sensorial properties of meat.

To evaluate the effect of the re-alimentation system, Perrier (1998) restricted at two levels (70% or 50% of voluntary intake) during the first period of growth (from weaning at 35 days until 56 days of age) and left all rabbits *ad libitum* during the fattening period. Notwithstanding the compensatory growth of the last period, feed restriction during post-weaning decreased final live weight, dressing percentage and carcass fat by 12% and 25% in the groups restricted at the two levels compared to rabbits fed *ad libitum*.

In other studies, feed restriction was achieved by limiting the access to the diet during some hours a day or some days a week (Cavani *et al.*, 1991; Castelló and Gurri, 1992; Jérôme *et al.*, 1998). Generally speaking, reducing the total amount of ingested feed significantly affects weight gain and carcass traits, whereas limiting the access to feeders during some hours per day or administering the quantity of feed corresponding to the voluntary feed intake in two or more times have no significant effect (Cavani *et al.*, 1991). A latter study, however, observed a reduced fattening state when limiting the access to feeders during 16 hours per day, even if neither daily growth nor slaughter dressing percentage changed (Jérôme *et al.*, 1998).

During the last 10 years, the appearance and the diffusion of epizootic rabbit enteropathy increased the use of feed restriction in commercial farms in view of controlling the economical impact of the illness. Among most significant studies to this regards, Gidenne *et al.* (2003) must be mentioned: restricting feed distribution (90%, 80%, 70% and 60% of voluntary feed intake) during the first period of growth from weaning until 54 days of age significantly reduced live weight of rabbits at the end of the trial, daily weight gain and feed intake in all experimental groups. During the second period of re-alimentation and until commercial slaughter (70 days of age), rabbits previously restricted at 90% recovered weight and performance, while other groups showed significantly lower live weight and weight gain compared to rabbits fed *ad libitum* (Table 4).

Table 4. Effect of feed restriction from weaning (54 d) on productive performance in re-feeding period (from 54 d to slaughter) (Gidenne *et al.*, 2003).

	ad libitum	90%	80%	70%	60%
Weight at 54 days, g	1799^{a}	1692^{b}	1624^{c}	1540^{d}	1431^{e}
Weight at slaughter, g	2468^{a}	2422^{ab}	2373^{bc}	2340^{c}	2279^{d}
Weight gain, g/d	46.1^{a}	49.7^{b}	51.1^{bc}	54.6^{cd}	58.4^{d}
Feed intake, g/d	136	135	130	131	128
Feed conversion	2.93^{a}	2.60^{b}	2.43^{b}	2.32^{bc}	2.02^{c}

^{a,b,c,d} Means having a common superscript are both different at the level P=0.05

During the period of feed restriction, daily weight gain decreased by 0.5 g/d per each percentage point of rationing (% RL) in comparison to the *ad libitum* group. Thanks to compensatory growth, final weight and daily weight gain from weaning to slaughter were less affected (-0.13 g/d per each % RL), but always negatively correlated with the restriction level (Gidenne *et al.*, 2003; Tudela, 2008).

According to Tudela (2008), the most interesting result, however, was the significant improvement of feed conversion ratio. This latter result may be irrelevant from an economic point of view especially when rabbits are slaughtered at heavy weights. In fact, feed conversion ratio improved only by 0.08 and 0.15 with feed restriction of 90% and 80% of voluntary intake and the advantage is likely annulled by the longer period of growth needed to reach a similar final weight.

According to Gidenne *et al.* (2003), in the first period of rearing feed restriction at and below 80% of voluntary intake significantly reduced the mortality during the same period (10.2% and 14.2% in control and 90% groups, and 5.5%, 5.4% and 2.8% in 80%, 70% and 60% groups, P<0.001), whereas morbidity decreased only at and below 70% of voluntary feed intake. A residual, even if less relevant, effect is still observed when the mortality during the whole trial is considered (15.9%, 19.2%, 12.4%, 15.0% and 11.9%, P<0.05).

The results of a further study in which rabbits were experimentally infected for ERE support the findings of the French researchers and outline a positive effect of feed restriction on the diffusion of digestive diseases (Boisot *et al.*, 2003). To make easy the management of feed restriction, the same French authors proposed a successful restriction of water to restrict indirectly also feed (Boisot *et al.*, 2005; Foubert *et al.*, 2008).

More recent studies proved that feed restriction, despite impairing performance, (Gidenne and Feugier, 2009; Gidenne *et al.*, 2009), reduced mortality during the period of restriction from 10.2% to 5.5% (P>0.001) at 80% of voluntary intake and morbidity from 12.4% to 5.4% from 70% of voluntary intake and compared to rabbits fed *ad libitum* (Gidenne *et al.*, 2009). The positive effect of health was maintained also when the whole experimental period was taken into account.

Therefore, the feeding level should be reduced at least by 20% and 30% compared to voluntary intake to obtain a significant reduction of mortality and morbidity, respectively (Gidenne *et al*., 2009). However, especially below certain levels (<80% of voluntary intake), feed restriction may stress rabbits and reduce their welfare and, as a consequence, should be used with prudence, especially when overlapping with other stressful events (Bovera *et al.*, 2008). On the other hand, other authors did not measure a positive effect of feed restriction (direct on diet or indirect on water) on animal health (Gualterio *et al.*, 2008; Matics *et al.*, 2008), but rather a negative effect (Bovera *et al.*, 2008). Differences in the restriction levels, in the type of nutrients which intake was restricted, in the type of farms in which trials were performed (commercial or experimental) and in the environmental conditions may partly explain the differences in results obtained by the different authors.

Some authors (Szendrő *et al.*, 2008; Metzger *et al.*, 2009) restricted only the amount of ingested DE at the same levels of ingestion for the other nutrients and found a positive effect of feed restriction on mortality which decreased from 29.5% to 28.1% to 4.8% ($P<0.05$) in rabbits fed *ad libitum* or restricted at 90% and 80% of voluntary DE intake. However, final live weight and daily growth rates significantly decreased while feed conversion improved. Slaughter dressing percentage did not change, whereas hind leg development was reduced, water content and final pH of meat increased, and cooking losses decreased; colour indexes (lightness, red and yellow indexes) changed as well as the fatty acids profile of the meat fat, in terms of decreasing amount of saturated and monounsaturated fatty acids and increased incidence of polyunsaturated fatty acids (Metzger *et al.*, 2009).

Moreover, feed restriction implies notable changes in feeding behaviour of rabbits (also of caecotrophy) both in the restriction period and on the re-alimentation period. During this latter one, the rabbit has now at its disposal the diet and it increases sharply the ingestion level before reaching stable and lower values. Besides, after stabilizing, feeding levels during re-alimentation are lower than what may be expected on the base of compensatory growth (Gidenne *et al.*, 2011), which maintain a favourable conversion index also in the same period of re-alimentation.

The changes in feeding behaviour during restriction may affect digestive physiology, with consequences on gastric traits and, especially, on caecal content fermentations and microbiota composition. The studies available until now however have not yet evidenced changes in the caecal fermentative activity in terms of pH, N-ammonia, total production of volatile fatty acids and their proportion or fibrolytic bacterial activity (Gidenne and Feugier, 2009). Besides, despite feed restriction immediately weaning could impair gut maturation, no effect was recorded on villi height and crypth depths or digestive enzyme secretion of rabbits restricted (75% of voluntary intake) during post-weaning (28-53 days of age) (Martignon *et al*., 2010). As a last point, feed restriction reduces the transit speed of digesta in the gut and may improve faecal digestibility both during the restriction and the re-alimentation period (Gidenne and Feugier, 2009; Gidenne *et al*., 2011).

According to Gidenne *et al*. (2011), feeding systems based on restriction during post-weaning are largely applied in France (95% of commercial intensive systems) with positive results on the reduction of digestive troubles and on feed efficiency. In Spain, the recent results published by Romero *et al*. (2010) point out a sharp impairment of growth and feed conversion after 2 weeks (35-49 days of age) of feed restriction (80% of voluntary intake), which arise the question whether this strategy is economically convenient in the Spanish system, in which the slaughter weight is the lowest (2.0-2.3 kg) and the fattening period the shortest (60-65 d) compared to the other main producers. In Italy, data on feed restriction in growing rabbits are rather outdated, referred to genetic types with slower growth rates compared to the current commercial hybrids and to a sanitary situation in which ERE was not yet present and digestive diseases easily controlled.

Objectives

The general objective of the present thesis was the development of nutritional strategies for improving feed efficiency and health status of growing rabbits. This goal was pursued by means of experimental activities with the specific objectives of *i*) defining requirements of soluble fibre in relation to dietary insoluble fibre and starch in rabbits during post-weaning and fattening (experiments 1 and 2); *ii*) measuring the effect of the rate and duration of feed restriction compared to an *ad libitum* feeding, in order to outline the most effective restriction technique to maximize performance and control digestive problems in growing and fattening rabbits (experiments 3 and 4).

The specific objectives have been pursued through the research activities in the four experiments of the doctoral thesis as summarized below:

Experiment 1. *Effect of increasing soluble fibre and starch and reducing protein in diets for growing rabbits.* The effect increasing soluble fibre (18%-20%-22%) and starch (10%-14%-18%) at the expenses of insoluble fibre and of reducing dietary crude protein (17% to 15%) was evaluated on health, performance, caecal fermentative activity, gut mucosa traits, nitrogen excretion and carcass and meat quality of growing rabbits.

Experiment 2. *Effect of dietary energy supply and protein level on health and efficiency of growing rabbits.* The effect of the administration of diets with increasing starch (from 14% to 20%) and soluble fibre (21% to 25%) levels and decreasing ADF supply (21% to 13%) at two protein levels (14% and 15%) was assessed on health, performance, caecal fermentative activity, gut mucosa traits, nitrogen excretion and carcass and meat quality in growing rabbits.

Experiment 3. *Effect of the level and duration of feed restriction in growing rabbits.* The effect of the level (90% and 80% *vs*. *ad libitum*) and duration (2 or 3 weeks after weaning) of feed restriction was measured on health, performance, caecal fermentative activity, body tissue balance and carcass and meat quality of growing rabbits.

Experiment 4. *Effect of feeding plan and feed restriction program in growing rabbits.* The use of feeding plans based on diets at moderate digestible energy concentration (10.7 MJ/kg) or high digestible energy concentration (11.1 MJ/kg) during the whole productive cycle (A-A; B-B) or in a sequence (B-A) was tested together with the use or not of a progressive feed restriction program which started from 80% of *ad libitum* and linearly reached *ad libitum* level after 3 weeks in order to avoid sudden changes in feed intake level during re-alimentation. The effect of the above mentioned feeding systems were evaluated on health, performance, caecal fermentative activity, body tissue balance and carcass and meat quality of growing rabbits.

Research groups and international collaborations

The present doctoral thesis was developed at the Department of Animal Science of the University of Padova, under the supervision of Prof. Gerolamo Xiccato.

Part of the thesis was realised in collaboration with Prof. Rosa Carabaño, Departamento de Producción Animal of the Politechnical University of Madrid, where a three-month stage was held.

The experimental activity was developed in four studies performed at the facilities of the Farm of the University of Padova.

The analyses of samples collected during the trials were performed at the laboratories of the Italian and Spanish Departments.

Experiment 1.

Effect of increasing soluble fibre and starch and reducing protein in diets for growing rabbits

Introduction

Following the results of other groups in the world, widely presented in the introduction section, previous studies of the Padova team have been devoted to individuate dietary fibre and protein levels in post-weaning and fattening rabbits capable of reducing the occurrence and severity of digestive problems and of limiting nitrogen excretion, guaranteeing however quantitative and qualitative performance (Trocino *et al*., 2010 and 2011; Xiccato *et al*., 2011). To this aim, different experimental diets characterized by divergent ratios in soluble fibre (SF) (pectins and hemicelluloses) and insoluble fibre (ADF and NDF), as well as different starch and protein concentrations have been tested. Main results showed that:

- increasing the soluble to insoluble fibre ratio reduces sanitary risk at farm;
- soluble fibre may replace starch in view of preventing digestive disorders and allows to maintain a suitable dietary energy concentration;
- reducing dietary protein, and as a consequence nitrogen excretion, may be pursued when suitable amino acidic supply is guaranteed and protein to energy ratio is maintained within a correct range;
- sunflower meal is a rather good protein source in rabbit feeding and may replace in part or fully soybean meal, supplying at the same time high insoluble fibre level;
- dehydrated alfalfa meal may be largely or totally replaced in diets without negative effects on performance of growing rabbits;
- dried beet pulps are an excellent source of soluble fibre, especially pectins, capable of providing high energy supply and replacing traditional fibrous raw materials, besides starch sources.

When considering technical and economical aspects of the different trials, the main factors that can affect the farmer income, at fixed prices, are mortality, morbidity and feed conversion. On the contrary, the slaughterhouse holder pays more attention to the animal weight and dressing out percentage. In this case, slaughtering at older age and higher live weights could be preferable. The early slaughtering of rabbits would allow improving feed conversion and reducing nitrogen excretion.

The present trial aimed to evaluate the effect of increasing starch and soluble fibre at the expenses of insoluble fibre and of reducing dietary protein on health, performance, feed conversion, nitrogen excretion, carcass and meat quality.

Materials and methods

Rearing conditions

The trial was realized in the experimental farm of the University of Padova in the period October-November 2008. Extraction fans and automatic heating system were used to control air circulation, temperature and humidity in the building (Figure 3).

Figure 3. Experimental facilities and equipments for air circulation and extraction fans.

Maximum temperature did not overcome 23°C and minimum temperature averaged 14°C (Figure 4). On average, minimum and maximum temperature measured 17° and 20°C, respectively. The maximum relative humidity averaged 75%, with a minimum value of 45% and a peak of 90% (Figure 4). The minimum relative humidity averaged 65% and moved in the range from 40% to 83%.

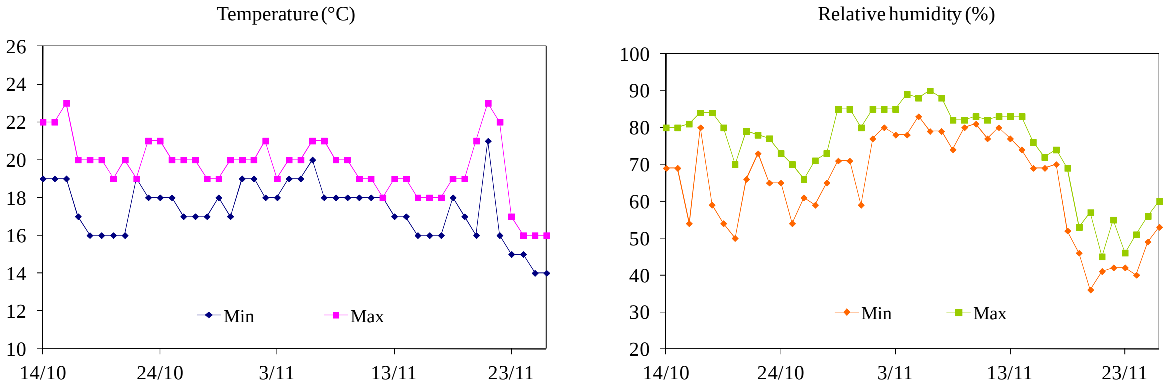


Figure 4. Daily values of minimum and maximum temperature and minimum and maximum relative humidity (RH) throughout the trial.

In the farm, both commercial cages for fattening and experimental cages for digestibility (25 x 44 x 28 cm) in galvanized iron net (Figure 5) were available. Both cages had movable feeders for manual distribution of feeds, placed outside of the cage and designed to avoid feed waste, and drinkers. Digestibility cages had a steel baffle to separate urine from faeces and a galvanized box with 3-mm net below the cage for faeces collection (Figure 5).

Before the arrival of rabbits, the building was kept empty for a long period (sanitary period) and then submitted to a disinfectant and fungicide treatment with enilconazole (Clinafarm, Janssen–Cilag Animal Health, Cologno Monzese, Italy).

Figure 5. Experimental cages for *in vivo* digestibility trials and details of digestibility cages with removable feeders and baskets for the faeces collection.

Animals and experimental groups

The research was performed outside of the application of D.L. 116/92 and was approved by the Ethical Committee of the University of Padova. The animals were selected in a commercial farm. At 36 days of age, 282 weaned rabbits of both genders from a hybrid line

(Grimaud Frères, France) were selected from multiparous does (3-6 kindling) with healthy litters and homogeneous kit weight.

The animals were transported to the experimental farm in an air-conditioned truck, inside collective cages at low density (10 rabbits per cage) to reduce the transport stress at minimum and they presented an optimal health status at their arrival. The rabbits were immediately weighted (951±31 g LW), put in individual cages and identified by ear tags. The animals were divided into six experimental groups of 47 units, homogeneous in average weight and variability, and fed *ad libitum* with six diets formulated according to a 2 x 3 factorial arrangement, with three (starch+soluble fibre) to ADF ratios (0.6, 1.2 and 2.0) and two protein levels (15% and 17%), as shown in the following scheme:

	Protein 15%	Protein 17%
A = 0.6: (Starch10%+SF5%)/ADF22%	Diet A15	Diet A17
B = 1.2: (Starch14%+SF7%)/ADF18%	Diet B15	Diet B17
C = 2.0: (Starch18%+SF9%)/ADF14%	Diet C15	Diet C17

Four diets (A15, C15, A17 and C17) were formulated and produced at the feed industry. Dilution technique was used to obtain two more diets: B15 (0.5 of A15+0.5 of C15) and B17 (0.5 of A17+0.5 of C17). No antibiotic or drugs were given in feed or water, apart from coccidiostat (Cycostat, Robenidine 66%).

The trial started the day after the arrival of the animals (at 37 days of age). Rabbits were controlled for 42 days and until commercial slaughter at 79 days of age. Rabbits were fed the experimental diets from 36 days of age until slaughter.

Experimental diets

The formulation of the diets is presented in Table 5. Diets were supplemented with synthetic amino acids, micro- and macro-minerals and vitamins to satisfy the nutritional needs of growing rabbits (De Blas and Mateos, 2010). All diets were in pellets with 3.5 mm of diameter and 1.0-1.1 cm of length.

The diets were formulated to present two protein levels (15% and 17%). The dietary protein level was increased by a higher inclusion rate of soybean meal 48% CP and sunflower meal 35% CP (+7/8 points) and barley (+4/5 points) at the expenses of wheat bran inclusion (-12/15 points). Within protein level, the diets presented increasing starch from 10% to 18% and soluble fibre from 5% to 9%, with a corresponding reduction in ADF concentration (22% to

14%). The contemporary increase of starch and soluble fibre at the expenses of ADF has been obtained by the inclusion rate of beet pulp (+30 points), barley (+26/28 points) and protein meals (+13/15 points) at the expenses of dehydrated alfalfa meal (-5/8 points) and wheat bran (-13/14 points).

Table 5. Ingredient composition (%) of experimental diets.

	Diet A15 CP 15% Starch 10% Soluble fibre 5% ADF 22%	Diet C15 CP 15% Starch 18% Soluble fibre 9% ADF 14%	Diet A17 CP 17% Starch 10% Soluble fibre 5% ADF 22%	Diet C17 CP 17% Starch 18% Soluble fibre 9% ADF 14%
Dehydrated alfalfa meal 16% CP	63.30	5.00	60.00	5.50
Wheat bran	32.00	18.00	18.00	5.00
Barley meal	0.00	27.00	8.00	33.50
Dried beet pulp	0.00	30.00	0.00	30.00
Soybean meal 48% CP	0.00	10.00	4.60	15.50
Sunflower meal 35% CP	0.00	5.00	5.00	6.00
Soybean oil	1.50	1.00	1.00	0.50
Cane molasses	1.50	1.50	1.50	1.50
Calcium carbonate	0.00	0.63	0.00	0.38
Dicalcium phosphate	0.28	0.70	0.73	1.15
Sodium chloride	0.40	0.40	0.40	0.40
L-lysine HCl	0.30	0.10	0.10	0.00
DL-methionine	0.15	0.10	0.10	0.00
Vitamin-mineral premix*	0.47	0.47	0.47	0.47
Coccidiostat	0.10	0.10	0.10	0.10

*Supplementation per kg of feed: vit. A, 12.000 UI; vit. D3, 1.000 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 largely different with aNDF from 27% to 40% and ADL from a minimum of 1.5% to a maximum of 5%. Soluble fibre ranged less from 4.4% to 10.0% and hemicelluloses from 13.9% to 16.6%. Starch level varied from 8.3% to 18.0%. As previewed when formulating the diets, the diet B15 resulted to be suitable to cover nutritional requirements of fattening rabbits and sufficiently safe, at least theoretically, to control digestive problems: crude protein level was in fact moderate in comparison with usual supplies around 16-16.5%, fibre was intermediate and consistent with requirements for the age of rabbits with ADF at 18%. The starch level (13.4%) guaranteed a sufficient energy supply to growing rabbits. The diet A15 was poorer from an energetic point of view, while diet C15 could be considered a challenge towards digestive pathologies because of the limited supply of insoluble fibre (ADF, 13.8%; ADL, 1.8%) and the relatively high level of starch (17.7%) (Lebas *et al*., 1998; Gidenne and García, 2006; Blas and Gidenne, 2010). The higher protein content in diets A17, B17 and C17 should have represented a further risk for the occurrence of digestive disorders (Carabaño *et al*., 2008;

Xiccato *et al*., 2011). All diets showed DP/DE ratio equal or higher than minimum recommended values (10 g DP/MJ DE) for growing rabbits, while diets A15 and B17, and especially diet A17 showed a high DP/DE ratio (>12 g PD/MJ ED) which was theoretically at risk for the occurrence of enteric diseases in post-weaning and growing rabbits (Table 6).

Table 6. Chemical composition (as fed) of experimental diets.

	Diets					
	A15	B15	C15	A17	B17	C17
Dry matter, %	90.3	89.5	89.0	90.0	89.6	89.1
Crude protein, %	14.9	15.3	15.2	16.9	17.0	16.7
Ether extract, %	4.0	3.4	3.2	3.2	2.8	2.4
Crude fibre, %	19.7	15.6	10.3	18.8	14.9	9.8
Ash, %	7.7	6.8	5.9	8.0	7.1	5.9
TDF, %	44.2	41.7	37.9	42.7	39.4	37.1
aNDF, %	39.9	34.8	29.1	37.5	32.1	27.1
ADF, %	23.3	18.0	13.8	22.6	17.9	13.2
Hemicelluloses (aNDF-ADF), %	16.6	15.5	15.3	14.9	14.2	13.9
Lignin (sa), %	5.1	3.2	1.8	4.8	3.1	1.5
Soluble fibre (TDF-aNDF), %	4.3	6.8	8.8	5.2	7.3	10.0
Starch, %	8.3	13.4	17.7	9.9	13.9	18.0
Gross energy, MJ/kg	17.00	16.64	16.33	16.73	16.46	16.13
Soluble fibre/ADF	0.19	0.38	0.64	0.23	0.41	0.76
Starch/ADF	0.36	0.75	1.29	0.44	0.78	1.36
(starch+soluble fibre)/ADF	0.54	1.13	1.93	0.67	1.18	2.12
Digestible energy, MJ/kg	8.33	9-85	11.47	8.64	9.93	11.48
Digestible protein/digestible energy	13.0	11.4	9.9	14.9	13.1	10.8

Controls on live animals

Individual live weight and feed intake were recorded three times a week. Health status was controlled daily to promptly detect the occurrence of disease, especially digestive problems. Rabbits were considered ill in case of diarrhoea, evident and lasting reduction of feed consumption (30% lower than the previous recording), or reduction in live weight (weight loss for two or more consecutive days). To calculate morbidity, each sick animal was considered only once even in case of new symptoms of illness after recovering, while dead animals were included only in the calculation of mortality. Sanitary risk was calculated as the sum of morbidity and mortality (Bennegadi *et al*., 2000).

Digestibility trial

The coefficients of total tract apparent digestibility (CTTAD) of dry matter and nutrients and the digestible energy (DE) concentration of the experimental diets were measured by an *in vivo* digestibility assay carried out on 72 rabbits among those on trial (12 animals of both sexes per diet) according to the European standardized method (Perez *et al*., 1995). The animals were kept in individual digestibility cages and given *ad libitum* access to the experimental diets. The digestibility trial started at the first day (49 days of age) with a 4-day collection period. Faeces were collected daily and stored in plastic bags at -18°C until analysis.

Sampling of caecal content and intestinal mucosa

Thirty-six rabbits out of the 282 initial rabbits were slaughtered at 45 days of age (6 rabbits per experimental diet) to sample caecal content and intestinal mucosa. The slaughtered animals were representative of the corresponding experimental group in terms of average live weight and variability. The rabbits were slaughtered between 08:00 to 12:00 a.m. They were weighed immediately before slaughter and slaughtered by cervical dislocation. Thereafter, the full gut and then the stomach and the caecum were separated and weighed. The caecum was removed and the pH of the caecal content was immediately measured. The caecal content was then diluted with a 15% HPO_3 solution (25% wt/wt), and stored at -20°C up until their chemical analyses (Trocino *et al*., 2011).

Two samples of mucosa were taken from the intermediate tract of jejunum and the distal tract of ileum, fixed in para-formaldehyde at 4% in PBS, then dehydrated and included in paraffin. Sections of 4 μm were obtained by microtome and used for morphometric evaluations on preparations coloured with haematoxylin/eosin. Villi length and the depth of the crypts were measured with an image-analysis software (DP-soft, Olympus Optical, Co., Hamburg, Germany), according to the procedure described by Hampson (1986), with an average of 30 measurements taken from five independent cross-sectional mucosa sections (on which at least six measurements were done) for each animal.

Chemical analyses

All samples were ground at 1 mm of diameter with a grinder (mod. ZM 100, Retsch, Haan, Germany). Diets and faeces were analyzed to determine the concentrations of dry matter (934.01), ash (967.05), crude protein (2001.11), and starch (amyloglucosidase-α-amylase method, 996.11), with AOAC (2000) methods following harmonized procedures (EGRAN, 2001). Ether extract was determined after acid-hydrolysis treatment (EC, 1998). Fibre fractions, that is aNDF (without sodium sulphite), ADF, and lignin (sa), were analyzed according to Mertens (2002), AOAC (2000, procedure 973.187), and Van Soest *et al*. (1991), respectively, using the sequential procedure and the filter bag system (Ankom Technology, New York).

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). 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 (PHM 84, Research pH-meter, Radiometer, Copenhagen, Denmark) equipped with ammonia-specific electrode (mod. 9512, Orion Research Incorporated, Boston, USA). Volatile fatty acid (VFA) concentration was measured on the supernatant with gas-chromatography (HRGC 5300 Carlo Erba, Milano, Italy) on a cross-bond capillary column (25 m x 0.32 mm I.D., 3.5 μm film thickness) (JRX, Mega, Milano, Italy) using the Osl method (1988).

Commercial slaughter, carcass dissection and meat quality analyses

At 79 days of age, 120 rabbits were selected (20 for each experimental group and representative in terms of average weight and variability) for slaughtering and dissection. Feed and water were available until loading (from 5:30 to 6:30 a.m.). The animals were transported inside cages (50 x 100 x 30 cm) with 6 rabbits per cage (one rabbit per diet) in a conditioned truck for about 60 minutes to a commercial slaughterhouse.

Slaughtering began at 09:30 and ended at 10:30 a.m. approximately. Rabbits were individually weighed, then stunned by electro-anaesthesia and killed by jugulating. Carcasses were chilled at 4° C for 2 hours and then transported to the laboratories of the Department of Animal Science of Padova for dissection. Slaughter recordings were performed according to the international scientific protocols (Blasco *et al*., 1993).

Twenty-four hours after slaughter, the pH was measured on two closed points of both the muscles *longissimus lumborum* and *biceps femoris,* using a pH-meter equipped with a combined Ingold electrode (406 M3) and with a thermal probe (Xiccato *et al*., 1994) (Figure 6). The lightness (L*), redness (a*) and yellowness (b*) were measured on the same muscles using a colorimeter Minolta Spectrophotometer CM-508 C (Minolta, Milano) (Figure 7), according to the method CIE L* a* b*(CIE, 1976). The chroma (C*) and hue indexes (H*) where calculated as [$C^{*}= (a^{*2}+b^{*2})^{0.5}$] and [H*=arc tang (b*/a*)], respectively.

The carcasses were then weighted and dissected according to the method of Blasco *et al*. (1993). The head, liver, lungs, thymus, trachea, oesophagus, heart and kidneys were removed from commercial carcasses so obtaining the "reference carcass". The dissectible fat (scapular and perirenal fat) was separated as fatness index of the whole carcass. The right hind leg meat was separated by bones to determinate meat to bone ratio, known as muscularity index of the whole carcass (Parigi Bini *et al*., 1992).

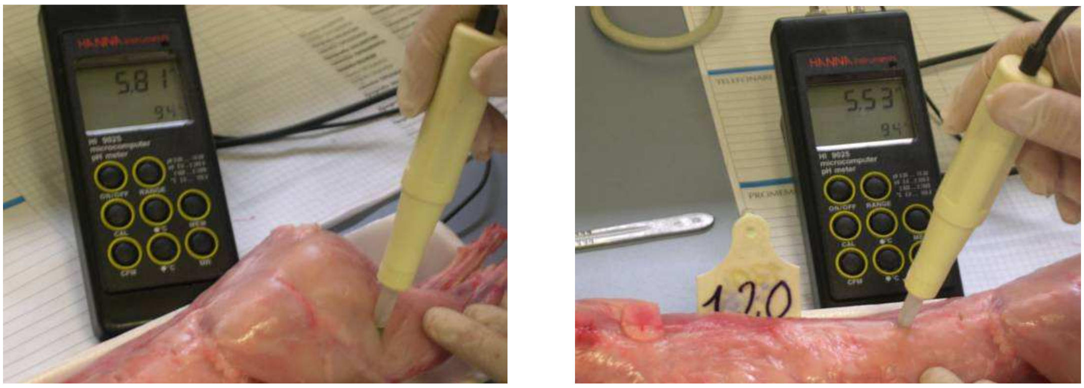


Figure 6. Measurement of pH on m. *longissimus dorsi* and *biceps femoris.*

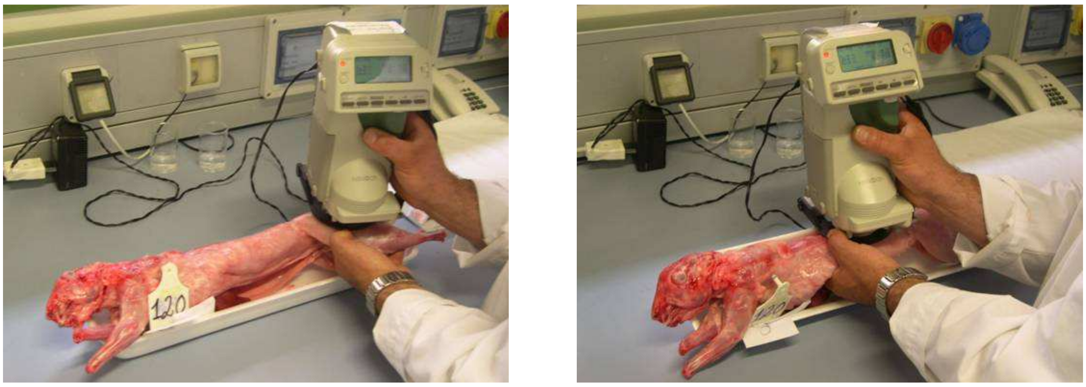


Figure 7. Measurement of color on the muscles *longissimus dorsi* and *biceps femoris.*

Nitrogen balance

Nitrogen balance was calculated on data recorded individually estimating the body N content at the various ages using the formula proposed by Szendro *et al*. (1998):

Body N (g/kg LW) = (28.3+0.93 x kg live weight)

Ingested nitrogen was calculated on the base of individual feed intake and dietary nitrogen (CP = N x 6.25). The amount of digested nitrogen was obtained by multiplying the amount of ingested nitrogen by crude protein digestibility of the corresponding diet. The amount of excreted nitrogen was obtained by subtracting ingested nitrogen to retained nitrogen, according to ERM (1999). The coefficients of protein retention were expressed both as retained N/ingested N (%) and retained N/digested N (%) (Parigi Bini *et al*., 1988).

Statistical analyses

The data recorded was analyzed with a three-way ANOVA [(starch+soluble fibre)/ADF ratio], protein level, sex with interactions. The GLM procedure of SAS (SAS, 1991) was used for all analyses. Orthogonal contrasts were used to test the linear and quadratic response to the increase of the (starch+soluble fibre)/ADF ratio. Differences among means with $P<0.05$ were accepted as representative of statistically significant differences. Differences among means with $0.05<P<0.10$ were accepted as representative of a tendency to differences. Mortality, morbidity and sanitary risk were analysed by the CATMOD SAS procedure.

Results

Diet digestibility

In Table 7, digestibility coefficients are reported in function of the main factors of variability. The apparent digestibility of dry matter greatly and linearly changed (significant linear component of variance, L<0.001) and increased with the raise of (starch+soluble fibre)/ADF ratio by about 10 points from diets with 0.6 ratio to diets with 1.2 ratio and from diets with 1.2 ratio to diets with 2.0 ratio, that is from 50.8% to 60.5% and 71.5%, respectively.

Table 7. Digestibility coefficients (%): effect of (starch+soluble fibre)/ADF ratio and protein level.

	(starch+soluble fibre)/ADF ratio					Protein level			RSD
	0.6	1.2	2.0	L[a]	Q[b]	15%	17%	Prob.	
Rabbits, n.	20	23	22			32	33		
Dry matter	50.8	60.5	71.5	<0.001	0.21	60.3	61.6	<0.01	2.0
Crude protein[1]	74.1	73.2	74.2	0.88	0.26	73.2	74.5	<0.01	1.9
Ether extract[2]	76.7	76.5	76.3	0.47	0.96	77.8	75.1	<0.001	1.7
Crude fibre	7.2	19.0	32.3	<0.001	0.49	19.4	19.6	0.87	4.7
TDF	22.4	38.3	56.9	<0.001	0.11	38.4	40.0	0.05	3.1
aNDF	16.1	29.0	48.1	<0.001	<0.01	30.6	31.6	0.30	3.7
ADF	8.9	18.2	34.8	<0.001	<0.01	19.9	21.4	0.17	4.3
Hemicelluloses[3]	26.6	42.1	60.5	<0.001	0.09	42.7	43.5	0.34	3.3
Soluble fibre	73.1	77.5	82.9	<0.001	0.91	77.5	78.2	0.59	5.5
Starch[4]	97.2	97.8	98.6	<0.001	0.42	97.8	97.9	0.13	0.4
Gross energy	50.3	59.7	70.7	<0.001	0.12	59.4	61.2	<0.01	2.1

[a] *L*: linear component of variance. [b] *Q*: quadratic component of variance.

[1] Probability of the interaction <0.01; crude protein digestibility: 72.3, 72.8, 74.8, 75.9, 74.4 and 73.9% in diets A15, B15, C15, A17, B17, and C17. [2] Probability of the interaction <0.001; ether extract digestibility: 76.3, 78.1, 79.0, 77.0, 74.8 and 73.5% in diets A15, B15, C15, A17, B17, and C17. [3] Probability of the interaction <0.001; hemicelluloses digestibility: 28.2, 41.8, 58.0, 24.9, 42.3 and 63.0% in diets A15, B15, C15, A17, B17, and C17. [4] Probability of the interaction <0.001; starch digestibility: 96.8, 98.0, 98.6, 97.6, 97.6 and 98.6% in diets A15, B15, C15, A17, B17, and C17.

The increase of (starch+soluble fibre)/ADF ratio did not affect digestibility of crude protein and ether extract, while digestibility of fibre and fibre fractions linearly and significantly increased (L<0.001). Digestibility of ADF and aNDF increased quadratically (Q<0.01), that is by reducing lignification degree, cellulose is more and more sensible to digestive and fermentative processes, while hemicelluloses and soluble fibre showed a linear improvement in their digestive utilization as (starch+soluble fibre)/ADF ratio increased.

The differences in formulation of diets with (starch/soluble fibre)/ADF ratios of 0.6, 1.2 and 2.0, with the reduction in the inclusion rate of alfalfa meal in favour of energy raw materials containing starch (barley) and high-soluble fibre raw materials (dried beet pulp) account for the increase in the digestibility of DM and fibrous fractions, even if differences between treatments were larger than what expected.

The effect of the protein level was less important and limited to the higher digestibility of DM, gross energy and crude protein ($P<0.01$) in the diets with a higher protein content. Also digestibility of TDF and soluble fibre was higher in diets at 17% CP compared to diets at 15% ($P<0.05$): this result may be ascribed to the lower inclusion rate of alfalfa meal in favour of soybean and sunflower meal. The apparent digestibility of ether extract decreased with the increase of the protein level, which could be explained by the lower inclusion of soybean oil (-0.5 points) in the more proteic diets, whereas digestibility of oils is notoriously higher (85-90%) compared to other types of fats (i.e., lipids of alfalfa meal with an average digestibility of 60%).

Health and productive performance

Mortality during the trial reached an average value of 8.5% and changed significantly with experimental factors (Table 8).

Table 8. Mortality, morbidity and sanitary risk: effect of (starch+soluble fibre)/ADF ratio and protein level.

	(starch+soluble fibre)/ADF ratio (R)				Protein level (P)			Prob.
	0.6	1.2	2.0	Prob.	15%	17%	Prob.	R x P
Mortality, %	15.8^b	4.5^a	4.9^a	0.02	10.6	6.5	0.16	0.64
Morbidity, %[1]	13.1	10.9	15.8	0.79	12.2	14.6	0.73	0.08
Sanitary risk, %	29.2^b	15.4^a	20.7^{ab}	0.09	22.8	21.1	0.66	0.13

[1] Morbidity 14.6%, 14.6%, 7.3%, 12.2%, 7.3% and 24.4% for diets A15, B15, C15, A17, B17 and C17.

Mortality was significantly higher (15.8% *vs*. 4.5% *vs*. 4.9%; P=0.02) when the (starch+soluble fibre)/ADF ratio was 0.6 compared to diets with the same ratio at 1.2 and 2.0. Similarly, even if at a lower level of significance (P=0.09), the increase of (starch+soluble fibre)/ADF ratio reduced the sanitary risk (mortality+morbidity).

Even if no significant effect of the protein level was measured, it is worthy to note that the administration of diet C17 produced the highest morbidity value due to digestive disorders of brief duration which occurred especially in the last 10 days of rearing before slaughter in rabbits fed with diets at high protein level and high ratio between soluble carbohydrates and ADF (probability of the interaction = 0.08).

Productive results during the trial are described in Table 9 according to the experimental factors. Regardless of the experimental treatments, the average live weight (2953 g) reached at 79 days of age, the average daily weight gain (46.g/d) with a feed conversion on individual data during the trial (from 37 to 79 days) on average equal to 3.40 may be considered a highly satisfying result which is consistent with the best expression of the genetic type used and in a favourable environmental condition.

Table 9. Performance from weaning to commercial slaughtering.

	(starch+soluble fibre)/ADF ratio (R)			Protein level (P)		Sex (S)		Probability					RSD
	0.6	1.2	2.0	15%	17%	Female	Male	R		P	S	R x P	
								L[a]	Q[b]				
Rabbits, n,	69	78	78	111	114	113	112						
Live weight, g													
At 37 d	1002	1010	1009	1006	1008	1012	1003	0.24	0.37	0.65	0.06	0.88	34
At 58 d	2083	2111	2126	2106	2108	2104	2110	0.22	0.82	0.94	0.83	0.12	211
At 79 d	2911	2953	2995	2943	2963	2945	2961	0.03	0.99	0.53	0.60	0.23	237
First period (37-58 d)													
Daily growth, g/d	51.4	52.4	53.2	52.4	52.4	52.0	52.7	0.28	0.93	0.99	0.59	0.09	9.8
Feed intake, g/d	163	141	121	144	140	142	141	<0.001	0.79	0.15	0.60	0.30	20
Feed conversion	3.21	2.46	2.30	2.81	2.50	2.83	2.48	<0.001	0.19	0.13	0.10	0.14	1.56
Second period (59-79 d)													
Daily growth, g/d	39.5	40.1	41.4	39.9	40.7	40.1	40.5	0.09	0.74	0.36	0.59	0.65	6.9
Feed intake, g/d	198	172	150	174	173	173	174	<0.001	0.44	0.60	0.72	0.24	19
Feed conversion	5.11	4.37	3.66	4.43	4.32	4.39	4.37	<0.001	0.81	0.12	0.74	0.38	0.56
Whole period													
Daily growth, g/d	46.4	46.3	47.3	46.1	46.5	46.0	46.6	0.05	0.90	0.56	0.42	0.19	5.5
Feed intake, g/d	181	157	135	159	156	158	157	<0.001	0.54	0.25	0.92	0.21	17
Feed conversion	3.98	3.40	2.87	3.47	3.37	3.45	3.39	<0.001	0.49	<0.001	0.05	0.74	0.23

[a] *L*: linear component of variance. [b] *Q*: quadratic component of variance.

The increase of the ratio (starch+soluble fibre)/ADF of the diet and, consequently, of the nutritive value modified significantly growth rate of rabbits during the whole trial (L=0.05) and rabbits fed the diets with the highest ratio showed also (L=0.03) the highest live weight at the end of the trial. However, the most evident effect was the linear reduction (L<0.001) of feed intake both during the first period (-26% in rabbits fed diets with 2.0 ratio compared to those fed diets with 0.6 ratio, with intermediate values for rabbits fed diets with 1.2 ratio) and the second period of growth (-24%), which resulted in a significant reduction of feed conversion in the two periods and in the whole trial: from 3.98 for rabbits fed diets A, to 3.40 for rabbits fed diets B, and until 2.87 for diets with 2.0 ratio (L<0.001), which corresponded to a 28% improvement in rabbits fed diets with 2.0 ratio compared to those fed diets with 0.6 ratio.

The increase in the dietary protein level did not modify productive results during the trial. Both in the first and in the second period, growth rates and feed intake were similar and the higher nutritive value of diets at 17% CP significantly improved feed conversion in the whole trial (from 3.47 to 3.37; P<0.001). The protein supply was therefore compatible with the expression of the potentiality of the genetic type used and protein ad amino acids requirements were satisfied even if at the lowest protein level used (15% CP) with a DP/DE ratio equal to 10 g/MJ. As a last point, no significant effect on health status was observed which could be due to the interaction of the dietary protein level with the ratio between soluble (starch and soluble fibre) carbohydrates and structural ones. The effect of the gender was weak and limited to a worse feed conversion in the whole trial of females compared to males (3.45 *vs*. 3.39, P=0.05).

The graphical representation of productive performance along the weeks of trials (Figures 8 and 9) confirms the above-described results: daily growth rate decreased from 60-65 g/d during the first week to 50-55 g/d in the second week to reach a plateau around 40-45 g/d which was maintained in the following 3 weeks and fell down to 35-40 g/d in the last week of trial. Feed intake showed an opposite trend and increasing at about 10 g/d per each week of trial, with two stationary phases during the third and the last week of the trial.

Diets with 2.0 ratio gave the lowest daily growth during the first week of trial, while permitted the best performance thereafter (Figure 8). During the last week, however, performance was similar among groups. Diets with 0.6 and 1.2 ratios produced similar growth rate during the whole trial. Differently, feed intake was clearly different since the first week of the trial according to the ratio (starch+soluble fibre)/ADF (Figure 8).

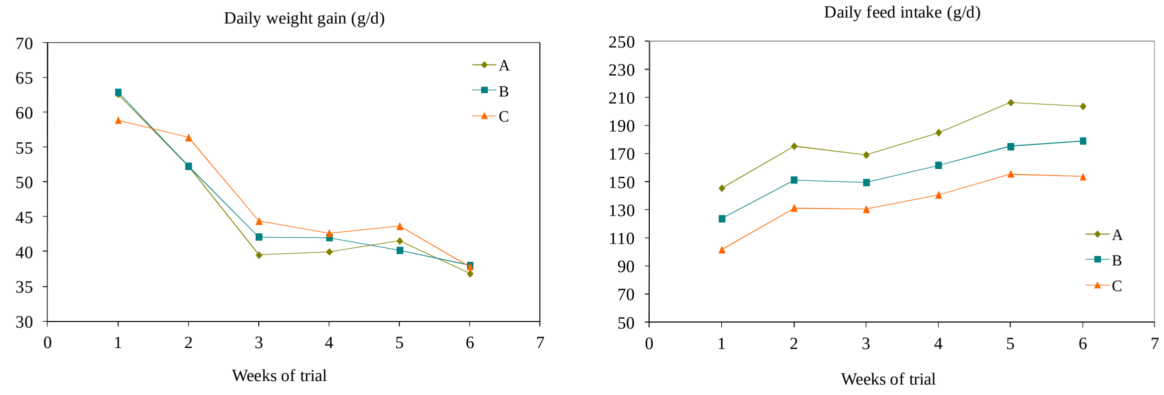


Figure 8. Weekly performance: effect of (starch+soluble fibre)/ADF ratio (diets A=0.6, diets B=1.2, diets C=2.0).

The protein level of the diets did not affect the shape of growth and feed intake curves during the different weeks of the trial (Figure 9).

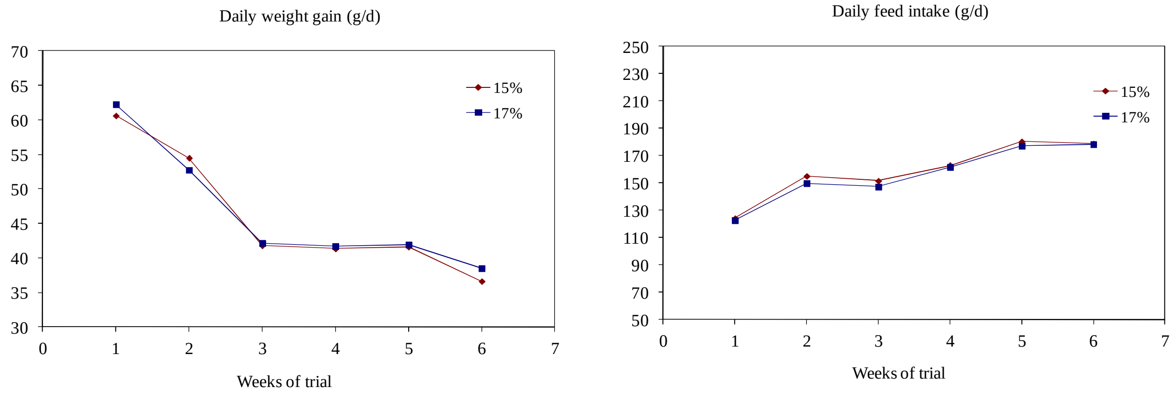


Figure 9. Weekly performance: effect of dietary protein level.

Caecal content and intestinal tissue characteristics

Table 10 reports data measured at 45 days of age on rabbits used for sampling caecal content and intestinal mucosa at jejunum and ileum. As discussed for performance, live weight at 45 days was similar among animals.

Table 10. Caecal content and intestinal mucosa characteristics in rabbits at 45 d of age.

	(starch+soluble fibre)/ ADF ratio (R)			Protein level (P)		Probability				RSD
	0.6	1.2	2.0	15%	17%	R		P	R x P	
						L[a]	*Q*[b]			
Rabbits, n	12	12	12	18	18					
Live weight (LW), g	1495	1511	1489	1494	1502	0.84	0.48	0.75	0.95	77
Full stomach, % LW	8.0	7.7	8.1	8.1	7.8	0.76	0.12	0.12	0.76	0.6
Full caecum, % LW	7.3	7.4	8.5	8.0	7.6	<0.01	0.10	0.61	0.79	0.9
Full gut, % LW	23.4	23.9	25.5	24.6	23.9	<0.001	0.26	0.17	0.82	1.4
Caecal content:										
pH	6.02	5.81	5.75	5.87	5.85	0.06	0.56	0.84	0.59	0.33
N-NH$_3$, mmol/L	7.45	5.28	5.59	5.27	6.95	0.10	0.21	0.07	0.34	2.72
Total VFA, mmol/L	63.0	79.2	78.5	71.8	75.5	0.02	0.13	0.47	0.78	15
C$_2$, % mol, VFA	84.9	84.1	85.7	84.4	85.5	0.36	0.13	0.14	0.80	2.2
C$_3$, % mol, VFA	4.0	4.2	3.5	3.9	3.9	0.04	0.12	0.98	0.27	0.7
C$_4$, % mol, VFA	10.4	11.2	10.4	11.2	10.1	0.93	0.29	0.14	0.49	2.1
C$_5$, % mol, VFA	0.54	0.48	0.41	0.50	0.45	0.08	0.95	0.40	0.80	0.18
C$_3$/C$_4$	0.41	0.39	0.35	0.36	0.40	0.23	0.73	0.33	0.17	0.12
Jeujunum mucosa										
Villi height, µm	565	531	485	496	557	0.06	0.87	0.07	0.44	98
Crypth depth, µm	136	137	136	134	138	0.99	0.88	0.55	0.29	20
Villi/crypth	4.18	3.89	3.57	3.72	4.04	0.03	0.96	0.16	0.88	0.66
Ileum mucosa										
Villi height, µm	390	400	375	382	395	0.72	0.63	0.71	0.91	98
Crypth depth, µm	165	112	121	148	117	0.24	0.33	0.29	0.38	86
Villi/crypth	3.08	3.60	3.13	3.13	3.41	0.89	0.11	0.33	0.97	0.81

[a]*L*: linear component of variance. [b]*Q*: quadratic component of variance.

The administration of diets at increasing ratio (starch+soluble fibre)/ADF significantly and linearly increased the proportion of full (with content) caecum (L<0.01) and of ful gut (L<0.001) on live weight and, therefore, the rate of filling of the digestive tract. The higher availability of fermentable carbohydrates linearly decreased caecal pH (from 6.02 to 5.75, L=0.06) and increased total volatile fatty acids (VFA) content at caecum (from 63.0 to 78.5 mmol/L; L=0.02), with a contemporary reduction of the molar proportion of propionic acid (from 4.0% to 3.5% mol VFA; L=0.04).

No significant effect of the protein level was measured at the comparative slaughter. Only the level of N-ammonia tended (P=0.07) to be higher in rabbits fed the diets with the highest protein level, which could be due to a higher ileal flux of protein to caecum.

Ileal mucosa did not change with experimental factors, while at jeujunum the increase of the (starch+soluble fibre)/ADF ratio reduced villi height (L=0.06) and the villi/crypth ratio (L=0.03). The increase of dietary protein tended (P=0.07) to increase villi height.

Slaughter results and meat quality

Live weight of rabbits at the final slaughtering linearly increased with the increase of the (starch+soluble fibre)/ADF ratio (L=0.03) (Table 11).

Table 11. Results at commercial slaughter and carcass quality.

	(starch+soluble fibre)/ ADF ratio (R)			Protein level (P)		Sex (S)		Probability					RSD
	0.6	1.2	2.0	15%	17%	Female	Male	R		P	G	R x P	
								L[a]	*Q*[b]				
Rabbits, n	40	40	40	60	60	55	65						
Slaughter weight (SW), g[2]	2821	2881	2911	2863	2878	2847	2895	0.03	0.67	0.66	0.16	0.27	182
Transport losses, % LW[3]	2.9	2.7	2.7	2.7	2.7	2.7	2.8	0.29	0.87	0.73	0.50	0.93	0.9
Gut incidence, % SW[4]	18.4	18.5	19.2	18.6	18.6	19.0	18.5	0.02	0.24	0.41	0.06	0.97	1.4
Cold carcass (CC), g	1712	1732	1746	1724	1736	1703	1757	0.22	0.90	0.60	0.02	0.17	123
Cold dressing, % CC	60.7	60.1	59.9	60.2	60.3	59.8	60.7	0.02	0.42	0.71	<0.01	0.38	1.4
Cold carcass dissection (% CC):													
Head	7.8	7.7	7.8	7.8	7.8	7.7	7.9	0.83	0.49	0.81	<0.01	0.07	0.4
Liver	5.3	5.2	5.1	5.2	5.2	5.2	5.2	0.20	0.93	0.81	0.89	0.78	0.8
Thoracic organs and kidneys	3.0	3.0	3.0	3.0	3.0	3.0	3.0	0.27	0.91	0.54	0.74	0.51	0.3
Reference carcass (RC), g	1432	1454	1466	1446	1456	1433	1469	0.16	0.80	0.61	0.07	0.13	107
Dissection of reference carcass:													
Dissectible fat, % RC	3.3	3.2	3.7	3.6	3.2	3.7	3.1	0.14	0.07	0.02	<0.01	0.36	1.0
Hind legs, % RC	33.0	32.8	32.4	32.7	32.8	32.8	32.7	0.15	0.65	0.62	0.84	0.66	1.0
Hind leg muscle/bone ratio	6.18	6.46	6.71	6.43	6.47	6.37	6.53	<0.01	0.90	0.80	0.27	0.42	0.53

[a] *L*: probability of the linear component of variance. [b] *Q*: probability of the quadratic component of variance. [2] Live weight at the slaughterhouse immediately before slaughter; [3] LW: live weight at the experimental farm; [4] Incidence of the full gastro-intestinal tract.

However, because of the contemporary increase of the gut incidence (from 18.4% to 19.2% LW, L=0.02), dressing percentage linearly decreased (from 60.7% to 59.9%, L=0.02) and, therefore, carcass weight was similar in the three groups (1730 g on average). The composition of the cold commercial carcass was similar in the three groups, while the muscle to bone ratio of the hind leg linearly increased (6.18 to 6.71; $P<0.01$) with the diets higher in (starch+soluble fibre)/ADF ratio.

The protein level of diets administered during rearing did not affect slaughter results of carcass characteristics, apart from the lower fattening status detected in rabbits fed the diets at 17% CP (P=0.02). This result is quite frequent: a high energy to protein ratio stimulates adipogenesis since it cannot fully satisfy the protein requirements for maximum muscular growth.

Differences detected according to sexes confirm previous observations: females showed a higher incidence of the full gut, which implied a worse slaughter dressing out percentage, a lower cold and reference carcass weight compared to males at the same age. The incidence of head is higher in males and the fattening state is greater in females.

Meat quality, in terms of pH and colour indexes of the muscles *longissimus lumborum* and *biceps femoris,* was not modified at a significant or at a perceivable level by feeding treatments or gender (Table 12).

Table 12. Meat quality: pH and colour of *longissimus lumborum* and *biceps femoris.*

	(starch+soluble fibre)/ ADF ratio (R)			Protein (P)		Sex (S)		Probability					RSD
	0.6	1.2	2.0	15	17	Females	Males	R		P	S	R x P	
								L^a	Q^b				
Rabbits, n	40	40	40	60	60	55	65						
Longissimus lumborum:													
pH	5.63	5.64	5.66	5.64	5.65	5.64	5.65	0.44	0.84	0.59	0.71	0.78	0.11
L*	49.6	50.0	50.4	50.4	49.6	50.1	49.8	0.22	0.95	0.12	0.49	0.46	2.69
a*	-1.80	-1.59	-1.82	+1.71	-1.76	-1.83	-1.65	0.91	0.22	0.74	0.30	0.13	0.92
b*	0.58	1.10	-0.05	0.36	0.73	0.29	0.79	0.15	0.03	0.29	0.16	0.56	1.90
C*	2.61	2.81	2.59	2.70	2.64	2.65	2.69	0.92	0.24	0.68	0.79	0.48	0.90
T*	-0.12	-0.12	-0.11	0.01	-0.10	0.01	-0.10	0.18	0.47	0.40	0.40	0.32	0.76
Biceps femoris													
pH	5.83	5.86	5.88	5.83	5.88	5.86	5.86	0.35	0.81	0.15	0.95	0.53	0.14
L*	47.8	47.9	48.2	47.7	48.3	47.7	48.2	0.40	0.70	0.13	0.18	0.27	1.94
a*	-2.56	-2.58	-2.70	-2.57	-2.66	-2.73	-2.49	0.28	0.63	0.43	0.03	0.17	0.59
b*	3.80	3.71	3.62	3.89	3.53	3.72	3.70	0.49	0.99	0.09	0.90	0.18	3.71
C*	4.66	4.63	4.58	4.75	4.49	4.68	4.56	0.72	0.96	0.15	0.49	0.11	0.96
T*	-0.89	-0.92	-0.91	-0.91	-0.90	-0.92	-0.90	0.69	0.70	0.92	0.76	0.04	0.29

[a]*L*: linear component of variance. [b]*Q*: quadratic component of variance.

Nitrogen excretion and efficiency of protein utilization

To evaluate the effect of nitrogen excretion in the environment, individual data recorded for measuring performance were used to calculate the nitrogen balance in function of the dietary treatment (Table 13). In both periods of trials a significant reduction of the nitrogen excretion was measured when the (starch+soluble fibre)/ADF ratio increased and the dietary protein level decreased. This reduction was mainly due to the reduction of the amount of ingested N, which depended on the lower feed ingestion in diets with increasing (starch+soluble fibre)/ADF ratio and the lower N concentration of less proteic diets, while the amount of retained N did not change (P>0.10).

Table 13. Nitrogen balance in rabbits fed diets at different protein level from 37 days of age until slaughter (79 days): effect of (starch+soluble fibre)/ADF ratio and protein level.

	(starch+soluble fibre) /ADF ratio (R)				Protein level (P)			Inter.	RSD
	0.6	1.2	2.0	Prob.	15%	17%	Prob.	R x P	
Rabbits, n	69	78	78		111	114			
Body N at 37 d[1], g	29.3	29.5	29.5	0.35	29.4	29.5	0.65	0.88	1.03
Body N at 58 d[1], g	63.0	64.0	64.4	0.44	63.8	63.8	0.93	0.12	6.72
Body N at 79 d[1], g	90.3	91.8	93.1	0.10	91.4	92.1	0.52	0.23	7.97
First period (37-58 d):									
Feed intake, g/d	163	141	121	<0.001	144	140	0.15	0.30	20
Ingested N, g	87.2	76.7	64.7	<0.001	73.1	79.3	<0.001	0.05	10.6
Retained N, g	33.7	34.4	34.9	0.54	34.3	34.3	0.99	0.09	6.56
Excreted N, g	53.5	42.3	29.8	<0.001	38.8	45.0	<0.001	0.05	5.58
Excreted N, g/d	2.55	2.01	1.42	<0.001	1.85	2.14	<0.001	0.05	0.27
Second period (58-79 d):									
Feed intake, g/d	198	173	150	<0.001	174	173	0.60	0.24	19
Ingested N, g	106.2	93.3	80.2	<0.001	88.5	97.9	<0.001	<0.01	10.6
Retained N, g	27.3	27.8	28.7	0.18	27.6	28.3	0.34	0.68	4.79
Excreted N, g	78.9	65.5	51.4	<0.001	60.9	69.2	<0.001	<0.001	8.12
Excreted N, g/d	3.76	3.12	2.45	<0.001	2.90	3.32	<0.001	<0.001	0.39
Whole trial (37-79 d):									
Feed intake, g/d	181	157	135	<0.001	159	156	0.25	0.21	16
Ingested N, g	193.4	169.9	144.9	<0.001	161.6	177.2	<0.001	<0.01	18.1
Retained N, g	61.0	62.2	63.6	0.12	61.97	62.6	0.55	0.20	7.79
Excreted N, g	132.4	107.7	81.2	<0.001	99.6	114.6	<0.001	<0.001	12.1
Excreted N, g/d	3.15	2.56	1.93	<0.001	2.37	2.73	<0.001	<0.001	0.29

[1] Body N (g/kg)= 28.3+0.93xLive weight (kg) (Szendro *et al.*, 1998).

The effect of the reduction of the (starch+soluble fibre)/ADF ratio was more evident compared to the effect of the dietary protein level (Figure 10), as a direct consequence of the great reduction in feed intake when increasing dietary starch and soluble fibre fractions.

During the whole trial, daily nitrogen excretion in rabbits fed diets with high (starch+soluble fibre)/ADF ratio (1.2 and 2.0) was 80% and 60% of that measured in rabbits fed diets A (ratio = 0.60) (Figure 10a and 11a). During the first period, nitrogen excretion tended to be lower when rabbits were fed diets with (starch+soluble fibre)/ADF ratio equal to 2.0.

Nitrogen excretion diminished by about 15% when decreasing dietary protein from 17% to 15% and without differences between the first and the second period of growth (Figure 10b and 11b). In other words, a clear improvement of feed conversion, like that provoked by a more energetic diet, permits to control nitrogen excretion in a more efficient way than 1-2 points reduction in crude protein of diets.

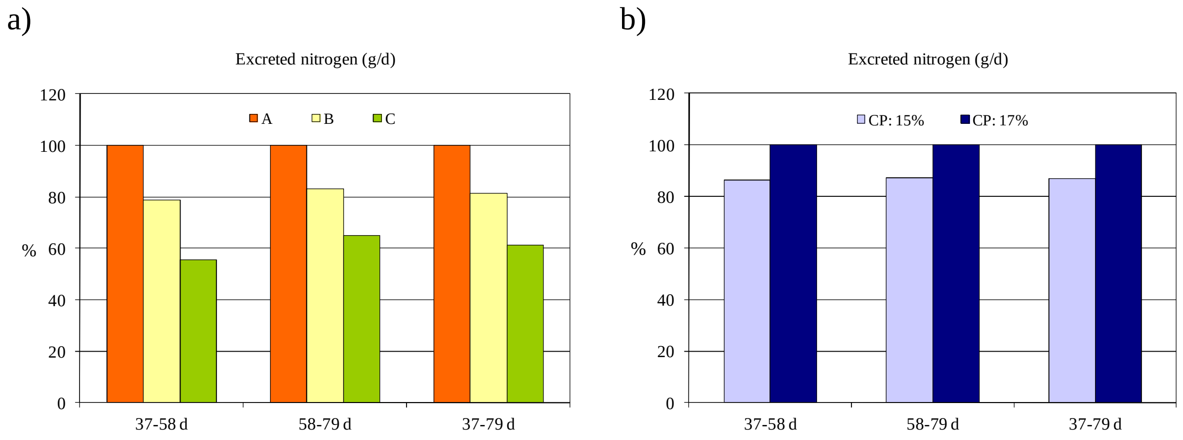


Figure 10. Nitrogen excretion during the trial: a) effect of (starch+soluble fibre)/ADF ratio (Diets A=100); b) effect of dietary protein level (CP 17%=100).

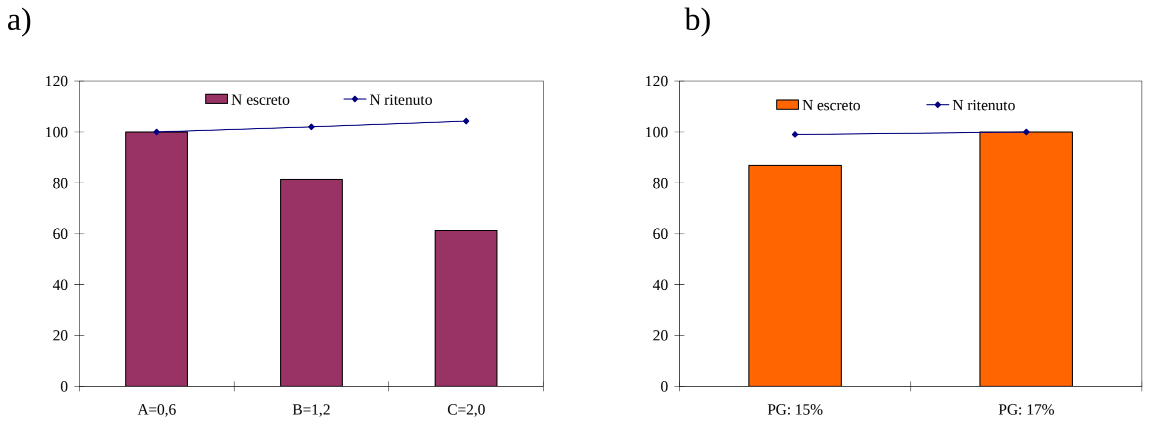


Figure 11. Excreted and retained N during the trial: a) effect of (starch+soluble fibre)/ADF ratio (Diets A=100); b) effect of dietary protein level (CP 17%=100).

Experiment 2.

Effect of a further increase of (starch+soluble fibre)/ADF ratio and decrease of protein level on diets for growing rabbits

Introduction

Both experimental studies and field results push towards the reduction of the use of high-insoluble fibre diets in growing rabbits, since they greatly limit growth performance, impair feed conversion and increase nitrogen excretion while do not control digestive disorders at an acceptable degree and do not prevent the use of prophylactic and therapeutic drugs.

Therefore, basing on the results of the previous experiment of this thesis (Experiment 1), the present trial aimed to evaluate the effect of a further increase of the (starch+soluble fibre)/ADF until 2.8 and a further reduction of the dietary protein until 14% on health, growth performance, feed conversion, nitrogen excretion and slaughter results of growing rabbits. To this aim, diets were formulated according to a bi-factorial design with (starch+soluble fibre)/ADF ratio increasing from about 1 to 3 and dietary protein decreasing from about 15% to 14%. Specific objectives were to reduce mortality and morbidity, to improve feed conversion, to reduce as much as possible the digestible protein to digestible energy ratio in view of optimizing protein efficiency and controlling nitrogen excretion, to obtain high dressing out percentage and meat to bone ratio. The effect of the weaning weight of kits was also taken into account.

Materials and methods

Rearing conditions

The trial was realized in the experimental farm of the University of Padova in the period March-April 2009. The characteristics of experimental facilities and cages have been detailed previously in the materials and methods of Experiment 1.

During the trial, the maximum temperature never exceeded 24°C and the minimum temperature remained within 17°C (Figure 12). Minimum and maximum temperature averaged 19°C and 21°C. The maximum relative humidity was on average 62%, ranging from 46% to 78% (Figure 12). Minimum relative humidity averaged 52% and changed from 37% to 70%.

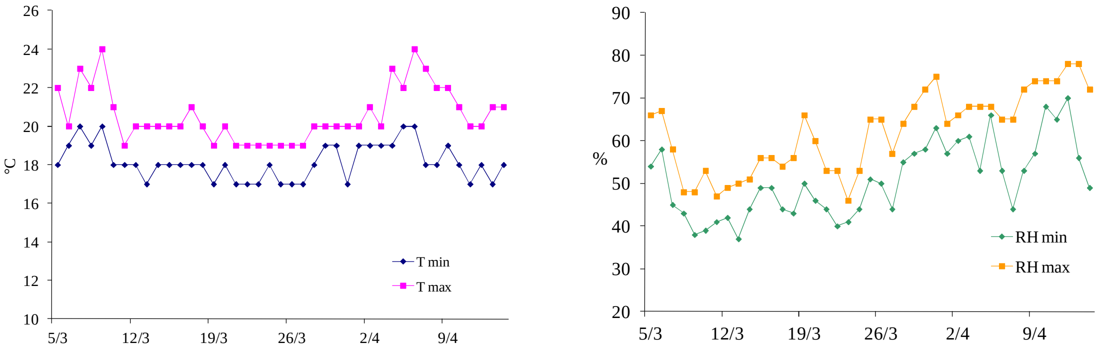


Figure 12. Daily values of minimum and maximum temperature and minimum and maximum relative humidity (RH) throughout the trial.

Animals, experimental groups and recordings

The research was approved by the Ethical Committee of the University of Padova for animal experimentation.

At 33 days, 306 rabbits (Grimaud Frères, France) with homogeneous weight were selected from healthy litters in a commercial farm and in such a way to be representative of the farm in terms of frequency of parity order and distribution of weaning weight. To this aim, rabbits used for the trial came from the full litters (of 9 kits each) of 34 rabbit does, previously selected and which did not present clinical signs of illness (stafilococcosis, pasteurellosis, mycosis, diarrhoea), according to the following scheme:

- 54 kits weaned from 6 does at their first parturition x 9 kits (P1);
- 54 kits weaned from 6 does at their second parturition x 9 kits (P2);
- 54 kits weaned from 6 does at their third parturition x 9 kits (P3);
- 54 kits weaned from 6 does at their fourth and fifth parturition x 9 kits (P4+5);
- 54 kits weaned from 6 does at their sixth and seventh parturition x 9 kits (P6+7);
- 36 kits weaned from 4 does with more than eight parturitions x 9 kits (P8).

Rabbits were transferred to the experimental facilities in collective cages and at their arrival, animals were healthy and weighed 975±97 g. Rabbits were controlled by sex, put into individual cages and marked using ear tags.

The animals were put in individual cages and divided into six groups of 51 units each. The first 6 rabbits of each doe were assigned to the different six experimental groups and alternating does of each parity order (that is first doe P1, second doe P2, etc.) and until 204 rabbits were assigned. Then, kits were assigned to the six experimental groups taking into account the parity order, but alternating the does (3 remaining kits per each doe). This way, a homogeneous distribution of rabbits according to the parity order and the litter origin was guaranteed within experimental group.

The six experimental groups were then given six diets formulated according to a bi-factorial arrangement [3 (starch+soluble fibre)/ADF ratio x 2 protein levels], as detailed in the scheme below:

	Protein 14%	Protein 15%
D = 0.6: (Starch14%+SF10%)/ADF20%	Diet D14	Diet D15
E = 1.2: (Starch17%+SF11%)/ADF16%	Diet E14	Diet E15
F = 2.0: (Starch20%+SF12%)/ADF12%	Diet F14	Diet F15

The diets were given *ad libitum* from the beginning of the trial until commercial slaughter (76 d). The trial started the day after rabbits' arrival at the experimental farm (at the age of 34 d): rabbits were reared during 42 days until commercial slaughter at 76 days of age.

Individual live weight and feed intake were recorded three times a week. Health status was controlled daily to detect promptly the occurrence of diseases, especially digestive problems, as described in the Experiment 1. At 42 d, 36 rabbits were slaughtered to sample caecal contents and mucosa of jejunum (intermediate tract). The *in vivo* digestibility trial was carried out on 72 animals from 54 to 58 days of age to evaluate the nutritive value of diets. Digestibility trial, histological analysis and chemical analyses of experimental diets, faeces and caecal contents were performed according to the methodologies previously detailed.

Experimental diets

All diets were pelleted with 3.5 mm of diameter and 1.0-1.1 cm of length. The diets D14, F14, D15 and F15 were produced, while E14 and E15 diets were obtained at the experimental farm by mixing diets D14 and F14 (0.5+0.5) and the diets D15 and F15 (0.5+0.5), respectively. Diets were formulated in such a way to present two protein levels, 14% and 15% CP. The protein level was increased thanks to a higher inclusion rate of soybean meal 49% CP (+2-2.5 points) (Table 14). The increase of soluble fibre and starch from diets with 1.2 ratio to diets with 2.8 ratio was obtained at the expenses of insoluble fibre (ADF) and through the higher inclusion of dried beet pulp (about +16 points), barley (+17/20 points) which replaced dehydrated alfalfa meal (-36/37 points). The protein level of diets with 2.8 ratio and with 1.2 ratio remained constant thanks to the increase of the soybean meal level (about +8 points in diets with 2.8 ratio compared to diets with 1.2 ratio).

Diets were supplemented with synthetic amino acids, micro- and macro-minerals and vitamins to satisfy the nutritional needs of young rabbits (De Blas and Mateos, 2010) and did not contain any antibiotic or coccidiostatic drugs. The results of chemical analyses on composition of diets are reported in Table 15.

Table 14. Ingredient composition (%) of experimental diets.

	Diet D14	Diet F14	Diet D15	Diet F15
Dehydrated alfalfa meal 16% CP	37.00	0.00	36.00	0.00
Wheat bran	21.00	16.00	30.00	20.00
Barley	16.00	33.00	10.50	30.00
Dried beet pulp	17.48	33.30	13.50	30.00
Soybean meal 49%	4.00	11.50	6.00	14.00
Sunflower oil	1.00	2.00	1.00	2.00
Molasses	1.50	1.50	1.50	1.50
Calcium carbonate	0.00	0.74	0.17	0.98
Dicalcium phosphate	0.70	0.80	0.20	0.55
Sodium chloride	0.40	0.40	0.40	0.40
L-lysine	0.25	0.10	0.14	0.00
DL-methionine	0.13	0.10	0.09	0.06
DL-threonine	0.07	0.09	0.03	0.04
Vitamin-mineral premix*	0.47	0.47	0.47	0.47

*Supplementation per kg of feed: vit. A, 12.000 UI; vit. D3, 1.000 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.

Table 15. Chemical composition (as fed) of experimental diets.

Diet	D14	E14	F14	D15	E15	F15
Dry matter, %	89.3	89.1	88.9	89.6	88.9	88.4
Crude protein, %	14.3	14.3	14.2	14.8	15.2	15.1
Ether extract, %	3.39	3.65	4.01	3.81	3.74	4.17
Crude fibre, %	15.5	13.1	9.35	15.6	12.5	8.94
Ash, %	6.86	6.04	5.27	6.95	6.69	5.27
TDF, %	42.7	40.2	37.3	43.7	40.3	35.9
aNDF, %	34.7	30.5	26.4	36.3	31.3	26.2
ADF, %	18.4	14.9	11.4	18.9	15.0	11.2
Hemicelluloses (NDF-ADF), %	16.3	15.6	15.0	17.4	16.3	15.0
Lignin (sa), %	2.7	1.7	0.8	2.9	1.8	0.8
Soluble fibre[1], %	10.0	11.1	12.0	9.1	10.0	11.2
Starch, %	14.0	17.8	21.2	12.4	16.2	19.6
Gross energy, MJ/kg	16.3	16.3	16.3	16.5	16.4	16.4
Starch/ADF ratio	0.8	1.2	1.9	0.7	1.1	1.8
Soluble fibre/ADF ratio	0.54	0.74	1.05	0.48	0.67	0.82
(Starch+soluble fibre)/ADF ratio	1.30	1.94	2.91	1.14	1.75	2.75
Digestible energy, MJ/kg	9.8	11.2	12.2	9.6	10.8	12.3
DP/DE, g/MJ	10.5	9.1	8.6	11.4	10.5	9.4

[1] TDF-aNDF after correction for protein and ash content (Van Soest *et al*., 2001).

As previewed during formulation, two groups of diets with a different protein level, 14.3% and 15.0% on average, resulted. Within protein level, TDF, aNDF and, especially, ADF and ADL decreased due to the decreasing inclusion (until absence) of alfalfa meal and the increasing rate of dried beet pulp (containing pectins) and barley (with a low content in insoluble fibre fractions). The (starch+soluble fibre)/ADF ratio increased from diets with 1.2 ratio (on average) to diets with 1.8 ratio to diets with 2.8 ratio. The content of digestible energy of diets strictly depended on changes in starch+soluble fibre/ADF ratio and increased from about 9.7 to 11.0 and 12.3 MJ/kg in diets with 1.2, 1.8 and 2.8 ratios, respectively. Differently, the nutritive value did not change with the protein level. The content of digestible protein (DP) was on average higher in diets at 15% CP than in those at 14% CP (103 *vs*. 113 g/kg) and the DP/DE ratio increased by about 1 g/MJ. When the (starch+soluble fibre)/ADF ratio increased, DP/DE ratio decreased by about 2.0 g/MJ from diets with 1.2 ratio to diets with 2.8 ratio (from 10.5 to 8.6 g/MJ within diets CP14 and from 11.4 to 9.4 g/MJ within diets CP15).

Health status

Rabbits were considered ill in presence of diarrhoea and mucus as well as when showing a clear and lasting reduction of feed intake (approximately -30% of the previous recordings) and of growth (weight loss during 2-3 consecutive days). Morbidity, mortality and sanitary risk were calculated according to Bennegadi *et al*. (2000). Moreover, the daily instantaneous mortality was calculated as the percentage of ill animals in respect to live animals in a fixed day. Finally, a “sanitary index” was calculated with the aim of weighing the degree of illness and or death. To calculate this index, each animal was scored according to the health condition as follows:

- no illness = 0
- brief illness (animal ill during 1 to 5 days) = 1
- long illness (animal ill for more than 5 days) = 2
- losses (loss of the animal because dead or discarded on the base of final live weight <2.0 kg) = 3.

About one week after their transfer to the experimental facilities, first symptoms of digestive diseases appeared: growth rate and feed intake decreased, and soon after diarrhoea and mucus in faeces appeared as well as first animals died. Morbidity and mortality were attributed to epizootic rabbit enteropathy, characterized by diarrhoea, mucoid enteritis, abdominal bloating and other pathognomic signs.

Due to the severity of the illness and to avoid an excessively high mortality which could have compromised also zootechnical performance, from 48 to 57 days of age, all rabbits were treated with an antibiotic in water (100 mL/100 L Tiamuline 10%, Vetem, Centralvet, Agrate Brianza, Milano, Italy, corresponding to 10 g tiamuline/100 L water, and 150 mL/100 L Colistine solphute 12% liquid, Dox-Al Italia S.p.A., Correzzana, Milano, Italy, corresponding to 18 g colistine/100 L). The first dead animals were delivered to the Istituto Zooprofilattico of Padova for the anatomic-pathological examination and the determination of the antibiogram which confirmed the veterinarian diagnosis for the presence of *Coli* and *Clostridia* and the “sensibility” of bacteria to the antibiotics used.

Final slaughter, carcass dissection and meat quality analyses

At 76 days of age, all rabbits were weighed. Out of all rabbits, 120 animals were selected (20 for each experimental group and representative in terms of average weight and variability) for commercial slaughter and dissection. Rabbits with live weight less than 2,0 kg were excluded from this set. Feed and water were available until loading. The animals were transported inside cages (50 x 100 x 30 cm) with 6 rabbits per cage (one rabbit per diet, 12 rabbits/m^2) in a conditioned truck for about 60 minutes to a commercial slaughterhouse.

At slaughterhouse rabbits were individually weighed, then stunned by electro-anaesthesia and killed by jugulating. Carcasses were chilled at 4°C for 2 h and then transported to the laboratories of the Department of Animal Science of Padova for dissection. Slaughter procedure, carcass dissection and meat quality analyses were performed by using the procedures and methodologies detailed in the first experiment.

Statistical analysis

The data of performance were analyzed using ANOVA and with (starch+soluble fibre)/ADF ratio, protein level, parity order, sex and the interaction protein level x (soluble fibre+starch)/ADF ratio as main factors. Weight at weaning was included in the model as a covariate. Data of digestibility coefficients, intestinal mucosa and caecal fermentative activity were submitted to analysis of variance with (soluble fibre+starch)/ADF ratio, protein level and their interaction as main factors. The GLM procedure of SAS (SAS, 1991) was used for all analyses. Orthogonal contrasts were used to test the linear and quadratic response to the increase of the ratio (starch+soluble fibre)/ADF. Differences among means with $P<0.05$ were accepted as representative of statistically significant differences. Differences among means with $0.05<P<0.10$ were accepted as representative of a tendency to differences. Mortality, morbidity, and sanitary risk were analysed by the CATMOD SAS procedure. The sanitary index was analysed using the GLM procedure of SAS.

Results

Digestibility and nutritive value of experimental diets

The digestibility coefficients of diets are reported in table 16 according to the experimental factor, ratio (starch+soluble fibre)/ADF and protein level.

Table 16. Digestibility coefficients (%): effect of (starch+soluble fibre)/ADF ratio and protein level.

	(starch+soluble fibre)/ADF ratio					Protein level			RSD
	1.2	1.8	2.8	L[a]	Q[b]	14%	15%	Prob.	
Rabbits, n	23	21	22			33	33		
Dry matter	58.2	66.4	75.6	<0.001	0.37	67.1	66.4	0.13	1.86
Crude protein	72.5	73.0	75.4	<0.001	0.08	72.5	74.8	<0.001	2.05
Ether extract	79.2	81.2	85.4	<0.001	<0.01	81.4	82.4	<0.01	2.32
TDF	37.4	49.6	61.6	<0.001	0.92	50.2	50.0	0.14	3.21
aNDF	27.0	37.6	51.0	<0.001	0.14	39.4	37.7	0.07	3.68
ADF	17.8	27.1	40.4	<0.001	0.09	28.3	28.6	0.83	4.41
Hemicelluloses	37.3	47.5	59.1	<0.001	0.46	49.9	46.0	<0.001	3.46
Soluble fibre[1]	85.5	89.4	88.7	0.12	0.20	85.0	90.8	<0.001	6.61
Starch	97.5	98.0	98.6	<0.001	0.47	98.1	98.0	0.03	0.23
Gross energy	59.0	66.6	71.1	<0.001	0.33	67.5	66.4	0.02	1.99

[a] *L*: linear component of variance. [b] *Q*: quadratic component of variance. [1] Significant interaction P=0.01, ether extract digestibility: 78.3%, 80.0%, 85.8%, 80.1%, 82.3%, and 84.9% in diets D14, E14, F14, D15, E15 and F15. [2] Significant interaction P=0.01, soluble fibre digestibility: 79.3%, 89.2%, 86.4%, 91.7%, 89.6%, and 90.9% in diets D14, E14, F14, D15, E15 and F15.

The digestibility of dry matter, energy and all other nutrients significantly and linearly increased with the increase of the ratio (starch+soluble fibre)/ADF and as a consequence of the higher inclusion rate of dried beet pulp and barley and the lower presence of alfalfa meal. Only digestibility of soluble fibre, which was always higher than 80%, was not affected.

The increase of structural carbohydrates with a lower lignification degree and a lower complexity of cell walls, characterized therefore by a higher susceptibility to the action of animal and bacterial enzymes, explain the increase of the apparent faecal digestibility of the fibrous fractions and dry matter. The effect of the protein level, even if significant, was less important and resulted in a moderate increase of crude protein digestibility, which could be explained by the higher rate of protein coming from soybean meal rather than alfalfa in the diets with the higher protein content. The digestibility of insoluble fibre fractions (ADF and lignin) did not change, whereas that of hemicelluloses and soluble fibre changed in a different way: lower coefficients were measured for hemicelluloses, while higher values were recorded for soluble fibre (pectins and β-glucans) without effects on the digestibility of total dietary fibre (TDF). The presence of significant interactions was limited to the digestibility of ether extract and soluble fibre.

Growth performance and health status

One week only after housing rabbits in the experimental farms, first symptoms of digestive disorders appeared (low feed intake, diarrhoea, mucus) which could be clearly referred to epizootic rabbit enteropathy.

The number of ill rabbits increased during the second week of trial, especially in rabbits fed diets with a (starch+soluble fibre)/ADF ratio of 2.8, with instantaneous morbidity equal to 15-18% of present and live rabbits, in front of maximum peaks at 12% for rabbits submitted to 1.2 and 1.8 ratios (Figure 13). Similarly, morbidity of rabbits fed diets with 15% CP reached daily peaks (13-15%) higher compared to rabbits fed diets at 14% CP (9-11%).

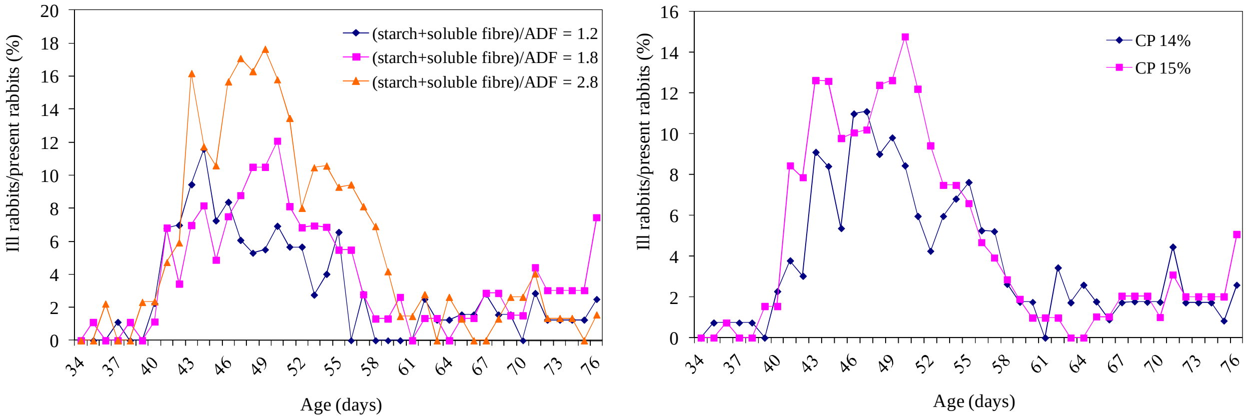


Figure 13. Morbidity (percentage of ill rabbits in respect of total live rabbits per day) according to the main experimental factors.

The first animals died during the second week of trial, when the number of ill rabbits increased rapidly (Figure 14). Daily mortality reached a peak during the third week and slowed down only after the antibiotic treatment which was administered from 48 until 57 days of age.

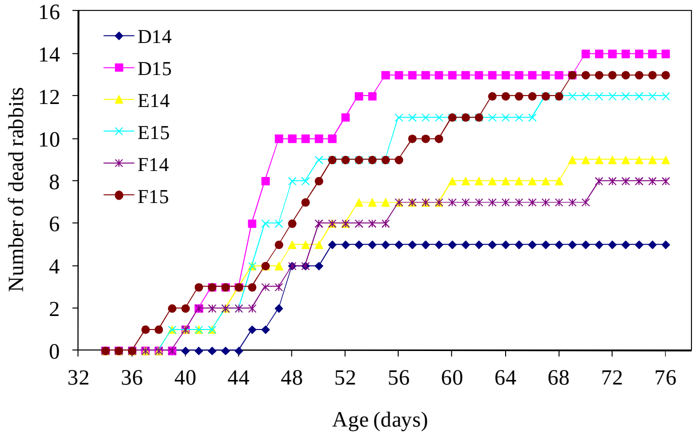


Figure14. Cumulative mortality (number of dead rabbits) per experimental diet.

In the whole trial, mortality averaged 22.6% corresponding to 61 dead animals of which 5, 9, 8, 14, 12 and 13 fed the diets D14, E14, F14, D15, E15 and F15, respectively. Mortality was mainly related to digestive problems due to ERE. Ten rabbits showed symptoms of respiratory diseases or other problems (cists, wounds of various type) (1, 3, 1, 2, 3 and 0 rabbits fed diets D14, E14, F14, D15, E15 and F15 respectively). Moreover, at the end of the trial, seven rabbits were excluded since they weighed less than 2.0 kg, which is the minimum commercial weight (2, 3 and 2 rabbits fed the diets E14, F14 and E15 respectively). The performance of these rabbits was not included in the data set of the trial to be statistically analysed.

Mortality did not vary according to the (starch+soluble fibre)/ADF ratio (Table 17). On the contrary, mortality was significantly higher (16.3% *vs*. 28.9%, P=0.01) in rabbits fed the diets with the highest protein content.

Table 17. Mortality, morbidity and sanitary risk: effect of (starch+soluble fibre)/ADF and protein level of diets.

	(starch+soluble fibre)/ADF ratio (R)				Protein level (CP)			Prob.
	1.2	1.8	2.8	Prob.	14%	15%	Prob.	R x CP
Mortality[1], %	21.1 (19)	23.3 (21)	23.3 (21)	0.79	16.3 (22)	28.9 (39)	0.01	0.48
Morbidity[1], %	17.8 (16)	20.0 (18)	31.1 (28)	0.09	25.9 (35)	20.0 (27)	0.32	0.12
Sanitary risk[1], %	38.9 (35)	43.3 (39)	54.4 (49)	0.10	42.2 (57)	48.9 (66)	0.26	0.28
Sanitary index	0.82	1.04	1.18	0.16	0.87	1.16	0.07	0.49

[1] Between parenthesis the number of dead and/or ill rabbits.

As what concerns the effect of the changes in structural and non structural carbohydrates (soluble and insoluble fibre and starch), the trend towards an increased morbidity (P=0.09) and, at similar mortality rates, the increase of the sanitary risk (P=0.10), when increasing the ratio (starch+soluble fibre)/ADF may be explained by the increase of starch and the reduction of ADF in diets with 1.2 ratio compared to diets with 2.8 ratio rather than with changes in soluble fibre fractions, which content slightly increased from 9-10% in diets with 1.2 ratio to 11-12% in diets with 2.8 ratio (see Table 17).

To find new indices of rabbit health a "sanitary index" was calculated with the aim of assessing also the severity of the illness and of taking into account the economic weight of discarded animals (slaughter live weight less than 2.0 kg). The value of this index ranged from 0.58 with the administration of diet D14 to 1.20 in rabbits fed diets E15 and F15. Even if differences among groups were not statistically significant, this index could express synthetically the average health status of animals belonging to different experimental groups, with a better economical value compared to the sanitary risk proposed by French researchers (Bennegadi *et al*., 2000).

As largely discussed, the diffusion of ERE not only increased rabbit mortality in farms, but also impaired growth performance of several ill rabbits (on average 23% of rabbits) which, even when recovered health thanks to the antibiotic treatment, did not reach the final requested commercial weight. Table 18 reports the frequency of dead+discarded rabbits that did not reach the end of the trial, rabbits below commercial weight (2.0-2.4 kg) and rabbits at the commercial weight (>2.4 kg), and how these latter animals were distributed in class of live weights (standard, heavy and very heavy). Within rabbits fed diets at 14% CP, a clear, even if not significant, reduction of rabbits with live weight within commercial standards (>2.4 kg), from 80% to 67%, was found when the (starch+soluble fibre)/ADF ratio increased (Table 18). When considering the effect of the main factors, the number of animals with a suitable commercial weight decreased significantly (74.1% *vs*. 62.2%; P=0.03) when the dietary protein level increased, as a consequence of both a higher number of dead and discarded animals (<2.0 kg) and light rabbits (2.0-2.4 kg) (Table 18). Among the different weight classes, the loss of animals was especially at the expenses of the most numerous classes, that is heavy rabbits with live weight from 2.7 to 3.0 kg.

Table 18. Distribution of rabbits according to live weight: effect of (starch+soluble fibre)/ADF and protein level of diets.

	(starch+soluble fibre)/ADF ratio (R)				Protein level (CP)			Prob.
	1.2	1.8	2.8	Prob.	14%	15%	Prob.	R x CP
Dead+discarded rabbits, %	21.1	27.8	26.7	0.40	20.0	30.4	0.04	0.32
Low weight (2.0-2.4 kg), %	6.7	3.3	10.0	0.22	5.9	7.4	0.62	-[1]
Commercial weight (>2.4 kg), %	72.2	68.9	63.3	0.39	74.1	62.2	0.03	0.73
- Standard (2.4-2.7 kg), %	16.7	14.4	15.6	0.90	15.6	15.6	0.96	0.55
- Heavy (2.7-3.0 kg), %	33.3	34.4	28.9	0.70	37.0	27.4	0.10	0.89
- Very heavy (>3.0 kg), %	22.2	20.0	18.9	0.86	21.5	19.3	0.66	0.94

[1] Data not analysed statistically.

Despite the presence of a pathology which severely affected rabbit performance during a quite large period of the trial, as described by the weekly variations of weight gain and feed intake (Figure 15), and with a morbidity rate which reached in the worse case (diet F14) the value of 40%, the antibiotic treatment permitted to overcome the illness and to reach the end of the trial with satisfying performance for the genetic type used and regardless of the experimental treatment (Table 19). In details, at 76 days of age rabbits weighed on average 2825 g, which corresponded to a daily weight gain of 43.9 g/d, a feed intake of 141 g/d and a feed conversion of 3.21 in the whole period of growth.

When the (starch+soluble fibre)/ADF ratio and the nutritive value of diets increased, growth rate and live weight did not change, whereas feed intake significantly and linearly decreased (P<0.001) both in the first and second period of the trial. As a consequence, feed conversion linearly improved (from 3.65 to 3.22 and 2.76; P<0.001) until values which were particularly favourable, especially for treatment with 2.8 ratio. The improvement of feed conversion was rather expected on the base of diets characteristics and the higher level of starch and soluble fibre in replacement of insoluble fibre. However, the composition with 2.8 ratio diets can be considered extreme and the recorded values of feed conversion (2.76) are hardly measurable not only in commercial rabbit farm but even in experimental conditions.

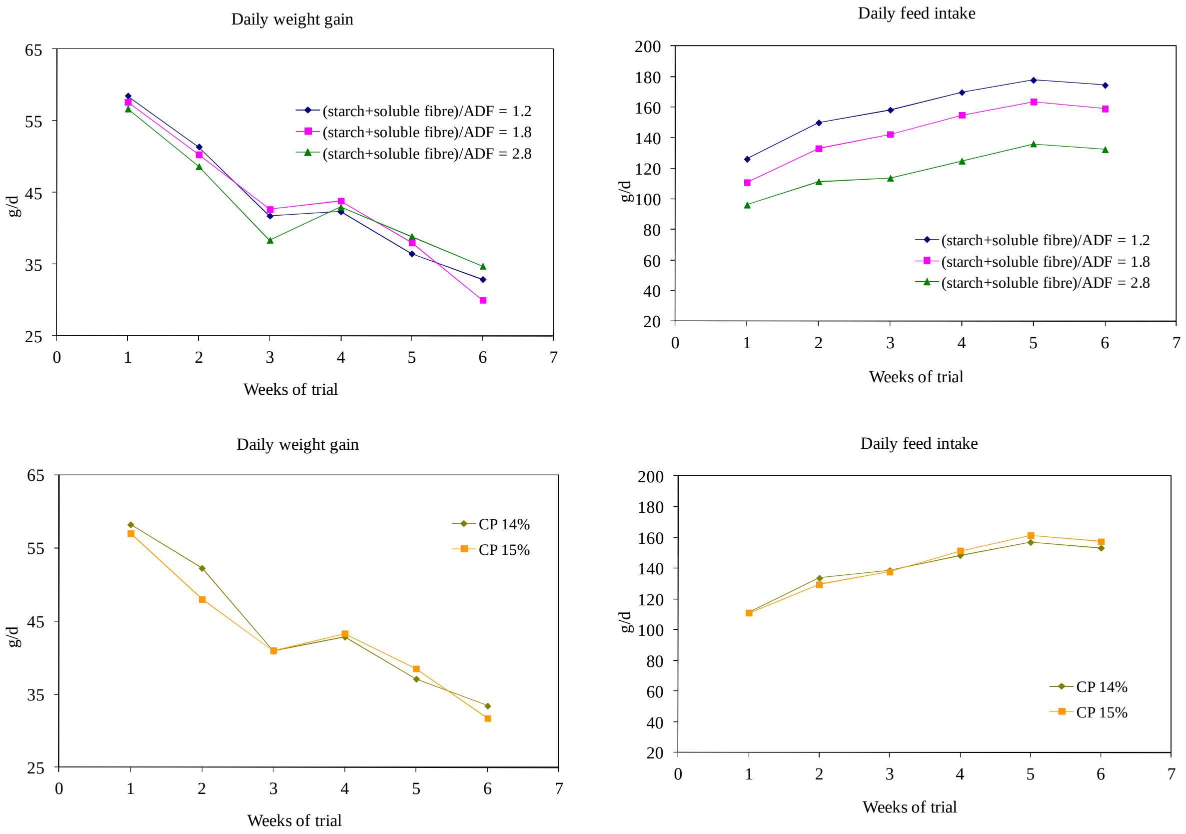


Figure 15. Weekly performance during the trial: effect of the (starch+soluble fibre)/ADF ratio and protein level of the experimental diets.

The increase of the protein level from 14% to 15% CP did not affect productive performance on the whole, whereas improved feed conversion during the first period (P=0.07) and increased growth and feed intake during the second period. The interaction between the two main experimental factors was never significant and, similarly, no effect of sex was recorded.

The effect of the parity order (K) was slightly appreciable at the start of the trial, with lighter rabbits from older does (P=0.11), and at 55 (P=0.07) and 76 (P=0.10) days: rabbits coming from does at their first kindling and from does with more than 8 kindling were lighter, showed lower growth rates during the first period of the trial (from 34 to 55 days of age). When the whole experimental period was considered, however, these differences were not detectable anymore.

From a statistical point of view, the effect of the weaning weight was more important ($P<0.001$) and affected live weight at 55 days and at the end of the trial, besides feed intake and feed conversion (Table 19). However, the graphical representations of the same regressions (Figure 16) show a weak correlation ($0.05 \leq r \leq 0.26$) between the initial weight (weaning weight) and the weight a 56 or 76 days, daily gain, feed intake and feed conversion.

Table 19. Performance from weaning until commercial slaughter.

	(starch+soluble fibre)/ADF ratio (R)			Protein level (CP)		Sex (S)		Probability						RSD
	1.2	1.8	2.8	14%	15%	Females	Males	R_L	R_Q	P	S	Weaning weight	RxCP	
Rabbits, n.	71	65	66	108	94	103	99							
Live weight, g														
At 34 d	974	987	972	986	970	982	974	0.92	0.34	0.27	0.57	-	0.85	99
At 55 d	2041	2034	1985	2036	2004	2029	2011	0.13	0.53	0.28	0.56	<0.001	0.37	212
At 76 d	2826	2862	2804	2829	2832	2833	2828	0.63	0.23	0.93	0.90	<0.001	0.38	255
First period (34-55 d)														
Daily growth, g/d	50.5	50.2	47.9	50.3	48.7	49.9	49.1	0.13	0.53	0.28	0.56	0.22	0.37	10.1
Feed intake, g/d	145	128	107	127	126	129	125	<0.001	0.54	0.76	0.18	<0.001	0.47	19
Feed conversion	2.89	2.69	2.38	2.56	2.75	2.59	2.71	<0.001	0.60	0.07	0.26	<0.001	0.38	0.72
Second period (56-76 d)														
Daily growth, g/d	37.4	39.4	39.0	37.8	39.5	38.3	38.9	0.19	0.26	0.10	0.55	0.36	0.78	7.2
Feed intake, g/d	175	159	132	152	158	155	155	<0.001	0.04	0.05	0.76	<0.001	0.86	20
Feed conversion	4.82	4.12	3.44	4.16	4.09	4.12	4.13	<0.001	0.90	0.51	0.91	0.06	0.42	0.79
Whole trial														
Daily growth, g/d	44.0	44.8	43.4	44.0	44.1	44.1	44.0	0.63	0.23	0.93	0.90	0.12	0.38	6.1
Feed intake, g/d	160	144	120	140	142	142	140	<0.001	0.12	0.33	0.56	<0.001	0.60	17
Feed conversion	3.65	3.22	2.76	3.19	3.23	3.22	3.20	<0.001	0.65	0.28	0.53	<0.001	0.40	0.25

R_L and R_Q: probability of the linear and quadratic component of variance for the effect of the (starch+soluble fibre)/ADF ratio.

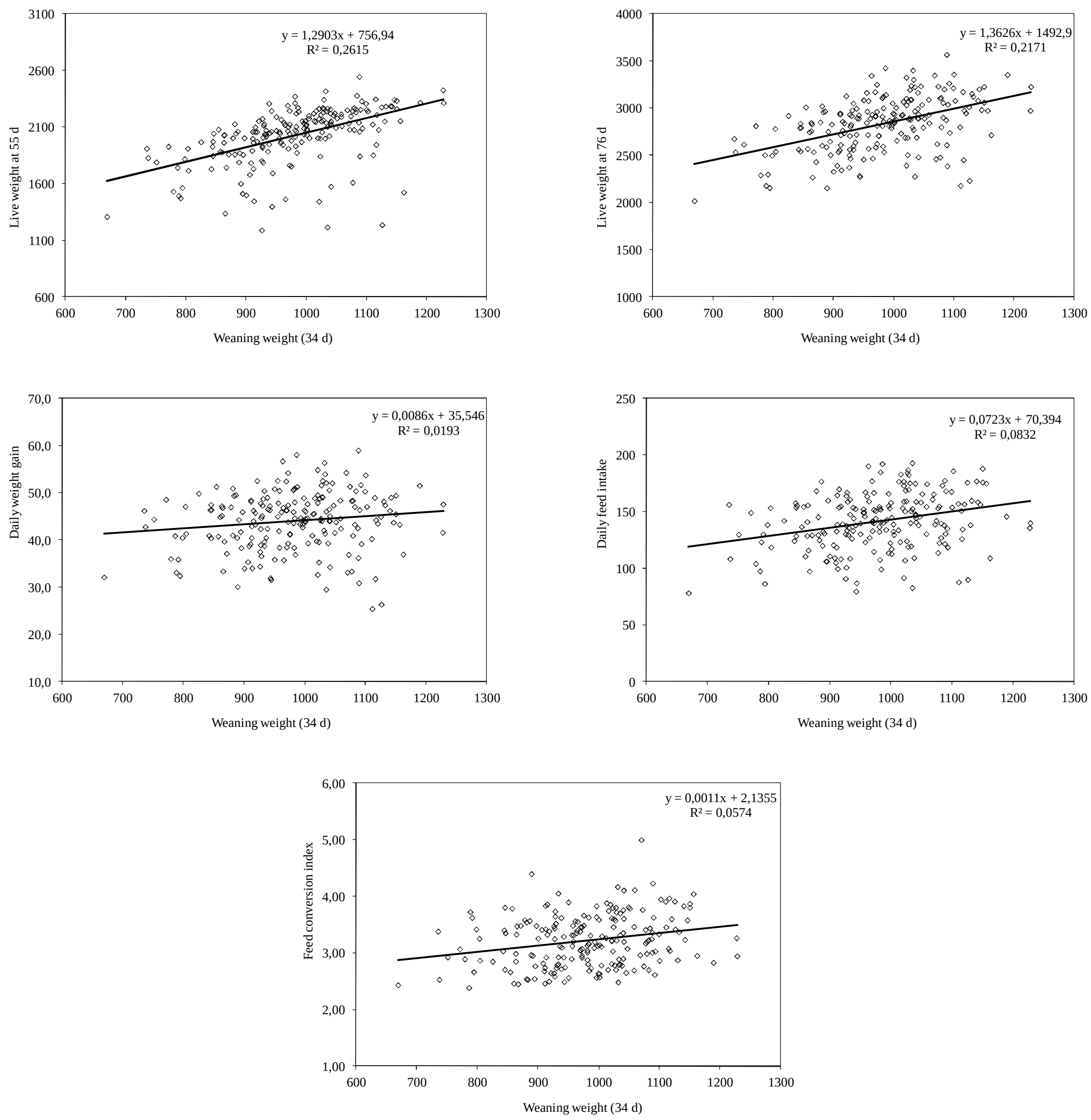


Figure 16. Correlation between weaning weight and live weight at 55 d, live weight at 76 d, daily weight gain, feed intake and feed conversion in the whole experimental period.

Caecal content and intestinal tissue characteristics

The administration of diets with a higher (starch+soluble fibre)/ADF ratio changed in a quadratic mode the incidence of the full stomach (quadratic component of variance, P=0.02) and the full gut (P=0.07) expressed on the base of live weight without effect on the filling of caecum (Table 20). At caecum, the increase of (starch+soluble fibre)/ADF ratio significantly and linearly stimulated VFA production which went side by side a reduction of pH value and a strong decrease in N-ammonia level (Table 20). Even if a lower rate of significance (P<0.10), the production of acetic acid (raising) and of propionic acid (decreasing) also changed.

Table 20. Caecal content and intestinal mucosa traits.

	(Starch+soluble fibre)/ADF (R)			Protein level (CP)		Probability				DSR
	1.2	1.8	2.8	14%	15%	R_L	R_Q	CP	R x CP	
Rabbits, n.	12	12	12	18	18					
Live weight (LW), g	1444	1446	1466	1454	1450	0.58	0.77	0.88	0.61	94.9
Full stomach, % LW	7.98^b	7.26^a	7.74^{ab}	7.93	7.39	0.41	0.02	0.03	0.37	0.71
Full caecum, % LW	8.21	7.79	8.84	7.85	8.47	0.52	0.14	0.09	0.54	1.05
Full gut, % LW	24.7	23.8	24.8	24.3	25.4	0.95	0.07	0.61	0.16	1.41
Caecal content										
pH	5.90	5.78	5.67	5.87	5.70	0.03	0.93	0.05	0.77	0.24
N-NH$_3$, mmol/L	4.85^B	2.59^{AB}	1.93^A	2.67	3.58	<0.01	0.32	0.24	0.33	2.23
Total VFA, mmol/L	52.1	61.4	61.9	56.3	60.6	0.03	0.24	0.23	0.84	10.5
C2, % mol.	81.8	82.3	84.6	82.9	82.8	0.07	0.45	0.99	0.91	3.63
C3, % mol.	5.19	4.52	4.36	4.95	4.44	0.09	0.54	0.20	0.08	1.15
C4, % mol.	12.5	12.7	10.5	11.6	12.2	0.18	0.32	0.65	0.87	3.51
C5, % mol.	0.57	0.55	0.56	0.57	0.55	0.87	0.85	0.81	0.91	0.21
C3/C4	0.45	0.37	0.49	0.44	0.43	0.66	0.20	0.85	0.28	0.21
Jejunum mucosa										
Villi height[1], µm	839	733	751	780	768	<0.01	0.01	0.58	0.03	64
Cripths depth, µm	192	194	192	188	197	0.99	0.69	0.14	0.31	19
Villi/crypths[1]	4.42	3.81	3.95	4.19	3.92	0.02	0.03	0.10	0.01	0.47
Ileum mucosa										
Villi height[1], µm	601	617	660	639	612	0.26	0.76	0.51	0.81	125
Cripths depth, µm	148	159	155	152	155	0.33	0.23	0.58	0.56	17
Villi/crypths[1]	4.09	3.97	4.27	4.24	3.97	0.63	0.52	0.38	0.97	0.91

R_L and R_Q: probability of the linear and quadratic component of variance for the effect of the (starch+soluble fibre)/ADF ratio. [1]Villi height: 867, 758, 715, 811, 706 and 786 µm in rabbits fed diets D14, E14, F14, D15, E15, F15; interaction R x P =0.03.

The effect of the protein level was less important and represented by a reduction of the full stomach incidence (P=0.03) and a trend to an increased full caecum incidence (P=0.09), besides a reduction of caecal pH (5.87 to 5.70) when the protein level increased from 14% to 15% CP. Moreover, total VFA production increased (56.3 to 60.6 mmol/L), even if at a non significant level. The interaction between the main experimental factors was never significant.

As what concerns the effect of the feeding treatments on intestinal mucosa traits, the administration of diets with increasing (starch+soluble fibre)/ADF ratio decreased in a non-linear way the villi height at jejunum (839 *vs*. 733 and 751 µm) and, as a consequence, the villi/crypths ratio (4.42 *vs*. 3.81 and 3.95).

Slaughter results and carcass and meat quality

The effect of the experimental factors was hardly perceivable on growth performance and absent on slaughter results, apart from a significant and quadratic change of the muscle to bone ratio (P=0.01) which was higher in rabbits fed diets with 2.8 ratio (Table 21). As what concerns colour and pH, only the yellow index of *biceps femoris* significantly and linearly increased (P=0.04) when the ratio (starch+soluble fibre)/ADF raised (Table 22). Mean values of the two muscles (*longissimus lumborum* and *biceps femoris*) within animals showed even less evident differences due to feeding treatments.

The gut incidence was higher in females rather than in males (P<001). As a consequence, dressing percentage in the same animals was lower (60.8% *vs*. 61.7%, P<0.01) (Table 21). Among the other variables, only the incidence of head on cold carcass was significantly higher in males than in females (P<0.01). On meat, a significant increase of pH on *longissimus lumborum*, even if in a strict range (from 5.51 to 5.56), in males compared to females; on the same muscle lower values of L* and higher tint were measured in males compared to females (Table 22).

At slaughter, the effect of the parity order was not detectable on carcass (Table 21) and hardly appreciable on meat (Table 22). On the other hand, the weaning weight significantly affected weight of the rabbits at slaughter, of the cold and reference carcasses (P<0.01), besides the incidence of hind legs (P=0.03) (Table 21). In other words, lighter animals at weaning also weighed less and developed less at the end of the rearing period. The different development of rabbits according to the weaning weight also significantly changed colour on *longissimus lumborum* (a*) and, especially, on *biceps femoris* (L*, b*, C*, T*) (Table 22).

Table 21. Results at commercial slaughter and carcass quality.

	(starch+soluble fibre)/ADF ratio (R)			Protein level (CP)		Sex (S)		Probability						RSD
	1.2	1.8	2.8	14%	15%	Females	Males	R_L	R_Q	P	S	Weaning weight	RxCP	
Rabbits, n	40	40	40	60	60	62	58							
Slaughter weight (SW), g[2]	2756	2788	2748	2765	2763	2759	2769	0.87	0.39	0.96	0.80	<0.01	0.81	213
Transport losses, % LW[3]	2.12	2.34	2.38	2.30	2.25	2.29	2.27	0.28	0.65	0.81	0.94	0.77	0.52	1.05
Gut incidence, % SW[4]	18.4	18.5	18.6	18.6	18.4	18.9	18.1	0.59	0.87	0.67	<0.01	0.62	0.40	1.53
Cold carcass (CC), g	1695	1703	1682	1691	1694	1676	1710	0.68	0.60	0.90	0.21	<0.01	0.85	142
Cold dressing, %SW	61.5	61.1	61.2	61.2	61.3	60.8	61.7	0.37	0.45	0.58	<0.01	0.33	0.98	1.63
CC dissection, %CC:														
Head	7.61	7.53	7.74	7.59	7.66	7.49	7.76	0.29	0.16	0.48	<0.01	0.12	0.73	0.53
Liver	5.09	5.19	4.99	5.08	5.10	5.09	5.09	0.55	0.27	0.85	0.94	0.42	0.76	0.70
Thoracic organs + kidneys	2.90	2.93	2.85	2.90	2.87	2.93	2.85	0.54	0.42	0.84	0.20	0.48	0.40	0.34
Reference carcass (RC), g	1429	1435	1417	1426	1428	1416	1438	0.69	0.64	0.92	0.36	<0.01	0.89	127
RC dissection, %RC:														
Dissectible fat	3.16	3.35	3.33	3.27	3.29	3.42	3.14	0.44	0.61	0.94	0.14	0.08	0.17	1.01
Hind legs	33.0	32.7	32.7	32.9	32.6	32.8	32.8	0.44	0.55	0.31	0.92	0.03	0.67	1.00
Hind leg muscle/bone ratio	6.07	6.60	6.13	6.15	6.36	6.26	6.25	0.76	0.01	0.25	0.97	0.83	0.95	0.61

R_L and R_Q: probability of the linear and quadratic component of variance for the effect of the (starch+soluble fibre)/ADF ratio.

[2] Live weight at the slaughterhouse immediately before slaughter; [3] LW: live weight at the experimental farm; [4] Incidence of the full gastro-intestinal tract.

Table 22. Meat quality: pH and colour of *longissimus lumborum* and *biceps femoris.*

	(starch+soluble fibre)/ADF ratio (R)			Protein level (CP)		Sex (S)		Probability						RSD
	1.2	1.8	2.8	14%	15%	Females	Males	R_L	R_Q	P	S	Weaning weight	RxCP	
Rabbits, n.	40	40	40	60	60	62	58							
Longissimus lumborum:														
pH	5.55	5.52	5.54	5.54	5.54	5.51	5.56	0.66	0.30	0.97	0.01	0.22	0.65	0.09
L*	53.3	54.1	53.7	53.5	54.0	54.4	53.0	0.50	0.23	0.37	<0.01	0.51	0.63	2.53
a*	–1.04	-1.41	-1.38	-1.21	-1.34	-1.42	-1.14	0.11	0.29	0.51	0.12	0.03	0.77	0.94
b*	1.65	1.58	1.78	1.37	1.96	1.76	1.58	0.77	0.74	0.12	0.65	0.86	0.32	2.01
C*	2.87	2.82	2.69	2.75	2.84	2.91	2.68	0.50	0.87	0.68	0.34	0.52	0.91	1.22
T*	-0.30	-0.56	-0.58	-0.34	-0.62	-0.64	-0.32	0.15	0.46	0.08	0.04	0.23	0.33	0.84
Biceps femoris														
pH	5.75	5.73	5.76	5.74	5.75	5.74	5.75	0.84	0.28	0.43	0.50	0.13	0.86	0.13
L*	50.3	51.1	50.8	50.9	50.6	51.0	50.5	0.26	0.14	0.56	0.16	0.05	0.94	2.09
a*	-2.40	-2.20	-2.36	-2.31	-2.32	-2.39	-2.24	0.79	0.20	0.92	0.25	0.35	0.94	0.67
b*	2.96	3.60	3.60	3.43	3.35	3.28	3.50	0.04	0.23	0.74	0.40	0.02	0.49	1.34
C*	3.99	4.36	4.42	4.27	4.25	4.26	4.25	0.06	0.45	0.90	0.95	0.03	0.51	1.01
T*	-0.84	-0.98	-0.95	-0.93	-0.92	-0.88	-0.97	0.10	0.14	0.76	0.08	0.04	0.60	0.29
Average of the two muscles														
pH	5.65	5.63	5.65	5.63	5.65	5.63	5.66	0.95	0.26	0.63	0.13	0.13	0.76	0.10
L*	51.8	52.6	52.2	52.2	52.3	52.7	51.7	0.27	0.10	0.78	<0.01	0.12	0.83	1.85
a*	-1.72	-1.81	-1.87	-1.77	-1.83	-1.90	-1.69	0.32	0.93	0.61	0.10	0.05	0.83	0.69
b*	2.30	2.59	2.69	2.40	2.66	2.52	2.54	0.21	0.72	0.33	0.94	0.19	0.23	1.36
C*	3.43	3.59	3.56	3.51	3.54	3.58	3.47	0.49	0.54	0.81	0.43	0.36	0.88	0.78
T*	-0.57	-0.77	-0.76	-0.64	-0.77	-0.76	-0.65	0.07	0.26	0.14	0.20	0.64	0.38	0.47

R_L and R_Q: probability of the linear and quadratic component of variance for the effect of the (starch+soluble fibre)/ADF ratio.

Discussion of experiments 1 and 2

Modulating nutrients in the diets for growing rabbits

The first and second experiments of the thesis were dedicated to the definition of requirements of soluble fibre and starch in relation to dietary insoluble fibre in rabbits during post-weaning and fattening. At the same time the role of dietary protein and its interaction with other nutrients was taken into account also in view of the relationships between dietary protein level and farm nitrogen efficiency and excretion.

Starch and soluble fibre to ADF ratio

The specific objectives above were pursued through the research activities of the first two experiments of the doctoral thesis. In details, in the first trial the effect of increasing the (starch+soluble fibre) to ADF ratio (from 0.6 to 1.2 to 2.0) was tested by using diets with increasing starch (9.1%, 13.7% and 17.9%) and soluble fibre (4.8%, 7.1% and 9.4%) at the expenses of insoluble fibre (ADF 23.0%, 18.0%, and 13.5%). In the second trial, the effect of the same ratio was tested at more extreme nutritional limits (from 1.2 to 1.8 to 2.8), by using diets in which soluble fibre was higher and less variable (10% to 12%), starch reached higher concentrations (13.2%, 17.0% and 20.4%) and ADF decreased at lower rates (18.7% to 14.9% to 11.3%) than in the Experiment n.1. In both trials, soluble fibre and starch increased at the expenses of insoluble fibre by replacing alfalfa meal and wheat bran with dried beet pulp and barley. The main goal of the two studies was to demonstrate the possibility of increasing the nutritive value of the diet for growing rabbits and improving rabbit digestive efficiency and feed conversion, without favouring the appearance and severity of digestive diseases. This result should have been obtained thanks to the positive role of soluble fibre that counterbalances the risk due to increased starch and reduced insoluble fibre levels.

In both trials, increasing the ratio between (starch+soluble fibre) and ADF content of the diet increased the digestive utilization of feeds and their nutritive value until figures rather unusual in diets for growing rabbits (apparent digestibility of dry matter reached 75.6% with the diet at the highest ratio). In fact, in Figure 17, the variability in DM digestibility is largely explained by the changes in (starch+soluble fibre)/ADF ratio from 0.6 to 2.8 (R^2=0.96).

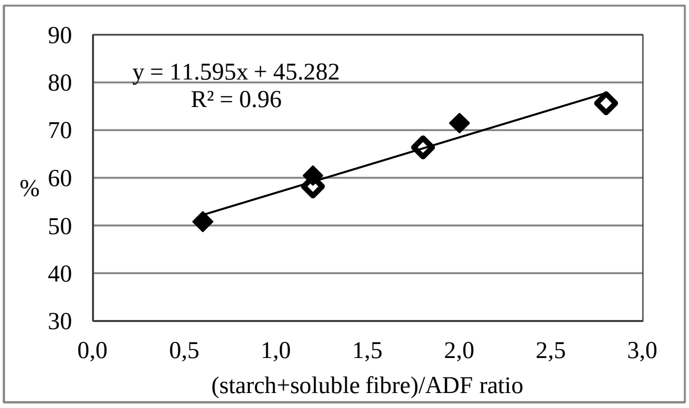


Figure 17. Correlation between (starch+soluble fibre)/ADF ratio and dry matter digestibility (average data of experiments 1-◆, and 2-◇).

The improvement in the nutrient apparent digestibility and the nutritive value of diets with increasing starch and decreasing insoluble fibre was quite obvious (Gidenne and Bellier, 2000). Similarly, increasing the levels of beet pulp and soluble fibre in replacement of alfalfa meal and insoluble fibre, as in the two experiments, has been often associated to increased diet digestibility and energy value (Trocino *et al.*, 1999, 2010, 2011; Falcão-e-Cunha *et al.*, 2004), which both depend on the higher amount of more digestible/fermentable fibre fractions, as well as on the increased digestibility of all fibre fractions (Carabaño *et al.*, 1997). In fact, digestibility of most soluble fractions (like those contained in dried beet pulp) may reach 60-70%, while that of insoluble fibre fractions (NDF), major constituent of alfalfa meal fibre, stands around 15-30% (Carabaño *et al.*, 2001; García *et al.*, 2009).

Productive performance was affected in a similar way in the two trials: feed intake decreased and feed conversion improved when starch and soluble fibre replaced insoluble fibre fractions of the diet. In Figure 18, the strict relationship between the level of feed intake and the changes in (starch+soluble fibre)/ADF ratio is clearly represented (R^2=0.98).

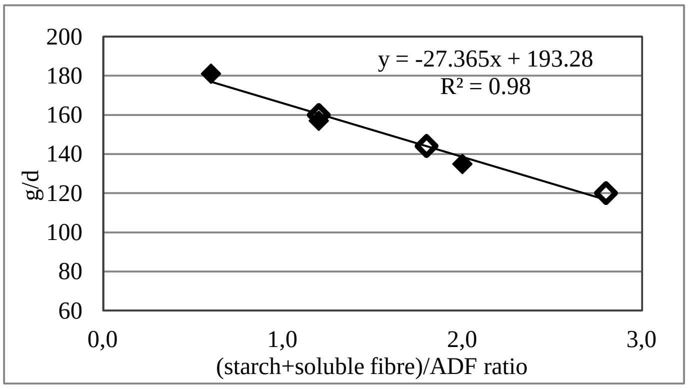


Figure 18. Correlation between (starch+soluble fibre)/ADF ratio and feed intake (average data of experiments 1-◆, and 2-◇).

In Figure 19, the variability of feed conversion data according to the (starch+soluble fibre)/ADF ratio is drawn, showing once again a high correlation between the two set of data (R^2=0.90).

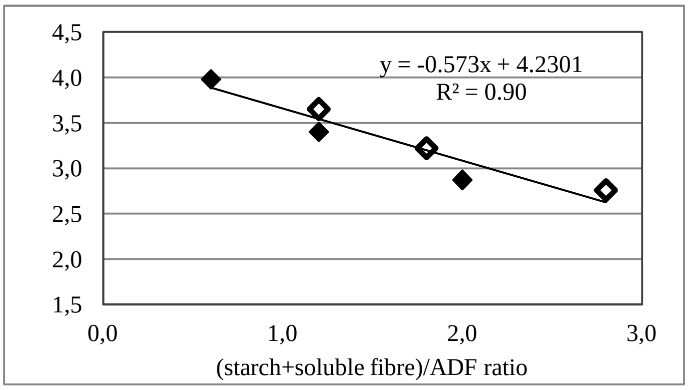


Figure 19. Correlation between (starch+soluble fibre)/ADF ratio and feed conversion index (average data of experiments 1-◆, and 2-◇).

During the first trial, also daily growth was positively affected by the increase of the (starch+soluble fibre)/ADF ratio, especially during the last period of fattening, while this trend was not observed in the second trial. In fact, when we put together the data of the experiments n. 1 and n. 2, the relationships between daily weight gain and dietary changes in (starch+soluble fibre)/ADF ratio was weak (R^2=0.19) (Figure 20).

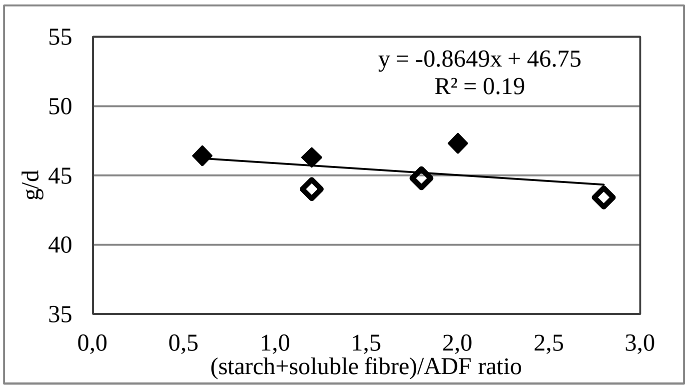


Figure 20. Correlation between (starch+soluble fibre)/ADF ratio and daily weight gain (average data of experiments 1-◆, and 2-◇).

Previously, Trocino *et al*. (2011) described that the replacement of insoluble with soluble fibre stimulated daily weight gain in the first period of growth, thanks to the high digestive utilization of fibre fractions in the young rabbits (Marounek *et al*., 1995). Differently, Martínez-Vallespín *et al*. (2011) found a depression of feed intake, and in this case, of milk production in rabbit does fed diets in which neutral detergent soluble fibre replaced insoluble fibre, and a reduction of litter feed intake and litter live weight gain during post-weaning. Other authors found that, at low levels of dried beet pulp inclusion (15%) in replacement of alfalfa meal, no differences were observed in daily weight gain or feed intake (Trocino *et al.*, 1999), while with higher inclusion rates (>30%), feed intake and daily growth rates were greatly depressed (Carabaño *et al.*, 1997; Falcão-e-Cunha *et al.*, 2004).

During the experiment n.1, health status was fairly good; mortality averaged 8.5% and decreased from 15.8% to 4.5% and 4.9% with (starch+soluble fibre)/ADF ratios from 0.6 to 1.2 and 2.0. Differently, during the second trial, health condition of animals was worse, epizootic rabbit enteropathy occurred and mortality averaged 22.6%. In these conditions, no positive effect of increasing (starch+soluble fibre)/ADF ratio was recorded and, on the opposite, morbidity tended ($P<0.10$) to be higher in rabbits fed the highest ratio. Previous studies showed that when only starch increased at the expenses of insoluble fibre, health condition of growing rabbits worsened (Carraro *et al.*, 2007; Blas and Gidenne, 2010). In young animals, however, some data showed a lower mortality when increasing the starch to ADF ratio (Debray *et al.*, 2002; Gutiérrez *et al.*, 2002; Feugier *et al.*, 2006). On the other hand, when using soluble fibre in substitution of insoluble fibre (Gómez-Conde *et al.*, 2007; Xiccato *et al.*, 2006; Martínez-Vallespín *et al.*, 2011) or of starch in iso-ADF diets (Jehl and Gidenne, 1996; Perez *et al.*, 2000; Soler *et al.*, 2004; Xiccato *et al.*, 2008) other authors found a positive effect on health especially in presence of ERE.

In both our trials, the higher availability of fermentable carbohydrates (either structural or not) decreased caecal pH, increased total volatile fatty acids (VFA) content, and decreased the molar proportion of propionate at caecum. When considering the data of the two trials, caecal pH linearly decreased with the increasing value of the (starch+soluble fibre)/ADF ($R^2=0.88$) (Figure 21), while the total concentration of caecal volatile fatty acids stand at different average values in the two trial and, therefore, changed differently within the two trials (Figure 22).

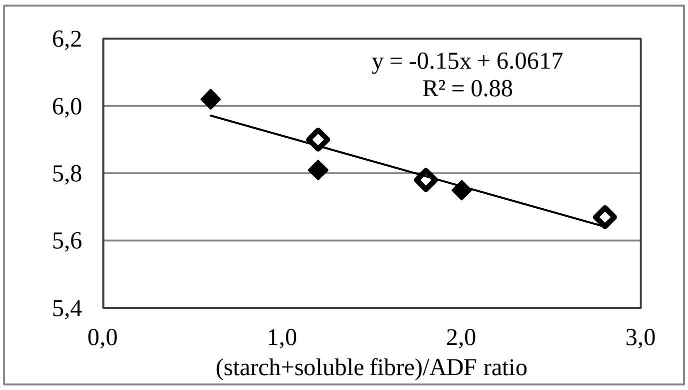


Figure 21. Correlation between (starch+soluble fibre)/ADF ratio and caecal pH (average data of experiments 1-◆, and 2-◇).

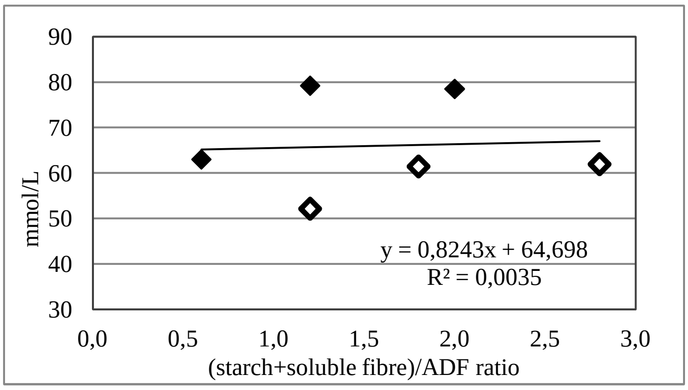


Figure 22. Correlation between (starch+soluble fibre)/ADF ratio and total volatile fatty acids in the caecal content (average data of experiments 1-◆, and 2-◇).

When speaking about starch supply, high dietary levels are usually expected to stimulate VFA production and, as a consequence of the enhanced activity of amylolitic bacteria, to reduce acetate production and favour butyrate and valerate rates (Parigi Bini *et al.*, 1990; Gidenne and Bellier, 2000; Blas and Gidenne, 2010). We observed a propionate reduction, that is considered favourable for the gut health of the rabbit, as already found by Trocino *et al.* (2011). Previous studies also showed a favourable and significant increase of caecal fermentative activity when the soluble fibre of diets increased at the expenses of insoluble fibre (García *et al.*, 2000; Falcão-e-Cunha *et al.*, 2004). On the other hand, the administration of diets with a raising rate of dried beet pulp, and therefore of soluble fibre, but in presence of a high quantity of raw materials rich in insoluble fibre (15-20%), did not change the fermentative caecal pattern (Carabaño *et al.*, 1997; Trocino *et al.*, 1999).

The variations in the ingredient composition of diets used in the present thesis, besides increasing dietary starch and soluble fibre and decreasing insoluble fibre, also affected the nature of insoluble fibre fractions (in terms of a lower lignification and complexity of the cell walls or different hemicellulosic constituents) and likely made fibre more susceptible to both digestive enzymes in the ileum and microflora fermentation in the caecum (Gidenne, 1992; Carabaño *et al.*, 2001; García *et al.*, 2002), and more oriented towards acetate rather than propionate production. In both the two trials, the major availability of fermentable carbohydrates also stimulated the synthesis of bacterial protein, therefore reducing the amount of caecal N-ammonia when the (starch+soluble fibre)/ADF ratio increased (Figure 23).

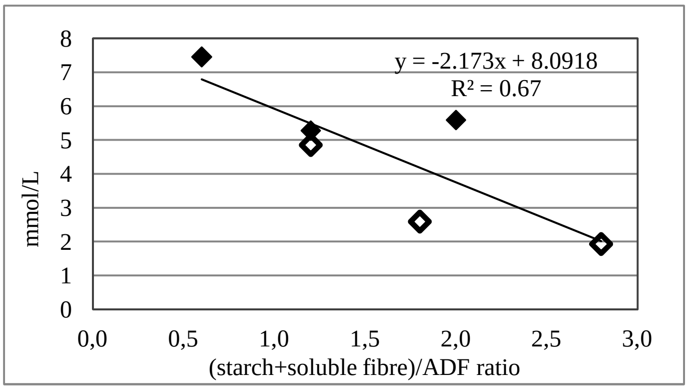


Figure 23. Correlation between (starch+soluble fibre)/ADF ratio and N-ammonia production at caecum (average data of experiments 1-◆, and 2-◇).

In both trials of the thesis, ileum mucosa was not affected, while the increase of the (starch+soluble fibre)/ADF ratio reduced villi height and villi/crypth ratio of jejunum mucosa. This result is usually considered negatively and related to an impaired functionality of the intestinal barrier and its capability of preventing and reacting to pathogens attacks as well as of contrasting the passages of toxins produced at an intestinal level (Van Dijk *et al.*, 2002). However, among previous studies in rabbits, the effect of the feeding treatment of intestinal mucosa traits are not conclusive: some authors did not observe any effect of increasing starch or starch to ADF ratio on mucosa morphology of young rabbits (Gutiérrez *et al.*, 2002), whereas other studies measured a significant reduction of villi height at ileum (Carraro *et al.*, 2007) or on the contrary an increase of villi height at jejunum (Álvarez *et al.*, 2007). With regards to the role of soluble fibre, various studies of Padova team did not find any effect on gut mucosa of growing rabbits (Xiccato *et al.*, 2008; Trocino *et al.*, 2010, 2011). Differently, other authors reported that the increase of most soluble fibre fractions (from 7% to 12%) at the expenses of insoluble fibre may favour the barrier function of the intestinal mucosa (Álvarez *et al.*, 2007; Gómez-Conde *et al.*, 2007).

At commercial slaughtering, gut weight and proportion on live weight increased with the (starch+soluble fibre)/ADF ratio in the first trial, while slaughter results or carcass traits did not vary in the second trial. Previous studies showed that rabbits fed with high-soluble fibre diets displayed higher gut proportion when contemporary wide variations in insoluble fibre (NDF, ADF) and starch levels were used (García *et al.*, 1993; Carabaño *et al.*, 1997; Falcão-e-Cunha *et al.*, 2004; Trocino *et al.*, 2011). On the contrary, when iso-ADF diets were compared, no real impact of soluble fibre level (or beet pulp inclusion) on the filling of digestive organs was found (Jehl and Gidenne, 1996; Gidenne and Perez, 2000; Trocino *et al.*, 2010).

The scarce influence of the chemical composition of the experimental diets on carcass and meat traits confirms a limited effect of feeding on the qualitative characteristics of rabbits when balanced diets are fed *ad libitum* (Xiccato, 1999; Hernandez and Gondret, 2006; Hernandez, 2008). On the other hand the variability degree in pH and colour of rabbit meat would require a higher number of measurements to reach statistically significant differences.

On the basis of the results of the two trials, we can conclude that the contemporary increase of starch (until 20%) and soluble fibre (until 12%) has positive effects on digestive physiology, health, weight gain and feed efficiency of rabbits without impairing slaughter results and carcass or meat quality. However, in presence of epizootic rabbit enteropathy, the contemporary decrease of ADF below 13% may negatively affect digestive health of growing rabbits.

Dietary protein

It is largely recognized that reducing dietary crude protein for fattening rabbits below the reference values of 16-16.5% as-fed would be beneficial to reduce the feeding costs and to control nitrogen excretion without affecting growth performance and meat quality (Maertens *et al.,* 1997; Xiccato and Trocino, 2010b).

In the conditions of the two first experiments of the present thesis, the reduction of dietary protein from 17% until 14% had no effect on growth performance and slaughter traits, while improved nitrogen utilization and reduced nitrogen excretion in the environment. Similarly, also Xiccato *et al*. (2011) did not observe any effect of reducing dietary CP from 16.2% to 15.2% on performance of growing rabbits. In fact, while about 15% of dietary CP may be considered to cover protein requirements for growing rabbits (De Blas and Mateos, 2010; Xiccato and Trocino, 2010b), a further reduction, until or below 14%, always requires that main limiting amino acids (lysine, methionine and threonine) are largely supplied (Trocino *et al*., 2000; García-Palomares *et al*., 2006b). With CP levels below 13.8%, daily weight gain was impaired (Maertens *et al*., 1997), especially in the first post-weaning period when protein requirements for growth are still high (Trocino *et al*., 2000; Feugier *et al*., 2006).

At caecal level, dietary CP contributes to gut health equilibrium and its lack (<12%) or excess (>18%) may modify caecal fermentation activity and microflora composition (Carabaño *et al.*, 2009). All microbial populations benefit from the protein available at caecal level for their development and proliferation, but some pathogenic strains of *E. coli* and *Clostridia* spp. seem to gain an advantage from protein unbalance and in particular from its excess. In fact, previous studies showed a significant reduction of mortality due to ERE when the dietary protein decreased (Xiccato *et al*., 2006, 2011) and ileal protein digestibility increased (Chamorro *et al*., 2007) as well as a reduction of *C. perfringens* (Chamorro *et al*., 2007) and total anaerobic bacteria at the ileum of growing rabbits (García-Palomares *et al*., 2006b). On the contrary, Feugier *et al*. (2006) did not find differences in the health condition of rabbits when dietary protein decreased from 21% to 15%.

On the other hand, Xiccato *et al*. (2011) found that the highest mortality was associated with the diet with high protein concentration and the lowest soluble fibre to starch ratio in comparison with all other diets. In fact, Martínez-Vallespín *et al.* (2011) found that reducing dietary protein from 17.5% to 14.5% had a positive effect on mortality (-19.9 percentage points) of rabbits affected by ERE during post-weaning period (28-49 days of age); also the replacement of starch by ADF and of insoluble fibre by soluble fibre had positive effects on rabbit health and these effects seemed to be additive with that of the reduction of dietary crude protein.

In the conditions of our trial, the dietary protein level did not clearly interact with the (starch+soluble fibre)/ADF ratio in modifying digestive health of rabbits, but the reduction of dietary protein improved health condition of rabbits by reducing morbidity during the first trial and mortality during the second trial, when ERE occurred.

In fact, at caecal level, the administration of diets with higher protein content increased the production of N-ammonia, which could be considered negatively for the rabbit digestive health. On the other hand, protein concentration did not affect the level and characteristics of VFA production at caecum. The effects on gut mucosa were rather weak and poorly correlated with the health status of rabbits. Also previous studies reported contradictory data about the effect of the protein level on the intestinal mucosa traits in rabbits. Neither the changes in dietary CP concentration (Chamorro *et al*., 2007; Xiccato *et al*., 2011) nor the dietary supplementation with specific amino acids (glutamine and arginine) (Chamorro *et al*., 2010), which may directly promote gastrointestinal integrity, modified mucosal histology of early-weaned rabbits.

As what concerns slaughter results, these trials confirm that the protein level has almost no effect on carcass characteristics or meat traits, whereas possible effects may be expected only with extreme CP concentrations (<130 g/kg or >180 g/kg) (Xiccato and Trocino, 2010b).

Sex

As already described by other authors (Lambertini *et al*., 1990; Parigi Bini *et al*., 1992; Petracci *et al*., 1999; Trocino *et al*., 2003: Lazzaroni *et al*., 2009), the gender of rabbit did not influence growth performance, while the gut incidence was higher in females rather than in males. As a consequence, dressing percentage in the same animals was lower. Among the other variables, only the incidence of head on cold carcass was significantly higher in males than in females. On meat, a significant increase of pH on *longissimus lumborum*, in males compared to females and some differences in colour (lower L* and higher tint were measured in males compared to females. Other authors observed a lower lightness (Trocino *et al.*, 2003) and less coloured meat (Carrilho *et al.*, 2009) in meat of females compared to meat of males. In rabbits slaughtered later than current slaughter age (>100 days), Lazzaroni *et al.* (2009) measured a lower lightness but higher red and yellow indexes in females than in males. Differently, Petracci *et al.* (1999) did not find any significant difference in colour or technological traits of meat (drip and cooking losses) depending on sex.

Conclusions

On the base of the results of the two experiments and the discussion above, it is possible to formulate diets for post-weaning and fattening rabbits capable of greatly improving feed conversion compared to actual standards, maintaining growth and slaughter results.

The use of diets with increasing levels of both starch and soluble fibre in replacement of insoluble fibre improved health condition of rabbits, increased diet utilization, especially of fibrous fractions, and nutritive value. Productive performance in term of live weight and weight gain were not influenced whereas feed intake decreased and feed conversion greatly improved. Thanks to the lower ingestion levels, nitrogen excretion deeply decreased with increasing dietary (starch+soluble fibre)/ratio. Caecal fermentations were more active and feed conversion improved both because of the starch increase and the insoluble fibre reduction, and because fibre had a different structure, that is, fibre was less lignified and more available for the enzymatic and bacterial attack.

At the final commercial slaughtering, the increase of the above ratio slightly stimulated live weight, but contemporary reduced dressing percentage because of the higher gut incidence. This last result, however, was counterbalanced by a significant increase of carcass muscularity as proved by the higher meat to bone ratio of hind leg.

Changes in protein level had less important effects, even if sometimes significant, compared to changes in dietary starch and fibrous fractions concentration. The reduction of CP from 17% to 14% permitted to reduce farm nitrogen excretion, while did not affect rabbit performance or health. However, rabbits fed with low dietary (starch+soluble fibre)/ADF showed a higher susceptibility to digestive disorders when CP increased from 14% to 15%. This result deserves to be further investigated also because it takes into account rather low levels of dietary protein compared to those currently used in field practice.

Experiment 3.

Effect of the level and duration of feed restriction in growing rabbits

Introduction

In commercial farms, growing rabbits are usually fed *ad libitum* (Maertens, 2009). However, feed distribution can be restricted during post-weaning period in order to avoid the risks of an excessive and not homogeneous feed intake and the possible negative consequences on intestinal health. Studies on feed restriction started long time ago and aimed firstly at evaluating growth performance and carcass and meat traits with the aim of improving feed efficiency and standardizing growth curves in rabbits with a different feed ingestion level (Ouhayoun *et al*., 1986; Ouhayoun, 1989; Cavani *et al*., 1991). During the last 10 years, the appearance and diffusion of epizootic rabbit enteropathy (ERE) pushed towards the use of feed restriction in commercial farms as a method to control the impact of the illness (Gidenne *et al*., 2003 and 2011). The present trial aimed to evaluate if and to what extent the level (90% and 80% *vs*. *ad libitum*) and the duration of feed restriction (2 or 3 weeks after weaning) might affect health status, growth performance, feed digestibility, body energy and protein balance, slaughter results and carcass and meat traits.

Materials and methods

Rearing conditions

The trial was realized in the experimental farm of the University of Padova in the period October-November 2009. The characteristics of experimental facilities and cages have been detailed previously in the Experiment 1.

Since the trial was held in autumn, the maximum temperature never overcame 21°C and the minimum temperature dropped until 13°C (Figure 24) and then was maintained at least around 15°C thanks to forced heating. The average values of the minimum and maximum temperature stood at 17°C and 19°C, respectively. The maximum relative humidity averaged 74%, with a minimum value equal to 56% and a maximum value of 86% (Figure 24). The minimum relative humidity averaged 65% and ranged from 36% to 76%.

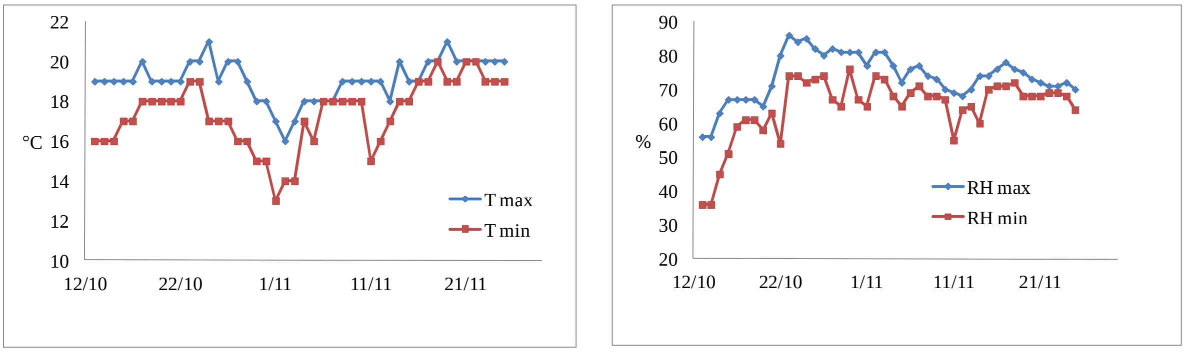


Figure 24. Daily values of minimum and maximum temperature and minimum and maximum relative humidity (RH) throughout the trial.

Animals, experimental groups and recordings

The research was approved by the Ethical Committee of the University of Padova for animal experimentation.

In a commercial farm, at 32 days of age, 264 rabbits of both genders from a hybrid line (Grimaud Frères, France) were selected from multiparous does (3-6 kindling) with healthy litters and homogeneous kit weight. The animals were transported to the experimental farm and, at their arrival, rabbits presented an optimal health status. The rabbits were immediately weighed (912±99 g LW), put into individual cages and identified using ear tags.

The day after, at 33 days of age, 18 rabbits were slaughtered according to the comparative slaughter techniques. The remaining 246 rabbits were divided in six experimental groups of 47 units, homogeneous in average weight and variability, and submitted to different feeding restriction levels according to a bi-factorial arrangement, with three restriction levels (*ad libitum*, 90% and 80% of *ad libitum*) during two different periods (2 weeks *vs*. 3 weeks after weaning), as shown in the following scheme:

	Feed restriction period (T)	
Feeding restriction level (R)	2 weeks (35-49 d of age) (T2)	3 weeks (35-56 d of age) (T3)
100% *ad libitum* (R100)	Group R100-T2	Group R100-T3
90% *ad libitum* (R90)	Group R90-T2	Group R90-T3
80% *ad libitum* (R80)	Group R80-T2	Group R80-T3

The trial lasted 6 weeks and was divided into two periods (Figure 25):

a) **post-weaning period** during which rabbits were fed a weaning diet (S) *ad libitum* (R100) or were restricted at 90% (R90) or 80% (R80) during 2 weeks (T2) or 3 weeks (T3);

b) **fattening period**, during which rabbits were fed always *ad libitum* with a fattening diet (I) during 4 weeks (groups T2) or 3 weeks (groups T3).

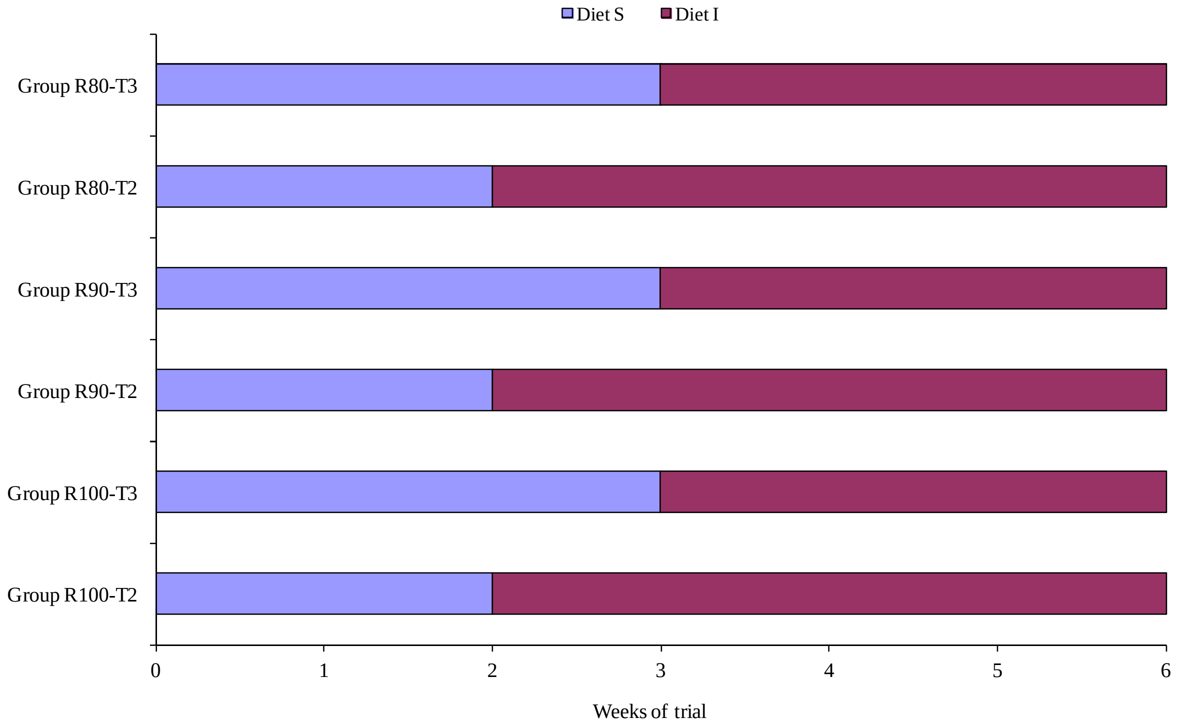


Figure 25. Duration of post-weaning period and fattening period in the different experimental groups.

The trial started the day after the arrival (33 days of age) of rabbits and lasted 42 days until commercial slaughter at 75 days of age.

Individual live weight was recorded three times a week. Individual feed intake was recorded daily from the arrival of rabbits until 60 days of age (that is some days after the end of the restriction period for the group T3, which was at 54 d). Thereafter, individual feed intake was recorded three times a week. Health status was daily controlled to detect promptly the occurrence of diseases, especially digestive problems, as described in the Experiment 1.

Experimental diets

All diets were pelleted with 3.5 mm of diameter and 1.0-1.1 cm of length. Diet S was supplemented with antibiotics (oxitetraciclin and apramicine, 1450 and 80 mg a.p./kg, respectively) and with coccidiostatic (diclazuril, 1 mg a.p./kg), at the rates currently used in the origin farm during post-weaning, where a severe risk of the presence of ERE have been detected.

The fattening diet (I), administered *ad libitum* during the second period, was characterized by a lower protein supply, a lower digestible protein to digestible energy ratio, and did not contain antibiotic or coccidiostatic drugs.

Diets were formulated consistently with commercial standards and supplemented with synthetic amino acids, micro- and macro-minerals in order to satisfy nutritional requirements of growing and fattening rabbits (De Blas and Mateos, 2010) (Table 23). As previewed, diet S had a higher protein content (16.8%) compared to diet I (15.6%) as well as a higher crude fibre and fibre fractions content, consistently with the higher protein and fibre requirements of young rabbits during post-weaning (Xiccato and Trocino, 2008; De Blas and Mateos, 2010) (Table 24).

Table 23. Ingredient composition (%) of experimental diets.

	Diet S	Diet I
Dehydrated alfalfa meal 16-17% CP	30.90	23.50
Wheat bran	24.00	24.00
Barley	12.00	22.00
Dried beet pulp	15.00	15.00
Soybean meal 49%	7.00	5.50
Sunflower meal 30%	7.00	5.50
Sunflower oil	1.00	1.50
Molasses	1.50	1.50
Calcium carbonate	0.25	0.28
Dicalcium phosphate	0.33	0.20
Sodium chloride	0.40	0.40
L-lysine HCl	0.05	0.10
DL-methionine	0.05	0.05
Vitamin-mineral premix*	0.47	0.47
Coccidiostat (Diclazuril)	0.05	--

*Supplementation per kg of feed: vit. A, 12.000 UI; vit. D3, 1.000 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.

Table 24. Chemical composition (as fed) of experimental diets.

	Diet S	Diet I
Dry matter, %	92.7	92.3
Crude protein, %	16.8	15.6
Ether extract, %	4.0	4.4
Crude fibre, %	15.9	14.3
Ash, %	7.4	6.8
TDF, %	42.1	41.3
aNDF, %	38.7	36.1
ADF, %	19.8	18.1
Lignin (sa), %	3.6	3.4
Starch, %	11.6	15.5
Gross energy, MJ/kg	16.8	17.0

Restriction procedure

The first day of trial, diet S was fed *ad libitum* to all rabbits. On the morning of the following day, feeders were weighed to establish the average feed intake of rabbits fed *ad libitum*. Restricted rabbits thus received in feeder an amount of diet equal to 90% (groups R90) and 80% (groups R80) of the average feed intake of the previous day. The same procedure was adopted during the following days, by weighing feeders and calculating the amount of diet to be fed to the different groups depending on the feed restriction level. By this method, however, the average feed intake of restricted groups during the first week of trial was systematically lower than expected 90% and 80% of the voluntary intake of groups R100. Both the rapidly increase of feed intake measured day by day in the *ad libitum* group, and the presence of rabbits in the restricted groups which consumed less than the administered quantity may account for this result. Therefore, in the second and third week of the trial, the quantity of diet to be fed to the restricted groups was raised to 95% (groups R90) and 85% (groups R80) of the intake measured the day before in rabbits of the group R100. This level was continuously corrected taking into account the average and real feed intake of restricted groups. At the end of the restriction period lasting 2 weeks (groups T2) or 3 weeks (groups T3), the diet S was changed by the diet I and animals were fed *ad libitum* until the end of the trial.

Health status

Health status was controlled as described for Experiment n.1 and mortality, morbidity and sanitary risk were calculated accordingly. During the trial, 24 rabbits died of which one rabbit of the group R100-T2, three rabbits submitted to R90-T2 treatment, three belonging to the group R80-T2, six in the group R100-T3, 6 in the group R90-T3 and 5 submitted to R80-T3 treatment. In most cases, ill and dead rabbits were hit by digestive troubles, characterized by the presence of diarrhoea and/or mucus, prostration and symptoms which may be ascribed to ERE.

Due to the quick impairment of health and the exponential increase in the number of ill and dead rabbits, notwithstanding the antibiotic inclusion in the diet, from 41 days of age and during 9 days, antibiotics were administered in water (Tiamuline 12,5%, 100 mL/100 L, Tiamvet, CEVA Santé Animale, France, and Colistine 12%, 150 mL/100 L, Colistine-solphate, Doxal, Sulbiate, Italy) to control the disease and maintain mortality within acceptable values in view of the aims of the trial.

Digestibility trials

Faecal apparent digestibility and nutritive value of diets were measured by two *in vivo* digestibility trials performed according to Perez *et al*. (1995), as detailed in Experiment n. 1, and according to the following scheme:

- diet S: 42 rabbits of T3 group (14 per restriction level) from 49 to 53 days of age (with rabbits submitted to the different experimental treatments, R100-T3, R90-T3 e R80-T3);
- diet I: 36 rabbits of T2 group (12 per restriction level) from 56 to 60 days of age (with rabbits fed *ad libitum* and belonging to groups R100-T2, R90-T2 and R80-T2).

Comparative slaughters

At 33 d, 18 rabbits, homogeneous in average live weight and variability and representative of all other rabbits, were submitted to an initial comparative slaughter to determine empty body weight (live weight minus gut content) and the empty body chemical composition (Xiccato *et al*., 2003). Moreover, at the end of the trial, the day before the commercial slaughter, at 74 days of age, 36 rabbits, homogeneous in average live weight and variability and representative of the six experimental groups (six rabbits per group) were slaughtered to determine their empty body weight as well as their body chemical composition. Rabbits were killed from 08:00 to 12:00 a.m. They were weighed immediately before slaughter and killed by cervical dislocation. The gut and the bladder were removed and emptied. Then, the fresh empty organs were joined to the whole body and weighed to determine the empty body weight (EBW). Gut content was calculated by difference (LW – EBW).

Final slaughter, carcass dissection and meat quality analyses

At 75 days of age, the remaining rabbits were slaughtered in a commercial slaughterhouse. Feed and water were available until loading (from 06:30 to 07.30 a.m.). The animals were transported inside cages (50 x 100 x 30 cm) with 6 rabbits per cage (12 rabbits/m^2) in a conditioned truck for about 60 minutes to a commercial slaughterhouse.

Slaughter began at 09:30 and ended at 11:00 a.m. approximately. Rabbits were individually weighed, then stunned by electro-anaesthesia and killed by jugulating. At evisceration, the weight of full gut and bladder was recorded. Carcasses were chilled at 4°C for 2 hours and then transported to the laboratories of the Department of Animal Science of Padova for dissection. Slaughter procedure, carcass dissection and meat quality analyses were performed by using the procedures and methodologies detailed in experiment n. 1.

The texture profile analysis (TPA) was performed on meat of hind leg by a TA.HDI dynamometer (Stable Micro System Ldt, United Kingdom). On the intact hind leg a constant compression was performed by a 2-cm diameter probe moving down at a speed rate of 2 mm/sec and along 5 mm since the contact with the surface to be analysed. Then, the force was stopped and the probe regained the initial position of contact with the surface. After 10 seconds, a second compression was exercised with the same conditions of the first one. The analysis was performed in the external medial area of the *biceps femoris* muscle.

The software Texture Export was used to acquire data and to treat them and the result was expressed as hardness (maximum force) which measured the peak of force at the first compression of the sample.

Chemical analyses

Chemical analyses of experimental diets, faeces and caecal contents were performed according to the methodologies previously detailed in Experiment n. 1. The empty bodies of the rabbits from comparative slaughters were frozen and then ground. The freeze-dried samples were analysed by AOAC (2000) methods for moisture, crude protein and ether extract. Energy concentration of empty bodies was measured by adiabatic bomb calorimeter

Statistical analysis

The data were analyzed using ANOVA and with the level and the duration of the feed restriction, as well as their interaction, and sex as main factors. The GLM procedure of SAS (SAS, 1991) was used for all analyses. Differences among means with $P<0.05$ were accepted as representative of statistically significant differences. Differences among means with $0.05<P<0.10$ were accepted as representative of a tendency to differences. Mortality, morbidity and sanitary risk were analysed by the CATMOD procedure of SAS.

Results

Digestibility and nutritive value of experimental diets

The digestibility of dry matter, gross energy and nutrients changed in the different experimental groups according to diets and restriction level (Table 25 and 26).

Due to the higher content of crude fibre and fibrous fractions, diet S showed a lower digestibility and nutritive value compared to diet I (on average 9.8 *vs.* 10.6 MJ DE/kg). The digestibility of diet S was measured during post-weaning when rabbits of groups R90 and R80 were restricted. In that case, a significant effect of feed restriction level was measured on digestibility of dry matter, energy and various nutrients.

In details, the digestibility of dry matter (56.6% *vs.* 58.6% *vs.* 60.3%, $P<0.001$) and gross energy (56.6% *vs.* 58.4% *vs.* 60.0%, $P<0.001$) significantly increased with the restriction level, in rabbits fed *ad libitum vs.* 90% *vs.* 80% respectively. Also digestibility of protein and ether extract changed accordingly. As what concerns crude fibre and fibre fractions, digestibility in rabbits of R90 group was intermediate between R100 and R80.

Digestibility of starch, even if in very narrow interval, decreased when the level of restriction increased ($P<0.001$).

During the re-alimentation period, when the digestibility of diet I fed ad *libitum* was observed, no important residual effect of the restriction treatment during post-weaning was measured. Only digestibility of ether extract, aNDF and ADF was higher in rabbits of groups R90 and R80 compared to R100 (Table 25).

The ratio digestible protein to digestible energy ranged from 12.9 g/MJ of diet S to 10.6 g/MJ of diet I. Even if protein requirements were fully satisfied during post-weaning and fattening, the ratio DP to DE was unbalanced and in favour of the protein supply, especially in the period soon after weaning. Recommendations, in fact, give optimal DP to DE ratio for growing rabbits at 11.0-11.5 g/MJ (De Blas and Mateos, 2010).

Table 25. Digestibility coefficients (%) and nutritive value of diets S.

Diet	S				
Treatment	R100	R90	R80	Prob.	RSD
Rabbits, n.	13	14	13		
Dry matter	56.6^{a}	58.6^{b}	60.3^{c}	<0.001	1.4
Crude protein	73.1^{a}	75.2^{b}	77.8^{c}	<0.001	1.3
Ether extract	78.0^{a}	82.2^{b}	83.3^{b}	<0.001	2.2
Crude fibre	14.1^{a}	16.8^{ab}	19.3^{bc}	<0.001	3.1
TDF	32.1^{a}	33.3^{ab}	35.1^{b}	<0.01	2.3
aNDF	26.6^{a}	28.0^{ab}	30.7^{b}	<0.001	2.5
ADF	16.7^{a}	18.3^{ab}	21.0^{b}	<0.001	2.7
Starch	98.8^{b}	97.7^{a}	97.8^{a}	<0.001	0.2
Gross energy	56.6^{a}	58.4^{b}	60.0^{c}	<0.001	1.5
Digestible energy (ED), MJ/kg	9.55	9.84	10.10		
Digestible protein (PD), g/kg	123	126	131		
DP to DE ratio, g/MJ	12.9	12.8	13.0		

Table 26. Digestibility coefficients (%) and nutritive value of diets I.

Diet	I				
Treatment	R100	R90	R80	Prob.	RSD
Rabbits, n.	9	10	11		
Dry matter	62.6	62.4	63.1	0.74	2.2
Crude protein	72.6	72.5	72.0	0.68	1.7
Ether extract	77.3^{a}	82.1^{b}	83.3^{b}	<0.001	1.9
Crude fibre	18.3	19.0	21.7	0.17	4.3
TDF	40.7	40.9	41.3	0.89	2.6
aNDF	33.1^{a}	34.7^{ab}	37.6^{b}	0.02	3.2
ADF	22.2^{a}	24.0^{ab}	26.9^{b}	0.03	3.7
Starch	98.8^{b}	98.8^{b}	97.9^{a}	<0.001	0.4
Gross energy	62.2	62.1	62.3	0.97	1.8
Digestible energy (DE), MJ/kg	10.58	10.56	10.60		
Digestible protein (DP), g/kg	114	113	112		
DP to DE ratio, g/MJ	10.7	10.7	10.6		

Growth performance and health status

As mentioned above, health of rabbits impaired during the second and third week of trial, with diarrhoeas and mucus in several rabbits of all treatments. The antibiotics included in the diet S were therefore considered insufficient to control ERE and an additional antibiotic treatment in water was offered during the third week of trial to maintain the sanitary status under control, with morbidity and mortality within acceptable values.

During the entire trial, mortality averaged 9.8% and morbidity 41.9%. The analysis of the effect of main factor (level and duration of feed restriction) on health evidenced a significant increase of mortality (from 5.7% to 13.8%; P=0.03) in rabbits restricted for the longer period (2 *vs.* 3 weeks) (Table 27), while the restriction level did not affect results.

Table 27. Mortality, morbidity and sanitary risk: effect of the level and the period of feed restriction.

	Restriction level (R)			Restriction period (T)		Probability		
	R100	R90	R80	T2	T3	R	T	R x T
Mortality, %	8.5 (7)	11.0 (9)	9.8 (8)	5.7 (7)	13.8 (17)	0.68	0.03	0.58
Morbidity, %	47.6 (39)	41.5 (34)	43.9 (36)	43.9 (54)	44.7 (55)	0.73	0.90	0.22
Sanitary risk, %	56.1 (46)	53.4 (43)	53.7 (44)	50.0 (61)	58.5 (72)	0.88	0.16	0.11

When looking at the evolution of mortality during the trial (Figure 26), rabbits started to die the third week of trial, from 47 days of age, and the number of dead rabbits clearly peaked during the fourth and fifth week of trial, after the change of diet (from S to I) and the end of feed restriction. The trend of mortality was, therefore, completely different from what usual under field conditions, where mortality reaches a peak during the first 2-3 weeks after weaning and decreases in the final fattening period.

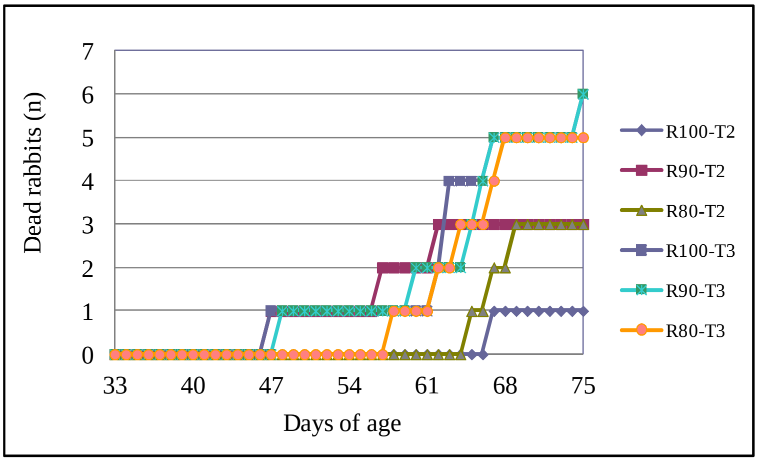


Figure 26. Cumulative mortality of rabbits within experimental groups.

On the other hand, the symptoms of the disease, even if not yet associated to mortality, were evident since the second week of the trial, with a peak in daily morbidity which hit 20-30% of present rabbits, depending on the experimental groups (Figure 27).

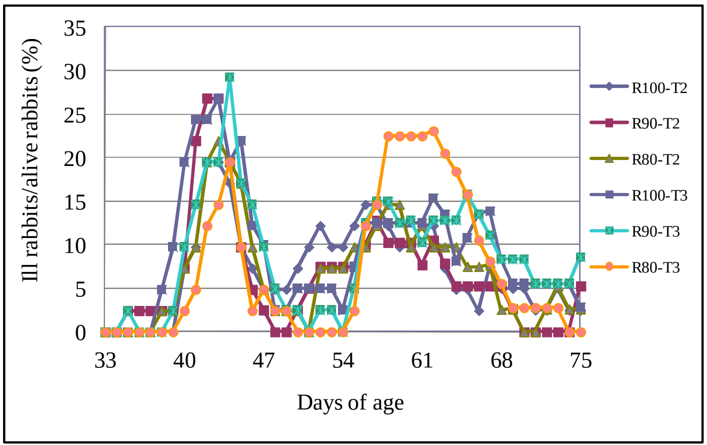


Figure 27. Instantaneous morbidity (percentage of illrabbits/present rabbits)within experimental groups.

The situation was rather stable during the third week, also because the antibiotic treatment in water was added, while a new peak of morbidity was recorded between the third and fourth week of the trial, when the group T3 started to be fed *ad libitum*. This peak was particularly high in rabbits of R80-T3 group which had showed a lower morbidity in the post-weaning period during which they were restricted at the maximum level.

The graphical representation of the same results within main factors simplifies the interpretation of results and data (Figures 28 and 29). During the second week of trial, rabbits submitted to the most severe restriction level (R80) fell ill less compared to rabbits of R100 and R90 groups and also recovered better during the third week of trial (Figure 28). During the fourth week, all rabbits showed a peak of instantaneous morbidity, which was however more evident (almost 20%) in rabbits previously submitted to the most severe feed restriction level (R80).

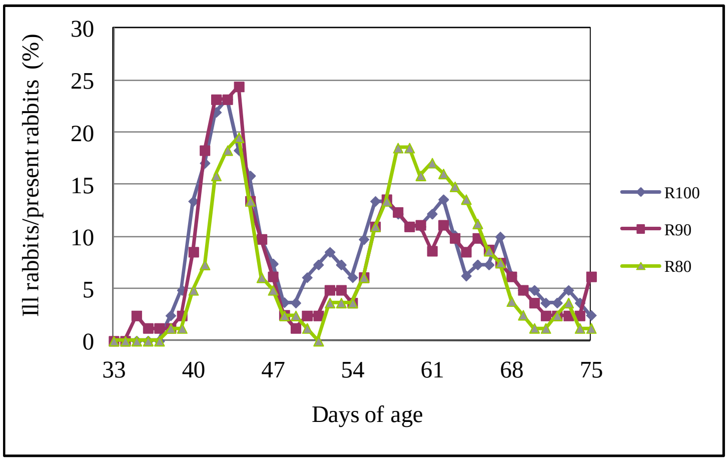


Figure 28. Instantaneous morbidity (percentage of ill/present rabbits): effect of the restriction level.

As what concerns the effect of the length of restriction period, the first peak of morbidity obviously affected in a similar way the rabbits of both T2 and T3 group (Figure 29). During the third week (47-54 days of age), the percentage of ill rabbits was higher in rabbits which were receiving diet I *ad libitum* (group T2), while during the fourth week the group restricted for a longer period (T3) showed more problems once left free of eating.

The differences observed in health and according to the experimental treatments during the various weeks of the trial show that the lower problems recorded during the first period of feed restriction were followed by a worsening of health during the fattening and re-alimentation period, and this negative trend was worse as stronger the restriction level and as longer the restriction period.

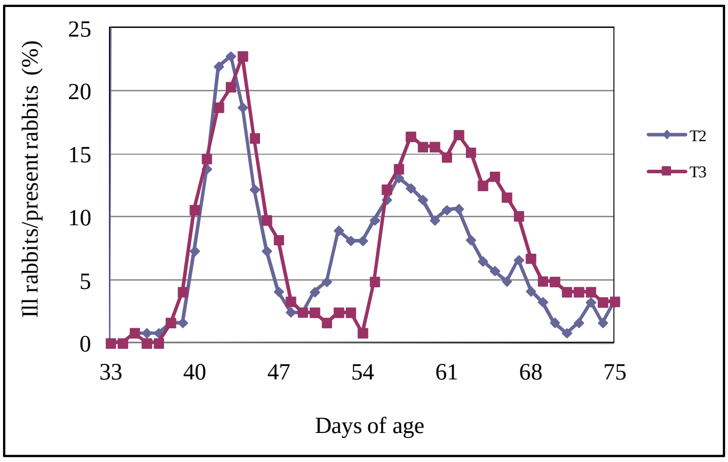


Figure 29. Instantaneous morbidity (percentage of ill/present rabbits): effect of the restriction period.

The trend in mortality and morbidity in our trial, with a worse health status in restricted rabbits, may be explained be the changes in daily feed intake (Figure 30). Firstly, we have to point out how difficult was to calculate the feed restriction curves basing on the amount of feed effectively ingested the day before by rabbits fed *ad libitum*. This fact was especially true during the second week of trial, when digestive problems appeared which reduced average feed intake of R100 rabbits. As a consequence, rabbits of R90 and R80 groups received an amount of diet higher than the theoretical 90% and 80% of *ad libitum* group. The correction in calculation used from 40 days of age to adjust average feed intakes suddenly decreased the amount of the diet administered at 41-43 days of age. Only at the end of the second week, intakes of restricted groups were in line with those previewed compared to *ad libitum* groups. A further factor must be taken into account, which is more correlated with the trend in mortality that paid the restricted groups, especially those of T3 group. This factor is the sudden increased in feed intake the day of transition from a restricted to a voluntary feeding (on 14th day of trial for T2 rabbits and on 21st day of trial for T3 rabbits). The sudden change of ingested feed corresponded to an increase of 20-25 g than the previous day on rabbits R90-T2 and doubled to 45-50 g on rabbits R80-T2, and further increased to 35 g on rabbits R90-T3 to reach +71 g on rabbits R80-T3, which were previously restricted at 80% during the first 21 days of trial. This sudden variations of ingestion level, as much higher as stronger the restriction level and longer the restriction period, which besides were contemporary to the change to a not-medicated and high-energy diet (with a higher starch/ADF ratio), may explain why animals were again susceptible to digestive diseases and mortality, especially in those animals previously submitted to a more severe restriction (80%) during a longer period (3 weeks).

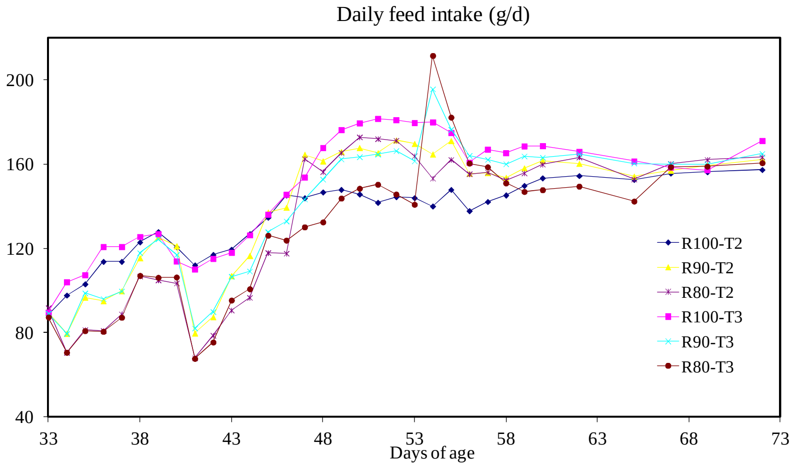


Figure 30. Evolution of daily feed intake in experimental groups.

Regardless of health and mortality (dead animals are not included in the analyses of zootechnical performance), the effect of the restriction system on productive results was clear and significant during the same period of restriction, but tended to decrease during the week in which they were free of feeding and even disappear when rabbits reached the end of the trial (Table 28). Live weight of rabbits, similar at the beginning of the trial, was significantly lower in rabbits submitted to the most severe restriction (R80) both after two weeks of restriction (at 47 days, 1646 g and 1592 g *vs.* 1507 g, P<0.001) and after three weeks (at 54 days, 2002 g and 1979 g *vs.* 1883 g, P<0.001; in this case the average values of R100, R90 and R80 groups include all animals, both those half still restricted and those half free of feeding). At the end of the experimental period, at 75 days of age, however, differences in live weights among the three groups were reduced and not statistically significant. When the whole experimental period was taken into account, therefore, daily weight gain only tended to be impaired with a stronger restriction level (P=0.08), feed intake was lower in animals submitted to the 80% restriction level (145 and 143 *vs.* 136 g/d; P<0.01), whereas feed conversion did not change significantly among restriction treatments (3.21, 3.14 and 3.14, P=0.13).

Table 28. Performance from weaning until commercial slaughter.

	Restriction level (R)			Restriction period (T)		Sex (S)		Probability				RSD
	R100	R90	R80	T2	T3	Females	Males	R	T	S	R x T	
Rabbits, no.	75	73	74	116	106	94	128					
Live weight, g												
at 33 d	915	912	911	911	914	906	919	0.98	0.81	0.38	0.99	100
at 47 d	1646^b	1592^b	1507^a	1579	1584	1585	1578	<0.001	0.84	0.75	0.95	165
at 54 d	2002^b	1979^b	1883^a	1977	1932	1942	1967	<0.001	0.09	0.37	<0.01	196
at 75 d	2817	2829	2742	2782	2809	2756	2835	0.12	0.48	<0.05	0.05	277
Performance from 33 to 75 d												
Daily growth, g/d	45.3	45.6	43.6	44.6	45.1	44.0	45.6	0.08	0.49	0.06	<0.05	5.99
Feed intake, g/d	145^b	143^b	136^a	139	143	139	143	<0.01	0.11	0.11	0.01	17.2
Feed conversion	3.21	3.14	3.14	3.14	3.19	3.18	3.14	0.13	0.28	0.28	0.74	0.27

When data were analysed week by week, the restriction treatment clearly reduced feed intake during the first two weeks of treatment (P<0.001), whereas since the third week (when all groups T2 were free of feeding *ad libitum*) this effect was weaker and attenuated by the change to a more energetic diet and did not affect feed intake until the end of the trial (Figure 31).

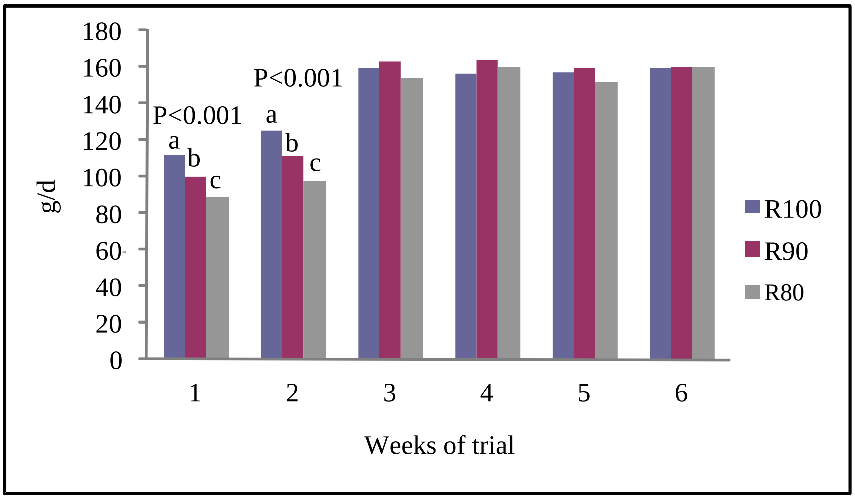


Figure 31. Daily feed intake: effect of restriction level.

During the third week, the probability of the interaction between the level and the period of feed restriction was significant (Figure 32; P<0.001) on feed intake which increased from rabbits of groups R100 to those of groups R80 (144, 166 and 166 g/d) for rabbits T2 now fed *ad libitum* with diet I. Clearly an opposite trend was recorded for the same restriction levels (174, 159 and 142 g/d) of T3 groups which were still restricted and fed diet S.

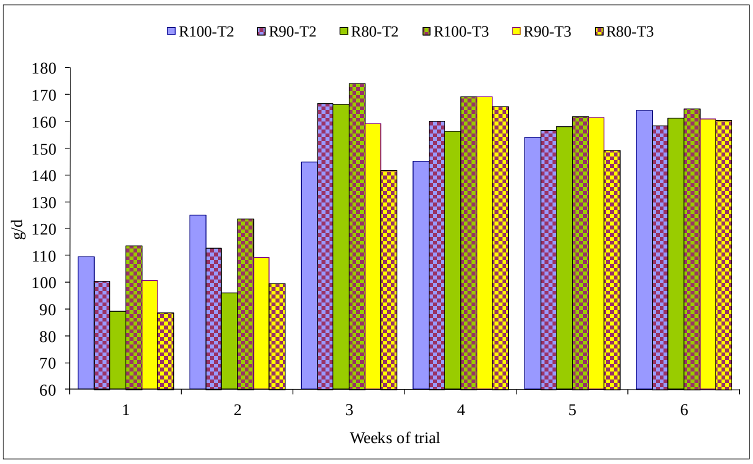


Figure 32. Daily feed intake: effect of the restriction treatment.

The level of feed restriction significantly reduced daily growth rate only during the first week of trial (Figure 33).

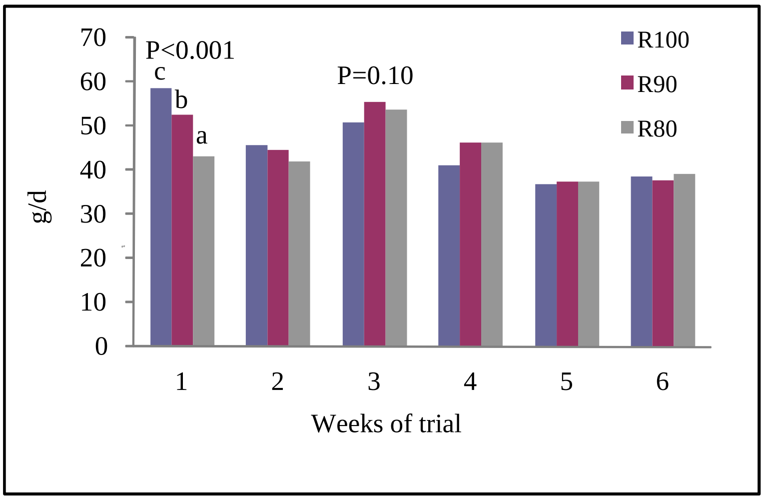


Figure 33. Daily growth rate: effect of restriction level.

The positive trend (P=0.10) of daily weight gain with the increase of feed restriction level which was recorded during the third week of trial is related to the increase in live weight of groups R90 and R80 during re-alimentation (groups T2). The same trend was measured during the fourth week, but was not significant at a statistical level. During the third week, the probability of the interaction between level and period of restriction was significant (P<0.001), and daily growth rate measured 45.8, 61.6 and 63.2 g/d in rabbits R100, R90 and R80 of group T2 (which were free of feeding *ad libitum*) and 56.0, 49.0 and 44.1 g/d for rabbits of the corresponding restriction levels but belonging to group T3 (still restricted) (Figure 34).

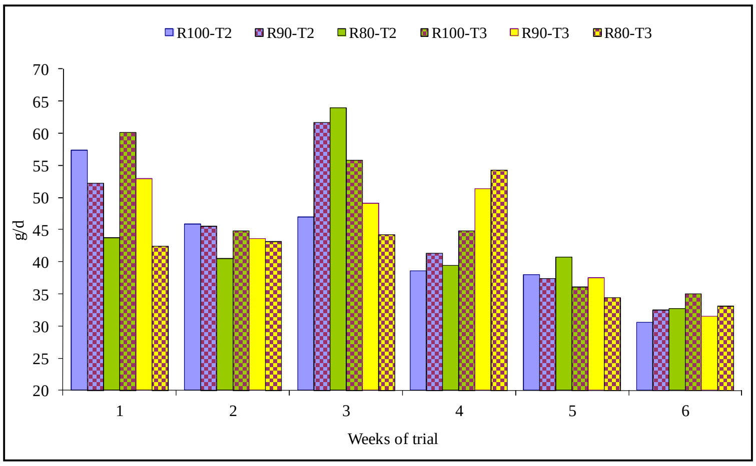


Figure 34. Daily growth rate: effect of interaction.

As what concerns the effect of the restriction period length on weekly performance, during the first two weeks ingestion of diets was obviously similar (Figure 35).

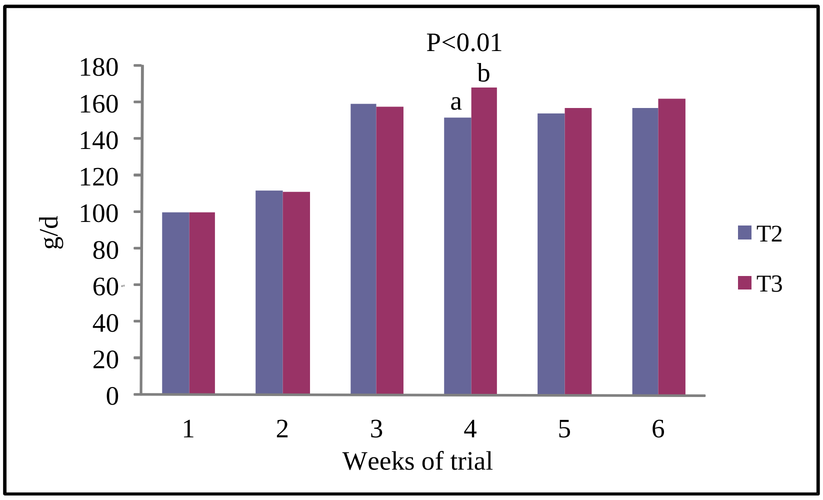


Figure 35. Daily feed intake: effect of the restriction period.

The similarity in feed intake of groups T1 and T2 during the third week of trial, when groups T2 were fed *ad libitum* while groups T3 were still restricted, could be explained by the different energy value of the diets that were used (diet I for T2 rabbits and diet S for T3 rabbits) and the chemiostatic regulation of appetite in rabbits. This hypothesis was supported by the different average feed intake measured in rabbits of R100-T2 (144 g/d) and R100-T3 (174 g/d) treatments, all fed *ad libitum*, during the third week (see Figure 35). For these reasons, groups T1 and T2 showed similar results in the same period and a certain effect of restriction only in the week immediately after the end of the restriction treatment (Figure 36).

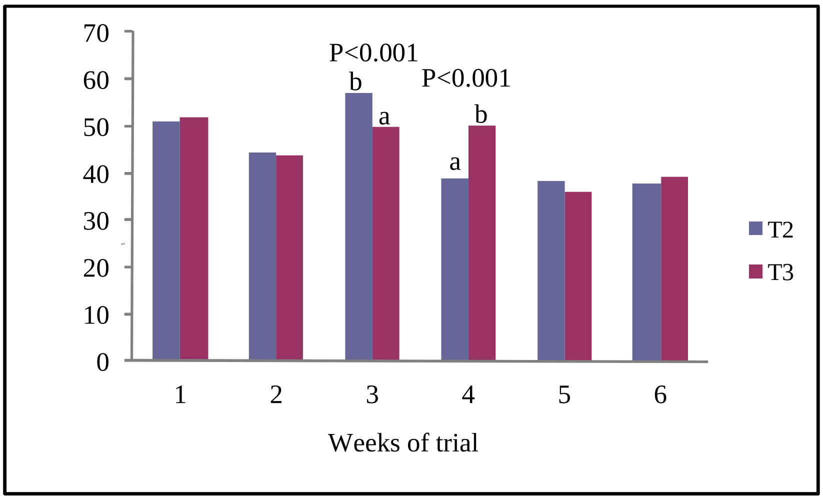


Figure 36. Daily growth rate: effect of the restriction period.

In details, during the third week of trial, daily growth rate significantly decreased in rabbits T3, still restricted, compared to rabbits T2, at that moment fed *ad libitum* (P<0.001), as evidenced also by the above-mentioned significant interaction “level x period”. During the fourth week of trial, when rabbits T2 and T3 were fed the same diet I, the already discussed peak in ingestion of rabbits T3 was measured due to the high ingestion level of groups R90-T3 and R80-T3. Therefore, daily growth rate during the fourth week was significantly higher in rabbits T3 than in rabbits T2.

As a last point, the effect of sex was rather weak and evident at the end of the rearing period with a higher final live weight in males than in females (2835 *vs.* 2756 g, $P<0.05$) due to a rather higher growth rate during the whole trial (45.6 *vs.* 44.0 g/d, $P=0.06$) (see Table 28).

Body composition and nitrogen balance

The changes in body composition during the trial and the composition of rabbits at the end of the trial were estimated on the base of the data recorded on animals slaughtered at the beginning and at the end of the trial by the comparative slaughter technique. By using the data of empty body weight and composition of reference rabbits slaughtered at 33 and 74 days of age, changes in composition of empty body were estimated for all rabbits that reached the end of the trial (75 days of age) and in function of the restriction treatments (Table 29).

In rabbits submitted to the most intensive restriction level (group R80), EBW gain was lower ($P=0.03$) than in R100 and R90 rabbits and, as a consequence, body gains in water, protein and ash were lower (Table 29). The gain of lipid was unexpected: the lowest values (220-240 g) were recorded in rabbits R100-T2, R90-T3 and R80-T3 and the highest values in other groups (significant interaction $P<0.001$). This result mainly depended on the significant interaction between level and period of restriction which penalized on one side rabbits fed *ad libitum* with a weaning diet during 2 weeks and on the other side rabbits submitted to restriction (80% and 90%) for a longer period (3 weeks) compared to the other animals, as already discussed for growth performance.

Data of body chemical composition were also used to measure the effect of the restriction level and period on nitrogen balance. The most severe feed restriction, 80% of *ad libitum*, significantly reduced the amount of ingested, retained and excreted nitrogen compared to not restricted rabbits or rabbits restricted at 90% (Table 30). The amount of ingested nitrogen decreased by 6% ($P<0.01$), that of retained nitrogen by 4% ($P<0.10$) and nitrogen excretion diminished by 8% ($P<0.001$) in R80 rabbits compared to *ad libitum* rabbits.

As what concerns the effect of the restriction period, an increase from 2 to 3 weeks impaired nitrogen balance with a significant increase of nitrogen ingestion (+4% in rabbits T3 compared to rabbits T2, $P=0.02$) and nitrogen excretion (+6% in rabbits T3 compared to rabbits T2, $P<0.01$), in front of a not significant change in the amount of retained nitrogen.

These results may be easily explained by the higher protein content of the diets fed during the restriction period and during re-alimentation (16.8% of diet S *vs.* 15.6% of diet I), especially in rabbits of groups R100-T3 and R90-T3, for which the increase of protein ingestion did not correspond to an increase of growth rate and, therefore, of nitrogen retention (significant interaction, $P<0.001$) (Table 30).

In other words, the administration of a diet with higher protein content and for a longer period impaired nitrogen balance, notwithstanding feed restriction. The lower energy value of diet S even accentuated this negative result, since ingestion level of diet S remained higher compared to diet I, especially during the third week of trial, as widely commented in the paragraph on growth performance.

Table 29. Variation of empty body weight (EBW) and composition of EBW gain from at 33 to 75 days of age: effect of the restriction level and period.

	Restriction level (R)			Restriction period (T)		Probability			RSD
	R100	R90	R80	T2	T3	R	T	R x T	
Rabbits, n.	75	73	74	116	106				
EBW at 33 d*, g	774	771	771	772	773	0.97	0.91	0.99	84
EBW at 75 d, g	2491	2490	2406	2449	2476	0.06	0.40	0.14	245
EBW gain, g	1716^{b}	1719^{b}	1636^{a}	1677	1703	0.03	0.38	0.10	223
Composition of EBW gain									
Water, g	1059^{ab}	1071^{b}	1004^{a}	1037	1052	0.01	0.44	0.60	145
Protein, g	351^{ab}	354^{b}	334^{a}	342	351	0.02	0.13	0.52	44
Ether extract[1], g	258	246	250	249	244	0.03	0.13	<0.001	27
Ash[2], g	49^{b}	49^{ab}	46^{a}	50	46	0.01	<0.001	0.05	7
Energy, MJ	18.7	18.5	18.4	18.6	19.1	0.20	0.04	0.30	2.2

*Data of the initial comparative slaughter of 18 rabbits at 33 d of age: EBW = 84% LW; EB composition: water, 73.7%; protein, 18.0%, ether extract, 4.6%; ash, 3.7%; gross energy, 6.57 MJ/kg.

[1] Interaction ($P<0.001$): 224, 261, 261, 292, 231 and 240 g in groups R100-T2, R90-T2, R80-T2, R100-T3, R90-T3 and R80-T3. [2] Interaction ($P=0.05$): 52, 51, 46, 46, 47 and 46 g in groups R100-T2, R90-T2, R80-T2, R100-T3, R90-T3 and R80-T3.

Table 30. Nitrogen excretion: effect of the restriction level and period.

	Restriction level (R)			Restriction period (T)		Probability			RSD
	R100	R90	R80	T2	T3	R	T	R x T	
Rabbits, n.	75	73	74	116	106				
Live weight at 33 d, g	915	912	911	911	914	0.97	0.81	0.99	100
Live weight at 75 d, g	2817	2829	2741	2782	2809	0.12	0.47	0.05	277
Body N at 33 d, g	26.7	26.6	26.6	26.6	26.7	0.98	0.81	0.99	3.0
Body N at 75 d, g	87.2	87.6	84.6	86.0	86.9	0.12	0.47	0.05	9.3
Feed intake, g/d	145^{b}	143^{b}	136^{a}	139	143	<0.01	0.11	0.01	17.2
Ingested N, g	156^{b}	154^{b}	146^{a}	149	155	<0.01	0.02	0.01	18.3
Retained N, g	60.5^{b}	61.0^{b}	58.1^{a}	59.4	60.3	0.08	0.48	0.03	8.4
Excreted N, g	95.5^{b}	92.7^{b}	87.5^{a}	89.3	94.5	<0.001	<0.01	0.02	12.1
Daily excreted N, g/d	2.27^{b}	2.21^{b}	2.08^{a}	2.13	2.25	<0.001	<0.01	0.02	0.3

Slaughter results, carcass and meat quality

The feeding treatment scarcely affected slaughter results, carcass and meat quality (Tables 31 and 32). As for performance, also slaughter results were scarcely affected by the level or period of feed restriction. In fact, it is notorious that when rabbits are fed *ad libitum* balanced diets, the feeding treatment cannot modify slaughter results if the live weight is not affected at an appreciable rate (Xiccato, 1999; Hernández, 2008). In the present trial, only the incidence of liver was significantly higher in rabbits submitted to the most severe restriction level (R80) and transport losses were higher in rabbits restricted for a longer period (T3). The amount of dissectible fat tended to be higher (P=0.09) in rabbits R90 than in R100 and R80 ones.

Table 31. Slaughter results and carcass quality.

	Restriction level (R)			Restriction period (T)		Sex (S)		Probability				RSD
	R100	R90	R80	T2	T3	Females	Males	R	T	S	R x T	
Rabbits, n	63	61	62	98	88	73	113					
Slaughter weight (SW), g[2]	2767	2782	2695	2741	2754	2717	2779	0.76	0.17	0.14	0.07	272
Transport losses, % LW[3]	1.7	1.7	1.7	1.5	1.9	1.7	1.7	0.98	0.04	0.90	0.09	1.2
Full gut incidence, % SW[4]	19.8	19.5	19.9	19.5	19.9	20.2	19.3	0.57	0.17	<0.01	0.14	2.2
Cold carcass (CC), g	1637	1655	1594	1621	1637	1594	1664	0.26	0.62	<0.05	0.24	170
Cold dressing, %	59.3	59.7	59.3	59.4	59.4	59.0	59.8	0.66	0.98	<0.05	0.66	2.0
Head, %CC	8.0	8.0	8.2	8.1	8.1	8.0	8.1	0.43	0.94	0.62	0.57	0.6
Liver, %CC	5.0	5.0	5.5	5.1	5.3	5.2	5.1	0.05	0.24	0.68	0.97	0.9
Reference carcass (RC), g	1368	1383	1321	1352	1362	1329	1386	0.17	0.72	0.06	0.28	152
Dissectible fat, % RC	3.1	3.6	3.2	3.3	3.3	3.4	3.2	0.09	0.74	0.23	0.12	1.0
Hind legs, % RC	32.3	32.8	33.3	33.0	32.6	32.5	33.1	0.17	0.36	0.24	0.30	1.7
Hind leg muscle/bone ratio	6.53	6.71	6.56	6.66	6.54	6.48	6.72	0.72	0.54	0.27	0.65	0.7
Hind leg meat hardness, N	11.5	12.7	12.3	12.7	11.6	13.3	11.0	0.82	0.47	0.17	0.25	5.6

As what concerns the effect of sex, females showed a higher incidence (P<0.01) of gut which impaired dressing out percentage (P<0.04) and decreased cold carcass weight (P<0.05) compared to males. Some changes due to sex were recorded for meat colour: *biceps femoris* showed a lower red index and a higher T index in females rather than in males.

Hardness of meat, measured as resistance to compression, did not change with feeding treatments or sex. From a methodological point of view, this type of measurement, that is resistance to compression of fresh meat of hind leg, evidenced some critical points of its application due to the composition of a product as the hind leg of rabbits for which also the classical measurement of share force is critical to be realized.

Table 32. Meat quality: pH and colour of *longissimus lumborum* and *biceps femoris*.

	Restriction level (R)			Restriction period (T)		Sex (S)		Probability				RSD
	R100	R90	R80	T2	T3	Females	Males	R	T	S	R x T	
Longissimus lumborum:												
pH	5.53	5.54	5.53	5.54	5.53	5.54	5.53	0.73	0.89	0.71	0.81	0.08
L*	49.9	50.2	49.5	49.6	50.2	49.7	50.1	0.46	0.15	0.37	0.15	2.48
a*	-2.12	-2.29	-2.23	-2.16	-2.26	-2.34	-2.08	0.62	0.52	0.09	0.99	0.78
b*	0.76	0.13	0.20	0.71	0.02	0.19	0.54	0.36	0.09	0.40	0.19	2.16
C*	3.00	2.93	2.99	2.88	3.06	2.80	3.15	0.96	0.41	0.11	0.59	1.13
T*	-0.23	-0.10	-0.06	-0.24	-0.02	-0.09	-0.17	0.53	0.09	0.56	0.14	0.67
Biceps femoris:												
pH	5.72	5.70	5.70	5.71	5.70	5.71	5.71	0.85	0.52	0.87	0.32	0.12
L*	48.3	48.6	48.4	48.3	48.5	48.5	48.4	0.80	0.50	0.83	0.11	2.15
a*	-2.61	-2.59	-2.81	-2.67	-2.67	-2.78	-2.56	0.11	0.99	0.03	0.48	0.53
b*	3.03	2.85	3.07	3.01	2.96	2.85	3.13	0.63	0.82	0.21	0.70	1.13
C*	4.09	3.97	4.27	4.11	4.11	4.08	4.13	0.30	0.99	0.74	0.55	0.85
T*	-0.83	-0.81	-0.79	-0.82	-0.80	-0.77	-0.86	0.69	0.64	0.05	0.86	0.24

Experiment 4.

Effect of feeding plan and feed restriction program in growing rabbits

Introduction

Results obtained during the first trial on feed restriction highlighted some problems and arose questions about the correct management of feed restriction in terms of restriction rate, times and modes of re-alimentation: reduction of voluntary intake during the second phase of growth with lower growth rates; impairment of health condition as a consequence of the great change in daily feed intake after re-alimentation; difficulty to calculate the daily feed restriction level on the base of feed intake of animals fed *ad libitum*.

As a consequence, the second study of the present study on feed restriction aimed at evaluating different combination of feeding plans and feed restriction programs: feeding plans were based on the administration of high digestible energy (DE diet A:11.1 MJ/kg) or moderate digestible energy (DE diet B: 10.7 MJ/kg)during the whole growing period (A-A; B-B) or in succession (B-A); feed restriction program started from 85-90% of voluntary intake and gradually increased until voluntary intake to avoid great changes in ingestion level during re-alimentation. The effects of the above described feeding programs were tested on health status, growth performance, digestive utilization of diets, and carcass and meat quality as well as body composition and nitrogen balance of rabbits.

Materials and methods

Rearing conditions

The trial was realized in the experimental farm of the University of Padova in the period April-June 2010. The characteristics of experimental facilities and cages have been detailed previously in the Experiment 1.

During the trial, the maximum temperature reached 27°C and the minimum temperature was 12°C (Figure 37). Average values of minimum and maximum temperature measured 18° and 23°C, respectively. The maximum relative humidity averaged 77%, with a minimum value of 55% and a maximum 89% (Figure 37). The minimum relative humidity averaged 52% and ranged from 31% to 76%. These environmental conditions may be tolerated without any problems by growing rabbits.

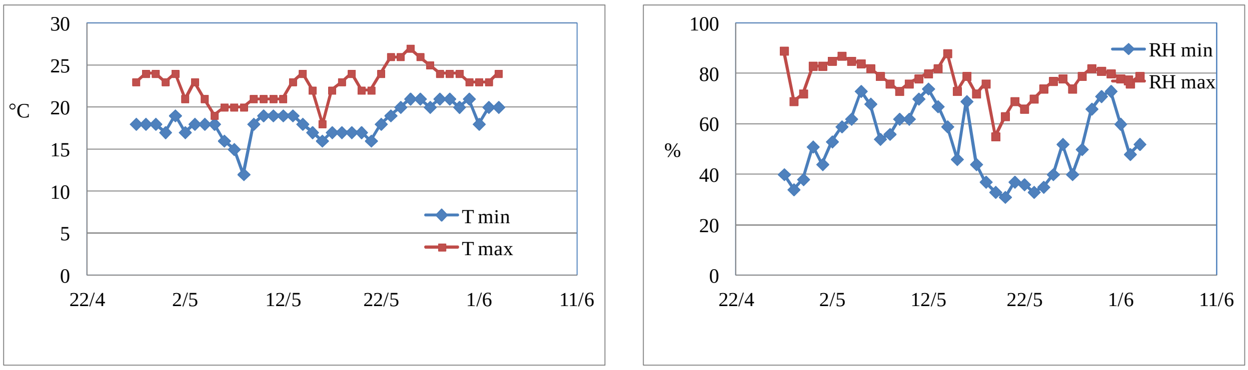


Figure 37. Daily values of minimum and maximum temperature and minimum and maximum relative humidity (RH) throughout the trial.

Animals, experimental groups and recordings

The research was approved by the Ethical Committee of the University of Padova for animal experimentation.

In a commercial farm, at 33 days of age, 300 rabbits of both genders from a hybrid line (Grimaud Frères, France) were selected (six rabbits per litter) from 50 multiparous does at their 3-6th kindling with healthy litters and homogeneous kit weight. The animals were transported to the experimental farm and at their arrival they presented an optimal health status. The rabbits were put in individual cages and identified using ear tags. The following day, 34 days of age, the trial started and the animals were weighed (953±110 g LW). The same day, 12 rabbits were selected, representative of all animals for average live weight and variability according to the comparative slaughter technique (Xiccato *et al.*, 2003) as described in Experiment n. 3. The remaining 288 rabbits were divided in six experimental groups of 48 units, homogeneous in average weight and variability, and submitted to three feeding plans per two restriction levels. The six rabbits from the same litter were assigned one per group to distribute the maternal effect over the treatments.

Two diets (diet A and diet B) with a different energy concentration and with the same DP/DE ratio (theoretical ratio: 11 g DP/MJ DE) were used for feeding rabbits. The feeding plans changed according to the diets administered during the first and second period of growth: plan A-A, rabbits were fed diet A during the whole trial (5 weeks); plan B-A, rabbits were fed diet B during the first three weeks and diet A during the last two weeks; plan B-B, rabbits were fed diet B during the whole trial (5 weeks).

Within the three feeding plans, rabbits were fed *ad libitum* during the whole trial; or restricted on average at 90% during the first three weeks of trial.

The trial lasted five weeks and was divided into two periods:

a) first period (3 weeks), post-weaning, , during which two groups of animals were fed with diets A and the remaining four groups with diet B. Within each feeding treatment, half of animals was restricted (R) and half was fed *ad libitum* (L);

b) second period (2 weeks) fattening, during which four out of the six experimental groups received diet A and the remaining two groups were fed diet B. All rabbits were fed *ad libitum* during this period (L) (Figure 38).

The trial started the day after the arrival of rabbits at the experimental farm (at the age of 34 d) and lasted 36 days until commercial slaughter at 70 days of age. Individual live weight and feed intake were recorded three times a week. Health status was checked daily to detect promptly the occurrence of diseases, especially digestive problems, as described in the Experiment 1.

An *in vivo* digestibility trial was carried out on 48 animals (12 rabbits fed *ad libitum* with diet A, 12 rabbits restricted fed with diet A, 12 rabbits fed *ad libitum* with diet B and 12 rabbits restricted fed with diet B) from 47 to 51 days of age following the procedure of Perez *et al.* (1995).

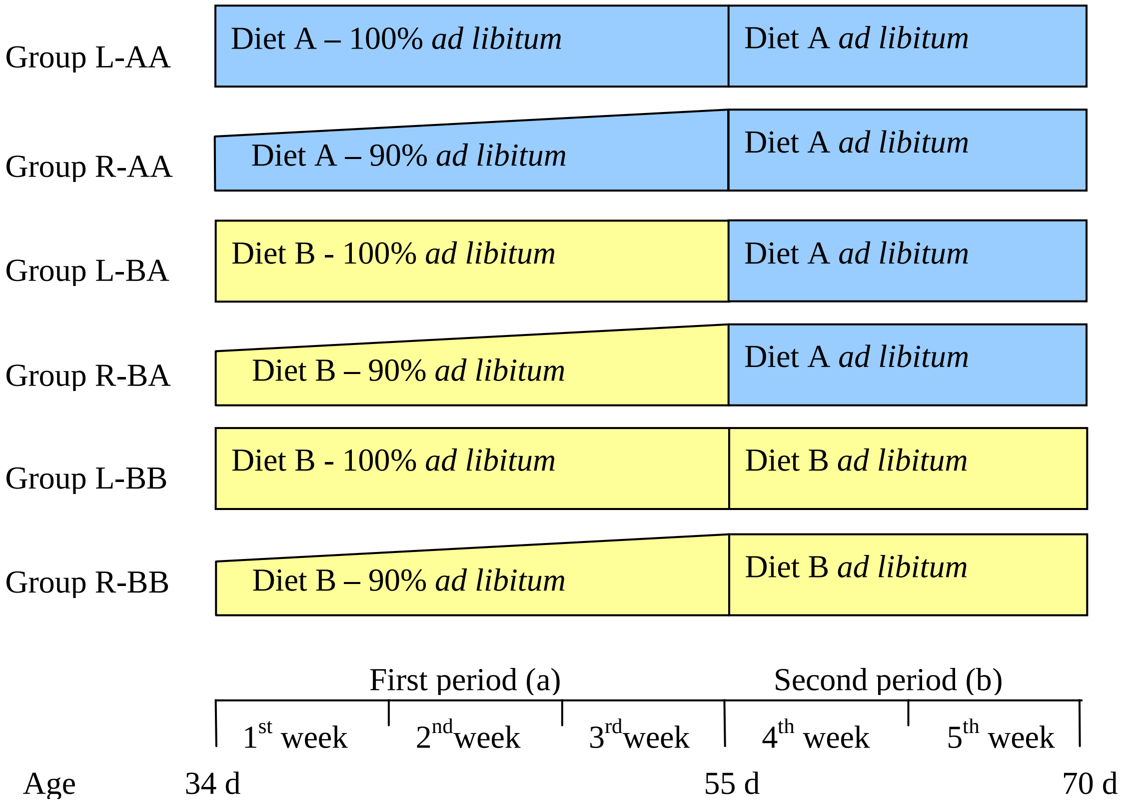


Figure 38. Scheme of experimental treatments.

Experimental diets

The diets were pelleted with 3.5 mm of diameter and 1.0-1.1 cm of length. The diets were similar to those used in commercial farms during post-weaning (diet B) and fattening (diet A) periods. Diets were supplemented with synthetic amino acids, micro- and macro-minerals and vitamins to satisfy the nutritional needs of young rabbits (De Blas and Mateos, 2010) and did not contain any antibiotic or coccidiostatic drugs. Main differences among diets were due to the inclusion rate of alfalfa (Table 33), higher in diet B, and barley, higher in diet A, whereas the other main raw materials changes in a restricted range.

Table 33. Ingredient composition (%) of experimental diets.

	Diet A	Diet B
Dehydrated alfalfa meal 16% CP	24.55	34.00
Wheat bran	20.00	24.00
Barley	20.00	12.00
Dried beet pulp	14.50	16.00
Soybean meal 47% CP	8.00	5.00
Sunflower meal 29% CP	8.00	5.00
Soybean oil	1.50	1.00
Molasses	1.50	1.50
Calcium carbonate	0.48	0.25
Dicalcium phosphate	0.50	0.28
Sodium chloride	0.40	0.40
L-lysine	0.05	0.05
DL-methionine	0.05	0.05
Vitamin - mineral premix*	0.47	0.47

*Supplementation per kg of feed: vit. A, 12.000 UI; vit. D3, 1.000 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.

Table 34 reports the chemical composition of diets measured in laboratory and their nutritive value determined in vivo. Differences between diets were lower than expected, especially for the starch content (13.8-13.6%) and fibre and fibrous fractions. Consistently with formulation criteria, diet A showed higher protein content compared to diet B (16.8% *vs.* 15.5%) due to the higher inclusion rate of soybean (+3 points) and sunflower (+3 points) meals. Therefore, diets had a different DP to DE ratio from 11.9 g/MJ in diet A to 11.0 g/MJ in diet B.

Table 34. Chemical composition (as fed) of experimental diet.

	Diet A	Diet B
Dry matter, %	88.9	89.4
Crude protein, %	16.8	15.5
Ether extract, %	4.3	3.5
Crude fibre, %	14.5	15.1
Ash, %	6.6	6.9
aNDF, %	32.0	34.4
ADF, %	17.3	18.5
Lignin (sa), %	4.1	4.3
Starch, %	13.8	13.6
Digestible protein (DP), g/kg	131.6	117.9
Digestible energy (DE), MJ/kg	11.1	10.7
DP to DE ratio, g/MJ	11.9	11.0

Restriction procedure

The amount of diet to administer daily to restricted rabbits was established on the base of a theoretical restriction curve according to 85% of theoretical voluntary intake was fed to rabbits on the first day of trial. The quantity of diet increased daily by 1% and reached 100% of theoretical voluntary intake on the 15th day of trial. Then, the amount of diet daily increased by a further 1% until the 28th day of the trial, when the administration of the diet was about 20 g higher than the theoretical voluntary feed intake. In our purpose, this restriction procedure would have restricted ingestion of 10% on average in the first 3 weeks of trial and, week by week, by 10-15% during the first week, 5-10% during the second week, and 0-5% during the third week thus permitting a progressive re-alimentation without great changes in feed intake levels since the 4th week of trial. The administration of diets was not corrected on the base of the real feed intake of not-restricted groups. The theoretical weekly curves of voluntary and restricted feed intake are reported in Table 35. The amount of feed to be offered during the first 28 days of trial was calculated as above described on the base of the theoretical voluntary feed intake and reported in Table 36 and Figure 39.

Table 35. Weekly theoretical curves of *ad libitum* and restricted feeding.

Group	DE1-3	DE4-5	Week 1	Week 2	Week 3	Week 4	Week 5	Weeks 1-3	Weeks 4-5
L-AA	10,0	11,0	110	140	155	160	160	135	158
L-BA	10,0	11,0	110	153	171	164	164	145	163
L-BB	10,0	10,0	110	153	171	178	185	145	184
R-AA	11,0	11,0	97	133	158	168	168	129	166
R-BA	10,0	11,0	97	146	174	171	172	139	170
R-BB	10,0	10,0	97	146	174	186	199	139	195

Table 36. Daily theoretical curves of *ad libitum* and restricted feeding.

Day Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
L-AA	92	100	105	110	115	121	125	129	133	137	140	144	146	148	150	152	154	155	156	157	158	159	160	160	161	161	161	161
L-BA	90	98	104	110	116	123	129	136	143	149	155	159	163	166	168	169	170	171	172	173	174	169	165	163	162	162	162	162
L-BB	90	98	104	110	116	123	129	136	143	149	155	159	163	166	168	169	170	171	172	173	174	175	176	177	178	179	180	181
R-AA	78	86	91	97	102	109	114	119	124	129	133	138	142	145	149	152	156	158	161	163	166	169	171	173	175	177	179	180
R-BA	77	84	91	97	103	111	117	125	133	140	147	153	158	163	166	169	172	174	177	180	183	179	177	176	177	178	180	181
R-BB	77	84	91	97	103	111	117	125	133	140	147	153	158	163	166	169	172	174	177	180	183	186	188	191	194	197	200	203

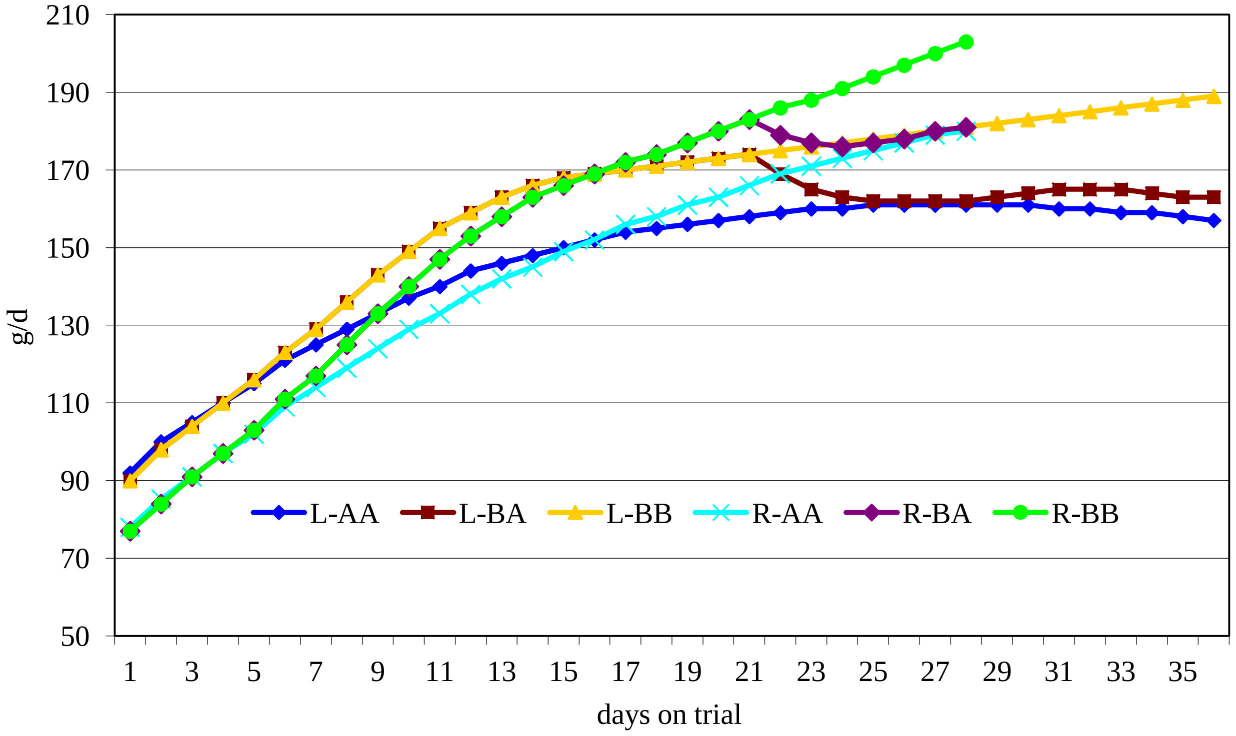


Figure 39. Theoretical voluntary intake and restriction curves.

Health status

During the trial, nine rabbits dead of which two belonging to the group L-AA, two submitted to the plan L-BA, two of the group L-BB, and three of the plan R-AA. In most cases, rabbits showed digestive problems, with symptoms which were ascribed to epizootic rabbit enteropathy. Moreover, at the end of the trial, further 11 rabbits were excluded from the dataset used in the statistical analyses because they weighed less than the minimum commercial live weight (2.0 kg): three out of 11 were from the group L-AA, one to L-BA, two to L-BB, two to R-AA, one to R-BA and two to R-BB.

Because of the quick impairment in health status, since 40 days of age and during 9 days, an antibiotic treatment in water was administered to rabbits with Tiamuline 12.5% (150 mL/100

L, Tiamvet, CEVA Santé Animale, France) and Colistine 12% (200 mL/100 L, Colistine-sulphate, Doxal, Sulbiate, Italy) to control illness and maintain mortality within acceptable rates in view of the scientific aims of the trial. The present study, in fact, intended to evaluate the effect of feed restriction on growth performance and body balance rather than on animal health.

Comparative and commercial slaughters, carcass dissection and meat quality analyses

As reported above, at the beginning of the trial, 12 rabbits were slaughtered (initial slaughter) according to the comparative slaughter technique, to determine the EBW and EB composition at the beginning of the trial. At 55 days of age, an intermediate slaughter was performed on 36 rabbits (6 per group) to evaluate the composition of rabbits at the end of the post-weaning period. Similarly, a final comparative slaughter was realized at 69 days of age on further 36 rabbits to evaluate EB composition at the end of the experiment. A total of 84 rabbits were slaughtered and analysed with the procedure described for the experiment 3.

At 70 days of age, 120 rabbits were selected (20 for each experimental group and representative in terms of average live weight and variability) for commercial slaughter and dissection. Feed and water were available until loading (from 06:30 to 07:30 a.m.). The animals were transported inside cages (50 x 100 x 30 cm) with 6 rabbits per cage (one rabbit per experimental group, 12 rabbits/m^2) in an air-conditioned truck for about 60 minutes to a commercial slaughterhouse.

Slaughter began at 08:50 and ended at 10:30 a.m. approximately. Rabbits were individually weighed, then stunned by electro-anaesthesia and killed by jugulating. Carcasses were chilled at 4°C for 2 hours and then transported to the laboratories of the Department of Animal Science of Padova for dissection. Slaughter procedure, carcass dissection and meat quality analyses were performed by using the procedures and methodologies detailed in the first experiment.

Chemical and statistical analyses

Chemical analyses of experimental diets, faeces and rabbit empty bodies were performed according to the methodologies previously detailed.

The data were analyzed using ANOVA and with feeding plan, feed restriction program, their interaction and sex as main factors. The GLM procedure of SAS (SAS, 1991) was used for all analyses. The Bonferroni *t*-test was used to compare means. Differences among means with $P<0.05$ were accepted as representative of statistically significant differences. Differences among means with 0.05<P<0.10 were accepted as representative of a tendency to differences. Mortality, morbidity and sanitary risk were analysed by the CATMOD procedure of SAS.

Results

Digestibility and nutritive value of the diets

Digestibility of nutrients was scarcely affected by feed restriction program: in fact, the digestibility trial was performed during the 3rd week of trial, when the restricted rabbits were consuming their diet almost at *ad libitum* level. Differently, digestibility and nutritive value changed greatly with the diet: digestibility of dry matter and energy, protein and fibre was significantly higher for diet A than diet B, clearly depending on inclusion in the diet A of a higher percentage of soybean and sunflower meal and barley in replacement of alfalfa (Table 37). The significant reduction of ether extract digestibility from diet A to diet B may be explained by the lower fat inclusion rate in diet B, whereas added fat have a higher digestibility compared to lipids contained in vegetable feeds (i.e., alfalfa meal) (Xiccato, 2010). The reduction of ether extract digestibility in restricted rabbits compared to those fed *ad libitum* is more difficult to be explained and depended especially on the lower digestibility of the diet in restricted rabbits fed diet A.

Table 37. Digestibility coefficients (%) and nutritive value (as fed): effect of diet and feed restriction.

Diet (D) Feed restriction (R)	A *ad libitum*	A Restricted	B *ad libitum*	B Restricted	Probability			RSD
Group	LA	RA	LB	RB	D	R	DxR	
Rabbits, n	12	12	12	12				
Dry matter	64.9	65.4	62.8	63.0	<0.001	0.56	0.73	1.9
Crude protein	78.2	78.6	76.1	75.9	<0.001	0.84	0.51	1.3
Ether extract	83.9	81.9	79.0	78.9	<0.001	<0.01	0.02	1.3
Crude fibre	22.8	24.3	19.7	19.4	<0.01	0.65	0.47	4.4
aNDF	31.1	32.1	30.3	31.1	0.38	0.43	0.92	3.7
ADF	18.7	20.0	17.6	17.8	0.20	0.56	0.68	4.5
Gross energy	66.3	66.9	64.3	64.3	<0.001	0.60	0.59	1.9

Health and productive performance

The evaluation of the effect of experimental treatments on health of rabbits was not among the main objectives of the present trial. Since soon, after one week, rabbits started to show signs of digestive diseases, an antibiotic treatment in water was administered to all rabbits. This treatment permitted to control the number of ill rabbits (Figure 40) and limiting dead rabbits to only 9 animals, even if further 8 rabbits were excluded at the end of the trial since they did not reach the minimum commercial weight (2.2 kg), so that total losses stayed at 6.8% (3.6% of dead rabbits and 3.2% of discarded rabbits).

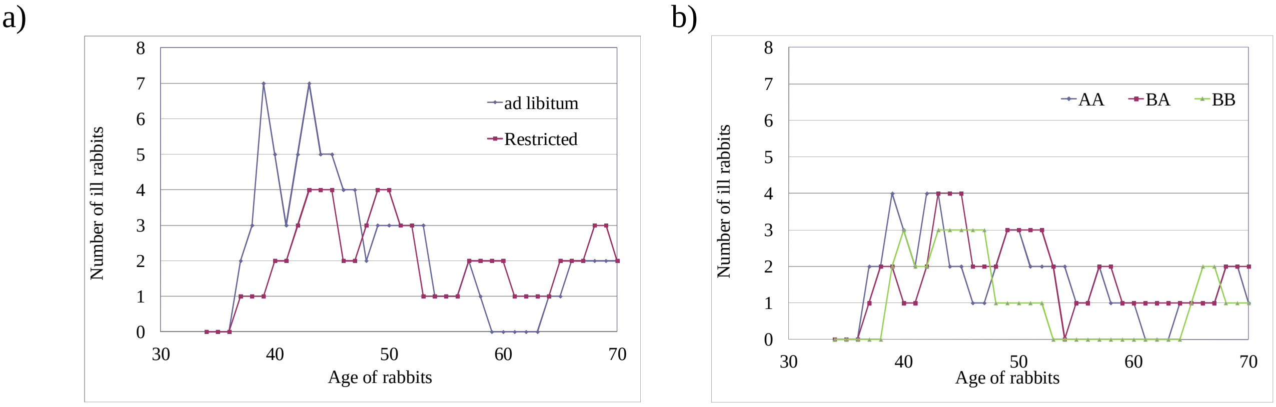


Figure 40. Number of ill rabbits per day during the trial (animals later dead or discarded are included) according to the feeding system (a) and the feeding plan (b).

The effect of experimental treatments, mortality and sanitary risk did not change either with the feeding system or with the feeding plan (Table 38), while morbidity was significantly affected by both factors. In details, the percentage of ill rabbits was higher in rabbits fed *ad libitum* rather than in those restricted.

Table 38. Effect of feeding system and feeding plan on health of rabbits from weaning until slaughter.

	Feeding (R)		Feeding plan (P)			Probability		
	ad libitum	Restricted	AA	BA	BB	R	P	R x P
Mortality[2], % (n)	7.9 (10)	5.6 (7)	9.5 (8)	4.8 (4)	6.0 (5)	0.35	0.39	0.70
Morbidity, % (n)	5.6 (7)	2.4 (3)	3.6 (3)	6.0 (5)	2.4 (2)	<0.001	<0.001	-[3]
Sanitary risk, % (n)	13.5 (17)	7.9 (10)	13.1 (11)	10.7 (9)	8.3 (7)	0.18	0.65	0.91

[1]Initial number of rabbits per group: 126 per Feeding group and 84 per Feeding plan group. [2]Dead and discarded rabbits. [3]Data not available because one group did not show morbidity.

This trend is easy to be highlighted when looking at the graphical representation of animals which fell ill daily in Figure 40a: during the period of feed restriction, more rabbits were ill in the *ad libitum* group already 4-5 days after the beginning of the trial.

The effect of the feeding plan on ill rabbits is less clear (Figure 40b) and more difficult to be explained, since it depended on the higher number of ill rabbits in animals submitted to the B-A feeding plan.

The daily changes in health status also explain differences in growth rates and feed intake during the different weeks of the trial (Table 39).

Table 39. Effect of feeding system and feeding plan on performance from weaning until slaughter: weekly data.

	Feeding (R)		Feeding plan (P)			Probability				RSD
	ad libitum	Restricted	AA	BA	BB	R	P	R x P	Sex	
Rabbits, n	116	119	76	80	79					
Growth rate, g/d										
Week 1	53.5	47.0	51.2	49.9	49.7	<0.001	0.35	0.74	0.57	6.9
Week 2	54.6	55.3	54.3	56.6	53.9	0.63	0.30	0.76	0.10	12.0
Week 3	48.3	51.0	49.2	49.7	50.1	0.05	0.84	0.53	0.99	10.1
Week 4	41.9	43.5	42.2	43.2	42.7	0.19	0.77	0.63	0.31	9.3
Week 5	37.5	37.1	37.5	37.0	37.5	0.77	0.90	0.48	0.57	8.7
Feed intake, g/d										
Week 1	108	93	101	100	101	<0.001	0.94	0.81	0.70	12
Week 2	134	129	128	135	131	0.05	0.16	0.95	0.92	20
Week 3	151	148	145	152	152	0.41	0.07	0.60	0.46	21
Week 4	149	149	145	150	152	0.87	0.11	0.90	0.21	21
Week 5	150	150	148	149	152	0.96	0.47	0.79	0.38	24

During the first week, growth rate was significantly impaired by feeding restriction (53.5 and 47.0 g/d in rabbits fed *ad libitum* and restricted, P<0.001) which depended on a 13% lower feed intake (108 *vs*. 93 g/d). During the second week of trial, the appearance of digestive disorders reduced feed intake in rabbits fed *ad libitum* so that differences in feed intake with the restricted groups decreased (134 *vs*. 129 g/d, P=0.05, which corresponded to a real restriction of 4%). For this reason, during the second week growth rate was similar between the groups, while during the third week rabbits fed *ad libitum* showed a similar feed intake (151 *vs*. 148 g/d, P>0.10) and even a lower growth rate compared to restricted rabbits (48.3 *vs*. 51.0 g/d, P=0.05). During the fourth and fifth week, neither growth rates nor feed intake differed between the two groups. As a consequence, productive performance in the whole period did not change with the experimental treatments.

Live weights of rabbits at the end of the first and second period were similar (Table 40), whereas feed intake was significantly higher in rabbits fed *ad libitum* during the first period (131 *vs*. 123 g/d, P<0.01), especially depending on feed intake of the first week of trial. This difference explains the worse conversion index of the same animals and in the same period (P<0.05). Feed intake and feed conversion measured in the first period explain the significant differences for the same traits in the whole trial between rabbits fed *ad libitum* and those restricted.

As what concerns the effect of the feeding plan, only feed conversion tended to impair from rabbits submitted to the plan A-A to those of the plan B-A until plan B-B in the first period (P<0.10). The differences in feed conversion among treatments reached a more significant level during the whole trial (2.89 *vs*. 2.93 *vs*. 2.97, P<0.05) (Table 40).

Table 40. Effect of feeding system and feeding plan on performance.

	Feeding (R)		Feeding plan (P)			Probability				RSD
	ad libitum	Restricted	AA	BA	BB	R	P	RxP	Sex	
Rabbits, n	116	119	76	80	79					
Live weight, g										
34 d	950	942	953	942	943	0.61	0.79	0.85	0.12	107
55 d	2044	2016	2036	2036	2019	0.27	0.83	0.78	0.99	201
70 d	2638	2618	2631	2634	2618	0.55	0.91	0.76	0.68	258
First period (34-55 d)										
Growth rate, g/d	52.1	51.1	51.6	52.1	51.2	0.28	0.77	0.87	0.28	7.2
Feed intake, g/d	131	123	125	129	128	<0.01	0.24	0.90	0.63	16
Feed conversion	2.52	2.45	2.43	2.48	2.53	0.04	0.06	0.57	<0.01	0.2
Second period (55-70 d)										
Growth rate, g/d	39.6	40.1	39.7	39.9	39.9	0.57	0.97	0.45	0.37	7.7
Feed intake, g/d	150	149	147	149	152	0.92	0.27	0.85	0.28	22
Feed conversion	3.85	3.79	3.75	3.80	3.90	0.35	0.20	0.31	0.79	0.5
Whole trial (34-70 d)										
Growth rate, g/d	46.9	46.5	46.6	47.0	46.5	0.63	0.86	0.73	0.77	5.8
Feed intake, g/d	139	134	139	137	138	0.04	0.25	0.99	0.39	16
Feed conversion	2.96	2.89	2.89	2.93	2.97	<0.01	0.03	0.36	0.05	0.2

Slaughter results and meat quality

Neither the feed restriction program, *ad libitum* or restricted, nor the feeding plan affected results (Tables 41 and 42). Rabbits showed an average slaughter weight of 2574 g, with a dressing percentage of 61.6%; the reference carcass (RC) weighed 1324 g on average, with dissectible fat at 2.9% RC, hind legs at 33.0% RC and meat to bone ratio of hind leg at 6.26. This performance was consistent with the genetic type used and the slaughter age of animals.

Table 41. Results at commercial slaughter and carcass quality.

	Feeding (R)		Feeding plan (P)			Probability				RSD
	ad libitum	Restricted	AA	BA	BB	R	P	RxP	Sex	
Rabbits, n	98	101	64	68	67					
Slaughter weight (SW), g	2585	2564	2579	2579	2564	0.55	0.92	0.86	0.54	247
Transport losses, % LW	1.96	2.06	1.93	2.08	2.01	0.47	0.67	0.02	0.29	0.9
Gut incidence, % SW	17.6	17.7	17.5	17.8	17.6	0.78	0.53	0.18	<0.001	1.3
Cold carcass (CC), g	1591	1582	1596	1582	1581	0.69	0.84	0.58	0.96	163
Cold dressing, %CC	61.5	61.7	61.9	61.3	61.6	0.51	0.07	0.09	0.01	1.5
Cold carcass dissection:										
Head, %CC	7.82	7.76	7.67	7.90	7.80	0.61	0.27	0.59	<0.01	0.6
Liver, %CC	5.33	5.19	5.32	5.22	5.24	0.35	0.85	0.62	0.10	0.8
Thor. org., kidneys, %CC	3.18	3.17	3.26	3.12	3.15	0.99	0.14	0.46	0.53	0.3
Reference carcass (RC), g	1328	1320	1325	1320	1326	0.77	0.98	0.54	0.17	142
Dissection of reference carcass:										
Dissectible fat, %RC	3.03	2.80	3.09	2.87	2.79	0.13	0.22	0.92	<0.01	0.8
Hind legs, %RC	32.8	33.2	32.7	33.3	33.1	0.52	0.67	0.53	0.23	2.2
Muscle/bone hind leg	6.24	6.29	6.46	6.22	6.11	0.77	0.16	0.14	0.77	0.6

Only sex affected data to some extents: gut incidence was higher in females than males (P<0.001) and dressing percentage lower in the former than in the latter rabbits (61.4 vs. 61.9, P=0.01). Also head incidence and dissectible fat of the reference carcass were affected by sex (P<0.01).

Table 42. Meat quality: pH and colour of *longissimus lumborum* and *biceps femoris.*

	Feeding (R)		Feeding plan (P)			Probability				RSD
	ad libitum	Restricted	AA	BA	BB	R	P	R x P	Sex	
Rabbits, n	60	60	40	40	40					
Longissimus lumborum										
pH	5.53	5.53	5.53	5.52	5.55	0.93	0.25	0.79	0.46	0.08
L*	53.5	53.7	53.2	53.6	54.1	0.59	0.28	0.24	0.26	2.5
a*	-1.53	-1.79	-1.74	-1.52	-1.72	0.09	0.41	0.07	0.24	0.81
b*	2.33	2.30	2.36	2.60	2.00	0.93	0.44	<0.01	0.56	2.07
C*	3.37	3.50	3.46	3.55	3.30	0.57	0.65	<0.01	0.06	1.22
T*	-0.53	-0.60	-0.60	-0.58	-0.52	0.66	0.90	0.74	0.54	0.81
Biceps femoris										
pH	5.75	5.74	5.75	5.74	5.75	0.96	0.93	0.76	0.16	0.12
L*	51.3	51.8	51.4	51.6	51.7	0.20	0.72	0.91	0.47	1.9
a*	-2.38	-2.44	-2.37	-2.37	-2.48	0.46	0.43	0.20	0.02	0.45
b*	3.40	2.97	3.36	3.18	3.01	0.07	0.49	0.26	0.88	1.28
C*	4.25	3.99	4.24	4.11	4.02	0.11	0.55	0.26	0.67	0.90
T*	-0.92	-0.83	-0.91	-0.87	-0.84	0.07	0.55	0.21	0.86	0.28

Body balance and nitrogen excretion

At intermediate slaughter (55 days of age), the EB crude protein content differed significantly according to the feed restriction program and was higher in rabbits fed *ad libitum* compared to those restricted (19.2% *vs*. 18.9%, respectively; P=0.05) (Table 43). A tendency to a significant interaction (P=0.06) was measured between the feed restriction program and the feeding plan, that is the EB protein content was higher in rabbits fed *ad libitum* during the whole trial with diet A, characterized by a higher DP to DE ratio, rather than *ad libitum* with diet B, whereas in restricted rabbits EB protein was similar regardless from the diet. At final slaughter (69 days of age) after two weeks of re-alimentation of previously restricted rabbits, no significant effects of the treatments were observed.

Restricted rabbits showed a lower EBW at 55 d, and the composition of the EB gain during the first period was characterized by lower gains of protein, lipid, ash and gross energy. In the same period, the different diets only affected EB gain of ash (Table 44). The significant interactions between the feed restriction program and the feeding plan to rabbits during the first period for EB gain of lipid and gross energy are due to the lowest value measured in restricted rabbits of plans R-BB and R-AA.

During the second period, when all rabbits were fed *ad libitum,* the effect of the feed restriction program was still evident, but restricted rabbits were favoured (Table 45). In details, between 55 and 69 days of age, the EB of previously restricted rabbits gained more protein (120 *vs*. 126 g), lipid (91 *vs*. 97 g), ash (18 *vs*. 19 g) and energy (6.8 *vs*. 7.1 MJ) than those fed *ad libitum*, confirming the existence of a compensative growth. In rabbits fed *ad libitum*, the EB gain of protein was lower in rabbits of plan L-BB (111 g) than those of plan L-BA (130 g), with intermediate values in rabbits of plan L-AA (121 g); differently, rabbits previously restricted showed a higher EB gain of protein in group R-AA (130 g) than in group R-BB (125 g), while those submitted to plan R-BA showed an intermediate protein gain (122 g). As what concerns the EB gain of lipid and energy, rabbits submitted to plan L-BB showed the most unfavourable results and those rabbits submitted to plan R-BB were in the most favourable condition, whereas rabbits always fed *ad libitum* or previously restricted and submitted to plans A-A and B-A showed similar and intermediate conditions.

On the base of results above described, rabbits of the group R-BB, previously submitted to the major constraint in energy intake, since they were restricted and were fed with the low-energy diet, utilized diet B during the second period with higher efficiency (higher gain in protein, lipid and energy) compared to rabbits submitted to the same plan (BB) but fed *ad libitum* during post-weaning. Rabbits fed diet A during the second period had an intermediate behaviour between the two groups L-BB and R-BB and regardless both from the feeding system (restricted or *ad libitum*) and the diet (A or B) received during the first period. As a consequence, when the whole trial is considered, differences among experimental groups decrease until disappearing and neither the composition of the empty body, nor the body balance were significantly different among groups, with the exception of the ash empty body gain (Table 46).

Table 43. Body composition of slaughtered rabbits at 55 and 69 d of age: effect of feeding system and feeding plan.

	Feeding (R)		Feeding plan (P)			Probability			RSD
	ad libitum	Restricted	AA	BA	BB	R	P	RxP	
Rabbits*, n	18	18	12	12	12				
EB composition at 55 d									
Water, %	67.9	68.6	68.3	68.2	68.3	0.31	0.99	0.81	1.9
Protein, %	19.2	18.9	19.1	19.1	19.0	0.05	0.94	0.06	0.50
Lipid, %	9.6	9.5	9.6	9.5	9.5	0.88	0.98	0.86	2.07
Ash, %	3.2	3.0	3.0	3.2	3.2	0.02	0.13	0.47	0.25
Gross energy, MJ/kg	8.52	8.35	8.51	8.45	8.36	-	-	-	-
EB composition at 69 d									
Water, %	65.9	66.2	65.8	66.3	66.0	0.65	0.75	0.92	1.6
Protein, %	19.7	19.5	19.8	19.5	19.5	0.31	0.33	0.80	0.6
Lipid, %	11.2	11.3	11.3	11.1	11.3	0.86	0.94	0.96	1.9
Ash, %	3.2	3.1	3.1	3.1	3.2	0.11	0.53	0.75	0.3
Gross energy, MJ/kg	9.35	9.24	9.40	9.32	9.27	-	-	-	-

*Number of rabbits slaughtered at each age

Table 44. EB weight gain composition of the EB gain between 34 and 55 days of age.

	Feeding (R)		Feeding plan (P)			Probability				RSD
	ad libitum	Restricted	AA	BA	BB	R	P	RxP	Sex	
Rabbits, n	116	119	76	80	79					
EBW at 34 d, g	802	797	805	797	797	0.61	0.79	0.85	0.11	90
EBW at 55 d, g	1738	1693	1725	1707	1713	0.05	0.80	0.76	0.98	170
EBW gain, g	936	897	920	911	916	0.02	0.91	0.82	0.28	128
EB gain composition										
Water, g	600	586	596	589	595	0.20	0.88	0.89	0.25	87
Protein, g	193	180	188	186	186	<0.001	0.77	0.15	0.32	25
Lipid, g	111	105	110	108	107	<0.001	0.24	<0.001	0.50	13
Ash, g	30	25	26	29	28	<0.001	<0.001	<0.001	0.29	4
Gross energy, MJ	9.1	8.5	9.0	8.8	8.7	<0.001	0.27	<0.01	0.40	1.1

*Data of the initial comparative slaughter on 12 rabbits at 34 days of age: Empty body weight = 84.5% live weight; EBW composition: water, 72.3%; protein, 17.6%, lipid, 6.9%; ash, 3.2%; gross energy 7.09 MJ/kg.

Table 45. EB weight gain composition of the EB gain between 55 and 69 days of age.

	Feeding (R)		Feeding plan (P)			Probability				RSD
	ad libitum	Restricted	AA	BA	BB	R	P	RxP	Sex	
Rabbits, n	116	119	76	80	79					
EBW at 55 d, g	1738	1693	1725	1707	1713	0.05	0.80	0.76	0.98	170
EBW at 69 d, g	2307	2286	2297	2312	2280	0.47	0.67	0.86	0.69	227
EBW gain, g	570	592	572	605	567	0.09	0.04	0.14	0.39	102
EB gain composition										
Water, g	340	350	334	368	334	0.24	<0.01	0.38	0.38	66
Protein, g	120	126	125	126	118	0.04	0.04	<0.01	0.40	20
Lipid, g	91	97	94	93	96	<0.001	0.46	<0.001	0.46	13
Ash, g	18	19	19	18	19	0.03	0.11	<0.001	0.41	3.2
Gross energy, MJ	6.8	7.1	6.9	7.1	6.8	<0.01	0.12	<0.001	0.45	1.0

Table 46. EB weight gain composition of the EB gain between 34 and 69 days of age.

	Feeding (R)		Feeding plan (P)			Probability				RSD
	ad libitum	Restricted	AA	BA	BB	R	P	RxP	Sex	
Rabbits, n	116	119	76	80	79					
EBW gain, g	1504	1489	1491	1516	1483	0.53	0.52	0.77	0.79	187
EB gain composition										
Water, g	941	936	929	957	929	0.78	0.26	0.83	0.73	122
Protein, g	314	306	314	311	305	0.11	0.29	0.47	0.85	37
Lipid, g	202	203	204	201	203	0.81	0.65	0.19	0.97	22
Ash, g	48	44	44	47	47	<0.001	<0.01	0.14	0.78	5.8
Gross energy, MJ	15.9	15.6	15.9	15.9	15.5	0.30	0.27	0.15	0.93	1.8

Table 47 reports the effect of the feeding treatments on the body nitrogen balance of rabbits during post-weaning and fattening periods and in the whole trial. Feed restriction significantly reduced body N at 55 days of age, without residual effects at 69 days of age. During the first period, feed restriction significantly reduced feed intake, ingested N as well as retained and excreted N. During the second period, a residual effect of feed restriction was measured only on retained nitrogen which was higher in previously restricted rabbits. During the whole trial, changes in retained N were not significant, as a consequence of the opposite trends in the first and second period, but significantly lower feed and N ingestion and N excretion were measured in restricted rabbits compared to those fed *ad libitum*.

The feeding plan did not affect body N at 55 or 69 days of age, but significantly affected the N balance. In details, N intake and excretion during the first period were significantly higher in rabbits fed diet A than in those fed diet B. During the second period, rabbits fed diet A showed on average higher N ingestion, retention and excretion than those fed diet B. As what concerns retained N, the lowest value was recorded for rabbits of group L-BB (significant interaction feeding system x feeding plan). During the whole experimental period, the effect of the feeding plan resulted in a reduction of N ingestion and excretion when comparing rabbits of plan A-A to those of plan B-A and the ones fed according to plan B-B.

Table 47. Effect of feeding system and feeding plan on body nitrogen balance from weaning to 69 d of age.

	Feeding (R)		Feeding plan (P)			Probability				RSD
	ad libitum	Restricted	AA	BA	BB	R	P	RxP	Sex	
Rabbits, n	116	119	76	80	79					
Body N at 34 d, g	22.6	22.4	22.6	22.4	22.4	0.61	0.79	0.85	0.12	2.5
Body N at 55 d, g	53.5	51.2	52.8	52.1	52.2	<0.001	0.68	0.42	0.98	5.2
Body N at 69 d, g	72.8	71.4	72.8	72.2	71.1	0.13	0.33	0.64	0.73	7.1
Balance from 34 to 55 d										
N ingested, g	69.9	66.1	70.4	67.1	66.5	<0.001	<0.01	0.88	0.63	8.3
N retained, g	30.9	28.8	30.1	29.7	29.8	<0.001	0.77	0.15	0.32	4.0
N excreted, g	39.0	37.3	40.3	37.4	36.7	0.01	<0.001	0.68	0.13	5.2
N excreted, g/d	1.86	1.77	1.92	1.78	1.75	0.01	<0.001	0.68	0.13	0.25
Balance from 55 to 69 d										
N ingested, g	55.3	55.2	55.7	56.8	53.4	0.93	0.02	0.76	0.28	8.0
N retained, g	19.3	20.2	20.1	20.1	18.9	0.04	0.04	<0.01	0.40	3.3
N excreted, g	36.1	35.1	35.6	36.7	34.5	0.18	0.04	0.49	0.30	5.6
N excreted, g/d	2.58	2.51	2.54	2.62	2.46	0.18	0.04	0.49	0.30	0.40
Balance from 34 to 69 d										
N ingested, g	125	121	126	124	120	0.04	0.03	0.99	0.39	4.8
N retained, g	50.2	49.0	50.2	49.8	48.7	0.11	0.29	0.47	0.85	6.0
N excreted, g	75.7	72.3	75.9	74.1	71.1	0.04	0.01	0.74	0.17	9.9
N excreted, g/d	2.15	2.07	2.17	2.12	2.03	0,04	0,01	0,74	0,17	0,28

Discussion of experiments 3 and 4

Modulating feeding strategies in growing rabbits

The third and fourth experiments of the thesis were dedicated to the development of feeding strategies which would permit a suitable control of rabbit feed intake during the post-weaning period, with the general aim of increasing global farm feed efficiency.

The third experiment was specifically devoted to evaluate the effect of the level (90 and 80% *vs. ad libitum*) and duration (2 or 3 weeks after weaning) of feed restriction. The fourth experiment compared *ad libitum vs.* restricted feeding which started from 80% of *ad libitum* and progressively reached voluntary intake level after 3 weeks. Both feeding levels were associated to feeding plans based on diets at moderate digestible energy concentration (10.7 MJ/kg) or high digestible energy concentration (11.1 MJ/kg).

In the condition of our trials, when the nutritive value of diets was tested during the restriction period, apparent digestibility of diets was higher in rabbits under restriction rather than in rabbits fed *ad libitum* (like in experiment 3). However, no residual effect of the restriction treatment during post-weaning was recorded when digestibility of diets was measured in the re-alimentation period (both in experiment 3 and 4). In their wide review on the effect of feed restriction in growing rabbits, also Gidenne *et al*. (2011) outlined a clear improvement of diet apparent digestibility during the restriction period, while during the following period, when rabbits are free of feeding, the residual effect of restriction are unclear and contradictory among studies. Theoretically, a better digestive efficiency would have been expected, since previously-restricted rabbits often grow at a higher rate compared to rabbits always fed *ad libitum*, but ingest a comparable amount of feed.

In both the two experiments of the thesis on feed restriction, the possibility of controlling the diffusion and severity of digestive diseases by modulating feed intake was considered a secondary objective and therefore antibiotics were included in the diets and/or water to adequately maintain under control digestive disease. This choice was oriented to avoid that a high incidence of ill and dead animals could dramatically alter growth performance, and energy and protein body balance. In the condition of the experiment 3, however, ERE occurred (average mortality at 9.8%; average morbidity at 41.9%) and a certain effect of the experimental treatments on health status was recorded. In details, health problems were lower during the first period of feed restriction, but a worsening of health was observed during the re-alimentation period, and this negative trend was worse as stronger the restriction level and as longer the restriction period were. The sudden raising of ingestion level accounted for the higher susceptibility to digestive diseases and mortality of those animals previously submitted to the most severe restriction (80%) and for the longest period (3 weeks). In the experiment 4, health conditions were more favourable, with mortality averaging 3.6%, even if some animals recovered from illness were discarded at the end of trial because they did not reach the minimum commercial weight (for a total of 6.5% of dead and discarded rabbits). In these conditions, mortality did not differ among groups whereas the percentage of ill rabbits was higher in rabbits fed *ad libitum* than in those restricted.

Since 10 year, French researchers have been testing the efficacy of feed restriction as a tool to control digestive disorders, and ERE in particular, in growing rabbits. Following the "promising" results of some studies, almost 95% of French rabbit farmers are now adopting feeding restriction programs (Gidenne *et al.*, 2011). Already in 2003, Gidenne *et al.* reported a reduction of post-weaning mortality and morbidity when restriction level reached 70% (or better 60%) of voluntary feed intake. At these restriction levels, the effect of feed restriction was evident and significant not only in the restriction period, but even in the whole experimental period, even if health condition in the second period tended to be worse in the rabbits previously submitted to restriction. The same authors, however, did not find significant differences in mortality and morbidity of animals in the whole trial, when restriction during post-weaning was less severe, ranging within 80% to 90% of voluntary intake.

Other French authors described a positive effect of feed restriction on the control digestive diseases in young rabbits (Boisot *et al.*, 2003, 2005; Foubert *et al.*, 2008). On the other hand, neither under an experimental infection of enteropathogenic *E. coli* (Martignon *et al.* 2010) nor in presence of a spontaneous colibacillosis (Martignon *et al.*, 2009), feed restriction had a positive effect of health of growing rabbits. Similarly, other studies did not find the above-mentioned positive effect (Bovera *et al.*, 2008; Gualterio *et al.*, 2008; Matics *et al.*, 2008). Differences in feed restriction levels and in farm hygiene conditions as well as in the duration of the rearing period may partly explain differences in results among studies.

When considering the effect of feed restriction on growth performance, a reduction of growth rate during the restriction period is an obvious consequence of feed restriction and the decrease in daily weight gain is estimated at -0.5 g/d per each percentage point of restriction in comparison to the *ad libitum* group (Gidenne *et al.*, 2003; Tudela, 2008). However, during the subsequent re-alimentation period and thanks to the compensatory growth, restricted rabbits are capable of growing more than rabbits always fed *ad libitum.* In fact, final weight and daily weight gain from weaning to slaughter are less affected (-0.13 g/d per each point of restriction) (Gidenne *et al.*, 2003; Tudela, 2008). For this reason, at the end of fattening, differences in live weight may be still evident, but at a lower rate depending on the restriction rate and the length of the restriction period as well as the length of the re-alimentation period.

Moreover, according to the review of Gidenne *et al.* (2011), during restriction, the rate of reduction in growth is lower than the applied rate of feed restriction, that is when reducing daily feed intake by 20% a reduction of about 15% is usually observed in daily growth rate. On the other hand, some authors (Romero *et al*., 2010) rather observed that the decrease in the growth rate was almost the double of the reduction in feed intake and, therefore, restricted animals still showed a significantly lower live weight at the end of the fattening trial compared to rabbits fed *ad libitum*.

In the conditions of the present thesis and during both the two experiments of feed restriction, the scarce effect of the level and period of feed restriction on performance of rabbits during the whole trial may be attributed to the moderate restriction level tested (80% and 90%) and the longer period of re-alimentation (3 to 4 weeks) compared to the French studies. In fact, by using more severe restriction rates (70% or 50% of voluntary intake) during the first period of growth (3 weeks, from weaning at 35 days until 56 days of age) followed by shorter re-alimentation periods (2 weeks), until slaughter at lower age (70 days) and live weight (about 2.4 kg) than in the present trial, Perrier (1998) found a significant reduction of live weight and slaughter results. Also Gidenne *et al*. (2003), in restricted rabbits (90%, 80%, 70% and 60% compared to *ad libitum* feeding) from weaning (34-38 d) to 54 days of age found a significant reduction of final live weight, growth rate and feed intake in all restricted rabbits. During the second period of re-alimentation and until commercial slaughter (68-72 days of age), rabbits restricted at 90% during the first period recovered weight and performance, as found also in our trial, whereas live weight and growth rate were significantly lower in all other groups compared to the control one.

The reduction of feed conversion index, which Tudela (2008) recognizes as the most interesting result of feed restriction, was hardly appreciable in our studies (especially in the experiment 3). In fact, restriction rates by 90% and 80% are expected to reduce conversion index by 0.08 and 0.15, respectively (Gidenne *et al*., 2003; Tudela, 2008). Gidenne *et al*. (2011) state that the overall feed conversion is improved by 10% to 20% and the margin of the feed cost improves by 2% to 10% when a feed restriction strategy is applied during post-weaning. However, this latter result may be irrelevant from an economic point of view when rabbits are slaughtered at heavy weights, when the advantage is scarce due the longer period of growth. In the present thesis, feed conversion during the whole index was not affected at all in the experiment 3, while some advantages were evidenced in the experiment 4. Also other authors

(Romero *et al.*, 2010) did not observe the claimed improvement of feed conversion when applying feed restriction and stated that this technique is not a useful strategy for improving farm efficiency in the condition of Spanish farms.

On the other hand, feed restriction favourably affected nitrogen excretion, by reducing it in both experiments. The effect was clearly and significant evident during the first period of growth (during which rabbits were restricted) and was significantly affected by the feed restriction level and by the feeding plan (rather than by the length of the restriction period itself). As a consequence, when the whole period from weaning to slaughter was considered, differences in nitrogen excretion were still significantly in favour of restricted rabbits.

As what concerns slaughter results and carcass and meat quality, the restriction treatment had a scarce effect on these traits during both experiments. In fact, it is widely known that when rabbits are fed *ad libitum* balanced diets, the feeding treatment cannot modify slaughter results if the live weight is not affected at an appreciable rate (Xiccato, 1999; Hernández, 2008). On the other hand, the feed restriction should bring about some differences in the growth of some organs and tissues compared to rabbits fed *ad libitum*, which however did not appear at the end of fattening in the conditions of out trials. The moderate restriction used and the somewhat long period of re-alimentation could have accounted for these results. On the other hand, several studies failed in finding a relationship between meat quality traits and feed restriction during post-weaning, as reviewed by Gidenne et al. (2011).

Conclusions

In the conditions of both experiments 3 and 4, feed restriction did not produce the expected results either on improved farm feed efficiency, or in terms of health or performance of growing rabbits. In the experiment 3, during post-weaning feed restriction permitted to reduce the number of ill and dead rabbits, but during re-alimentation the health condition worsened in previously restricted rabbits, and this result was more evident as more severe the restriction was and, especially, as longer the period of restriction was. In fact, during the first days of re-alimentation a great raising and variability in feed intake was recorded, which contributed to challenge digestive equilibrium of rabbits.

In the experiment 4, restricting rabbits on the base of a theoretical feed intake curve was rather easy to be applied and the progressive turn to a voluntary intake during the third week of trial prevented the occurrence of great variations in feed intake levels and possible negative consequences on health.

In both trials, thanks to the compensatory growth during the re-alimentation period, the negative effect of feed restriction on performance during post-weaning was diluted in animals reaching the end of the rearing period and differences in final live weight and body composition among experimental groups were not significant. This result is to be considered typical of the Italian production system, which market requires that rabbits are slaughtered at age and weights higher than the other European markets.

Main conclusions

The general objective of the present thesis was the development of nutritional strategies for improving feed efficiency and health status of growing rabbits. This goal was pursued by means of four experiments with the specific objectives of *i*) defining requirements of soluble fibre in relation to dietary insoluble fibre and starch in rabbits during post-weaning and fattening (experiments 1 and 2); *ii*) measuring the effect of the rate and duration of feed restriction compared to feeding voluntary feed intake, in order to define the most effective restriction technique capable of maximizing growth performance and control digestive problems in growing and fattening rabbits (experiments 3 and 4).

Basing on the results obtained in the different trials, the following main conclusions can be drawn:

- it is possible to formulate diets for post-weaning and fattening rabbits capable of greatly improving feed conversion compared to actual standards, maintaining growth and slaughter results;
- the contemporary increase of starch (until 20%) and soluble fibre (until 12%) has positive effects on digestive physiology, weight gain and feed efficiency of rabbits without impairing slaughter results and carcass or meat quality;
- the increase of both starch and soluble fibre in replacement to insoluble fibre seems to improve the health status of rabbits, but further studies need to confirm this positive effect;
- in presence of epizootic rabbit enteropathy, the contemporary decrease of ADF below 13% may negatively affect digestive health of growing rabbits;
- dietary crude protein may be reduced from 17% until 14%, if balanced supplementation in essential amino acids is provided, without consequences on growth and slaughter performance, but with a reduction of farm nitrogen excretion;
- high-protein diets associated to low (starch+soluble fibre)/ADF ratio tend to increase susceptibility of rabbits to digestive diseases;
- in the conditions of our trials (environment, genetic, restriction conditions, use of antimicrobials, previous sanitary period, high market carcass weight), feed restriction until 80% of the voluntary feed intake does not produce the expected results on improved farm feed efficiency, in terms of either health or performance of growing rabbits when the whole productive cycle is considered;

- feed restriction usually improves health during the restriction period itself, but health condition worsens in previously restricted rabbits during the re-alimentation phase to an extent which is more pronounced as more severe the restriction was and, especially, as longer the period of restriction was;
- when adopting feed restriction technique, a suitable management strategy has to be applied with two main aims, i) avoiding sudden changes in feed intake level of growing rabbits at the beginning of the re-alimentation period; ii) reducing labour at the farm. To these aims, a theoretical progressive feed intake curve is easy to be applied for restricted rabbits which permits a controlled turn to a voluntary intake during the re-alimentation period. In this way great variations in feed intake levels and possible negative consequences on health are prevented.

References

Álvarez, J.L., Margüenda, I., García–Rebollar, P., Carabaño, R., De Blas, C., Corujo, A., García–Ruiz, A.I. *(2007). Effects of type and level of fibre on digestive physiology and performance in reproducing and growing rabbits*. World Rabbit Science 15, 9-17.

AOAC *(1980). Official Method of Analysis of the Association of Official Analytical Chemist, 11th Edition. Assoc*. Off. Analyst. Chemist, Washington, DC.

AOAC *(2000). Official Method of Analysis of the Association of Official Analytical Chemist, 17th Edition. Assoc*. Off. Analyst. Chemist, Arlington, VA.

Bennegadi, N., Gidenne, T., Licois, D. *(2000). Non specific enteritis in the growing rabbit: detailed description and incidence according to fibre deficiency and sanitary status*. In: Proceedings 7th World Rabbit Congress, Valencia, Spain, Vol. C, 109-117.

Blas, E., Gidenne, T. *(1998). Digestion of starch and sugar.* In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 1st Edition. CABI Publishing, Wallingford Oxon, UK. pp. 17-38.

Blas, E., Gidenne, T. *(2010). Digestion of starch and sugars*. In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 2nd Edition. CABI Publishing, Wallingford Oxon, UK. pp. 19-38.

Blasco, A., Ouhayoun, J., Masoero, G. *(1993). Harmonization of criteria and terminology in rabbit meat research*. World Rabbit Science 1, 3–10.

Boisot, P., Licois, D., Gidenne, T., *(2003). Une restriction alimentaire réduit l'impact sanitaire d'une reproduction expérimentale de l'entéropathie épizootique (EEL) chez le lapin en croissance*. In: Proceedings 10èmes Journées Recherche Cunicole, Paris, France. pp. 267–270.

Boisot, P., Duperray, J., Guyonvarch, A., *(2005). Intérêt d'une restriction hydrique en comparaison au rationnement alimentaire en bonnes conditions sanitaires et lors d'une reproduction expérimentaler de l'Entéropathie Epizootique du Lapin (EEL).* In: Proceedings 11èmes Journées de la Recherche Cunicole, Paris, France. pp. 133–136.

Bovera F., Di Meo C., Marono S., Vella N., Nizza A., *(2008). Feed restriction during summer: effect on rabbit growth performance*. In: Proceedings 9th World Rabbit Congress, Verona, Italy. pp. 567–572. Available at: http://world-rabbit-science.com/WRSA-Proceedings/Congress-2008-Verona/Papers/N-Bovera.pdf. Accessed December 1, 2011.

Carabaño, R., Piquer, J., Menoyo D., Badiola, I. *(2010). The digestive system of the rabbit*. In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 2nd Edition. CABI Publishing, Wallingford Oxon, UK. pp. 1–18.

Carabaño, R., Motta–Ferreira, W., De Blas, C., Fraga, M.J. *(1997). Substitution of sugarbeet pulp for alfalfa hay in diets for growing rabbits*. Animal Feed Science and Technology 65, 249–256.

Carabaño, R., García, J., De Blas, C. *(2001). Effect of fibre source on ileal apparent digestibility of non–starch polysaccharides in rabbits*. Animal Science 72, 343–350.

Carabaño, R., García, J., Nicodemus, N., De Blas, C. *(2005). Nutrizione e funzione barriera intestinale nel coniglio da carne*. In: Atti delle Giornate di Coniglicoltura ASIC 2005, Forlì, Italy. pp. 1–16.

CARABAÑO, R., BADIOLA, I., LICOIS, D., GIDENNE, T. *(2006). The digestive ecosystem and its control through nutritional or feeding strategies*. In: Coudert P., Maertens L. (Eds.) Recent Advances in Rabbit Sciences. ILVO, Melle, Belgium. pp. 211–228.

CARABAÑO, R., BADIOLA, I., CHAMORRO, S., GARCÍA, J., GARCÍA–RUIZ, A.I., GARCÍA–REBOLLAR, P., GÓMEZ–CONDE, M.S., GUTIÉRREZ, I., NICODEMUS, N., VILLAMIDE, M.J., DE BLAS, C. *(2008). Review. New trends in rabbit feeding: influence of nutrition on intestinal health.* Spanish Journal of Agricultural Research 6, 15-25.

CARABAÑO, R., VILLAMIDE, M.J., GARCÍA, J., NICODEMUS, N., LLORENTE, A., CHAMORRO, S., MENOYO, D., GARCÍA–REBOLLAR, P., GARCÍA–RUIZ, A.I., DE BLAS, C. *(2009). New concepts and objectives for protein–amino acid nutrition in rabbits. Review.* World Rabbit Science 17, 1–14.

CARRARO, L., TROCINO, A., FRAGKIADAKIS, M., XICCATO, G., RADAELLI, G. *(2007). Digestible fibre to ADF ratio and starch level in diets for growing rabbits.* Italian Journal of Animal Science 6 (Suppl. 1), 752–754.

CARRÉ, B., BRILLOUET, J.M. *(1989).* Determination of water insoluble cell walls in feed: Interlaboratory study. J. AOAC Int. 72:463–467.

CARRILHO, M.C., CAMPO, M.M., OLLETA, J.L., BELTRAN, J.A., LOPEZ, M. *(2009). Effect of diet, slaughter weight and sex on instrumental and sensory meat characteristics in rabbits.* Meat Science 82, 37–43.

CASTELLÓ, J.A., GURRI, A., *(1992). Effects of the energy levels in the feed and the feeding program on performances of growing rabbits*. J. Appl. Rabbit Res. 15, 958–964.

CAVANI, C., BIANCONI, L., URRAI, G.F., *(1991). Distribuzione automatizzata e frazionata degli alimenti nel coniglio in accrescimento: 1. Influenza della modalità di distribuzione e del livello alimentare.* In: Proceedings IX Congresso ASPA, Rome, Italy. pp. 857–864.

CEROLINI, S. *(2008). Avicoltura. Nutrizione e alimentazione degli avicoli*. In: Romboli, I., Marzoni Fecia di Cossato, M., Schiavone, A., Zaniboni, L., Cerolini, S. (Eds.) Avicoltura e Coniglicoltura. Point Veterinaire Italie, Milano, Italy. pp. 179–262.

CHAMORRO, S., GÓMEZ–CONDE, M.S., PÉREZ DE ROZAS, A.M., BADIOLA, I., CARABAÑO, R., DE BLAS, C. *(2007). Effect on digestion and performance of dietary protein content and of increased substitution of lucerne hay with soya–bean protein concentrate in starter diets for young rabbits.* Animal 1, 651–659.

CHAMORRO, S., DE BLAS, C., GRANT, G., BADIOLA, I., MENOYO, D., CARABAÑO, R. *(2010). Effect of dietary supplementation with glutamine and a combination of glutamine–arginine on intestinal health in twenty–five–day–old weaned rabbits*. Journal of Animal Science 88, 170–180.

CHEEKE, P.R. *(1987). Rabbit Feeding and Nutrition*. Academic Press, Orlando, Florida. 376 p.

DAZA, A., RODRÍGUEZ, I., OVEJERO, I., LÓPEZ-BOTE, C.J. *(2003). Effect of pig performance of feed restriction during the growth period.* Spanish Journal of Agricultural Research. 1 (4), pp 3-8.

DE BLAS, C., MATEOS, G.G. *(1998). Feed formulation.* In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 1st Edition. CABI Publishing, Wallingford Oxon, UK. pp. 241–253.

DE BLAS, C., MATEOS, G.G. *(2010). Feed formulation*. In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 2nd Edition. CABI Publishing, Wallingford Oxon, UK. pp. 222–232.

DEBRAY, L., FORTUN–LAMOTHE, L., GIDENNE, T. *(2002). Influence of low dietary starch/fibre ratio around weaning on intake behaviour, performance and health status of young and rabbit does*. Animal Research 51, 63–75.

DEWRÉE, R., MEULEMANS, L., LASSENCE, C., DESMECHT, D., DUCATELLE, R., MAST, J., LICOIS, D., VINDEVOGEL, H., MARLIER, D. *(2007). Experimentally induced epizootic rabbit enteropathy: clinical, histopathological, ultrastructural, bacteriological and haematological findings.* World Rabbit Science 15, 91–102.

EC. *(1998). Commission Directive 98/64/EC of 3 September 1998 establishing Community methods of analysis for the determination of amino acids, crude oils and fats, and olaquindox in feeding stuffs and amending Directive 71/393/EEC.* Official Journal of the European Union 19.9.1998, L257/14.

EGRAN, EUROPEAN GROUP ON RABBIT NUTRITION (GIDENNE, T., PEREZ, J.M., XICCATO, G., TROCINO, A., CARABAÑO, R., VILLAMIDE, M.J., BLAS, E., CERVERA, C., FALCAO–E–CUNHA, L., MAERTENS, L.) *(2001). Technical note: attempts to harmonize chemical analyses of feeds and faeces for rabbit feed evaluation.* World Rabbit Science 9, 57–64.

ERM/AB-DLO. *(1999). Establishment of Criteria for the assessment of the nitrogen content of animal manures.* European Commission, Final Report, Luxembourg.

FALCÃO E CUNHA, L., PERES, H., FREIRE, J.P.B., CASTRO–SOLLA, L. *(2004). Effects of alfalfa, wheat bran or beet pulp, with or without sunflower oil, on caecal fermentation and on digestibility in the rabbit.* Animal Feed Science and Technology 117, 131–149.

FEUGIER, A., SMIT, M.N., FORTUN–LAMOTHE, L., GIDENNE, T. *(2006). Fibre and protein requirements of early weaned rabbits and the interaction with weaning age: effects on digestive health and growth performance.* Animal Science 82, 493-500.

FOUBERT, C., DUPERRAY, J., BOISOT, P., GUYONVARCH, A., *(2008). Effect of feed restriction with or without free access to drinking water on performance of growing rabbits in healthy or epizootic rabbit enteropathy conditions.* In: Proceedings 9th World Rabbit Congress, Verona, Italy. 667-672. Available at: http://world-rabbit-science.com/WRSA-Proceedings/Congress-2008-Verona/Papers/N-Foubert.pdf. Accessed December 5, 2011.

GARCÍA, G., GALVEZ, J.F., DE BLAS, C. *(1993). Effect of substitution of sugar beet pulp for barley in diets for finishing rabbits on growth performance and on energy and nitrogen efficiency.* Journal of Animal Science 71, 1823–1830.

GARCÍA, G., DE BLAS, C., CARABAÑO, R., GARCÍA, P. *(1995). Effect of type of lucerne hay on caecal fermentation and nitrogen contribution trough caecotrophy in rabbits.* Reproduction Nutrition Development 35, 267-275.

GARCÍA, J., CARABAÑO, R., PEREZ–ALBA, L., DE BLAS, C. *(2000). Effect of fibre source on caecal fermentation and nitrogen recycled trough caecotrophy in rabbits.* Journal of Animal Science 78, 638–646.

GARCÍA, J., GIDENNE, T., FALCAO-E-CUNHA, L., DE BLAS, C. (*2002). Identification of the main factors that influence caecal fermentation traits in growing rabbits.* Animal Research 51, 165–173.

GARCÍA, J., GÓMEZ–CONDE, M.S., PÉREZ DE ROZAS, A., BADIOLA, I., VILLAMIDE, M.J., DE BLAS, C., CARABAÑO, R. *(2009). Role of type of fibre on intestinal microbiota and performance in rabbits.* In: Atti delle Giornate di Coniglicoltura ASIC 2009, Forlì, Italy. pp. 11–23.

GARCÍA–PALOMARES, J., CARABAÑO, R., GARCÍA–REBOLLAR, P., DE BLAS, C., GARCÍA A.I. *(2006a). Effects of dietary protein reduction during weaning on the performance of does and suckling rabbits.* World Rabbit Science 14, 23–26.

GARCÍA–PALOMARES, J., CARABAÑO, R., GARCÍA–REBOLLAR, P., DE BLAS, C., CORUJO, A., GARCÍA–RUIZ, A.I. *(2006b). Effects of a dietary protein reduction and enzyme supplementation on growth performance in the fattening period.* World Rabbit Science 14, 231–236.

GARCÍA–RUIZ, A.I., DE BLAS, C., CARABAÑO, R. *(2005). Comparison of different methods for nitrogen and amino acid evaluation in rabbit diets.* Animal Science 80, 169–178.

GIDENNE, T. *(1992). Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit*. British Journal of Nutrition 61, 133–146.

GIDENNE, T. *(1996). Nutritional and ontogenic factors affecting caecocolic digestive physiology.* In: Proceedings 6th World Rabbit Congress, Toulouse, France. Vol.1, pp. 13–28.

GIDENNE, T. *(2003). Fibres in rabbit feeding for digestive trouble prevention: respective role of low-digested and digestible fibre.* Livestock Production Science 81, 105–117.

GIDENNE, T., BELLIER, R. *(2000). Use of digestible fibre in replacement of available carbohydrates – Effect on digestion, rate of passage and caecal fermentation pattern during the growth of the rabbit.* Livestock Production Science 63, 141–152.

GIDENNE, T., COMBES S., FEUGIER A., JEHL N., ARVEUX P., BOISOT P., BRIENS C., CORRENT E., FORTUNE H:, MONTESSURY S., VERDELHAN S. *(2009). Feed restriction strategy in the growing rabbit. 2. Impact on digestive health, growth and carcass characteristics.* Animal 3:4, 509-515.

GIDENNE, T., CARABAÑO, R., GARCÌA, J., DE BLAS, C. *(2010). Fibre Digestion*. In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 2nd Edition. CABI Publishing, Wallingford Oxon, UK. pp.66–82.

GIDENNE, T., FORTUN-LAMOTHE, L., COMBES, S. *(2011). Feed restriction strategies, implications on physiology, growth and health of the growing rabbit.* In: Proceedings Giornate di Coniglicoltura ASIC 2011, Forlì, Italy. pp. 1–19. Available at: http://www.asic-wrsa.it/documenti/giornate2011/lavori/L01_Gidenne.pdf. Accessed December 5, 2011.

GIDENNE, T., GARCÍA, J. *(2006). Nutritional strategies improving the digestive health of the weaned rabbit.* In: Maertens, L. and Coudert, P. (eds.) Recent Advances in Rabbit Science. ILVO, Melle, Belgium. pp. 229-238.

GIDENNE, T., FEUGIER, A.*, (2009). Feed restriction strategy in the growing rabbit. 1. Impact on digestion, rate of passage and microbial activity.* Animal 3:4, 501-508.

GIDENNE, T., FEUGIER, A., JEHL, N., ARVEUX, P., BOISOT, P., BRIENS, C., CORRENT, E., FORTUNE, H., MONTESSUY, S,., VERDELHAN, S. *(2003). Un rationnement alimentaire quantitatif post-sevrage permet de réduire la fréquence des diarrhées, sans dégradation importante des performances de croissance: résultats d'une étude multi-site*. In: Proceedings 10èmes Journées de la Recherche Cunicole, Paris, France. pp. 29-32.

GIDENNE, T., LEBAS, F. *(2005). Le comportement alimentaire du lapin.* In: Proceedings 11èmes Journées de la Recherche Cunicole, Paris, France. pp. 29–32.

GIDENNE, T., LICOIS, *(2005). Effect of high fibre intake on the resistance of the growing rabbit to an experimental inoculation with an enteropathogenic strain of Escherichia coli.* Animal Science 80, 281–288.

GIDENNE, T., PEREZ, J.M. *(2000). Replacement of digestible fibre by starch in the diet of growing rabbits. I. Effects on digestion, rate of passage and retention of nutrients.* Annales des Zootechnie 49, 357–368.

GIDENNE, T., JEHL, N. *(1996). Replacement of starch by digestible fibre in the feed for the growing rabbit. 1. Consequences for digestibility and rate of passage.* Animal Feed Science and Technology 61,183–192.

GIDENNE, T., JEHL, N., LAPANOUSE, A., SEGURA, M. *(2004a). Inter–relationship of microbial activity, digestion and gut health in the rabbit: effect of substituting fibre by starch in diets having a high proportion of rapidly fermentable polysaccharides*. British Journal of Nutrition 92, 95–104.

GIDENNE, T., MIRABITO, L., JEHL, N., PEREZ, J.M., ARVEUX, P., BOURDILLON, A., BRIENS, C., DUPERRAY, J., CORRENT, E. *(2004b). Impact of replacing starch by digestible fibre, at two levels of lignocellulose, on digestion, growth and digestive health of the rabbit.* Animal Science 78, 389–398.

GÓMEZ–CONDE, M.S., GARCÍA, J., CHAMORRO, S., EIRAS, P., GARCÍA–REBOLLAR, P.G., PEREZ DE ROZAS, A., BADIOLA, I., DE BLAS, C., CARABAÑO, R. *(2007). Neutral detergent–soluble fiber improves gut barrier function in 25 old weaned rabbits.* Journal of Animal Science 85, 3313–3321.

GUALTERIO, L., GONZÁLEZ–REDONDO, P., NEGRETTI, P., FINZI, A., *(2008). Rationing of drinking water supply in relationship with growth and sanitary performance of growing rabbits.* In: Proceedings 9th World Rabbit Congress, Verona, Italy. pp. 959–964. Available at: http://world-rabbit-science.com/WRSA-Proceedings/Congress-2008-Verona/Papers/P-Gualterio.pdf. Accessed December 1, 2011.

GUTIÉRREZ, I., GARCÍA P., CARABAÑO R., DE BLAS C. *(2000). Effect of supplementation with animal plasma and antibiotics on jejunal morphology of early–weaned rabbits*. World Rabbit Science 8 (Suppl. 1), 263–267.

GUTIÉRREZ, I., ESPINOSA, A., GARCÍA J., CARABAÑO, R., DE BLAS, C. *(2002). Effect of levels of starch, fiber, and lactose on digestion and growth performance of early weaned rabbits.* Journal of Animal Science 80, 1029–1037.

GUTIÉRREZ, I., ESPINOSA, A., GARCÍA, J., CARABAÑO, R., DE BLAS, C. *(2003). Effect of protein source on digestion and growth performance of early–weaned rabbits.* Animal Research 52, 461–471.

HALL, M.B. *(2003). Challenges with nonfiber carbohydrate methods*. Journal of Animal Science 81, 3226–3232.

HALL, M.B., LEWIS, B.A., VAN SOEST, P.J. *(1997). A simple method for estimation of neutral detergent–soluble fibre*. Journal of the Science of Food and Agriculture 74, 441–449.

HAMPSON, D.J. (1986). *Alteration in piglet small intestinal structure at weaning.* Res. Vet. Sci. 40, 32–40.

HAMPSON, D.J., PLUSKE, J.R., PETHIC, D.W. *(2001). Dietary manipulation of enteric disease.* In: Lindberg, J.E., Ogle, B. (Eds.) Digestive Physiology of Pigs. CABI Publishing, Wallingford Oxon, UK. pp. 247–260.

HERNÁNDEZ, P. *(2008). Enhancement of nutritional quality and safety in rabbit meat.* In: Proceedings 9th World Rabbit Congress, Verona. Italy. pp. 1287–1300. Available at: http://world-rabbit-science.com/WRSA-Proceedings/Congress-2008-Verona/Papers/Q0-Hernandez.pdf. Accessed December 5, 2011.

HERNÁNDEZ, P., GONDRET, F. *(2006). Rabbit meat quality.* In: Maertens, L., Coudert, P. (Eds.) Recent Advances in Rabbit Sciences. ILVO, Merelbeke, Belgium. pp. 269–290.

JEHL, N., GIDENNE, T. *(1996). Replacement of starch by digestible fibre in feed for the growing rabbit. 2. Consequences for microbial activity in the caecum and on incidence of digestive disorders*. Animal Feed Science and Technology 61,193–204.

JEROME, N., MOUSSET, J.L., MESSAGER, B., DEGLAIRE, I., MARIE, P., *(1998). Influence de différentes méthodes de rationnement sur les performances de croissance et d'abattage du lapin.* In: Proceedings 7èmes Journées de la Recherche Cunicole, Lyon, France, 175–178.

LAMBERTINI, L., BENASSI, M.C., ZAGHINI, G. *(1990). Effetto di sesso e peso sulle caratteristiche qualitative della carcassa di coniglio*. Rivista di Coniglicoltura 27(4), 33–39.

LAVRENČIČ, A. *(2007). The effect of rabbit age on in vitro caecal fermentation of starch, pectin, xylan, cellulose, compound feed and its fibre*. Animal 1, 241–248.

LAZZARONI, C., BIAGINI, D., LUSSIANA, C. *(2009). Different rearing systems for fattening rabbits: Performance and carcass characteristics.* Meat Science 82, 200–204.

LEBAS, F. *(1989). Besoins nutritionnels des lapins. Revue bibliographique et perspectives.* Cuni–Sciences 5(2), 1-28.

LEBAS, F., *(2007). Productivité des élevages cunicoles professionnels en 2006. Résultats de RENELAP et RENACEB*. Cuniculture Magazine 34, 31–39.

LEBAS, F., GIDENNE, T., PEREZ, J.M., LICOIS, D. *(1998). Nutrition and pathology*. In: De Blas, C., Wiseman, J. (Eds.) The Nutrition of the Rabbit CABI Publishing. CAB International, Wallingford Oxon, UK. pp. 197–213.

LICOIS, D. *(2004). Domestic rabbit enteropathies*. In: Proceedings 8th World Rabbit Congress, Puebla, Mexico. Becerril, C., Pro, A. (Eds.), Colegio de Postgraduados for WRSA publ. pp 385–403.

LICOIS, D., DEWREE, R., COUDERT, P., VINDEVOGEL, H., MARLIER, D. *(2003). Experimental reproduction of Epizootic Rabbit Enteropathy (ERE) trial with inoculums originating from Belgium and Holland and with bacterial strains isolated from these inoculums and also from INRA TEC2 and TEC3 inoculums*. In: Proceedings 10èmes Journées de la Recherche Cunicole, Paris, France. pp. 255–258.

LICOIS, D., WYERS, M., COUDERT, P. *(2005). Epizootic Rabbit Enteropathy: experimental transmission and clinical characterization*. Veterinary Research 36, 601–613.

LLORENTE, A., GARCÍA, A.I., NICODEMUS, N., VILLAMIDE, M.J., CARABAÑO, R. (2006). *Digestibilidad ileal parente y verdadera de aminoácidos de harinas de girasol, productos de soja y guisante en conejos*. In: Proceedings XXXI Symposium Cunicultura, Lorca, Spain. pp. 117–124.

MAERTENS, L. *(1992). Rabbit nutrition and feeding: a review of some recent developments*. Journal of Applied Rabbit Research 15, 889–913.

MAERTENS, L., *(2009). Possibilities to reduce the feed conversion in rabbit production.* In: Proceedings Giornate di Coniglicoltura ASIC 2009, Forlì, Italy, pp. 1–10. Available at: http://www.asic–wrsa.it/documenti/giornate2009/01_Maertens.pdf. Accessed December 1, 2011.

MAERTENS, L. *(2010). Feeding systems for intensive production.* In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 2nd Edition. CABI Publishing, Wallingford Oxon, UK. pp. 253–266.

MAERTENS, L., DE GROOTE, G. *(1988). The influence of the dietary energy content on the performances of post–partum breeding does.* In: Proceedings 4th World Rabbit Congress, Budapest, Hungary. Sandor Holdas, Hercegalom, Hungary. Vol. 3, pp. 42–52.

MAERTENS, L., LUZI, F., DE GROOTE, G. *(1997). Effect of dietary protein and amino acids on the performance, carcass composition and N–excretion of growing rabbits.* Annales des Zootechnie 46, 255–268.

MARLIER, D., DEWREE, R., LICOIS, D., COUDERT, P., LASSENCE, C., POULIPOULIS, A., VINDEVOGEL, H. *(2003). Rabbit Epizootic Enteropathy: a preliminary assessment of results after a 20 months research period.* In: Proceedings 10èmes Journées de la Recherche Cunicole, Paris, France. pp. 247–250.

MARLIER, D., DEWREE, R., LASSENCE, C., LICOIS, D., MAINIL, J., COUDERT, P., MEULEMANS, L., DUCATELLE, R., VINDEVOGEL, H. *(2006). Infectious agents associated with epizootic rabbit enteropathy: isolation and attempts to reproduce the syndrome.* Veterinary Journal 172, 493–500.

MAROUNEK, M., VOVK, S.J., SKRIVANOVA, V. *(1995). Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits*. British Journal of Nutrition 73, 463–469.

MARTÍNEZ-VALLESPÍN, B., MARTÍNEZ-PAREDES, E., RÓDENAS, L., CERVERA, C., PASCUAL, J.J., BLAS, E. *(2011). Combined feeding of rabbit female and young: Partial replacement of starch with acid detergent fibre or/and neutral detergent soluble fibre at two protein levels.* Livestock Science 141, 155-165.

MATICS, ZS., DALLE ZOTTE, A., RADNAI, I., KOVÁCS, M., METZGER, ZS., SZENDRO, ZS. *(2008). Effect of restricted feeding after weaning on the productive and carcass traits of growing rabbits.* In: Proceedings 9th World Rabbit Congress, Verona, Italy. pp. 741–746. Available at: http://world-rabbit-science.com/WRSA-Proceedings/Congress-2008-Verona/Papers/N-Matics.pdf. Accessed December 1, 2011.

MARTIGNON, M.H., COMBES, S., GIDENNE, T. *(2009). Effect of the feed distribution mode in a strategy of feed restriction: effect on the feed intake pattern, growth and digestive health in the rabbit*. In: Proc. 13èmes J. Rech. Cunicoles, Le Mans, France. pp. 39-42.

MARTIGNON, M.H., COMBES, S., GIDENNE, T. *(2010). Digestive physiology and hindgut bacterial community of the young rabbit (Oryctolagus cuniculus): Effects of age and short-term intake limitation*. Comparative. Biochemistry Physiology- Part A 156, 156–162.

MERTENS, D.R. *(2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds using refluxing in beakers or crucibles: collaborative study.* Journal of AOAC 85, 1217– 1240.

MERTENS, D.R. *(2003). Challenges in measuring insoluble dietary fiber*. Journal of Animal Science 81, 3233–3249.

METZGER, SZ., SZENDRŐ, SZ., BIANCHI, M., HULLÁR, I., FÉBEL, H., ., MAERTENS, L., CAVANI, C., PETRACCI, M., RADNAI, I., BIRÓ-NÉMETH, E. *(2009). Effect of energy restriction in interaction with genotype on the performance of growing rabbits: II. Carcass traits and meat quality.* Livestock Science 126, 221-228.

MORISSE, J.P., BOILLETOT, E., MAURICE, R. *(1985). Alimentation et modification du milieu intestinal chez le lapin (AGV, NH3, pH, flora).* Rec. Med. Vet., 161(5), 443-449.

NICODEMUS, N., PÉREZ–ALBA, L., CARABAÑO, R., DE BLAS, C., BADIOLA, I., PÉREZ DE ROZAS, A., GARCÍA, J. *(2004). Effect of fibre and level of ground of fibre sources on digestion and ileal and caecal characterization of microbiota of early weaned rabbits.* In: Proceedings 8th World Rabbit Congress, Puebla, Mexico. Becerril, C., Pro, A. (Eds.), Colegio de Postgraduados for WRSA publ. pp 928–929.

NICODEMUS, N., REDONDO, R., CARABAÑO, R., DE BLAS, C., GARCÍA, J. *(2003). Effect of level of fibre and type of ground of fibre sources on digestion and performance of growing rabbits.* In: Proceedings of the 3rd Meeting of nutrition and pathology and meat quality working groups of COST ACTION 848, Prague, Czech Republic. p. 22.

OSL, F. *(1988). Bestimmung der niederen freien Fettsauren im Hart- und Schnittkäse mit der Head-Space Gaschromatographie*. Deutsche Molkerei Zeitung 109, 1516–1518.

OUHAYOUN, J., *(1989). La composition corporelle du lapin. Facteurs de variations.* INRA Prod. Anim. 2, 215–226.

OUHAYOUN, J., POUJARDIEU, B., DELMAS, D., *(1986). Influence des conditions d'élevage et du rationnement sur la vitesse de croissance du lapin entre 11 et 20 semaines. 2 – Composition corporelle.* In: Proceedings 4èmes Journées de la Recherche Cunicole, Paris, France, Communication No. 24.

PARIGI BINI, R., XICCATO, G., *(1986). Utilizzazione dell'energia e della proteina digeribile nel coniglio in accrescimento*. Rivista di Coniglicoltura 23(4), 54–56.

PARIGI BINI, R., XICCATO, G., *(1998). Energy metabolism and requirements.* In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 1st Edition. CABI Publishing, Wallingford Oxon, UK. pp.103–131.

PARIGI BINI, R., XICCATO, G., CINETTO, M. *(1988). Integrazione con lisina e metionina di sintesi di un mangime per conigli in accrescimento.* (Lavoro sottoposto al controllo scientifico di Zoot. Nutr. Anim.). "Coniglicoltura", 25 (8), 33-38.

PARIGI BINI, R., XICCATO, G., CINETTO, M. *(1990). Utilisation des protéines et de l'énergie alimentaire par le lapin traité au clenbutérol.* In: Proceedings 5èmes Journées de la Recherche Cunicole en France. Vol. 2, Communication 43, 1–6.

PARIGI BINI, R., XICCATO, G., CINETTO, M., DALLE ZOTTE, A. *(1992). Effetto dell'età, del peso di macellazione e del sesso sulla qualità della carcassa e della carne cunicola. 1. Rilievi di macellazione e qualità della carcassa.* Zootecnica e Nutrizione Animale 18, 157–172.

PEETERS, J.E. *(1992) La patología digestiva en la explotación cunícola industrial. Reproductores, lactantes, engorde y reposición.* V Jorn. T&. Cunicultura. Barcelona, España: 7-22.

PEREZ DE ROZAS, A.M., CARABAÑO, R., GARCÍA, J., ROSSELL, J., DIAZ, J.V., BARBÉ, J., PASCUAL, J.J., BADIOLA, I. *(2005). Etiopatogenia de la Enteropatia Epizootica del Conejo*. In: Actas XXX Symposium de Cunicultura, Valladolid, Spain. pp. 167–174.

PEREZ, J.M., LEBAS, F., GIDENNE, T., MAERTENS, L., XICCATO, G., PARIGI BINI, R., DALLE ZOTTE, A., COSSU, M.E., CARAZZOLO, A., VILLAMIDE, M.J., CARABAÑO, R., FRAGA, M.J., RAMOS, M.A., CERVERA, C., BLAS, E., FERNÁNDEZ, J., FALCAO–E–CUNHA, L., BENGALA FREIRE, J. *(1995). European reference method for vivo determination of diet digestibility in rabbits.* World Rabbit Science 3, 41–43.

PEREZ, J.M., GIDENNE, T., BOUVAREL, I., ARVEAUX, P., BOURDILLON, A., BRIENS, C., LA NAOUR, J., MESSAGER, B., MIRABITO, L. *(2000). Replacement of digestible fibre by starch in the diet of growing rabbits. II. effects on performances and mortality by diarrhoea.* Annales de Zootechnie 49, 369–377.

PERRIER, G., *(1998). Influence de deux niveaux et deux durées de restriction alimentaire sur l'efficacité productive du lapin et les caractéristiques bouchères de la carcasse.* In: Proceedings 7èmes Journées de la Recherche Cunicole, Lyon, France. ITAVI, Paris. pp. 179–182.

PERRIER, G., OUHAYOUN, J., *(1996). Growth and carcass traits of the rabbit. A comparative study of three modes of feed rationing during fattening.* In: Proceedings 6th World Rabbit Congress, Toulouse, France. ITAVI, Paris, Vol. 3, pp. 225–232.

PETRACCI, M., CAPOZZI, F., CAVANI, C., CREMONINI, M.A., MINELLI, G. *(1999). Influence of slaughter weight and sex on meat quality of rabbits slaughtered at the same age.* In: Piva, G., Bertoni, G., Masoero, F., Bani, P., Calamari, L. (Eds.) Recent Progress in Animal Production Science. 1. Proceedings ASPA XIII Congress, Piacenza Italy. Franco Angeli, Milano, Italy. pp. 650–652.

REBOLLAR, P.G., PÉREZ-CABAL, M.A., PEREDA, N., LORENZO, P.L., ARIAS-ÁLVAREZ, M., GARCÍA-REBOLLAR, P. *(2009). Effects of parity order and reproductive management on the efficiency of rabbit productive systems.* Livestock Science 121, 227–233.

ROBERTSON, J.B., VAN SOEST, P.J. *(1981). The detergent system of analysis and its application to human foods*. In: James, W.P.T., Theander, O. (Eds.). The analysis of dietary fiber in food. Marcel Dekker, Inc., New York and Basel. pp. 142–143.

ROMERO, C., CUESTA, S., ASTILLERO, J.R., NICODEMUS, N., DE BLAS, C., *(2010). Effect of early feed restriction on performance and health status in growing rabbits slaughtered at 2 kg live-weight.* World Rabbit Science 18, 211-218.

ROMERO, C., NICODEMUS, N., GARCÍA–REBOLLAR, P., GARCÍA–RUIZ, A.I., IBÁÑEZ, M.A., DE BLAS C. *(2009). Dietary level of fibre and age at weaning affect the proliferation of Clostridium perfringens in the caecum, the incidence of Epizootic Rabbit Enteropathy and the performance of fattening rabbits.* Animal Feed Science and Technology 153, 131–140.

ROSELL, J.M. *(2003). Health status of commercial rabbitries in the Iberian peninsula. A practitioner's study during 2002.* World Rabbit Science 11, 157–169.

ROSELL, J.M., GONZÀLEZ, F.J., (2007). *Resultados de Gestiòn Técnica 2006*. Cunicultura 189, 285–287.

SAS, STATISTICAL ANALYSIS SYSTEM INSTITUTE INC., *(1991). User's guide, Statistics, version 6.03. Edition.* SAS Institute Inc., Cary, NC. 1028 p.

SOLER, M.D., BLAS, E., CANO, J.L., PASCUAL, J.J., CERVERA, C., FERNÁNDEZ–CARMONA, J. *(2004). Effect of digestible fibre/starch ratio and animal fat level in diets around weaning on mortality rate of rabbits.* In: Proceedings 8th World Rabbit Congress, Puebla, Mexico. Becerril, C., Pro, A. (Eds.), Colegio de Postgraduados for WRSA publ. pp 996–1001.

SZALO, I.M., LASSENCE, C., LICOIS, D., COUDERT, P., POULIPOULIS, A., VINDEVOGEL, H., MARLIER, D. *(2007). Fractionation of the reference inoculum of epizootic rabbit enteropathy in discontinuous sucrose gradient identifies aetiological agents in high density fractions.* The Veterinary Journal 173, 652–657.

SZENDRŐ, ZS., KENESSEY, A., JENSEN, J.F., JENSEN, N.E., CSAPO, J., ROMVARI, R., MILITSITS, G. *(1998). Effect of genotype, age, body weight and sex on the body composition of growing rabbits*. World Rabbit Science 6, 277–284.

SZENDRŐ, SZ., METZGER, SZ., FÉBEL, H., HULLÁR, I., MAERTENS, L., BIANCHI, M., CAVANI, C., PETRACCI, M., BIRÓ-NÉMETH, E., RADNAI, I., *(2008). Effect of energy restriction in interaction with genotype on the performance of growing rabbits I: Productive traits.* Livestock Science. 118, 123-131.

TROCINO, A., FRAGKIADAKIS, M., RADAELLI, G., XICCATO, G., *(2010). Effect of dietary soluble fibre level and protein source on growth, digestion, caecal activity and health of fattening rabbits.* World Rabbit Science 18, 199–210.

TROCINO, A., FRAGKIADAKIS, M., MAJOLINI, D.,. CARABAÑO, R, XICCATO, G. *(2011). Effect of the increase of dietary starch and soluble fibre on digestive efficiency and growth performance of meat rabbits.* Animal Feed Science and Technology 165, 265–277

TROCINO, A., XICCATO, G., QUEAQUE, P.I., SARTORI, A., *(1999). Effect of feeding plans based on dried beet pulp on performance and meat quality of growing rabbits.* In: Piva, G., Bertoni, G., Masoero, F., Bani, P., Calamari, L. (Eds.) Recent Progress in Animal Production Science. 1. In: Proceedings ASPA XIII Congress, Piacenza Italy. Franco Angeli, Milano, Italy. pp. 713–715.

TROCINO, A., XICCATO, G., SARTORI, A., QUEAQUE, P.I. *(2000). Feeding plans at different protein levels: effects on growth performance, meat quality and nitrogen excretion in rabbits*. In: Proceedings 7th World Rabbit Congress, Valencia, Spain. Vol. C, pp. 467–474.

TROCINO, A., XICCATO, G., QUEAQUE, P.I., SARTORI, A. *(2003). Effect of transports duration and gender on rabbit carcass and meat quality.* World Rabbit Science 11, 23–32.

TROWELL, H. *(1972). Ischemic heart disease and dietary fiber.* American Journal of Clinical Nutrition 25, 926–932.

TROWELL, H. *(1978). The development of the concept of dietary fiber in human nutrition*. American Journal of Clinical Nutrition 31, 3–11.

TUDELA, F. *(2008). Producción de conejos con restricciones alimentarias.* In: Proceedings XXXIII Symposium de ASESCU, Sevilla, Spain. pp. 14–21.

TŮMOVÁ, E., SKŘIVAN, M., SKŘIVANOVÁ, V., KACEROVSKÁ, L., *(2002). Effect of early feed restriction on growth in broiler chickens, turkeys and rabbits.* Czech J. Animal Science 47, pp 418–428.

UDÉN, P., ROBINSON, P.H., WISEMAN, J. *(2005). Use of detergent system terminology and criteria for submission of manuscripts on new or revised, analytical methods as well as descriptive information on feed analysis and/or variability*. Animal Feed Science and Technology 118, 181–186.

VAN DIJK, J.E., HUISMAN, J., KONINKX, J.F.J.G. *(2002). Structural and functional aspects of healthy gastrointestinal tract*. In: Blok, M.C. (Ed.) Nutrition and Health of the Gastrointestinal Tract. Wageningen Academic Publishers, Wageningen, The Netherlands. pp. 71–96.

VAN SOEST, P. J., ROBERTSON, J. B., LEWIS, B. A., *(1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition*. J. Dairy Sci. 74, 3583–3597.

VILLAMIDE, M.J., MAERTENS, L., DE BLAS, C. *(2010). Feed evaluation. In:* De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 2nd Edition. CABI Publishing, Wallingford Oxon, UK. pp. 151–162.

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

XICCATO, G., PARIGI BINI, R., DALLE ZOTTE, A., CARAZZOLO, A. *(1994). Effect of age, sex and transportation on the composition and sensory properties of rabbit meat*. In: Proceedings 40th International Congress Meat Science and Technology, The Hague. W–2.02.

XICCATO, G., TROCINO, A. *(2007). Italia: un sistema integrato di produzione cunicola.* Rivista di Coniglicoltura XLIV(5), 10–15.

XICCATO, G., TROCINO, A. *(2008). Coniglicoltura. Apparato digerente*. In: Romboli, I., Marzoni Fecia di Cossato, M., Schiavone, A., Zaniboni, L., Cerolini, S. (Eds.) Avicoltura e Coniglicoltura. Point Veterinaire Italie, Milano, Italy. pp. 404–411.

XICCATO, G., TROCINO, A. *(2010a). Energy and protein metabolism and requirements.* In: De Blas, C. and Wiseman, J. (Eds.) The Nutrition of the Rabbit, 2nd Edition. CABI Publishing, Wallingford Oxon, UK. pp. 83–118.

XICCATO, G., TROCINO, A. *(2010b). Feed and energy intake in rabbits and consequences on farm global efficiency*. In: Proceedings 6th International Conference on Rabbit Production in Hot Climates, Assiut, Egypt. pp. 1-18.

XICCATO, G., TROCINO, A., CARRARO, L., FRAGKIADAKIS, M. *(2006). Digestible fibre to ADF ratio and protein concentration in diets for early–weaned rabbits.* In: Proceedings 3rd Rabbit Congress of America, Maringá, Brasil. Comm. 35, pp 1–6.

XICCATO, G., TROCINO, A., CARRARO, L., FRAGKIADAKIS, M. *(2007). Digestible fibre to ADF ratio and protein concentration in diets for early-weaned rabbits.* World Rabbit Science, 15, 55.

XICCATO, G., TROCINO, A., CARRARO, L., FRAGKIADAKIS, M., MAJOLINI, D. *(2008). Digestible fibre to starch ratio and antibiotic treatment time in growing rabbits affected by epizootic rabbit enteropathy.* In: Proceedings 9th World Rabbit Congress, Verona, Italy. pp. 847–851. Available at: http://world-rabbit-science.com/WRSA-Proceedings/Congress-2008-Verona/Papers/N-Xiccato.pdf. Accessed on December 2, 2011.

XICCATO, G., TROCINO, MAJOLINI, D., L., FRAGKIADAKIS, M., TAZZOLI, M. *(2011). Effect of decreasing dietary protein level and replacing starch with soluble fibre on digestive physiology and performance of growing rabbits.* Animal 5:8, 1179-1187.

XICCATO, G., TROCINO, A., SARTORI, A., QUEAQUE, P.I. *(2002). Effect of dietary starch level and source on performance, caecal fermentation and meat quality in growing rabbits.* World Rabbit Science 10, 147–157.

XICCATO, G., TROCINO, A., SARTORI, A., QUEAQUE, P.I. *(2003). Effect of weaning diet and weaning age on growth, body composition and caecal fermentation of young rabbits.* Animal Science 77, 101–111.