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THE ROLE OF DIGESTIBLE FIBRE, STARCH AND PROTEIN ON HEALTH STATUS AND PERFORMANCE IN DIETS FOR GROWING RABBITS

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Introduzione e obiettivi

Negli ultimi 10 anni, la diffusione dell'enteropatia epizootica del coniglio (Epizootic rabbit enteropathy, ERE) negli allevamenti europei ha aumentato la mortalità e la morbilità e, quindi, peggiorato in maniera significativa la conversione alimentare realizzata a livello aziendale. La mancata identificazione di un agente eziologico della malattia e l'attribuzione di un carattere multifattoriale alla stessa, così come i limiti imposti dalla normativa europea sull'uso di antibiotici, hanno favorito gli studi e le ricerche in materia di alimentazione e gestione degli animali per individuare strategie in grado di prevenire, o quantomeno limitare, l'impatto dell'enteropatia. Con questo obiettivo specifico, gli studi degli ultimi anni hanno inteso definire i fabbisogni nutrizionali nei conigli durante lo svezzamento ed il post-svezzamento.

Tra i diversi principi nutritivi, un aumento della concentrazione di frazioni fibrose digeribili (fibra digeribile, FD = pectine ed emicellulose) è stato associato a un miglioramento dello stato di salute degli animali, oltre che a un incremento dell'attività fermentativa a livello ciecale e un miglioramento della conversione alimentare (Gidenne e García, 2006). Una maggiore concentrazione di fibra solubile nelle diete per lo svezzamento ha ridotto la presenza a livello ciecale di *C. perfringens* e di altri agenti patogeni opportunisti, così come la mortalità causata da diarrea (Soler e coll., 2003; Gómez-Conde e coll., 2007). Con un contenuto costante di ADF, la sostituzione di amido e proteina con fibra digeribile ha ridotto l'incidenza di disturbi digestivi e migliorato, quindi, lo stato di salute nel coniglio (Perez e coll., 2000; Gidenne e coll., 2001; Marguenda e coll., 2006). La riduzione dell'apporto di fibra insolubile (Gutiérrez e coll., 2002; Alvarez e coll., 2007) e l'aumento delle frazioni più solubili (García-Ruiz e coll., 1997; Carabaño e coll., 2008) hanno anche migliorato l'integrità, e quindi la funzionalità, della mucosa intestinale dei giovani conigli.

Le informazioni sulle interazioni tra proteina e fibra digeribile sono ancora limitate. Una carenza (<12%) o un eccesso di proteina (>18%) possono favorire i disturbi digestivi e aumentare la mortalità, indurre uno squilibrio nelle attività fermentative ciecali e, quindi, nella composizione della microflora intestinale (Maertens e De Groote, 1988; Lebas, 1989; Carabaño e coll., 2008, 2009). La riduzione del livello proteico dal 18 al 16% (in diete con la stessa digeribilità ileale della proteina) ha significativamente diminuito la presenza di *Clostridium perfringens* e la mortalità da enteropatia epizootica (Chamorro e coll., 2007), mentre un'ulteriore riduzione dal 16 al 14% non ha avuto alcun effetto sulla mortalità, pur avendo indotto la riduzione dei batteri anaerobi a livello ciecale (García-Palomares e coll., 2006a, 2006b; Carabaño e coll., 2009). Anche l'impiego di fonti proteiche meno digeribili può aumentare il flusso di azoto a livello ciecale e favorire lo sviluppo di agenti patogeni, come *E. coli* e *Clostridium spp.*, comportando così un aumento del rischio per la salute degli animali e un peggioramento delle prestazioni produttive (Gutiérrez e coll., 2003; Chamorro e coll., 2005, 2007; Carabaño e coll., 2008, 2009). Considerata l'attenzione dei mass media e dei consumatori per l'uso di alimenti non modificati geneticamente, esiste un certo interesse nel valutare fonti proteiche alternative alla soia, sicuramente non modificate a livello biotecnologico. Tra queste, la farina di semi di girasole è ampiamente utilizzata nell'alimentazione del coniglio solitamente in combinazione con la farina di soia. L'uso di diete basate sull'utilizzo esclusivo di farina di girasole come fonte proteica richiede, tuttavia, una valutazione più attenta.

Le attività sperimentali realizzate nella presente Tesi di dottorato hanno inteso, in generale, definire i fabbisogni nutrizionali del coniglio in post-svezzamento e ingrasso, con particolare riguardo a frazioni fibrose, soprattutto le più digeribili (pectine, emicellulose), contenuto di amido, e livello di proteine. Nel dettaglio, obiettivi specifici della presente tesi sono stati: 1) mantenere l'equilibrio delle fermentazioni a livello ciecale; 2) ridurre la frequenza e la gravità delle patologie digestive; 3) migliorare l'efficienza di conversione alimentare e di ritenzione azotata; e 4) garantire elevate prestazioni di crescita e ottimali qualità della carcassa e della carne. Tali obiettivi sono stati perseguiti mediante la realizzazione delle seguenti quattro attività sperimentali:

Contributo sperimentale n. 1

Livello di fibra digeribile e sostituzione di farina di soia con farina di girasole in diete per conigli in accrescimento.

A 34 giorni di età, 216 conigli ibridi (837±48 g) di entrambi i sessi sono stati divisi in sei gruppi sperimentali da 36 unità ciascuno e alimentati *ad libitum* con sei diete isoproteiche (15,9% PG) formulate in base ad un disegno bi-fattoriale, con due fonti proteiche (farina di soia *vs*. farina di girasole) e tre rapporti FD/ADF (1,0, 1,1 e 1,2). La digeribilità apparente ed il valore energetico delle diete sono stati determinati *in vivo* (Perez e coll., 1995). A 56 giorni di età, è stato effettuato un campionamento dei contenuti ciecali e della mucosa intestinale per la misura dell'attività fermentativa ciecale e la misurazione dei villi e delle cripte, rispettivamente. I campioni di feci e mangime sono stati analizzati secondo AOAC (2000) e le procedure armonizzate a livello europeo (EGRAN, 2001). Il contenuto di fibra alimentare totale (TDF) è stato determinato attraverso procedura enzimatico-gravimetrica, previo trattamento con α-amilasi, proteasi e aminoglucosidasi

(Megazyme int. Ireland Ltd., Wicklow, Irlanda). Il contenuto di fibra digeribile (FD) è stato calcolato come differenza tra TDF e l'ADF. La concentrazione degli acidi grassi volatili nel contenuto ciecale è stata misurata mediante gascromatografia (Osl, 1988). A 76 giorni, i conigli sono stati macellati e le carcasse sezionate secondo i protocolli scientifici internazionali (Blasco e coll., 1993). Sono stati misurati pH (Xiccato e coll., 1994) e colore (CIE, 1976) dei muscoli *longissimus lumborum* e *biceps femoris*. I dati sono stati analizzati con ANOVA utilizzando la procedura GLM (SAS Inst. Inc., Cary, NC), e considerando gli effetti del rapporto FD/ADF e delle fonti proteiche. L'effetto del sesso è stato incluso per analizzare la variabilità di dati di accrescimento, risultati di macellazione, qualità delle carcasse e della carne. La mortalità, la morbilità e il rischio sanitario sono stati analizzati con la procedura CATMOD del SAS.

La digeribilità delle diete è significativamente aumentata con il rapporto FD/ADF, a causa della maggiore inclusione delle polpe di bietola a scapito dell'erba medica. Il valore nutritivo delle diete è aumentato non solo per la maggiore presenza di FD, ma anche per la maggiore digeribilità (9-10 punti) di tutte le frazioni fibrose: la digeribilità dell'ADF è aumentata dal 14,6 al 25,6%; la digeribilità delle emicellulose dal 40,3 al 49,1%; la digeribilità delle pectine dall'85,0 al 93,8% ($P<0,01$). Questo incremento può essere associato con il minor grado di lignificazione e complessità delle pareti cellulari e con la maggior suscettibilità dei carboidrati strutturali alle azioni enzimatiche sia animali sia batteriche. Le fonti proteiche hanno invece avuto un effetto più limitato sulla digeribilità degli alimenti. Nelle diete contenenti solo girasole, la digeribilità della proteina grezza è risultata tendenzialmente ($P=0,06$) inferiore a quelle delle diete a base di soia. La digeribilità dell'estratto etereo e delle emicellulose è stata maggiore ($P<0,001$) nelle diete a base di girasole rispetto a quelle a base di soia, mentre ADF e pectine sono risultati meno digeribili. Il valore nutritivo delle diete è stato moderato, e migliorato dall'aumento del rapporto FD/ADF, indipendentemente dalla fonte proteica, e in linea con le attuali raccomandazioni per l'alimentazione del coniglio in post-svezzamento.

I problemi sanitari sono stati limitati e non influenzati dal trattamento alimentare. Le caratteristiche della mucosa intestinale e le attività fermentative ciecali non sono state influenzate dal trattamento, mentre il pH del contenuto ciecale è diminuito significativamente all'aumentare del rapporto FD/ADF ($P=0,04$). L'accrescimento giornaliero, il peso vivo dei conigli e, di conseguenza, i risultati di macellazione non sono variati con il trattamento alimentare, mentre, soprattutto nel primo periodo (34-55 d di età), il consumo di alimento è diminuito e la conversione alimentare migliorata linearmente all'aumentare del rapporto FD/ADF e del valore nutritivo delle diete. Né le prestazioni né i risultati di macellazione sono stati modificati dalla fonte proteica utilizzata per la formulazione delle diete.

Contributo sperimentale n. 2

Rapporto fibra digeribile/amido e livello proteico nell'alimentazione di conigli in accrescimento.

A 32 giorni di età, 246 conigli ibridi (837±48 g) di entrambi i sessi sono stati divisi in sei gruppi sperimentali da 36 unità ciascuno e alimentati *ad libitum* con sei diete formulate con un livello di ADF costante (18%), ma differenti per livello di proteina (15 *vs*. 17%) e rapporto FD/amido (0,8, 1,5 e 2,8) secondo un disegno sperimentale bi-fattoriale. La prova di digeribilità *in vivo* (su 60 animali da 52 a 56 d di età), il campionamento dei contenuti ciecali e della mucosa intestinale (su 36 conigli a 55 d), l'analisi istologica della mucosa intestinale e le analisi chimiche delle diete sperimentali, delle feci e dei contenuti cecali, nonché la macellazione, la dissezione delle carcasse e le analisi di qualità della carne sono stati effettuati secondo le metodologie riportate per il primo contributo sperimentale. I dati sono stati analizzati statisticamente come riportato per il primo contributo e considerando gli effetti del rapporto FD/amido e del livello di proteina. L'effetto del sesso è stato incluso nel modello per analizzare la variabilità di dati di accrescimento, risultati di macellazione, qualità della carcassa e della carne.

L'aumento del livello di proteina alimentare ha significativamente migliorato la digeribilità della sostanza secca (circa 1 punto) e dei diversi principi nutritivi. Il contenuto di energia digeribile è variato poco e il rapporto proteina digeribile (PD)/ED è risultato maggiore nelle diete al 16% PG rispetto a quelle al 15% PG. L'aumento del rapporto FD/amido ha migliorato la digeribilità apparente dell'amido (anche se in maniera limitata in valore assoluto) e, in maniera considerevole, la digeribilità della fibra e delle frazioni fibrose, mentre non ha modificato la digeribilità della sostanza secca e dell'energia lorda. Il valore nutritivo e il contenuto di ED delle diete non sono cambiati con il rapporto FD/amido, poiché l'energia fornita dalla FD è stata simile a quella fornita dall'amido. Questo risultato può essere ascritto soprattutto alla completa digeribilità delle pectine (100%) e, in misura minore, al generale aumento della digeribilità di tutte le frazioni fibrose, anche quelle meno digeribili (per esempio, ADF).

Durante la prova, sebbene mortalità e morbilità siano risultate basse, si è rilevato un effetto significativo del trattamento alimentare: mortalità e rischio sanitario sono risultati tendenzialmente (P=0,10-0,11) superiori nei conigli alimentati con le diete a maggiore tenore proteico. La somministrazione di diete a basso rapporto FD/amido ha aumentato la mortalità (10 vs. 1,4 e 1,4%, P=0,04), ma anche diminuito il numero dei conigli malati che sono arrivati alla fine della prova, seppure non significativamente, di modo che il rischio sanitario associato alla dieta non è stato differente tra i trattamenti. Per i conigli alimentati con la dieta a più basso rapporto FD/amido e maggiore livello proteico, sono state misurate maggiore mortalità (17,1 *vs*. 1,5%) e rischio sanitario (20,0 *vs*. 8,1%) rispetto ai conigli alimentati con le altre diete.

Il livello proteico delle diete non ha modificato l'attività fermentativa ciecale, mentre la profondità delle cripte (P=0,02) e il rapporto villi/cripte (P=0,11) sono risultati inferiori nei conigli alimentati con le diete a minore tenore proteico. L’aumento del rapporto FD/amido non ha modificato le caratteristiche della mucosa intestinale, mentre ha aumentato la quantità totale di acidi grassi volatili, anche se in modo non significativo, e l’incidenza dell’acido acetico (P=0,02), mentre ha ridotto la percentuale di acido valerianico (P<0,01).

Le prestazioni produttive sono state soddisfacenti per l’età di macellazione ed il tipo genetico usato, e non influenzate dai trattamenti sperimentali, che hanno prodotto variazioni minori delle caratteristiche della carcassa e della qualità della carne.

Contributo sperimentale n. 3

Riduzione dal contenuto di proteina e aumento del rapporto fibra digeribile/amido in diete per conigli in accrescimento.

A 29 giorni di età, 282 conigli ibridi (596±58 g) di entrambi i sessi sono stati assegnati a sei gruppi da 47 unità ciascuno e alimentati *ad libitum* con sei diete al 18% di ADF e formulate secondo un disegno bifattoriale con tre livelli di proteina grezza (15,0%, 15,5% e 17,0%) e due diversi rapporti FD/amido (1,1 *vs.* 2.0). Sono state realizzate due prove di digeribilità *in vivo* da 35 a 39 d e da 56 a 60 d di età. Le prove digeribilità, il campionamento dei contenuto ciecali e delle mucose intestinali su 36 conigli a 38 d, l’analisi dei tessuti, le analisi chimiche di diete sperimentali, feci e contenuto ciecale sono stati effettuati secondo le metodologie precedentemente descritte. A 78 giorni di età, i conigli sono stati macellati e le carcasse sezionate. Il bilancio azotato è stato calcolato sui dati individuali stimando il contenuto di N del corpo a diverse età (Szendro e coll., 1998). I risultati sono stati analizzati statisticamente considerando gli effetti di livello di proteina, rapporto FD/ amido e loro interazione. L’effetto del sesso è stato incluso nel modello per analizzare la variabilità di dati di accrescimento, risultati di macellazione, caratteristiche della carcassa e della carne. I coefficienti di digeribilità delle diete nelle due prove di digeribilità sono stati analizzati con un modello che considerava l’effetto di età, livello di proteina, rapporto FD/ amido e relative interazioni.

L’incremento del livello proteico della dieta ha aumentato significativamente la digeribilità dei principi nutrienti, ad eccezione di estratto etereo e amido, e quindi il valore nutritivo delle diete. La sostituzione parziale di farina di erba medica con farine di soia e girasole e l'aumento del livello di inclusione di orzo e polpe secche di bietola hanno consentito un livello costante di fibra digeribile e ridotto il livello delle frazioni fibrose meno digeribili, riducendo così il transito di alimento e aumentando l'efficienza di utilizzazione digestiva.

La digeribilità dell'energia lorda e della sostanza secca non è cambiata con l'età degli animali. L'utilizzazione digestiva della proteina è significativamente diminuita (da 79,2 a 75,7%, P<0,001), mentre è aumentata la digeribilità di amido (da 96,4 a 97,9%, P<0,001), fibra grezza (da 16,7 a 22,2%, P<0,001) e frazioni fibrose (TDF, NDF, ADF, emicellulose) ad eccezione di FD.

La riduzione del livello proteico delle diete somministrate nel primo periodo (29-50 d) sotto il 15,5% ha significativamente (P<0.001) diminuito accrescimento giornaliero (da 53,1 e 55,1 g/d nelle diete con il 15,5% e il 17% PG a 49,3 g/d nelle diete al 14% PG) e peso vivo a 50 d di età (1712 e 1755 *vs*. 1631 g), senza modificare il consumo alimentare. Nel secondo periodo (50-78 d), le prestazioni produttive non sono state influenzate dal livello proteico della dieta. In tutto il periodo, i conigli alimentati con diete a basso contenuto di proteina sono cresciuti meno rispetto a quelli alimentati con diete a maggiore contenuto proteico, mentre apporti intermedi di PG (15,5%) non hanno prodotto risultati significativamente diversi dagli altri gruppi. La conversione alimentare è variata in base al ritmo di accrescimento ed è migliorata significativamente nel post-svezzamento e nell'intero periodo di prova con i maggiori apporti proteici. Un aumento del contenuto proteico delle diete, inoltre, pur stimolando la ritenzione di azoto, ha aumentato l'escrezione di N da 80,4 a 95,0 e 115,0 g, corrispondenti a 1,64, 1,94 e 2,35 g di N escreto al giorno. Riportando i risultati alla dieta a maggiore contenuto proteico (100), l'escrezione di N è stata diminuita del 17 e del 30% passando dal 17 al 15,5 e 14,0% di PG, mentre la ritenzione di N è diminuita solo del 3 e del 6%, rispettivamente. L'escrezione di N è stata maggiormente ridotta nel secondo piuttosto che nel primo periodo di crescita.

L'aumento del rapporto FD/amido, ha significativamente migliorato l'accrescimento medio giornaliero durante il post-svezzamento, diminuito il consumo di alimento e, di conseguenza, migliorato la conversione alimentare. Da 50 giorni di età fino alla macellazione, l'accrescimento è stato simile in tutti gruppi sperimentali, mentre gli effetti sul consumo alimentare e l'indice di conversione sono rimasti evidenti e significativi. Pertanto, un maggiore rapporto FD/amido ha aumentato il peso vivo a 50 giorni di età, anche se le differenze non sono risultate più significative alla fine del ciclo produttivo, riducendo l'assunzione di alimento e la conversione (P <0.001) nel periodo di prova. Inoltre, è stata ridotta l'escrezione di N del 5,5%, ma senza effetti sulla ritenzione a livello corporeo.

Lo stato sanitario è stato soddisfacente in tutti i gruppi e non influenzata dai trattamenti alimentari.

L’aumento del livello proteico dal 14 al 17,0% ha aumentato la produzione di acidi grassi volatili e ridotto il valore di pH ciecale, mentre non è cambiata la concentrazione di ammoniaca. Le maggiori inclusioni di orzo e polpe secche di bietola, a parità di FD, in diete con alto tenore proteico potrebbero spiegare l’intensa attività fermentativa ciecale. Aumentando il rapporto FD/amido, il transito intestinale è stato rallentato e il peso dell’apparato digerente è risultato aumentato, soprattutto per il maggiore riempimento del cieco. L’attività fermentativa ciecale è stata stimolata come evidenziato dalla maggiore produzione totale di acidi grassi volatili (da 64,0 a 78,4 mmol/l, P<0,001), accompagnata da riduzione di pH (5,87-5,71) e di produzione di ammoniaca (da 4,7 a 2,3 mmol/l, P<0,001). La morfometria della mucosa del digiuno non è stata influenzata dal contenuto in proteina o dal livello di fibra digeribile della dieta.

L’effetto del livello proteico della dieta sul peso vivo e sul peso di macellazione spiega le variazioni osservate nelle altre variabili correlate con il peso degli animali: l’aumento del contenuto proteico nella dieta ha aumentato il peso della carcassa in modo significativo e tendenzialmente (P=0,09) migliorato la resa di macellazione. Altre caratteristiche della carcassa e della carne non sono state influenzate dal trattamento. Così come per le prestazioni produttive, il rapporto FD/amido ha influenzato poco i risultati di macellazione e le caratteristiche della carcassa, con piccole variazioni (P<0,10) del grasso separabile e del rapporto muscolo/ossa misurato sull’arto posteriore.

Contributo sperimentale n. 4

Livelli di amido e pectine in diete per conigli in accrescimento: effetti su stato di salute, fisiologia digestiva, prestazioni produttive e qualità della carcassa e della carne.

A 34 giorni di età, 240 conigli ibridi (827±26 g) di entrambi i sessi sono stati assegnati a sei gruppi sperimentali di 40 unità ciascuno e alimentati *ad libitum* con sei diete formulate secondo un disegno bifattoriale, con tre livelli di amido (5, 10 e 15%) e due livelli di pectine (5 e 10%). La prova di digeribilità *in vivo* da 52 a 56 d di età, il campionamento dei contenuti ciecali e della mucosa intestinale a 51 d, le analisi morfometriche della mucosa intestinale e le analisi chimiche di diete sperimentali, feci e contenuti ciecali sono state effettuate secondo le metodologie precedentemente descritte. A 75 d, i conigli sono stati macellati e le carcasse sezionate. I dati sono stati analizzati considerando l’effetto di livello di amido, livello di pectine e loro interazione. L’effetto del sesso è stato incluso nel modello per analizzare la variabilità di dati di accrescimento, risultati di macellazione, caratteristiche della carcassa e qualità della carne.

L’aumento del contenuto di amido alimentare ha migliorato la digeribilità della sostanza secca (dal 54,7% al 62,9%) e dei diversi principi nutritivi. L'inclusione di una maggiore quantità di polpe di bietola, superiore al 30%, in sostituzione della farina di erba medica nelle diete ricche di pectine, spiega la maggiore digeribilità della fibra grezza (dall'8,1 al 26,4%) e delle frazioni fibrose.

Le diete caratterizzate da valori estremi del rapporto pectine/amido (0,5 *vs*. 2,0) hanno mostrato digeribilità e valore nutritivo (ED: 9,5 MJ/kg) molto simili: a parità di erba medica, la sostituzione di farina d'orzo con polpe secche di bietola ha permesso di mantenere elevata la digeribilità delle diete. L'amido dell'orzo è stato sostituito dalla fibra (pectine in particolare) delle polpe di bietola. La digeribilità delle frazioni fibrose è stata cambiata dal livello di pectine: nelle diete con il 5% di pectine, la digeribilità della fibra grezza non è cambiata (da 7,2% a 9,6%, P>0,05), a differenza di quanto misurato per le frazioni fibrose; nelle diete con il 10% pectine, la digeribilità è significativamente aumentata per fibra grezza (da 23,5 a 30,3%), NDF (da 34,3 a 45,1%), ADF (da 25,7 a 32,1%) e emicellulose (da 45,4 a 60,8%).

L'aumento dell'apporto di amido ha determinato un significativo incremento di peso finale a 75 d e accrescimento giornaliero e ha ridotto il consumo di alimento con relativo miglioramento della conversione alimentare (da 3,52 a 3,31 e 3,13, P<0,001) nell'intero periodo. Durante il post-svezzamento l'accrescimento giornaliero è stato aumentato (P<0,001), mentre il consumo di alimento ha mostrato una tendenziale riduzione con la somministrazione di diete ricche di amido (P=0,06); nel secondo periodo, l'accrescimento giornaliero è stato simile fra i gruppi, mentre è rimasta evidente la riduzione del consumo di alimento (da 172 a 159 g/d, P <0,001).

Un maggiore livello di pectine ha stimolato l'accrescimento degli animali (soprattutto in fase di post-svezzamento), ridotto il consumo e la conversione alimentare (3,59 *vs*. 3,05, P<0,001). Il peso vivo finale è risultato maggiore negli animali alimentati con diete contenenti il 10% pectine rispetto a quelli alimentati con diete al 5% di pectine, sia a 55 giorni di età che alla fine ciclo produttivo.

Lo stato di salute è stato buono per tutti i conigli, con soli due animali morti nell'intero periodo sperimentale, e le fermentazioni ciecali sono state poco modificate dal trattamento alimentare: la produzione di N-ammoniacale a livello ciecale è stata maggiore negli animali alimentati con diete a basso contenuto di amido, probabilmente a causa della maggiore assunzione di proteine (PD/ED: 12,9, 12,2 e 11,6 g/MJ, rispettivamente, per le diete con 5, 10, e 15% di amido). La produzione totale di acidi grassi volatili non è cambiata con l'aumento dell'amido alimentare, mentre si è riscontrato un inatteso aumento dell'incidenza di acido acetico (P=0,12) e una riduzione dell'acido propionico (P<0,01). Le caratteristiche chimiche delle diete possono spiegare i risultati ottenuti: 1) anche il più alto livello di amido utilizzato (circa il 16%, valore medio delle diete L1 e H1) era compatibile con la capacità digestiva dell'animale all'età considerata (51 d), e non ha comportato un apporto consistente di amido non digerito al cieco; 2) inoltre, le diete avevano un tenore di FD simile (24,7, 23,6 e 23,8% per le diete con 5, 10 e 15% di amido), in grado di garantire un'attività fermentativa comparabile e orientata più alla produzione di acetato che di propionato e butirrato. L'aumento del livello di pectine ha ridotto il valore di pH ciecale (P=0,02), ma ha avuto un debole effetto sull'incidenza di propionato e butirrato. Soltanto la produzione totale di acidi grassi volatili ha mostrato un tendenziale aumento (73,8 *vs*. 82,8 mmol/l, P=0,10) e quella di valerato è aumentata (0,43 vs. 0,35%, P<0,01) con la somministrazione di diete al 10% di pectine.

L'analisi morfometrica di ileo e digiuno non ha evidenziato significative differenze nello sviluppo dei villi e delle cripte, sebbene l'altezza dei villi sia del digiuno che del cieco abbiano evidenziato una tendenziale riduzione (P=0,09 e 0,11) nei conigli alimentati con diete contenenti livelli di amido (circa il 10%) raccomandati per il post-svezzamento, rispetto ai conigli alimentati con diete contenenti livelli inferiori (5%) o superiori (15%).

L'effetto del livello di amido sui risultati di macellazione è dipeso principalmente dalle differenze nel peso vivo degli animali. I conigli più pesanti, che avevano ricevuto le diete a maggiore contenuto di amido, hanno mostrato maggiori pesi alla macellazione, pesi delle carcasse e rese di macellazione, minori perdite di trasporto e maggiore incidenza del grasso sperabile (P<0,01).

L'aumento della concentrazione di pectine della dieta ha avuto un effetto debole ed il maggiore peso di macellazione (2628 *vs*. 2687 g, P<0,05) nei conigli alimentati con le diete al 10% di pectine non è stato associato ad una maggiore resa di macellazione. L'incidenza dell'apparato digerente è stata significativamente maggiore nei conigli alimentati con diete al 10% piuttosto che al 5% di pectine (18,5 *vs*. 19,0%, P<0,05).

Il trattamento alimentare non ha prodotto variazioni significative della qualità della carne, in termini di pH o colore misurati sui muscoli *longissimus lumborum e biceps femoris* 24 ore dalla macellazione.

Discussione

In tutti i contributi sperimentali, quando l'aumento del rapporto FD/ADF a parità di amido è stato ottenuto sostituendo la farina di erba medica con le polpe di bietola, la **digeribilità** di sostanza secca, energia lorda, fibra grezza e frazioni fibrose è aumentata significativamente. La digeribilità fecale apparente delle frazioni fibrose più solubili e digeribili (come quelle contenute nelle polpe) può, in effetti, raggiungere il 60-70%, mentre quella delle frazioni insolubili, principale costituente della fibra dell'erba medica, varia dal 15 al 30% (Carabaño e coll., 2001; García e coll., 2009).

Senza dubbio, la digeribilità e il valore nutritivo delle diete sono aumentati con il rapporto DF/ADF, non solo per la maggiore presenza fibra digeribile, ma anche per la maggior digeribilità (9-10 punti) di tutte le frazioni fibrose. Diversamente, quando la FD ha sostituito l'amido (crescente rapporto FD/amido da 0,8 fino a 2,8) e con limitate differenze a livello di ADF (rapporto FD/ADF da 0,9 a 1,2) (Contributi sperimentali n. 1 e 2), la digeribilità di sostanza secca e energia lorda e, di conseguenza, il valore nutritivo delle diete non sono cambiati, poiché l'energia fornita dalla FD è stata simile a quella offerta dall'amido, come osservato anche da altri autori (De Blas e Carabaño, 1996; Gidenne e Bellier, 2000). Tuttavia, nei Contributi sperimentali n. 3 e 4, il valore nutritivo delle diete è cresciuto all'aumentare del rapporto FD/amido da 1,1-1,7 a 1,9-4,2. L'aumento contemporaneo del rapporto FD/ADF (da 0,9-1,0 a 1,4-1,5) e il livello molto basso di inclusione di polpe nelle diete con il più basso rapporto FD/amido (anche 0% nel Contributo sperimentale n. 3 rispetto ad un massimo del 33-34%) potrebbero spiegare questo risultato. Nel Contributo sperimentale n. 4, le diete caratterizzate da valori estremi del rapporto pectine/amido (5% pectine, 15% amido=0,5% e 10% pectine, 5% amido=2), hanno mostrato digeribilità e valori nutritivi molto simili: con lo stesso livello di inclusione di farina di erba medica, la sostituzione di orzo con polpe di bietola ha mantenuto la digeribilità a livelli elevati. La dieta con il più alto contenuto di FD e amido ha evidenziato il più alto valore nutritivo.

Confrontando diverse fonti proteiche, per le diete contenenti solo girasole è stata misurata una minore digeribilità della proteina grezza (Contributo sperimentale n. 1), coerentemente con la minore digeribilità della proteina della farina di girasole rispetto alla farina di soia (Maertens e coll., 2002). Tuttavia, la fonte proteica non ha modificato le fermentazioni ciecali, lo stato di salute, le prestazioni o i risultati di macellazione.

L'aumento nella dieta della concentrazione di PG (dal 15 al 16% nel Contributo sperimentale n. 2 e dal 14 al 17% nel Contributo sperimentale n. 3), in generale, ha determinato una maggiore digeribilità delle proteine e dei principi nutritivi a causa della parziale sostituzione della proteina dell'erba medica con quelle delle farine di soia e girasole.

Le conseguenze dei trattamenti alimentari sulle **prestazioni produttive** sono una diretta conseguenza del valore nutritivo delle diete. L'aumento del rapporto FD/ADF e, quindi, del valore nutritivo delle diete ha ridotto il consumo di alimento, per la regolazione chemiostatica dell'appetito (Gidenne e Lebas, 2005; Xiccato e Trocino, 2010b), e migliorato linearmente la conversione alimentare, pur senza differenze nel peso vivo finale e nell'accrescimento giornaliero (Contributo sperimentale n. 1). Il miglioramento della conversione alimentare all'aumentare del contenuto di FD può essere messo in relazione con il più lento transito intestinale e l'aumento della digeribilità e del valore nutritivo delle diete che è stato osservato anche in altri studi (Xiccato e coll., 2006a, 2008;

Carraro e coll., 2007; Fragkiadakis e coll., 2007). Nel Contributo sperimentale n. 2, l'aumento del rapporto FD/amido non ha avuto alcun effetto apprezzabile sulle prestazioni produttive. Anche García e coll. (1993) avevano osservato che diete contenenti polpe di bietola non modificavano le prestazioni di accrescimento quanto utilizzate in sostituzione di materie prime apportatrici di amido, a causa del loro simile valore nutritivo. Nel Contributo sperimentale n. 3, durante il post-svezzamento, l'accrescimento giornaliero è stato stimolato e il consumo di alimento ridotto. Di conseguenza, la conversione alimentare è migliorata all'aumentare del rapporto FD/amido. Nelle ultime settimane prima della macellazione, le prestazioni di crescita sono risultate simili fra i diversi gruppi. Nel Contributo sperimentale n. 4, l'aumento delle pectine nelle diete dal 5 al 10% (con un rapporto FD/amido da 2,4 a 3,1) ha stimolato significativamente l'accrescimento giornaliero (soprattutto nel post-svezzamento), aumentato il peso vivo finale, ridotto il consumo e migliorato la conversione alimentare. L'aumento del rapporto FD/amido ha anche diminuito l'escrezione azotata, senza modificare la ritenzione, soprattutto nel primo periodo piuttosto che nel secondo (Contributo sperimentale n. 3).

La riduzione dell'apporto proteico con la dieta al di sotto del 15% ha peggiorato l'accrescimento e il peso degli animali nel post-svezzamento (prime tre settimane dopo lo svezzamento) (Contributi sperimentali n. 2 e 3). Questi risultati confermano precedenti osservazioni relative ad un contenuto proteico nelle diete commerciali per lo svezzamento e l'accrescimento superiore rispetto ai reali fabbisogni degli animali (Maertens e coll., 1997; Trocino e coll., 2000, 2001; García-Palomares e coll., 2006a, 2006b; Eiben e coll., 2008). In conigli macellati a 63 giorni di età e 2,35 kg di peso vivo, condizioni tipiche del mercato spagnolo, la riduzione del livello proteico delle diete dal 16 al 14% non ha influenzato le prestazioni produttive (García-Palomares e coll., 2006b). In conigli macellati dopo (75-90 d) e a pesi maggiori (2,5-3,0 kg), la riduzione del livello proteico dal primo al secondo periodo ha permesso di soddisfare meglio i fabbisogni proteici nella prima fase di accrescimento e di ridurre l'escrezione azotata durante la seconda fase. In effetti, in questo ultimo periodo prima della macellazione, il consumo di alimento è maggiore e il livello proteico delle diete può essere diminuito senza conseguenze negative sulle prestazioni o sulla qualità della carcassa e della carne (Maertens e coll., 1997; Maertens e Luzi, 1998; Trocino e coll., 2000, 2001).

L'equilibrio delle **fermentazioni ciecali** è considerato un indicatore del possibile effetto dei fattori nutrizionali sulla salute dell'apparato digerente nel coniglio. Nel Contributo sperimentale n. 1, l'aumento del rapporto FD/ADF nell'intervallo considerato (da 1,0 a 1,2) non ha modificato la produzione totale di acidi grassi volatili, sebbene sia significativamente diminuito il pH del contenuto ciecale. Questa variazione, sebbene non associata ad alcun aumento degli acidi grassi volatili totali come osservato anche da Carabaño e coll. (1997), può essere considerato favorevolmente per il mantenimento di un equilibrio della popolazione ciecale e delle fermentazioni. L'aumento del contenuto di FD e la riduzione dell'amido possono rallentare il transito intestinale e aumentare l'incidenza dell'apparato digerente, soprattutto per il maggiore riempimento del cieco. Di conseguenza, le fermentazioni sono state stimolate, la produzione totale di AGV è stata aumentata così come l'incidenza di acido acetico, mentre la percentuale di acido valerianico, di solito associata con l'attività dei batteri amilolitici, è risultata ridotta; anche il pH del contenuto ciecale e la concentrazione di ammoniaca sono risultate diminuite (Contributi sperimentali n. 2 e 3). L'aumento della concentrazione di pectine (Contributo sperimentale n. 4) è risultato in una riduzione del pH ciecale, un tendenziale aumento della produzione totale di AGV e una riduzione della percentuale di acido valerianico. Studi realizzati in precedenza avevano mostrato un favorevole e significativo aumento dell'attività fermentativa ciecale all'aumentare del contenuto di fibra digeribile e/o solubile (García coll., 2000; Falcao-e-Cunha coll., 2004). I bassi livelli di azoto ammoniacale riscontrati a livello ciecale in tutti i contributi sperimentali e la tendenza ad una riduzione con l'aumento del rapporto FD/amido dimostrano che la FD rappresenta un apporto di carboidrati capaci di favorire la fermentazione e la fissazione dell'ammoniaca nella proteina batterica.

Un solo punto percentuale di differenza nel contenuto proteico delle diete (dal 15 al 16%) non è stato sufficiente per modificare in maniera significativa l'attività fermentativa ciecale (Contributo sperimentale n. 2), mentre quando la proteina grezza è stata aumentata dal 14 al 17%, le fermentazioni ciecali sono state stimolate (Contributo sperimentale n. 3). La maggiore inclusione di orzo e polpe di bietola, a livelli costanti di FD, può spiegare anche la più intensa attività fermentativa ciecale negli animali alimentati con le diete a più alto contenuto di proteina.

In tutte le attività sperimentali della presente tesi, è stato dato rilievo e dedicata una quota importante di lavoro alla valutazione delle condizioni della **mucosa intestinale** come strumento per valutare lo stato di salute dei conigli e/o la loro suscettibilità alle malattie e ai disturbi digestivi. Ricerche precedenti avevano indicato un possibile effetto positivo dell'aumento del contenuto di frazioni fibrose solubili, piuttosto che della riduzione del contenuto di NDF, sull'integrità della mucosa intestinale, con una riduzione della mortalità ed un miglioramento delle prestazioni dei giovani conigli (Gutiérrez e coll., 2002; Feugier e coll., 2006; Álvarez e coll., 2007; Gómez-Conde e coll., 2007). Nella presente tesi, tuttavia, né l'aumento del rapporto FD/ADF (Contributo sperimentale n. 1) o FD/amido (Contributi sperimentali n. 2, 3 e 4) o la concentrazione di pectine (Contributo sperimentale n. 4) hanno modificato in maniera apprezzabile la struttura della mucosa intestinale a livello di ileo o digiuno. Solo nel Contributo sperimentale n. 4, l'altezza dei villi a livello sia di ileo sia di digiuno è risultata tendenzialmente minore (P=0,09 e 0,11) nei conigli alimentati con le diete al 10% di amido, livello raccomandato per il periodo post-svezzamento, piuttosto che in quelli che avevano ricevuto le diete a minore (5%) o maggiore (15%) contenuto. La riduzione nella profondità delle cripte trovata all'aumentare del livello proteico delle diete (Contributo sperimentale n. 2) potrebbe essere associata a una minore capacità della mucosa di riparare i danni a livello dei villi e, quindi, indirettamente, potrebbe spiegare la maggiore suscettibilità ai disturbi digestivi nei conigli alimentati con le diete ad elevato contenuto proteico. Altre ricerche realizzate in precedenza hanno evidenziato un effetto minimo o nullo della fonte di proteina, a parità di apporto proteico, anche se era stata osservata una significativa riduzione della mortalità da enteropatia epizootica all'aumentare della digeribilità ileale della proteina (Gutiérrez e coll., 2002, 2003; Chamorro e coll., 2007). Un effetto positivo sull'integrità della mucosa è stato riportato in conigli svezzati precocemente e alimentati con diete contenenti plasma animale piuttosto che soia (Gutiérrez e coll., 2000), mentre gli effetti negativi di fattori anti-nutrizionali presenti nelle leguminose sono solo stati ipotizzati (Gutiérrez e coll., 2003; Cano e coll., 2004).

L'uniformità osservata per equilibrio delle fermentazioni ciecali e struttura della mucosa intestinale è stata accompagnata da uno **stato di salute** relativamente buono in tutte le sperimentazioni realizzate. In effetti, questa situazione così favorevole non ha permesso di verificare correttamente l'effetto dei trattamenti alimentari sulle condizioni di salute dei conigli e la possibilità di ridurre e controllare la diffusione e l'importanza dei disturbi digestivi, o meglio dell'enteropatia epizootica. Né l'aumento del rapporto FD/ADF (Contributo sperimentale n. 1) o il rapporto FD/amido o il livello di pectine (Contributi sperimentali n. 3 e 4) hanno modificato significativamente lo stato di salute degli animali. Solo nell'ambito del Contributo sperimentale n. 2, anche se mortalità e morbilità sono state piuttosto basse, è stato misurato un effetto significativo del trattamento alimentare: il più basso rapporto FD/amido ha aumentato la mortalità, ma allo stesso tempo ha ridotto il numero di animali malati che hanno comunque raggiunto la fine della prova rispetto agli altri trattamenti sperimentali. La mortalità e il rischio sanitario più alti sono stati associati alla dieta con il minore rapporto FD/amido e il maggiore contenuto proteico (Contributo sperimentale n. 2). In passato, era stato evidenziato un effetto positivo sullo stato di salute, con una riduzione della mortalità da ERE e dei disturbi digestivi, all'aumentare del rapporto FD/amido (Gómez-Conde e coll., 2004; 2007; Xiccato e coll., 2006a; Carraro, 2006) o quando amido e proteina erano state sostituite da fibra digeribile in diete con lo stesso livello di ADF (Perez e coll., 2000; Soler e coll., 2004). Un eccesso proteico potrebbe peggiorare la salute degli animali, alterando l'equilibrio ciecale e favorendo l'utilizzazione della proteina a scopo energetico, aumentando la concentrazione di N ammoniacale e il valore di pH, e favorendo così lo sviluppo di popolazioni batteriche patogene (Lebas e coll., 1998). In effetti, la mortalità da ERE è stata significativamente ridotta (Gutiérrez e coll., 2002, 2003; Chamorro e coll., 2007) dalla riduzione del livello di proteina alimentare e dall'aumento della digeribilità ileale della proteina o dall'aumento del livello di fibra digeribile (Xiccato e coll., 2006a; Gómez-Conde e coll., 2007).

Nei quattro contributi sperimentali, il trattamento alimentare ha avuto un effetto molto debole sui **risultati di macellazione** e le **caratteristiche delle carcasse** o **la qualità della carne**, confermando il ruolo minore della nutrizione sulla qualità della carcassa e della carne in animali alimentati *ad libitum* con diete bilanciate e che raggiungono pesi vivi finali simili (Xiccato, 1999; Hernández e Gondret, 2006; Hernández, 2008; Xiccato e Trocino, 2010b). Alcune differenze sono state misurate quando il livello proteico della dieta ha modificato le prestazioni e il peso vivo finale degli animali, e quindi con la più bassa concentrazione proteica (14-15%) (Contributi sperimentali n. 2 e 3), confermando i risultati di Maertens e coll. (1997) che avevano riportato un peggioramento dei risultati di macellazione con diete contenenti il 13% di proteina grezza. Solo la somministrazione di diete con diversi livelli di grasso, da fonti alimentari diverse, avrebbe potuto modificare in maniera apprezzabile le caratteristiche della carcassa e della carne (Hernández, 2008).

Conclusioni

Le conclusioni che si possono trarre dai risultati e dalla discussione di cui sopra sono fatte partendo dall'intento generale della tesi, vale a dire la definizione dei fabbisogni nutrizionali dei conigli nelle fasi di post-svezzamento e ingrasso con riguardo alle frazioni fibrose, al contenuto di amido e il suo rapporto con la fibra, e il contenuto di proteina con diversi obiettivi specifici:

***1*) mantenere l'equilibrio delle fermentazioni a livello ciecale.** Le variazioni dei più importanti principi nutritivi (fibra digeribile, ADF, amido e proteina) negli intervalli di concentrazione testati possono modificare la condizione intestinale e l'equilibrio ciecale nel coniglio: l'aumento del contenuto di FD in sostituzione dell'amido o l'aumento del livello proteico in diete con un buon contenuto di fibra digeribile stimolano la fermentazione ciecale, limitano la produzione di azoto ammoniacale e mantengono il pH ciecale a valori utili per lo sviluppo e l'attività della normale popolazione batterica ciecale. Né il livello di FD, di amido o di ADF, tuttavia, possono modificare la morfologia e l'integrità della mucosa intestinale.

***2)* ridurre la frequenza e la gravità delle patologie digestive**. Nonostante le ampie variazioni di composizione chimica delle diete sperimentali, non sono stati osservati effetti sullo state di salute degli animali e, piuttosto, i livelli di amido, pectine e proteine utilizzati sono sempre stati associati ad uno stato di salute ottimale. Pertanto, non siamo in grado di valutare adeguatamente l'effetto e la possibile influenza dei trattamenti alimentari studiati sullo stato di salute degli animali in un allevamento colpito da ERE o da altri disturbi di tipo digestivo. Tuttavia, c'è una certa evidenza che un basso rapporto FD/amido aumenta la mortalità. Quest'andamento è accentuato quando un basso rapporto FD/amido della dieta (<1) è associato ad un alto contenuto proteico (>16%). Diete con alti rapporti FD/amido (1-1,5) e livelli proteici moderati (circa il 15%) sono raccomandati. In altre parole, l'aumento del livello energetico della dieta mediante l'inclusione di fibra digeribile, piuttosto che di amido, combinato con un basso flusso di proteina indigerita a livello ciecale, potrebbe ridurre il rischio sanitario.

3) **migliorare l'efficienza di conversione alimentare e di ritenzione azotata.** L'aumento del contenuto di FD in sostituzione delle frazioni meno digeribili o dell'amido (a livelli costanti di ADF) migliora la conversione alimentare, riducendo il consumo di alimento, e permette una buona riduzione dell'escrezione azotata. L'amido e la fibra digeribile possono essere considerati alternativi nell'alimentazione del coniglio e, potenzialmente, additivi: l'associazione di elevati livelli di amido e fibra digeribile potrebbe perfino dare migliori risultati per conversione alimentare e convenienza economica.

4) **garantire elevate prestazioni di crescita e ottimali qualità della carcassa e della carne**. L'aumento del rapporto FD/ADF migliora il valore nutritivo delle diete e la loro efficienza di utilizzazione per l'accrescimento. L'aumento del rapporto FD/amido non modifica il valore nutritivo delle diete o, nella maggior parte dei casi, le prestazioni produttive e i risultati di macellazione. A volte, l'accrescimento può essere stimolato, soprattutto nelle prime settimane dopo lo svezzamento, quando i conigli stanno ancora sviluppando la loro capacità di utilizzazione digestiva degli alimenti.

Per massimizzare le prestazioni produttive, il livello proteico delle diete non dovrebbe scendere sotto il 15,5% nelle prime settimane del ciclo di produzione, poiché il successivo accrescimento compensativo che i conigli mostrano nelle ultime settimane prima della macellazione non permetterebbe il recupero delle differenze di peso. L'apporto proteico deve essere controllato sia nel primo sia nel secondo periodo di crescita, durante il quale i fabbisogni proteici sono minori, allo scopo di aumentare l'efficienza di utilizzazione e ridurre l'escrezione dell'azoto.

Variazioni nell'apporto di FD, amido o proteina inducono differenze nei risultati di macellazione solo quando sono a livelli tali da modificare il peso di macellazione e, quindi, tutte le variabili direttamente correlate con il peso vivo. Il trattamento alimentare non è mai in grado di produrre differenze nelle caratteristiche della carne, pH e colore, dei principali muscoli che possano essere apprezzabili a livello commerciale.

RESUMEN

El papel de la fibra digestible, el almidón y la proteína sobre la salud y los rendimientos productivos en dietas para conejos en crecimiento

Introducción y objetivos

Durante los últimos 10 años, la diseminación de la enteropatía epizoótica (EEC) en toda Europa ha incrementado notablemente la mortalidad y la morbilidad en las granjas de conejos. Además de las pérdidas económicas debidas a la pérdida de animales, la enfermedad ha generado un empeoramiento del índice de conversión y un aumento del gasto en veterinario que ha incrementado los costes y reducido el beneficio de las explotaciones. La falta de conocimiento sobre el agente etiológico de esta enfermedad, el acuerdo científico en cuanto a su carácter multifactorial, así como, las limitaciones impuestas por la legislación Europea sobre la utilización de antibióticos, han estimulado la búsqueda de estrategias de alimentación y manejo capaces de prevenir o, al menos, limitar el impacto de la EEC. Los estudios realizados en los últimos años se han centrado en especificar las necesidades de los animales en el periodo post destete para reducir o limitar los problemas causados por la enfermedad.

Entre los nutrientes estudiados, la fracción mas digestible de la fibra (FD= fibra soluble o pectinas y hemicelulosas) parece que mejora el estado sanitario de los animales y su eficacia alimenticia por su efecto positivo en el control de la microbiota intestinal (Gidenne and García, 2006). Aunmentado la fibra soluble de la dieta se reduce la presencia de *C. perfringens* y otros patógenos oportunistas en el ciego y la mortalidad por EEC (Soler *et al.*, 2003; Gómez-Conde *et al.*, 2007). Además, este tipo de fibra parece también mejorar la integridad de la mucosa intestinal (García-Riuz *et al.*, 1997; Carabaño *et al.*, 2008). Manteniendo constante el contenido de FAD y reemplazando almidón y proteína por FD también se han conseguido efectos positivos (Perez *et al.*, 2000; Gidenne *et al.*, 2001; Margüenda *et al.*, 2006), así como reduciendo el nivel de fibra insoluble (FND) y reemplazándola por fibra soluble (Gutiérrez *et al.*, 2002; Alvarez *et al.*, 2007). Sin embargo, todavía se conoce poco sobre la interacción de la inclusión de FD y la utilización de otros nutrientes como la proteína, también relacionados con la aparición de enfermedades digestivas. El déficit (<12%) o el exceso de proteína (>8%) aumenta la mortalidad, modificando la actividad fermentativa y la composición de la microbiota intestinal (Maertens e De Groote, 1988; Lebas, 1989; Carabaño *et al.*, 2008, 2009). Reduciendo los niveles de proteína del 18 al 16%, en dietas con la misma digestibilidad ileal, se reduce la presencia de *Clostridium perfringens* y la mortalidad por EEC (Chamorro *et al.*, 2007), mientras que reducciones mas acusadas (del 16 al 14%) no tienen un

efecto claro sobre la mortalidad aunque reducen las bacterias anaerobias en el íleon (García-Palomares *et al*., 2006a, 2006b; Carabaño *et al*., 2009). El uso de fuentes de proteína mas digestibles (como la harina de girasol) pueden también reducir el flujo de proteína al ciego y la presencia de patógenos como *E. coli* y *Clostridium*, mejorando la salud intestinal y los rendimientos (Gutiérrez *et al.*, 2003; Chamorro *et al.*, 2005, 2007; Carabaño *et al.*, 2008, 2009). Teniendo en cuenta la preocupación de los consumidores sobre el uso de materias primas genéticamente modificadas, actualmente hay un interés en la evaluación de fuentes alternativas a la soja. Entre ellas, la harina de girasol, en combinación con soja, se está utilizando de manera general en los piensos de conejos. Sin embargo, la sustitución total de la soja por la harina de girasol demanda, en la práctica, una cuidadosa evaluación del equilibrio de aminoácidos para mantener los rendimientos de los animales. Las actividades experimentales realizadas en esta tesis se encuadran en los objetivos generales de definir las necesidades nutricionales de los gazapos en el período del post destete, centrándose en las distintas fracciones de la fracción fibrosa y más concretamente en la parte más digestible (fibra digestible, pectinas y hemicelulosas), el contenido en almidón en relación con la fibra y el suministro de proteína con el objetivo de 1) mejorar las condiciones intestinales, 2) reducir la aparición e intensidad de los problemas digestivos, 3) mejorar la eficacia de utilización del alimento, y 4) garantizar rendimientos elevados y la calidad final de la carne. Los objetivos de esta tesis se han desarrollado en 4 experimentos que se detallan a continuación.

Experimento 1

Nivel de fibra digestible y la substitución de harina de soja por harina de girasol en dietas de conejos en crecimiento.

Con una edad de 34 d, 216 gazapos (837±48 g de peso vivo) de ambos géneros, de una línea hibrida, se asignaron a seis grupos experimentales (36 animales por grupo). Cada grupo experimental recibió una de seis dietas experimentales que se suministraron *ad libitum*. Las dietas se formularon de acuerdo con un diseño factorial 2 x 3 con dos fuentes de proteína (harina de soja vs harina de girasol) y tres relaciones FD/FAD (1,0, 1,1 y 1,2). Todas las dietas fueron iso-proteícas (15,9% CP). Se determinó *in vivo* la digestibilidad aparente de los nutrientes y la energía digestible (ED) de las dietas (Pérez *et al*., 1995). Se muestreó el contenido cecal para la determinación de los ácidos grasos volátiles (AGV) y la mucosa intestinal para la caracterización de la longitud de los villi y las criptas en gazapos de 56 d. Las heces y el pienso se analizaron de acuerdo con los métodos de la AOAC (2000) y los procedimientos armonizados de EGRAN (2001). El contenido en fibra dietética total (FDT) se determinó por el método gravimétrico-enzimático (α-amylasa, proteasa and aminoglucosidasa; Megazyme Int. Ireland Ltd., Wicklow, Ireland). El contenido en FD se calculó por diferencia entre el contenido en FDT y FAD, que teóricamente incluye pectinas y hemicelulosas. La concentración de AGV se determinó mediante cromatografía de gases de acuerdo con el método de Osl (1998). A los 76 d de edad, los animales se sacrificaron en un matadero comercial y la canal se diseccionó en el Departamento de acuerdo con el protocolo internacional (Blasco *et al.*, 1993). Se midió el pH (Xiccato *et al.*, 1994) y el color (CIE, 1976) en los múlculos *longissimus lumborum* y *biceps femoris.* La varianza de los resultados fueron analizados con el procedimiento GLM (SAS Inst. Inc., Cary, NC), considerando la relación FD/FAD y fuente de proteína como fuentes de variación. El efecto del género también se incluyó en el modelo para analizar la variabilidad de los datos de crecimiento y los resultados del sacrificio, canal y carne. La mortalidad, morbilidad y el riesgo sanitario se analizaron con el procedimiento CATMOD del paquete estadístico SAS.

La digestibilidad de la materia seca, energía bruta, fibra bruta y otras facciones fibrosas se incrementó en paralelo al incremento de la relación FD/FAD, como consecuencia de la mayor inclusión de pulpa de remolacha en detrimento de la alfalfa. La digestibilidad y el valor nutritivo de las dietas también siguieron la misma tendencia, no sólo por la mayor presencia de constituyentes con FD, sino también por la mayor digestibilidad de todas las fracciones de la fibra (9-10 puntos; P<0,01): de 4,6 a 25,6% para la FAD, de 40,3 a 49,1% para las hemicelulosas y de 85,0 a 93,8% para las pectinas. Este incremento puede estar asociado con el menor grado de lignificación de las paredes celulares y a la mayor susceptibilidad de los hidratos de carbono a la acción de los enzimas digestivos del animal y las bacterias. La fuente de proteína tuvo un efecto mas limitado sobre la digestibilidad de los nutrientes. En las dietas de girasol, la digestibilidad de la proteína tendió (P=0,06) a disminuir, de acuerdo con la menor digestibilidad de la harina de girasol respecto a la de la soja. La digestibilidad del extracto etéreo y las hemicelulosas fueron mayores (P<0,001) en las dietas girasol que en las de soja; mientras que la FAD y las pectinas fueron menos digestibles. El valor energético de las dietas fue relativamente bajo y se incrementó con la relación FD/FAD (alrededor de 9-10 MJ/kg) con independencia de la fuente de proteína y siguieron las recomendaciones actuales para la alimentación en post destete.

Durante el experimento, no hubo apenas problemas sanitarios y no se observó efecto de la dietas sobre la salud de los animales. Ni los parámetros medidos en la mucosa, ni la fermentación cecal se modificaron con los tratamientos estudiados. Sin embargo, el pH del ciego se redujo (P=0,04) con el aumento de la relación FD/FAD.

El incremento de la relación FD/FAD y del valor energético de las dietas no afectó al crecimiento diario ni al peso vivo final de los animales, consecuentemente, tampoco hubo efecto en los parámetros del matadero. Sin embargo, el consumo tendió a reducirse, principalmente en la primera parte del periodo de crecimiento (de 34 a 55 d), y en consecuencia se redujo linealmente el índice de conversión (de 3,55 a 3,30 en el total del cebo; P<0,001). No se detectaron efectos de la fuente de proteína ni en los rendimientos productivos ni en los parámetros de sacrificio y calidad de carne.

Experimento 2

Relación fibra digestible/almidón y nivel proteína para la alimentación de conejos en crecimiento.

Con una edad de 32 d, 246 conejos (837±48 g LW) de ambos géneros, de una línea hibrida, se asignaron a seis grupos experimentales (36 animales por grupo). Cada grupo experimenta recibió una de las seis dietas experimentales que se suministraron *ad libitum.* Las dietas se formularon de acuerdo con un diseño factorial 2 x 3 con dos niveles de proteína (15 vs 17%) y tres relaciones FD/almidón (0,8, 1,5 y 2,8). Todas las dietas tuvieron el mismo nivel de FAD (18%). Se determinó la digestibilidad fecal (60 animales de 52 a 56 d de edad), la morfología de la mucosa intestinal y la fermentación cecal (con 36 conejos de 55 d de edad), los análisis químicos del pienso, heces y ciego, los parámetros de crecimiento, sacrificio, canal y calidad de la carne siguiendo los procedimientos descritos en el Experimento 1.

El aumento del contenido en proteína mejoró significativamente la digestibilidad de materias seca (alrededor de 1 punto) y de los nutrientes. El contenido en energía digestible varió con el nivel de proteína, sin embargo la relación PD/ED fue mayor en las dietas con 16 que con 14% de proteína. En ambos caso los aportes de proteína de las dietas cubrían las necesidades para gazapos en crecimiento (De Blas and Mateos, 1998). Al incrementar la relación FD/almidón descendió la digestibilidad aparente del almidón (si bien el descenso fue pequeño en valor absoluto), mientras que se incremento la digestibilidad de las fracciones fibrosas. Ni la digestibilidad de la materia seca ni la de la energía, ni la ED de la dieta variaron con los cambios en la relación FD/almidón. Esto se debió a que la energía que aportó la FD fue similar a la del almidón, en dietas con igual contenido en fibra bruta y FND. Debido a que las hemicelulosas permanecieron constantes este resultado puede ser atribuido mayoritariamente a la completa digestión de las pectinas (100%) y en una menor extensión al aumento general de todas las fracciones fibrosas, incluidas las menos digestibles (e.g. ADF).

Durante el desarrollo del experimento tanto la mortalidad como la morbilidad fue baja, pese a ello, se observó un efecto significativo del nivel de proteína en estas variables. La mortalidad y el riesgo sanitario tendieron a incrementarse (P=0,10-0,11) en las dietas con mayor contenido en proteína. La administración de dietas con menor relación FD/almidón mostraron mayor mortalidad

(10 *vs*. 1,4 y 1,4%, P=0,04), pero redujeron el número de animales enfermos al final del periodo de cebo en comparación con las otras dietas, aunque no de manera significativa. Por ello no se observaron diferencias significativas en el riesgo sanitario de los tres grupos. Los conejos alimentados con la relación FD/almidón más baja y el mayor nivel de proteína mostraron mayor mortalidad (17,1 vs. 1,5%) y riesgo sanitario (20,0 vs. 8,1%) que el resto de los tratamientos.

El nivel de proteína no afectó ni al llenado de los órganos ni a la actividad fermentativa del ciego, sin embargo, la profundidad de las criptas (P=0,02) y la relación villi/cripta (P=0,11) descendieron con las dietas bajas en proteína. El incremento de la relación FD/almidón no modificó la morfología de mucosa ni la producción total de AGV pero se incrementó (P=0,02) la proporción de acético, principal producto de la fermentación, mientras que se redujo la proporción de valérico (P<0,01).

Los rendimientos en cebo fueron los esperados para la edad al sacrificio y el tipo genético utilizado, no observándose efecto de los tratamientos excepto para parámetros de menor importancia en la calidad de la carne.

Experimento 3

Reducción del nivel de proteína e incremento de la relación fibra digestible/almidón en la dieta de conejos en crecimiento.

Con una edad de 29 d, 282 conejos (596±58 g LW) de ambos géneros, de una línea hibrida, se asignaron a seis grupos experimentales (47 animales por grupo). Cada grupo experimental recibió una de seis dietas experimentales que se suministraron *ad libitum*. Las dietas se formularon de acuerdo con un diseño factorial 3 x 2 con tres niveles de proteína (15, 15,5, 17,0% CP) y dos relaciones FD/almidón (1,1, 2,0). Todas las dietas tuvieron el mismo nivel de FAD (18%). Se realizaron dos ensayos de digestibilidad con un total de 60 animales (10 por dieta) desde los 35 a 39 d y desde 56 a 60 d de edad; a los 38 d se muestreó el contenido del ciego y la mucosa del yeyuno utilizando 36 animales (6 por tratamiento). Los ensayos de digestibilidad, los muestreos en el contenido cecal y la mucosa, los análisis del pienso, heces y ciego se realizaron según los procedimientos descritos en el Experimento 1. A 78 d de edad, los animales fueron sacrificados y se diseccionaron las canales de acuerdo con los procedimientos descritos en el Experimento 1. Se calculó el balance de nitrógeno sobre los datos recolectados individualmente, estimando el contenido en N del cuerpo a varias edades usando la fórmula propuesta por Szendro *et al*. (1998). Los datos obtenidos fueron analizados estadísticamente para evaluar el efecto de los tratamientos sobre la retención, ingestión y excreción de nitrógeno. Se realizó un análisis del varianza utilizando el procedimiento GLM (SAS), considerando los efectos principales (nivel de proteína y relación

FD/almidón) y su interacción. El efecto del sexo también se incluyó en el modelo para analizar la variabilidad de los rendimientos productivos, rendimientos en matadero, canal y carne. Los coeficientes de digestibilidad de los nutrientes obtenidos en los dos ensayos de digestibilidad se analizaron como un análisis de varianza de tres vías usando el procedimiento GLM, considerando el nivel de proteína, la relación FD/almidón y la edad, así como sus interacciones como efectos.

El incremento del nivel de proteína de la dieta produjo un aumento significativo en la digestibilidad de los nutrientes y, por ello de su valor energético, excepto para la digestibilidad del extracto etéreo y el almidón. La substitución parcial de alfalfa por harina de soja y girasol y el aumento de la inclusión de cebada y pulpa de remolacha mantuvo constante el nivel de FD y produjo un descenso el nivel de las fracciones fibrosas menos digestibles. Esto cambios pudieron reducir la velocidad de transito y mejorar la digestibilidad. La eficacia digestiva de los nutrientes también mejoró con el aumento de la relación FD/almidón. La reducción de la alfalfa y el incremento de la inclusión de cebada y pulpa de remolacha, a niveles constantes de FD, produjo un aumento de pectinas y una reducción de las fracciones fibrosas menos digestibles (principalmente hemicelulosas) que puede explicar la mejora observada para la digestibilidad.

La digestibilidad de la material seca y de la energía bruta no varió con la edad de los animales. La digestibilidad de la proteína se redujo (de 79,2 a 75,7%, $P<0,001$), mientras que se encontraron mejoras con la edad en la digestibilidad del almidón (de 96,4 a 97,9%, $P<0,001$), de la fibra bruta (de 16,7 a 22,2%, $P<0,001$) y de las del resto de la fracciones fibrosas (FDT, FND, FAD, hemicelulosas), excepto la de la FD.

En el primer periodo de crecimiento (29-50 d de edad), la reducción de la proteína por debajo de 15,5% redujo ($P<0,001$) la velocidad de crecimiento (de 53,1 and 55,1 g/d con dietas con 15,5% y 17% de proteína a 49,3 g/d con dietas con 14% proteína) y el peso vivo a 50 días de edad (1712 y 1755 *vs*. 1631 g), sin cambios en el consumo. En el segundo periodo (50-78 d) no se detectaron diferencias significativas en los rendimientos productivos con el nivel de proteína. En el período global, los animales alimentados con dietas con el menor nivel de proteína mostraron menores rendimientos productivos que los animales alimentados con las dietas de más alto contenido, mientras que los alimentados con dietas de contenidos medios mostraron resultados intermedios, no observándose diferencias significativas respecto a los otros dos grupos. La eficacia alimenticia varió en paralelo a la velocidad de crecimiento, mejorando significativamente, tanto en el primer periodo y en el periodo global, con el incremento de proteína en la dieta, mientras que no hubo efecto en el segundo periodo. El incremento de proteína en la dieta, a pesar de incrementar la retención de nitrógeno, incrementó la excreción nitrogenada de 80,4 a 95,0 y 115,0 g, que corresponde a 1,64, 1,94 y 2,35 g N excretados por día. Cuando estos datos se refieren a las dietas mas altas de proteína (=100) la excreción nitrogenada se redujo en un 17 y 30% en las dietas de contenido medio y bajo en proteína, mientras que el N retenido solo desciende en un 3 y un 6%, respectivamente. La excreción de nitrógeno se redujo más en el primer que en el segundo periodo del cebo.

Cuando se incrementó la relación FD/almidón, mejoró la velocidad de crecimiento durante el periodo post destete, se redujo la ingestión y como consecuencia mejoró la eficacia alimenticia. De 50 d de edad hasta el sacrificio no se observaron diferencias en crecimiento, pero se mantuvieron los efectos sobre el consumo y la eficacia alimenticia. Como consecuencia, el incremento de la relación FD/almidón mejoró el peso vivo a 50 d de edad y, aunque las diferencias no llegaron a ser significativas, al final del ensayo se redujeron el consumo y el índice de conversión (P<0,001) en el conjunto del periodo de cebo. El aumento de la relación FD/almidón también redujo la excreción de nitrógeno (5,5%), pero sin efectos en la retención nitrogenada que mejoró un 2%. El estado sanitario de los animales fue bueno y no se observó efecto de los tratamientos estudiados.

Cuando aumentó el contenido de proteína de la dieta de 14 a 17%, la producción AGV se incrementó y el pH cecal descendió, mientras que no se observaron cambios en la concentración de amoníaco. La mayor inclusión de cebada y pulpa de remolacha, a FD constante, en las dietas con mayor contenido en proteína podrían explicar la mayor actividad fermentativa cecal. Cuando se incrementó la FD a costa de reducir el contenido de almidón de las dietas, el tránsito intestinal pudo descender y el peso del aparato digestivo tendió a incrementarse y, en especial, el ciego. La actividad fermentativa se incrementó, subiendo la concentración de AGV (de 64,0 a 78,4 mmol/l; P<0,001), bajando el pH (de 5,87 a 5,71) y la concentración de amoníaco (de 4,7 a 2,3 mmol/l; P<0,001). La morfología de la mucosa no se vio afectada ni por el nivel de proteína ni por la fibra digestible de la dieta.

Los cambios producidos en el peso al sacrificio con el nivel de proteína de la dieta condujeron a cambios en todas las variables correlacionadas. Así, un incremento del contenido en proteína de la dieta incrementó significativamente el peso vivo al sacrificio y el peso de la canal y tendió (P=0,09) a mejorar el rendimiento a la canal. Otras características de la canal y de la carne no se vieron afectadas. Como para las variables de crecimiento, la relación FD/almidón afectó ligeramente a los parámetros del sacrificio y la canal, con pequeñas variaciones (P<0,10) de la grasa de fácil separación y a la relación musculo/ hueso en el lomo y las patas.

Experimento 4

Niveles de almidón y pectinas en dietas de conejos en crecimiento: efecto sobre la salud, la fisiología digestiva, los parámetros productivos, y la calidad de la canal y de la carne.

Con una edad de 34 d, 240 conejos (827±26g LW) de ambos géneros, de una línea híbrida, se asignaron a seis grupos experimentales (40 animales por grupo). Cada grupo recibió una de las seis dietas experimentales que se suministraron *ad libitum*. Las dietas se formularon de acuerdo con un diseño factorial 3 x 2 con tres niveles de almidón (5, 10 y 15%) y dos niveles de pectinas (5, 10%). El estado sanitario de los animales se controló diariamente para poder detectar tempranamente cualquier enfermedad y especialmente problemas digestivos. Se realizaron ensayos de digestibilidad (60 animales de 52 a 56 d de edad), muestro del ciego y la mucosa intestinal (36 conejos de 51 d), estudios morfológicos de la mucosa y los análisis químicos de los piensos experimentales, heces y ciego siguiendo los procedimientos descritos en el Experimento 1. A los 75 d de edad, los conejos se sacrificaron y la canal se diseccionó siguiendo los procedimientos descritos anteriormente. El análisis de varianza de los datos se realizó utilizan el procedimiento GLM, considerando como efectos principales los niveles de almidón y pectinas y las interacciones. Se incluyó el efecto del sexo en el modelo para analizar los datos de crecimiento, sacrificio y los parámetros de canal y carne.

El incremento del contenido en almidón de las dietas mejoró la digestibilidad de la materia seca y de los nutrientes (de 54,7% a 62,9%). La inclusión de altas cantidades de pulpa de remolacha, mayores del 30%, en substitución de la alfalfa en las dietas altas en pectinas, mejoró la digestibilidad de la fibra bruta (de 8,1 a 26,4%) y de todas las fracciones fibrosas.

Las dietas con valores extremos de la relación pectinas/almidón (0,5 vs. 2,0) mostraron valores energéticos similares (ED: 9,5 MJ/kg). Con la misma cantidad de alfalfa, la sustitución de cebada por pulpa de remolacha mantuvo la digestibilidad de la dieta en valores elevados. El almidón de la cebada fue reemplazado por fibra digestible (especialmente pectinas) de la pulpa de remolacha. La digestibilidad de las fracciones fibrosas cambió de acuerdo con los cambios en el nivel de pectinas. En las dietas con 5% de pectinas, la digestibilidad de la fibra bruta varió poco (de 7,2% a 9,6%, $P>0,05$), mientras que esta variación fue mayor en las dietas con un 10% de pectinas para las distintas fracciones fibrosas: la digestibilidad se incrementó para la fibra bruta (de 23,5 a 30,3%), FND (de 34,3 a 45,1%), FAD (de 25,7 a 32,1%) y hemicelulosas (de 45,4 a 60,8%), sin cambios significativos para la digestibilidad de las pectinas.

El incremento del nivel de almidón aumentó significativamente el peso final a 75 d y la velocidad de crecimiento y redujo la ingestión y el índice de conversión (de 3,52 a 3,31 y 3,13, $P<0,001$) en el período global del cebo. Sin embargo, en el período post destete la velocidad de crecimiento fue mayor (P<0,001) y el consumo tendió a ser menor (P=0,06) con las dietas altas en almidón. En el segundo período el crecimiento los resultados fueron similares entre tratamientos pero la ingestión se redujo (de 172 a 159 g/d; P<0,001), probablemente por una mejor regulación del apetito.

El nivel de pectinas en la dieta afectó significativamente a los parámetros de crecimiento: cuando se incrementó el nivel de pectinas mejoró la velocidad de crecimiento, especialmente en la etapa post destete, se redujo la ingestión y mejoró el índice de conversión (3,59 vs. 3,05; P<0,001). El peso final fue mayor in los animales alimentados con dietas altas en pectinas (10%) comparado con aquellos que recibieron dietas de menor contenido (5%), tanto a 55 d de edad como al final del cebo.

El estado de salud de los animales fue bueno, sólo dos animales murieron. La fermentación cecal se vio afectada por los tratamientos: la concentración de amoníaco fue mayor en los animales alimentados con dietas bajas en almidón, probablemente por una mayor ingestión de proteína (PD/ED: 12,9, 12,2 and 11,6 g/MJ respectivamente para dietas con 5, 10, y 15% de almidón). Con el aumento del almidón no hubo cambios en la concentración total de AGV, pero, inesperadamente, tendió a incrementarse (P=0,12) la proporción de acético y se redujo la de propiónico (P<0,01). La composición química de las dietas puede explicar los resultados obtenidos en este ensayo: 1) dietas con alto contenido en almidón (cerca de 16%, como media de las dietas L1 y H1) parecen compatibles con la capacidad digestiva del animal a la edad considerada (51 d) y no produjeron un flujo significativo de almidón sin digerir al ciego; 2) además, las dietas que tuvieron un contenido en DF similar (24,7, 23,6 y 23,8% para las dietas con 5, 10 y 15% almidón) para asegurar una actividad fermentativa comparable mostraron una fermentación mas orientadas a la producción de acético que a la de butírico y propiónico. El aumento del nivel de pectinas redujo el pH (P=0,02), pero tuvieron poco efecto sobre las proporciones de propiónico y butírico. Con la administración de dietas con 10% de pectinas, sólo la concentración total de AGV tendió a incrementarse (73,8 *vs*. 82,8 mmol/l; P=0,10) y la proporción de valérico a disminuir (0,43 *vs*. 0,35%; P<0,01).

La morfología de la mucosa del yeyuno y del íleon no mostró cambios significativos, sólo se observó una menor longitud de los villi (P=0,09 y 0,10), tanto en yeyuno como en íleon, en animales alimentados con dietas de contenido intermedio de almidón (10%) respecto a la niveles mas bajos (5%) o mas altos (15%).

El efecto del nivel de almidón en la dieta parece estar ligado a las diferencias que se producen en el peso al sacrificio. Los animales de mayor peso, que recibieron dietas con más alto contenido en almidón, mostraron mayores pesos al sacrificio y de la canal y mayores rendimientos y menores pérdidas en el sacrificio y mayores proporciones de grasa diseccionable (P<0.01). El efecto de incrementar el contenido en pectinas fue pequeño y el aumento del peso al sacrifico (2628 *vs*. 2687 g; P<0,05) no estuvo asociado a un mayor rendimiento a la canal, debido a que el peso de aparato digestivo fue mayor en las dietas altas en pectinas respecto a las de menor contenido (18,5 *vs*. 19,0%, P<0,05).

Los tratamientos no afectaron a la calidad de la carne en lo referente a pH o el color medidos en los músculos *longissimus lumborum* y *biceps femoris* 24 horas tras el sacrificio.

Discusión general

En todos los experimentos donde se incrementó la relación FD/FAD, con niveles similares de almidón, se realizó sustituyendo alfalfa por pulpa de remolacha esto conllevó una mejora de la **digestibilidad** de la material seca, energía bruta, fibra bruta y demás fracciones fibrosas. La digestibilidad aparente fecal de la fracción mas digestible y soluble (como la contenida en la pulpa de remolacha) puede llegar a ser del 60-70% mientras que las fracciones mas insolubles, principales constituyentes de la fibra de la alfalfa, varia entre 15-30% (Carabaño *et al.*, 2001; García *et al.*, 2009).

La digestibilidad y el valor energético de las dietas con mayores relaciones FD/FAD se incrementa, no sólo por la elevada presencia de constituyentes característicos de la fibra digestible, sino también por la mayor digestibilidad (9-10 puntos) de todas las fracciones fibrosas. De manera diferente, cuando la FD reemplaza al almidón (relación FD/almidón desde 0,8 hasta 2,8), con cambios limitados en el nivel de FAD (FD/FAD de 0,9 a 1,2) (Experimentos 1, 2), la digestibilidad de la materia seca y de la energía bruta y, por lo tanto, el valor energético no varían, ya que la energía suministrada por la FD fue similar a la aportada por el almidón como ya ha sido mencionado por otros autores (De Blas y Carabaño, 1996; Gidenne y Bellier, 2000). En los experimentos 3 y 4, el valor energético aumentó cuando la relación FD/almidón aumentó de 1,1-1,7 a 1,9-4,2. El aumento de la relación FD/FAD (de 0,9-1,0 a 1,4-1,5) con bajos contenidos en pulpa de remolacha en dietas con la relación FD/almidón más baja (0% de inclusión en el Experimento 3 en comparación con un máximo del 33-34%) podrían explicar estos resultados. En el Experimento 4, las dietas con valores extremos de la relación pectinas/almidón (5% pectinas, almidón 15%=0,5 y 10% pectinas, almidón 5%=2) mostraron una digestibilidad y un valor energético similares: con la misma inclusión de alfalfa, la sustitución de cebada por pulpa de remolacha dio lugar a altas digestibilidades. La dieta con los niveles mas altos de FD y almidón mostraron el valor energético mas elevado.

Comparando distintas fuentes de proteína, los animales que consumieron las dietas con harina de girasol mostraron menores digestibilidades de la proteína que las dietas con harina de soja

(Experimento 1), de acuerdo con lo observado anteriormente Maertens *et al.* (2002). El tipo de proteína no afectó ni al patrón de fermentación cecal ni al estado sanitario o los resultados de crecimiento o sacrificio.

El aumento del nivel de proteína (de 15 a 16%, Experimento 2, y de14 a 17%, Experimento 3) mejoró la digestibilidad de los nutrientes y de la proteína como consecuencia de la sustitución de proteína de alfalfa por la de harina de soja y girasol.

Los efectos de los tratamientos sobre los **parámetros de crecimiento** dependieron del valor energético de las dietas. Un aumento de la relación FD/FAD, y por lo tanto del valor energético de las dietas, produjeron un descenso en el consumo para mantener un consumo energético constante (Gidenne y Lebas, 2005; Xiccato y Trocino, 2010b) y una mejora en el índice de conversión (especialmente en el primer periodo del cebo) sin afectar a la velocidad de crecimiento o al peso final de los animales (Experimento 1). La mejora del índice de conversión con el aumento de la FD puede estar relacionado con un menor tránsito digestivo y un aumento de la digestibilidad de las dietas como ya ha sido observado en estudios previos (Xiccato *et al*., 2006a, 2008; Carraro *et al*., 2007; Fragkiadakis *et al*., 2007). En el Experimento 2, un aumento de la relación FD/almidón tuvo pocos o ningún efecto sobre los parámetros de crecimiento. También García *et al*. (1993) observaron que la sustitución de cebada por pulpa de remolacha no empeoró los resultados de crecimiento debido a que ambas materias primas tuvieron un valor similar en energía.

En el Experimento 3, durante el periodo post destete, un aumento de la relación FD/almidón produjo un aumento de la velocidad de crecimiento y una reducción del consumo y, como consecuencia, una mejora en el índice de conversión. Durante el periodo final de cebo no se observaron diferencias entre tratamientos. En el Experimento 4, el aumento de pectinas en la dieta del 5 al 10% (con relaciones FD/almidón entre 2.4 y 3.1) mejoró el crecimiento (especialmente en post destete) y el peso vivo final de los animales, se redujo el consumo y mejoró el índice de conversión. El incremento de la relación FD/almidón también redujo la excreción de N, sin afectar a la retención corporal de N. Este efecto fue más notable durante la primera parte del cebo que en el periodo final (Experimento 3).

Una reducción del nivel de proteína por debajo de 15% empeoró la velocidad de crecimiento y el peso vivo en el periodo post destete (Experimentos 2 y 3). Estos resultados están en línea con trabajos previos que sugieren que el contenido en proteína de las dietas comerciales para el post destete y el periodo final del cebo son mayores que las necesidades de los animales (Maertens *et al.*, 1997; Trocino *et al.*, 2000, 2001; García-Palomares *et al.*, 2006a, 2006b; Eiben *et al.*, 2008). En animales sacrificados a 63 d de edad y 2,35 kg de peso vivo, una reducción del nivel de proteína del pienso del 16 al 14% no afectó al crecimiento (García-Palomares *et al.*, 2006b). En animales sacrificados mas tarde (75-90 d) y con mas peso (2,5-3,0 kg), un descenso de proteína en el segundo periodo del cebo respecto a los suministrados en el post destete, permitió satisfacer mejor las necesidades de ambos periodos y reducir la excreción de N en la fase final. De hecho, durante el periodo final la ingestión es mayor y los niveles de proteína del pienso se pueden reducir sin consecuencias negativas sobre los rendimientos o la calidad de la carne (Maertens *et al.*, 1997; Maertens y Luzi, 1998; Trocino *et al.*, 2000, 2001).

El equilibrio de las **fermentaciones cecales** se considera un indicador clave de los posibles efectos de los nutrientes sobre la salud digestiva. En el Experimento 1, un aumento de la relación FD/FAD (de 1,0 a 1,2) no modificó la concentración de AGV aunque cuando se redujo el pH. Esta variación, no asociada a un incremento en la concentración de AGV fue también observada por Carabaño *et al*. (1997) y podría considerarse como favorable para el mantenimiento del equilibrio de la microbiota y la fermentación cecal. Un aumento de la FD junto con un descenso en el contenido de almidón puede ralentizar el tránsito intestinal y producir retenciones de digesta en el ciego aumentando los problemas intestinales. Como consecuencia, la fermentación se estimula, la concentración de AGV y acético aumentan mientras que la de valérico, asociado generalmente con la actividad de las bacterias amilolíticas, el pH y la concentración de amoníaco también descendió (Experimentos 2 y 3). Un aumento de la concentración de pectinas (Experimento 4) redujo el pH cecal, tendió a aumentar la concentración de AGV totales y redujo la proporción de valérico. Estudios previos han mostrado que un aumento de la fibra digestible y/o soluble aumenta la actividad fermentativa (García *et al.,* 2000; Falcao-e-Cunha *et al.,* 2004). Los bajos niveles de amoníaco observados en todos los experimentos y la tendencia a reducirse con el aumento de la relación FD/almidón sugieren que la FD hidratos de carbono que favorece la fijación de amoníaco en la proteína bacteriana.

Un punto de diferencia en la proteína de la dieta (de 15 a 16%) no fue suficiente para afectar la actividad fermentativa cecal (Experimento 2), mientras que cuando la proteína subió de 14 a 17%, se estimuló la actividad fermentativa (Experimento 3). La mayor inclusión de cebada y pulpa de remolacha, con niveles constates de FD, pueden también explicar la mayor actividad fermentativa cuando los animales consumen dietas con mayores contenidos en proteína.

En todos los experimentos de la presente tesis, se puso gran atención y una parte importante del trabajo para evaluar las condiciones de la **mucosa intestinal** para evaluar el estado sanitario y/o la susceptibilidad de enfermedad. Los estudios previos habían mostrado que, mas que un descenso del contenido en FND, el incremento en las fracciones mas solubles de la fibra pueden favorecer la integridad de la mucosa, reducir la mortalidad y mejorar el crecimiento de los animales (Gutiérrez *et al.,* 2002; Feugier *et al.,* 2006; Álvarez *et al.,* 2007; Gómez-Conde *et al.,* 2007). En la presente tesis, el aumento de la relación FD/FAD (Experimento 1), FD/almidón (Experimentos 2, 3 y 4) y la concentración de pectinas (Experimento 4) no produjeron ningún cambio importante en la estructura de la mucosa intestinal, tanto en el íleon como en el yeyuno. Sólo en el Experimento 4, la altura de los villi, en los dos tramos estudiados, tendieron a ser mas cortos (P=0,09 y 0,10) cuando los animales consumieron dietas con los contenidos en almidón (10%) recomendados para dietas de post destete que con dietas de niveles de inclusión mayores (15%) o menores (5%). La menor profundidad de las criptas observada con el aumento del contenido en proteína de la dieta (Experimento 2) puede estar asociada a una menor capacidad de la mucosa de reparar los daños en los villi y por tanto, indirectamente, podrían explicar la mayor susceptibilidad a la aparición de problemas digestivos observada con dietas altas en proteína. Además, trabajos previos han mostrado efectos limitados o nulos de la fuente de proteína, aunque las dietas con proteína mas digestibles a nivel ileal disminuyan la mortalidad debida a la EEC (Gutiérrez *et al.,* 2002, 2003; Chamorro *et al.,* 2007).

La inclusión en la dieta de plasma en lugar de soja mejora la integridad de la mucosa en gazapos destetados tempranamente (Gutiérrez *et al*., 2000), sugiriéndose que los factores antinutritivos de las leguminosas podrían tener efectos negativos (Gutiérrez *et al*., 2003; Cano *et al*., 2004).

La relativa uniformidad en el equilibrio cecal y la estructura de la mucosa fue acompañada por un relativamente buen **estado sanitario** de los animales en todos los experimentos. De hecho, estas buenas condiciones sanitarias no permitieron valorar correctamente el efecto de los tratamientos sobre la condición sanitaria o sobre la posibilidad de reducir y controlar la difusión e importancia de los trastornos digestivos por la EEC. Un aumento de la relación FD/FAD (Experimento 1), FD/almidón y el nivel de pectinas (Experimentos 3 y 4) no afectó significativamente a la salud de los conejos. Sólo en el Experimento 2, a pesar de tener una baja mortalidad y morbilidad, se observó un efecto significativo de los tratamientos: las dietas con la menor relación FD/almidón mostraron un aparición aguda de enteropatía y un aumento de la mortalidad, aunque los animales afectados se recuperar más rápido que en otros grupos. Las mayores tasas de mortalidad y de riesgo sanitario se asociaron con dietas con una relación FD/almidón bajas y altos niveles de proteína in comparación con otros tratamientos (Experimento 2). Estudios previos han observado un efecto positivo de aumentar la relación FD/FAD (Gómez-Conde *et al.,* 2004; 2007; Xiccato *et al.,* 2006a; Carraro, 2006) o reemplazar el almidón o la proteína por FD in dietas iso-FAD (Perez *et al.,* 2000; Soler *et al.,* 2004) sobre las condiciones sanitarias (reducción de la mortalidad) de los animales con enteropatía o trastornos digestivos. De hecho, un exceso de proteína en la dieta podría empeorar la salud de los animales, alterando el equilibrio cecal y promoviendo la utilización de la proteína para la producción de energía, aumentar la concentración de amoníaco y el pH, y favorecer el desarrollo de bacterias patógenas (Lebas *et al.*, 1998). En este sentido, la mortalidad causada por la EEC se reduce (Gutiérrez *et al*., 2002, 2003; Chamorro *et al*., 2007) cuando se reduce el nivel de proteína o se incrementa su digestibilidad ileal o incrementándose la fibra digestible (Xiccato *et al*., 2006a; Gómez-Conde *et al*., 2007).

En los cuatro ensayos, los tratamientos ensayados casi no tuvieron efectos sobre los **resultados del sacrificio y las características de la canal y la carne**, confirmando el escaso papel de la nutrición sobre estas variables cuando los animales son alimentados *ad libitum* con dietas equilibradas y no hay diferencias en el peso final de los animales (Xiccato, 1999; Hernández y Gondret, 2006; Hernández, 2008; Xiccato y Trocino, 2010b). Sólo la administración de dietas con diferentes niveles o tipos de grasa podrían cambiar apreciablemente las características de la canal y la carne (Hernández, 2008). Solamente cuando el nivel de proteína de la dieta afectó al peso final (dietas con los niveles más bajos de proteína, 14-15%, Experimentos 2 y 3) se detectaron algunas diferencias significativas, confirmando los resultados de Maertens *et al*. (1997) quienes observaron peores resultados en matadero con dietas por debajo del 13% de proteína.

Conclusiones

De los resultados obtenidos se pueden sacar las siguientes conclusiones teniendo en cuenta el objetivo general de esta tesis que fue: definir las necesidades nutricionales de los conejos en el período post destete y final del cebo, con especial énfasis en las diferentes fracciones fibrosas, sobretodo las mas digestible (pectinas, hemicelulosas), el contenido en almidón en relación a la fibra y el suministro de proteína con diferentes objetivos:

***1*) mejorar las condiciones intestinales de los conejos**. Dentro de los intervalos estudiados, los cambios en FD, FAD, almidón y proteína pueden afectar a las condiciones intestinales y el equilibrio cecal: el aumento de la FD a expensas del almidón o el aumento la proteína, en dietas con cantidades apreciables de FD, estimula la fermentación cecal, limita la concentración de amoníaco y cambia el pH cecal, creando un medio favorable a la población bacteriana. El nivel de almidón, FD y FAD, no afectó a la morfología y la integridad de la mucosa intestinal.

***2)* reducir la incidencia y severidad de los trastornos digestivos**. Los experimentos se realizaron bajo condiciones óptimas de salud. Como consecuencia, a pesar de la amplia variación en la composición química de las dietas experimentales no se detectaron efectos sobre la salud de los animales. Por lo tanto, no fuimos capaces de evaluar adecuadamente el efecto de los tratamientos en una granja afectada por la EEC. Sin embargo, si se puso de manifiesto que dietas con bajas relaciones FD/almidón aumentaron la mortalidad y el tiempo de recuperación de los animales afectados, que fue más rápida. Esta tendencia se refuerza si las dietas con una baja relación FD/almidón (<1) se asocian con dietas altas en proteína (>16%). Por ello la recomendación es suministrar dietas con una relación FD/almidón alta (1-1,5) y niveles moderados en proteína (entorno al 15%). Sustituyendo almidón por FD se mantiene el nivel energético con un bajo flujo de proteína no digerida al ciego, que puede reducir el riesgo sanitario.

3) **incrementar la eficiencia alimenticia y la retención de nitrógeno**. El aumento de FD a expensa de las fracciones fibrosas menos digestibles o almidón (con niveles constantes en FAD) mejora la conversión alimenticia, reduciendo la ingestión, y permite una apreciable reducción de la excreción nitrogenada. El almidón y la fibra digestible pueden considerarse como alternativas en la alimentación del conejo y potencialmente aditivas: la asociación de altos niveles de almidón y FD pueden dar aun mejores resultados de conversión alimenticia y rentabilidad.

4) **garantizar altos rendimientos y la calidad del producto final**. Incrementar la relación FD/FAD mejora el valor energético de la dieta y su eficiencia de utilización para el crecimiento. Incrementar la relación FD/almidón no modifica el valor energético de la dieta y, en la mayor parte de los casos, tampoco el crecimiento o los resultados del sacrificio. En algunos casos, el crecimiento puede mejorarse, especialmente durante las primeras semanas del cebo, cuando los animales todavía están desarrollando su capacidad digestiva.

Para maximizar los rendimientos de crecimiento, los niveles de proteína no deberían reducirse por debajo del 15,5% en las primeras semanas del cebo, ya que el crecimiento compensatorio posterior no permite recuperar las pérdidas de peso. La proteína de la dieta debe ser controlada tanto en la primera como, especialmente, en la última parte del cebo donde las necesidades de proteína son menores, para mejorar la eficacia nitrogenada y limitar su excreción.

Los cambios en FD, almidón o proteína producen diferencias en los resultados del sacrificio si son capaces de modificar el peso final y, por lo tanto, los parámetros de la canal relacionados con el peso vivo. Las características de la carne, pH y color, de los principales músculos no variaron con los tratamientos estudiados o en una extensión apreciable desde un punto de vista comercial.

SUMMARY

The role of digestible fibre, starch and protein on health status and performance in diets for growing rabbits

Introduction and objectives

Since 1996, the spread-out of Epizootic Rabbit Enteropathy (ERE) in European rabbit breeding stock has remarkably increased mortality and losses and impaired feed conversion, due to the large number of morbid animals. The lack of identification of an etiological agent responsible for the syndrome and the attribution of a multifactorial character to it, as well as the limitations imposed by EU legislation on the use of antibiotics, have stimulated research on feeding and management strategies capable of preventing or at least limiting the impact of enteropathy. In particular, studies in the past few years intended to specify the nutritional requirements in rabbits during weaning and post-weaning, in relation to the possibility of reducing the damages caused by enteropathy.

Among the various nutrients, the most digestible fibre fractions (DF: pectins and hemicelluloses) seem to favour the health status of the animals, in addition to enhance caecal the fermentative activity and feed efficiency (Gidenne and García, 2006). By increasing soluble fibre level in starter weaning diets, *C. perfringens* and other opportunistic pathogens at caecal level were reduced, followed by the mortality caused by diarrhoea (Soler *et al.,* 2003; Gómez-Conde *et al*., 2007). By keeping a constant ADF content and replacing starch and protein by digestible fibre (Perez *et al*., 2000; Gidenne *et al*., 2001; Marguenda *et al*., 2006), digestive disorders decreased and rabbit health status improved. Reducing insoluble fibre (Gutiérrez *et al*., 2002; Alvarez *et al*., 2007) and increasing the more soluble fractions (García-Ruiz *et al*., 1997; Carabaño *et al*., 2008) had also positive effects on intestinal mucosa integrity.

Few are yet known nowadays on the interaction between digestible fibre and protein. Lack (<12%) or excess of protein (>18%) may favour digestive disorders and mortality, modifying caecal fermentative activity and microflora composition (Maertens e De Groote, 1988; Lebas, 1989; Carabaño *et al*., 2008, 2009). Reducing protein level from 18 to 16% (in diets with the same ileal digestibility) significantly reduced the presence of *Clostridium perfringens* and mortality by epizootic rabbit enteropathy (Chamorro *et al*., 2007), while further reduction from 16 to 14% did not have any effect on mortality, though reducing anaerobic bacteria at ileum (García-Palomares *et al*., 2006a, 2006b; Carabaño *et al*., 2009). The use of less digestible protein sources may also increase the nitrogen flux at caecal level and favour the development of pathogens such as *E. coli* and *Clostridia spp*, thus impairing animal health and performance (Gutiérrez *et al.*, 2003; Chamorro *et al.*, 2005, 2007; Carabaño *et al.*, 2008, 2009). Given the late attention of the mass media and consumers on the use of not genetically modified raw materials, a certain interest exists in evaluating protein sources alternative to soybean, which is certainly not modified biotechnologically. Among these, sunflower meal is widely used in rabbit nutrition, usually combined with soybean meal. The use of diets based only on sunflower meal demands a careful evaluation of the aminoacidic equilibrium.

The experimental activities realized in the frame of the present PhD thesis followed the general objectives of defining rabbit nutritional needs during post-weaning and fattening with special regards to the different fibrous fractions, mostly the more digestible (pectins, hemicelluloses), the starch content in relation to fibre, as well as the dietary protein supply with the aim of *1*) improving rabbits intestinal conditions *2)* reducing the incidence and severity of digestive pathologies *3)* increasing feed efficiency, and *4)* guaranteeing high growth performance and final meat quality. The objectives of the present thesis were pursued along the following four studies.

Experiment 1

Digestible fibre level and substitution of soybean with sunflower meal in diets for growing rabbits.

At 34 d, 216 rabbits (837±48 g LW) of both genders from a hybrid line were divided in six experimental groups of 36 units and fed *ad libitum* with six isoproteic diets (15.9% CP) formulated according to a 2 x 3 factorial design, with two protein sources (soybean *vs*. sunflower meal) and three levels of DF to ADF ratio (1.0, 1.1 and 1.2). The apparent digestibility of nutrients and the digestible energy (DE) concentration of the diets were determined *in vivo* (Perez *et al.*, 1995). Caecal contents and intestinal mucosa for villi and crypts measurements were sampled at 56 d of age. Faeces and feed samples were analysed according to A.O.A.C methods (2000) and the European harmonized procedures EGRAN (2001). The content of Total Dietary Fibre (TDF) was determined through a gravimetric-enzymatic procedure that requires the treatment with α-amylase, protease and aminoglucosidase (Megazyme Int. Ireland Ltd., Wicklow, Ireland). The content of digestible fibre (DF) was calculated as the difference between the TDF and the ADF content, thus theoretically including, pectins and hemicelluloses. Volatile fatty acid concentration was measured on the supernatant by gas chromatography using the method of Osl (1988). At 76 d of age, rabbits were slaughtered in a commercial slaughterhouse and carcasses were dissected at the Department according to the international scientific protocols (Blasco *et al.*, 1993). The pH (Xiccato *et al.*, 1994) and the colour (CIE, 1976) of *longissimus lumborum* and *biceps femoris* were measured. The data were analyzed by ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC) and considering the effects of DF to ADF ratio and of the protein source. The effects of gender was also included in the model to analyze variability of data of growth performance, slaughter results, carcass and meat traits. Mortality, morbidity and sanitary risk were analyzed by the CATMOD procedure of SAS.

The digestibility of dry matter, gross energy, crude fibre and fibrous fractions significantly increased with DF to ADF ratio, due to the higher inclusion of beet pulp and the lower inclusion of alfalfa. The digestibility and nutritive value of the diets increases with DF to ADF ratio not only for the higher presence of digestible fibre constituents, but also for the increased digestibility (9-10 points) of all fibre fractions: ADF digestibility increased from 14.6 to 25.6%; hemicelluloses digestibility from 40.3 to 49.1%; pectins digestibility from 85.0 to 93.8% ($P<0.01$). This increment may be associated with the lower grade of lignification and complexity of cell walls and to the higher susceptibility of structural carbohydrates to the action of digestive enzymes, animal or bacterial ones. Protein source had a more limited effect on the digestibility of nutrients. In the sunflower diets, crude protein digestibility tended to decrease ($P=0.06$), consistently with the lower digestibility of crude protein in sunflower meal rather than soybean meal. Ether extract and hemicelluloses digestibility were higher ($P<0.001$) in sunflower diets than in soybean diets, while ADF and pectins were less digestible. Nutritive value of diets was moderate and increased by DF to ADF ratio (from about 9-10 MJ/kg) regardless to the protein source and follows the current recommendations for rabbit nutrition during post-weaning.

During the trial, sanitary problems were limited and unaffected by the feeding treatment. Nor intestinal mucosa traits or caecal fermentation activity were influenced by feeding treatments, while pH of caecal content was significantly reduced by increasing the DF/ADF ratio ($P=0.04$). By increasing DF/ADF ratio and the nutritive value of the diets, daily growth rate, live weight of rabbits and, consequently, slaughter results did not vary, while feed consumption tended to decrease, mainly in the first period of the trial, from 34 to 55 days of age, and feed conversion linearly improved (from 3.55 to 3.42 and 3.30 in the whole trial; $P<0.001$). Neither growth performance nor slaughter results and meat traits were affected by protein source.

Experiment 2

Digestible fibre/starch ratio and protein level for feeding growing rabbits.

At 32 d, 246 rabbits (837±48 g LW) of both genders from a hybrid line were divided in six experimental groups of 36 units and fed *ad libitum* with six diets formulated with a constant ADF level (18%), but differing in protein levels (15 *vs.* 17%) and DF/starch ratio (0.8, 1.5 and 2.8), according to a bi-factorial arrangement. The *in vivo* digestibility trial (on 60 animals from 52 to 56 d of age), the sampling of caecal content and intestinal mucosa (on 36 rabbits at 55 d), histological analyses of gut mucosa and chemical analyses of experimental diets, faeces and caecal content as well as slaughter and dissection procedure and meat quality analyses were performed according to the methodologies detailed in the first experiment. The data were analyzed by ANOVA using the GLM procedure and considering the effects of DF/starch ratio and protein level. Sex effects were also included in the model to analyze variability of data of growth performance, slaughter results, carcass and meat traits.

Increasing dietary protein significantly improved dry matter (about +1 point) and nutrient digestibility. The digestible energy content varied little depending on dietary protein, however, and, therefore, DP/DE ratio was higher in diets at 16% CP than those at 15% CP. In both cases, protein supplies satisfy requirements of growing rabbits (De Blas and Mateos, 1998). Increasing DF/starch ratio, starch apparent digestibility significantly decreased (although to a low extent in absolute value), while greatly increased fibre and fibrous fractions digestibility. Dry matter and gross energy digestibility did not change significantly, however. The nutritive value of diets, that is the digestible energy (DE) content, did not change with DF/starch ratio, since energy supplied by DF was similar to that provided by starch within constant crude fibre and NDF levels. Since hemicelluloses did not change appreciably, this result may be largely ascribable to the complete digestibility of pectins (100%) and, to a lesser extent, to the general increase in digestibility of all fibrous fractions, also the less digestible ones (e.g. ADF).

Even though mortality and morbidity were low during the trial, a significant effect of dietary treatment was detected. Mortality and sanitary risk tended (P=0.10-0.11) to increase in rabbits fed with the highest protein content diets. Lower DF/starch ratio diets provoke acute ERE and increased mortality (10 *vs*. 1.4 and 1.4%, P=0.04), though affected animals recovered faster, so the sanitary risk did not differ among the three groups. Rabbits fed the diet with the lowest DF/starch and highest protein level showed significantly higher mortality (17.1 vs. 1.5%) and sanitary risk (20.0 vs. 8.1%) compared to the other groups.

The protein level did not affect neither filling of single compartment nor caecal fermentation activity, while crypt depth (P=0.02) and villi/crypt ratio (P=0.11) decreased with low protein diets. Increasing DF/starch ratio did not modify any morphometric variable, while increased the total amount of volatile fatty acids, even though at a non-significant level, and rose (P=0.02) the acetate proportion, main fermentation product, while reduced valerate proportion (P<0.01).

Growth performance was satisfying for the slaughter age and the genetic type used, but not significantly affected by the experimental treatments, which only modified minor traits of carcasses and meat quality.

Experiment 3

Reducing dietary protein and increasing digestible fibre/starch ratio in diets for growing rabbits.

At 29 d, 282 rabbits (596±58 g LW) of both genders from a hybrid line were divided in six experimental groups of 47 units and fed *ad libitum* with six diets formulated with similar content of ADF (18%) and differing in crude protein level (15.0, 15.5, 17.0% CP) and DF/starch ratio (1.1, 2.0), according to a bi-factorial arrangement. Two *in vivo* digestibility trials were performed on 60 animals (10 per diet) from 35 to 39 d and from 56 to 60 d of age; at 38 d caecal content and jejunum mucosa were sampled from 36 rabbits (6 animals per group). Digestibility trials, caecal and gut mucosa sampling, histological analysis and chemical analyses of experimental diets, faeces and caecal contents were performed according to the methodologies previously described. At 78 d of age, rabbits were slaughtered and carcasses dissected according to the procedures described above. Nitrogen balance was also calculated on data recorded individually estimating the body N content at the various ages using the formula proposed by Szendro *et al*. (1998). The same data were statistically analysed in order to evaluate the effect of dietary factors on nitrogen retention, intake and excretion. The data were analyzed by ANOVA using the GLM procedure and considering the effects of protein level, DF/starch ratio as well as their interaction. The effects of sex was also included in the model to analyze variability of data of growth performance, slaughter results, carcass and meat traits. The digestibility coefficients of nutrients measured in the two digestibility trials were analyzed by a three-way ANOVA using the GLM procedure and considering the effects of age, protein level, DF/starch ratio as well as their interactions.

Increasing the protein level significantly increased nutrients digestibility, and thus the nutritive value, but decreased ether extract and starch digestive utilization. The partial replacement of alfalfa by soybean and sunflower meal and the increase in barley and dried beet pulp maintained a constant digestible fibre level and decreased the less digestible fibrous fractions, thus reducing the feed transit and increasing the digestibility. Nutrient digestive efficiency significantly improved by increasing DF/starch ratio. The reduction of alfalfa, the increase of barley and dried beet pulps, at constant digestible fibre content, may explain the increase in digestibility due to the slower feed transit caused by the reduction of less digestible fibre (mainly hemicelluloses) and the increase of pectins content.

Dry matter and gross energy digestibility did not change with the age of animals. Digestive utilization of crude protein significantly decreased (from 79.2 to 75.7%, P<0.001), while digestibility of starch (from 96.4 to 97.9%, P<0.001), crude fibre (from 16.7 to 22.2%, P<0.001) and of fibrous fractions (TDF, NDF, ADF, hemicelluloses) increased, except for DF.

In the first period of growth (29-50 d of age), reducing the dietary protein below 15.5% significantly reduced (P<0.001) daily weight gain (from 53.1 and 55.1 g/d in diets with 15.5% and 17% protein to 49.3 g/d in diets with 14% protein) and live weight at 50 d of age (1712 and 1755 *vs*. 1631 g), without affecting feed intake. In the second period (50-78 d) performance was not influenced by protein level. In the overall period, rabbits fed low-protein diets showed lower growth performance than those fed high-protein diets; while those fed medium-protein diets did not differ significantly from the other groups. Feed conversion varied according to the growth rate and significantly improved in the post-weaning and whole period by increasing protein, while was not influenced in the second period. When increasing dietary protein, despite stimulating nitrogen retention, total nitrogen excretion increased from 80.4 to 95.0 and 115.0 g, corresponding to 1.64, 1.94 and 2.35 g N excreted per day. When referring to high-protein diets (=100), nitrogen excretion was reduced by 17 and 30% by decreasing the protein level in medium- and low-protein diets, while retained N decreased only by 3 and 6%, respectively. The N excretion was reduced more in the first rather than in the second period of growth.

By increasing DF/starch ratio, daily growth rate during post-weaning was significantly stimulated, feed intake reduced and as a consequence feed conversion improved. From 50 days of age until slaughter, growth was similar, but the effects on feed intake and conversion remained evident and significant. As a consequence, higher DF/starch ratio increased live weight at 50 days of age, even though the differences were no longer significant at the end of the trial, reducing feed intake and conversion (P<0.001) in the whole experiment. Increasing DF/starch ratio also reduced N excretion by 5.5%, but without effecting body N retention that improved by 2%. Health status was satisfactory in all groups and not influenced by feeding treatments.

By increasing the protein content from 14 to 17.0%, VFA production increased and caecal pH decreased, while ammonia did not change. Higher inclusion of barley and dried beet pulps, at constant DF content, in diets with higher protein content may explain the more intense caecal fermentative activity. By increasing DF and reducing starch, intestinal transit got slower and gut incidence tended to increase, mainly due to caecal filling. Caecal fermentative activity increased together with VFA production (from 64.0 to 78.4 mmol/l; P<0.001) reducing pH (from 5.87 to 5.71) and ammonia production (from 4.7 to 2.3 mmol/l; P<0.001). Jejunum mucosa morphometry was not influenced by dietary protein or digestible fibre level.

Protein effect on live and slaughter weight reflected changes in all correlated variables: increasing dietary protein content significantly increased live weight and carcass weight and tended (P=0.09) to improve slaughter dressing percentage. Other carcass and meat characteristics were not affected. Regarding growth performance, DF/starch ratio slightly affected slaughter and carcass traits, with small variations (P<0.10) of dissectible fat and hind leg muscle/bone ratio.

Experiment 4

Starch and pectin levels in diets for growing rabbit: effects on health status, digestive physiology, growth performance and carcass and meat quality.

At 34 d, 240 rabbits (827±26 g LW) of both genders from a hybrid line were divided in six experimental groups of 40 units and fed *ad libitum* with six diets formulated according to a bi-factorial design 2 x 3, with three levels of starch (5, 10 and 15%) and two levels of pectins (5 and 10%). Health status was controlled daily to detect promptly the occurrence of diseases, especially digestive problems. The *in vivo* digestibility trial on 60 animals from 52 to 56 d of age, the sampling of caecal content and intestinal mucosa on 36 rabbits at 51 d, histological analyses of gut mucosa and chemical analyses of experimental diets, faeces and caecal content were performed according to the methodologies previously described. At 75 d of age, rabbits were slaughtered and carcasses dissected following procedures described above. The data were analyzed by ANOVA using the GLM procedure and considering the effects of starch and pectins level, as well as their interaction. Sex effects were also included in the model to analyze variability of data of growth performance, slaughter results, carcass and meat traits.

Increasing dietary starch improved dry matter and nutrient digestibility (from 54.7% of low-starch diets to 62.9% of high-starch diets). In high-pectins diets crude fibre (8.1 to 26.4%) and all fibre fractions digestibility was higher.

Extreme pectin/starch ratio diets (0.5 vs. 2.0) showed very similar digestibility and nutritive values (DE: 9.5 MJ/kg). Diet digestibility was maintained at high level by replacing barley meal with dried beet pulp, without changing the inclusion rate of alfalfa meal. The digestibility of fibre fractions changed according to the dietary pectin level: within diets at 5% pectins, digestibility of crude fibre changed in a restricted range (from 7.2% to 9.6%, P>0.05), while significant differences were found for fibre fractions; within diets at 10% pectins, digestibility increased for crude fibre (23.5 to 30.3%), NDF (34.3 to 45.1%), ADF (25.7 to 32.1%) and hemicelluloses (45.4 to 60.8%), without significant differences for pectins digestibility.

Increasing dietary starch significantly increased final live weight at 75 d and daily growth and reduced feed intake with a corresponding improvement in feed conversion (3.52 to 3.31 and

3.13, P<0.001) in the whole period. During post-weaning, daily weight gain was higher (P<0.001) and feed intake tended to be lower with high-starch diets (P=0.06), while in the second period daily growth was similar, but feed intake was significantly reduced (172 to 159 g/d; P<0.001) probably due to a better chemiostatic regulation of appetite.

Dietary pectins level significantly affected growth performance: increasing concentrations stimulated daily weight gain (especially during post-weaning), reduced feed intake and improved feed conversion (3.59 vs. 3.05; P<0.001). Final live weight was higher in animals fed diets containing 10% pectins compared to those fed diets with 5% pectins, both at 55 days of age and at the end of the trial.

Health status was good for all rabbits (only two animals died) and caecal fermentation activity changed little with dietary treatment: N-ammonia production was higher in the caecum of animals fed low-starch diets, likely because of the higher protein intake (DP/DE: 12.9, 12.2 and 11.6 g/MJ respectively for the diets with 5, 10, and 15% starch). With increasing dietary starch, total VFA production did not change, while the increase of acetate (P=0.12) and the reduction of propionate (P<0.01) were unexpected. The chemical characteristics of the diets may explain the result obtained in this experiment: 1) even the highest level of starch used (about 16%, average value of diets L1 and H1) was compatible with the digestive capacity of the animal at the age considered (51 d) and did not produce a significant flow of undigested starch to the caecum; 2) besides, diets had a similar DF content (24.7, 23.6 and 23.8% for diets with 5, 10 and 15% starch) to ensure a comparable fermentative activity and more oriented to acetate production rather than propionate and butyrate. The increase in pectins level reduced pH (P=0.02), but had little effect on propionate and butyrate proportions. Only total VFA production tended to increase (73.8 *vs*. 82.8 mmol/l; P=0.10) and percentage of valerate decreased (0.43 *vs*. 0.35%; P<0.01) with the administration of diets containing 10% pectins.

Jejunum and ileum mucosa revealed no significant differences in the villi and crypts development, but the villi height at both ileum and jejunum tended to be lower (P=0.09 and 0.10) in rabbits fed diets containing levels of starch (about 10%) recommended for the post-weaning than in those fed diets containing less (5%) or higher (15%) concentrations.

The effect of dietary starch on slaughter results mainly depended on differences in animal live weight. Heavier rabbits, that received the diets with higher starch content showed higher slaughter and carcass weights and dressing percentage, lower incidence of slaughter losses and greater incidence of dissectible fat (P<0.01). The effect of increasing dietary pectins was fair and the higher slaughter weight (2628 *vs*. 2687 g; P<0.05) was not associated with higher slaughter dressing percentage. Gut incidence in rabbits fed high-pectins diets was significantly higher (18.5 *vs*. 19.0%, $P<0.05$) than in rabbit fed low-pectins diets.

Dietary treatments did not affect meat quality, in terms of pH or colour measured on the *longissimus lumborum* and *biceps femoris* muscles 24 hours after slaughter.

General discussion

In all experiments, where increasing DF to ADF ratio at similar starch level were obtained by replacing alfalfa meal with beet pulp, the **digestibility** of dry matter, gross energy, crude fibre and fibrous fractions significantly increased. Apparent faecal digestibility of more digestible and soluble fibrous fractions (like those contained in beet pulp) may indeed reach 60-70%, while that of insoluble fractions, main constituent of alfalfa fibre, ranges from 15 to 30% (Carabaño *et al.*, 2001; García *et al.*, 2009).

Undoubtedly, digestibility and the nutritive value of the diets increase with DF/ADF ratio, not only for the higher presence of digestible fibre constituents, but also for the increased digestibility (9-10 points) of all fibre fractions. Differently, when DF replaces starch (increasing DF/starch ratio from 0.8 until 2.8) within limited changes in ADF level (DF/ADF ratio from 0.9 to 1.2) (Experiments 1, 2), dry matter and gross energy digestibility and, therefore, the diet nutritive value did not change, since energy supplied by DF was similar to that provided by starch as also observed by others (De Blas and Carabaño, 1996; Gidenne and Bellier, 2000). In the experiments 3 and 4, the nutritive value of diets increased by increasing DF/starch ratio from 1.1-1.7 to 1.9-4.2. The contemporary increase in DF/ADF ratio (from 0.9-1.0 to 1.4-1.5) and the very low beet pulp inclusion rate in the diets with the lowest DF/starch ratio (even 0% in Experiment 3 in comparison with maximum 33-34%) could account for this result. In Experiment 4, the diets characterized by extreme values of pectins/starch ratio (5% pectin, 15% starch=0.5 and 10% pectin, 5% starch=2), showed very similar digestibility and nutritive values: with the same inclusion rate of alfalfa meal, replacing barley meal with dried beet pulp maintained diet digestibility at high level. The diet with the highest DF and starch content also showed the highest nutritive value.

Comparing different protein sources, when fed the sunflower diets, rabbits showed lower crude protein digestibility (Experiment 1), consistently with the lower CP digestibility in sunflower meal than in soybean meal found by Maertens *et al.* (2002). The pattern of caecal fermentations did not change; neither health status nor growth performance and slaughter results were affected.

The increase of dietary CP concentration (from 15 to 16% in Experiment 2 and from 14 to 17% in Experiment 3) resulted in a higher nutrient and protein digestibility because of the partial replacement of alfalfa protein by protein from soybean and sunflower meal.

Effects of dietary treatments on **growth performance** depend on the nutritive value of diets. Increasing the DF/ADF ratio and, therefore, the nutritive value of diets, due to the chemiostatic regulation of appetite, feed consumption decreases (Gidenne and Lebas, 2005; Xiccato and Trocino, 2010b), especially in the first period of the trial and feed conversion linearly improves, even without differences in final live weight and daily weight gain (Experiment 1). The improvement of feed conversion with increasing DF content may be related to the slower intestinal transit and the increase of digestibility and nutritive value of the diets that has been also found in other previous studies (Xiccato *et al*., 2006a, 2008; Carraro *et al*., 2007; Fragkiadakis *et al*., 2007). In Experiment 2, increasing the DF/starch ratio has null or little effect on growth performance. Also García *et al*. (1993) observed that diets based on dried beet pulp did not impair growth performance when substituting starch-raw materials because of their similar nutritive value. In Experiment 3, during post-weaning, daily growth rate was significantly stimulated and feed intake reduced; as a consequence feed conversion improved by increasing DF/starch ratio. During the last weeks before slaughter, growth was similar among groups. In Experiment 4, increasing dietary pectins from 5 to 10% (with DF/starch ratio raising from 2.4 to 3.1) significantly stimulated daily weight gain (especially during post-weaning) and increased final live weight, reduced feed intake and improved feed conversion. Increasing DF/starch ratio also reduced N excretion, without effecting body N retention especially during the first rather that the second period of growth (Experiment 3).

Reducing dietary protein below 15% impaired daily weight gain and live weight immediately after post-weaning (first three weeks after weaning) (Experiments 2 and 3). These results confirm previous observation showing that protein content in commercial diets for weaning and growth is higher than animal requirements (Maertens *et al.*, 1997; Trocino *et al.*, 2000, 2001; García-Palomares *et al.*, 2006a, 2006b; Eiben *et al.*, 2008). In rabbits slaughtered at 63 days of age and 2.35 kg of live weight, the reduction in dietary protein from 16 to 14% did not influence growth performance (García-Palomares *et al.*, 2006b). In animals slaughtered later (75-90 d) and at heavier weight (2.5-3.0 kg), decreasing protein levels from the first to the second period permitted to satisfy better the protein needs during the first growth phase and reduced N excretion during the second one. In fact, during the latter period, feed intake is higher and dietary protein may be reduced without negative consequences on performance and meat quality (Maertens *et al.*, 1997; Maertens and Luzi, 1998; Trocino *et al.*, 2000, 2001).

Equilibrium of **fermentations at caecum** is considered a key indicator of possible effects of nutrients and levels on rabbit digestive health. In Experiment 1, increasing DF/ADF ratio within the tested range (from 1.0 to 1.2) did not modify the VFA production, even though caecal pH was significantly reduced. This variation, even though not associated to an increase in the total VFA production as found also by Carabaño *et al*. (1997), may be seen favourably in view of the maintenance of an equilibrium in the caecal bacterial population and fermentation. Increasing DF and reducing starch may slow down intestinal transit and increase gut incidence, mainly due to a higher caecal repletion. As a consequence, fermentations were stimulated, total production of VFAs increased as well as the acetate proportion, while valerate incidence usually associated to the activity of amylolitic bacteria, decreased; pH and ammonia concentration also decreased (Experiments 2 and 3). Increasing the dietary pectins concentration (Experiment 4) reduced caecal pH, tended to increase total volatile fatty acids and reduced valerate incidence. Previous studies have shown a favourable and significant increase of the caecal fermentative activity by increasing the dietary content of digestible and/or soluble fibre (García *et al.*, 2000; Falcao-e-Cunha *et al.*, 2004). The limited levels of caecal ammonia found in all experiments and the trend to a reduction with DF/starch ratio demonstrate that DF supplies suitable carbohydrates capable of favouring the ammonia fixation into bacterial protein.

One-point difference in protein between diets (from 15 to 16%) was not sufficient to significantly affect caecal fermentative activity (Experiment 2), while when dietary protein raised from 14 to 17.0%, caecal fermentation was stimulated (Experiment 3). The higher inclusion of barley and dried beet pulps, at constant DF content, may also explain the more intense caecal fermentative activity in diets with higher protein content.

In all experiments of the present thesis, great attention and an important part of work were devoted to the evaluation of the conditions of **intestinal mucosa** in order to evaluate rabbit health status and/or susceptibility to illness. Previous studies showed that rather than the decrease in NDF content, the increase in more soluble fibrous fractions may favour the integrity of intestinal mucosa, reduce mortality and improve growth performance in young rabbits (Gutiérrez *et al.*, 2002; Feugier *et al.*, 2006; Álvarez *et al.*, 2007; Gómez-Conde *et al.*, 2007). In the present thesis, increasing DF/ADF ratio (Experiment 1), DF/starch ratio (Experiments 2, 3 and 4) and pectin concentration (Experiment 4) did not produce any remarkable alterations to the intestinal mucosa structure, both at ileum and jejunum level. Only in Experiment 4, the height of the villi at both ileum and jejunum tended to be lower (P=0.09 and 0.10) in rabbits fed diets containing levels of starch (about 10%) recommended for the post-weaning than in those fed diets containing less (5%) or higher (15%) concentrations. The lower crypt depth found with increasing dietary protein (Experiment 2) may be associated with a lower capacity of the mucosa of repairing villi damages and therefore, indirectly, may explain the higher susceptibility to digestive disorders found in rabbits fed high-protein diets. Also previous studies showed a modest or null effect of protein source, at similar protein contents, even though significant decrease of mortality due to epizootic enteropathy was observed when increasing ileal protein digestibility (Gutiérrez *et al.,* 2002, 2003; Chamorro *et al.,* 2007).

A positive effect on the mucosa integrity has been observed in early-weaned rabbits fed diets containing animal plasma rather than soybean (Gutiérrez *et al*., 2000), while negative effects of legumes anti-nutritional factors have been only hypothesized (Gutiérrez *et al*., 2003; Cano *et al*., 2004).

The relative uniformity in caecal equilibrium and the structure of intestinal mucosa observed was accompanied by a relatively high **sanitary status** in all trials. In fact, this good condition did not permit to assess correctly the effect of the dietary treatments on health condition in rabbits or on the possibility of reducing and controlling diffusion and importance of digestive disorders by epizootic rabbit enteropathy. Increasing dietary DF/ADF ratio (Experiment 1), DF/starch ratio and pectins level (Experiment 3 and 4) did not significantly affect rabbit health. Only in Experiment 2, even though mortality and morbidity were low, a significant effect of dietary treatment was detected: the lowest DF/starch ratio diets provoked acute ERE and increased mortality, though affected animals recovered faster than in other groups. The highest mortality and sanitary risk were associated to the diet with low DF/starch and high protein level in comparison with other groups (Experiment 2). Previous studies showed a positive effect of increasing DF/ADF ratio (Gómez-Conde *et al.,* 2004; 2007; Xiccato *et al.,* 2006a; Carraro, 2006) or replacing starch or protein with digestible fibre in iso-ADF diets (Perez *et al.,* 2000; Soler *et al.,* 2004) on health condition in terms of a reduction of mortality due to ERE and digestive disorders. In fact, a dietary protein excess could impair animal health, altering caecal equilibrium and promoting protein utilization for energy production, increasing N-ammonia and pH, so favouring the development of pathogen bacterial populations (Lebas *et al.,* 1998). In fact, mortality caused by ERE significantly decreased (Gutiérrez *et al*., 2002, 2003; Chamorro *et al*., 2007) by reducing protein level and increasing ileal protein digestibility or increasing digestible fibre (Xiccato *et al*., 2006a; Gómez-Conde *et al*., 2007).

In all four trials the feeding treatment hardly affected **slaughter results** and **carcass characteristics** or **meat traits**, confirming the little role of nutrition treatment over carcass and meat quality, since the animals are fed *ad libitum* balanced diets and there were no relevant differences in the final body weight (Xiccato, 1999; Hernández and Gondret, 2006; Hernández, 2008; Xiccato and Trocino, 2010b). Only the administration of diets with different levels of fat, from different sources, could change appreciably the carcass and meat characteristics (Hernández, 2008). Only when dietary protein affected growth performance and final live weight, that is at the lowest protein concentrations (14-15%) (Experiments 2 and 3), some differences were measured, confirming the results of Maertens *et al*. (1997) who found worse slaughter results only below 13% dietary CP.

Conclusions

Some major conclusions may be drawn starting from the general objectives of the present thesis, that is defining rabbit nutritional needs during post-weaning and fattening with special regards to the different fibrous fractions, mostly the more digestible ones (pectins, hemicelluloses), the starch content in relation to fibre, as well as the dietary protein supply with different aims:

***1*) improving rabbits intestinal conditions**. Changes in main nutrients (digestible fibre, ADF, starch and protein) within the tested intervals may affect the intestinal condition of rabbits and caecum equilibrium: increasing DF at the expenses of starch or increasing protein in diets with appreciable DF supply stimulate caecal fermentation, limit ammonia production and influence caecal pH to a favourable extent for caecal bacterial population. Starch, DF and ADF level, did not affect the morphology and integrity of rabbits intestinal mucosa.

***2)* reducing the incidence and severity of digestive disorders**. The trials were performed under optimal health status. Thus, despite the wide variations in the chemical composition of experimental diets, no effects on animal health were noticed. Therefore, we are not able to adequately evaluate the effect of the tested dietary treatments in a farm stroked by ERE. However, there is evidence that low DF/starch ratio increases mortality and affected animals recover faster. This trend is enhanced if a low DF/starch ratio (<1) in the diet is associated with high dietary protein (>16%), so diets with high DF/starch ratio (1-1.5) and moderate protein level (about 15%) are recommended. By substituting starch with DF, high dietary energy level is maintained and combined with a low flux of undigested protein at caecal level; it may reduce the sanitary risk.

***3)* increasing feed efficiency and N retention**. The increase of dietary DF at the expenses of less digestible fibre fractions or starch (at constant ADF level) improves feed conversion, by reducing feed intake, and allows an appreciable reduction of nitrogen excretion. Starch and digestible fibre may be considered alternative in rabbit feeding and, potentially, additive: the association of high starch and DF level could results in even better results on feed conversion and profitability.

***4)* guaranteeing high performance and final product quality**. Increasing DF/ADF ratio improves diet nutritive value and their efficiency of utilization for growth. Increasing DF/starch does not modify the nutritive value of diets and, in most cases, nor growth performance or slaughter results. Sometimes, growth may be rather stimulated, especially during the first weeks of growth when young rabbits are still developing their digestive capacity.

To maximize growth performance, dietary crude protein should not be reduced below 15.5% in the first weeks of growth since the subsequent compensatory growth that rabbits show in fattening period before slaughter does not permit to recover the weight loss. Dietary protein has to be controlled both in the first and especially in the second period of growth, during which protein requirements are lower, to increase N efficiency and limit its excretion.

Changes in DF, starch or protein produce significant differences in slaughter results only when capable of modifying slaughter live weight and, therefore, carcass traits directly correlated to live weight. Meat traits, pH and colour, of main muscles never change with the dietary treatment to an extent appreciable form a commercial point of view.

INTRODUCTION

Some notes on rabbit meat production

Rabbit meat is classified in the category of ‘white meat’ and is characterized by a low content in lipids, cholesterol, sodium and by the absence of allergenic and anti-nutritional factors (Hernández, 2008). Regarding the industrial production of rabbit meat, apart from China, the market is limited to a few countries in the Mediterranean zone. The world production of rabbit meat is estimated to be about 1.750.000 tones in the year 2007 (FAO, 2010). About 60% of the world rabbit meat is produced in China, Italy, Spain and France (Figure 1).

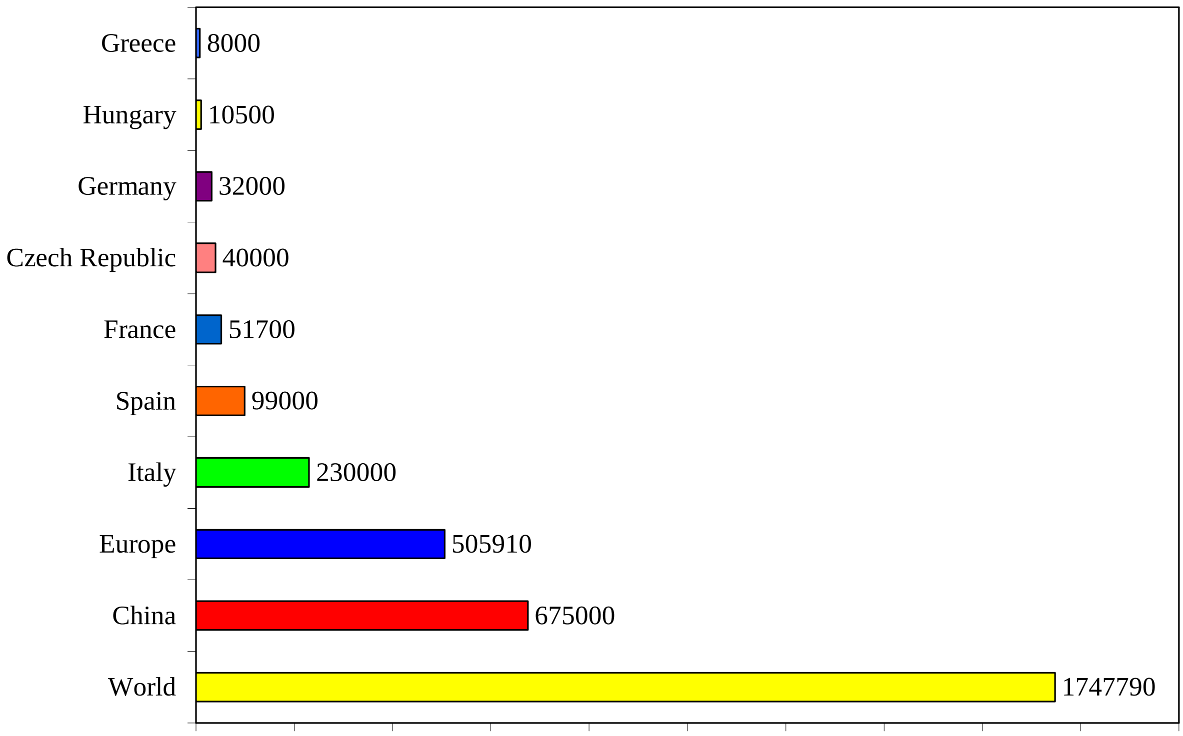


Figure 1. Rabbit meat annual production year 2007 (FAO, 2010)

Italy is the leading producer of industrial rabbit meat in Europe with 230,000 tons produced. Rabbit production represents in Italy the fourth sector of animal production, coming after beef, swine and poultry. The annual consumption of rabbit meat in Italy is very high, reaching 4 kg/person/year on average. Fifty per cent of the does are located in small farms covering the traditional market. The rest of the farms are intensive and well organized, with about 900 producing does per farm on average. Live rabbits may weight at slaughter from 2.2 to 3.0 kg, with an average live weight of 2.5 kg and average carcass weight of 1.6 kg. This weight is higher than other European Countries compelling the Italian farmers to prolong the fattening period, until 80-85 days, in comparison with the 65-70 days needed in Spain and France (Xiccato and Trocino, 2007).

Differently from poultry, rabbit chain integration with feed industry is low (15-20%). This fact does not permit an adequate organization and programming of the production. However, this sector takes other advantages from the characteristics of the animal itself: fast growth rate and short reproductive rhythm and easy adaptation to intensive breeding, are the strength points of rabbit production.

In the years 1980-90, intensive rabbit production developed. Thanks to the continuous studies and knowledge advancement on genetics, reproductive physiology, nutritional and environmental needs of the rabbit, new breeding techniques and management systems have been developed: highly productive hybrids, artificial insemination, feeding programs for each productive stage, all-in/all-out system, environmental control, specialized cages for does, litter and fatteners, improved the organization of the farm and increased the productivity and economic outcome of the producer.

In this context, nutrition remains a very important factor for optimizing rabbit performance and animal health. At least 60% of the total cost of production is represented by feeding. In order to reduce this cost and improve feed conversion, the nutritional needs of the animals at different productive stages must be satisfied. Moreover, nutrition plays an important role on the health status of the rabbits, by guarantying the proper digestive physiological functions and microbial fermentation in the caecum. This role is even more important in the modern and intensive rabbit production, where the highly productive genetic types used seem to be more susceptible to digestive troubles and pathologies and the use of antibiotics is limited by European rules and consumers’ concerns about meat safety.

Main features of the digestive apparatus and physiology in rabbits

According to its feeding behaviour, the rabbit is classified as an herbivore selector of concentrates. Its dietetic regime is based exclusively on vegetable feed and is constituted by those parts of the plants characterized by low fibre, high protein and digestible carbohydrates content, such as young leaves, tubers and sprouts (Cheeke, 1987).

Like most herbivores, the rabbit hosts in its digestive apparatus a symbiotic microbial population, consisting of bacteria that ferment the non digestible substances that reach the large intestine, and protozoa. This population is mainly found in the caecum, the largest fermentative site in rabbit, and its composition is yet scarcely known (Pérez de Rozas *et al.*, 2005; Carabaño *et al.*, 2006, 2008). Unlike other herbivores, the rabbit is characterized by a particular adaptation of feeding behaviour, called caecotrophy, that is the ingestion of the caecal content which is excreted in the form of caecotrophes, the so-called ‘soft faeces’. This behaviour enables the rabbit to exhibit a major digestive utilization of the nutrients through i) a fast removal of the less digestible constituents *via* the hard faeces; ii) the re-ingestion and digestion of the soft faeces, that are enriched by the microbial flora in protein and vitamins; iii) the direct absorbance through the caecal mucosa of the volatile fatty acids (VFAs) produced by the fermentative activity. The mechanism of caecotrophy and a normal digestive physiology are guaranteed by an adequate content of dietary fibre (lignin, cellulose and hemicelluloses).

The digestive apparatus of the rabbit is typical of monogastrics, consisting of mouth, pharynx, oesophagus, stomach, small and large intestine (Figure 2).

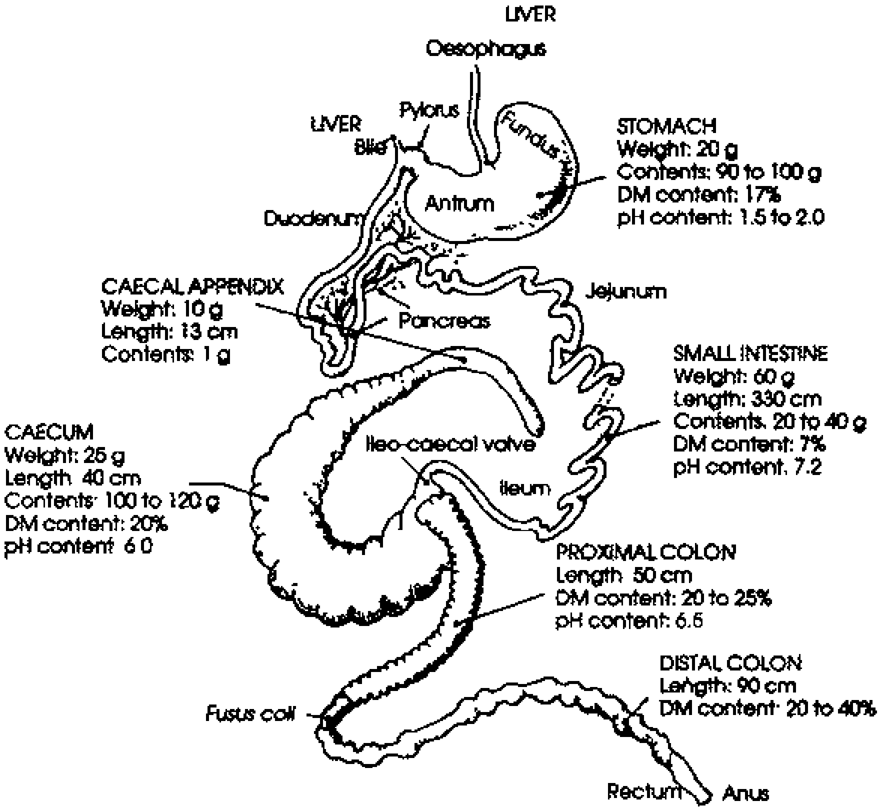


Figure 2. Digestive apparatus of rabbits (Cheeke, 1987)

Apart from caecotrophy, the digestive physiology, digestion and absorbance of the nutrients are substantially similar to other monogastrics (Cheeke, 1987; Xiccato and Trocino, 2008). The stomach is the second largest compartment of the digestive apparatus, after caecum, representing around 30-35% of the total gut weight. Gastric pH may reach extremely low values and ranges from 1.0-1.5 to 5.0 according to the measuring site (fundus or pyloros) and the presence or not of soft faeces. Through pyloros, chylos passes from the stomach to the small intestine. This compartment is 3-meter long and is divided in duodenum, jejunum and ileum, where the main processes of digestion and absorbance of the nutrients take place. The digested substances are absorbed by the mucosa in various tracts of the small intestine. The absorbance of lipids begins from the distal part of duodenum and is concluded at the proximal part of jejunum, while that of proteins (oligopeptides and amino acids) and disaccharides and monosaccharides occurs in various parts of the intestine.

As in other animals, villi are the principal structures of the intestinal mucosa dedicated to the absorbance of nutrients (Figure 3). The products of digestion penetrate into the villi by diffusion, active transport or pinocytosis, and may take the route of the central cheliferous vessel or the vein system. Sugars, long chain fatty acids, immunoglobins and cholesterol get into the central cheliferous vessel and through that pass into the vein system. Water, inorganic salts, amino acids, monosaccharides, short chain fatty acids and glycerine pass through the villous blood circulation and arrive to the liver (Bortolami *et al.*, 1985).

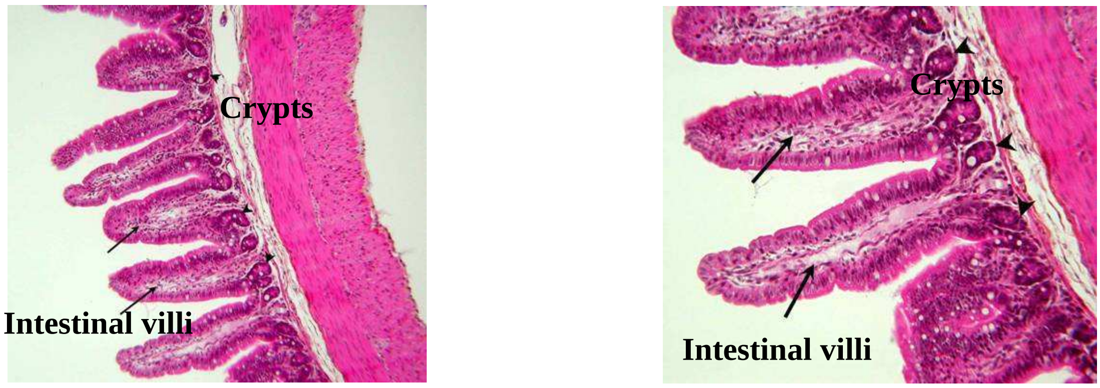
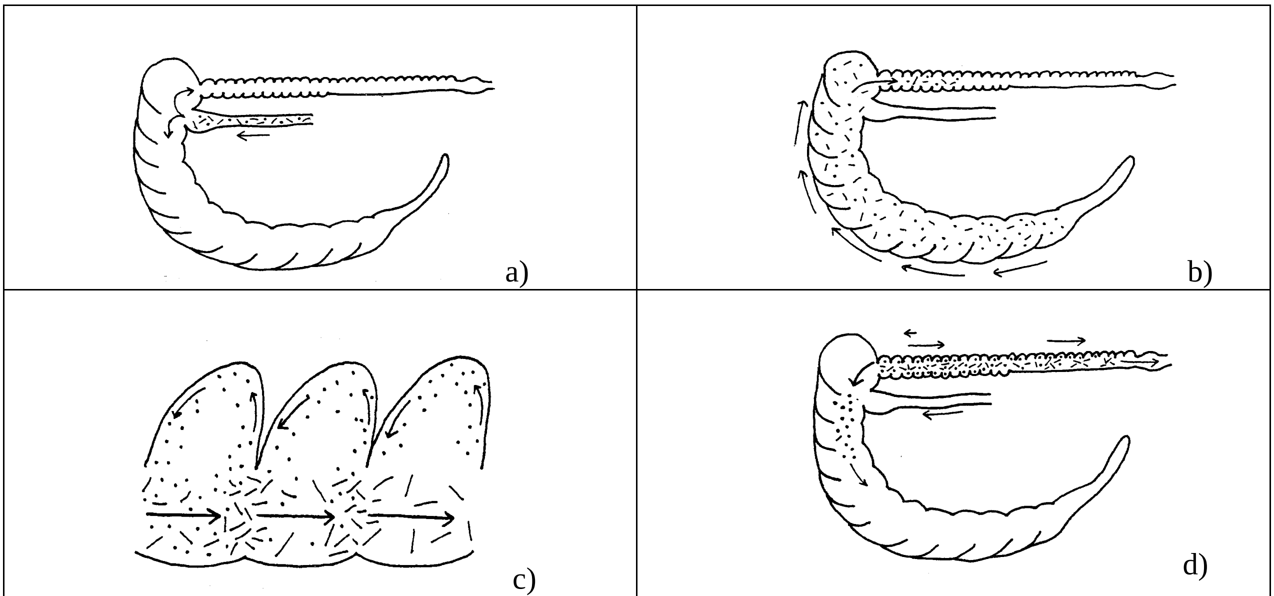


Figure 3. Fine structure of rabbit small intestine (20 and 40X) H-E

The large intestine consists of caecum, colon, rectum, and anus, and is connected to the small intestine through the ileum-caecal valve. The caecum is the most voluminous organ of the digestive apparatus of the rabbit. It is 45-50 cm long and has a filling capacity almost 40-50% of the total digestive apparatus. It has the shape of a closed bag divided in sectors, ending with the caecal appendix (vermiform appendix), a lymphoid organ with specific functions. Caecal appendix produces an alkaline juice rich in bicarbonate ions used as buffer to stabilize caecal content pH and influences microbial fermentation and production of vitamin B_{12} (Cheeke, 1987).

Colon is divided into two parts, proximal (40-50 cm long) and distal colon (80-100 cm). In the proximal colon, three tracts may be distinguished: the first one presents three taeniae and between those three series of haustra; the second tract has only one taeniae and one series of haustra; the third tract, also called *fusus coli*, has no taeniae or haustra and has the function of pace-maker for the control of the colon contractions during the formation of the faeces. Distal colon has no haustra and concludes with rectum and anus.

Indigested material deriving from the small intestine passes through the ileum-caecal valve in the caecum and the colon where it is continuously mixed and moved due to peristaltic and anti-peristaltic movements and retrograde flow, which separate liquids and fine particles from large particles (Figure 4a). Fast horizontal contractions of the caecum in both directions mix and push the content towards proximal colon (Figure 4 b and c). At the same time, contractions of the haustra determine the backward movement of the material towards the caecum (Figure 4d).



a) Entrance of intestinal content and dispersion in the caecum.
b) Caecum contraction and material transit in colon.
c) Peristaltic movement of colon push coarse fibrous particles towards distant colon to form hard faeces. Anti-peristaltic movements and retrograde flow push small particles and liquids towards caecum.
d) Small particles and liquids are separated from fibre and re-enter in the caecum as to be fermented.

Figure 4. Entrance of digesta in caecum, formation and excretion of hard faeces, caecal fermentation of small particles (from Cheek, 1987)

Both in colon and caecum, long particles, more than 0.3 mm, tend to be accumulated in the lumen, due to their lower density. Due to their higher density, particles smaller than 0.3 mm tend to deposit on the intestinal walls and follow the retrograde flow towards the caecum. As a consequence, small and dense particles tend to remain longer inside the caecum and ferment, while larger and less dense particles are rapidly removed to form hard faeces. The formation of hard faeces is followed by a mechanical separation of water, caused by the contractions of the proximal colon walls and the presence of segmental contractions that separate the faeces. The formation process of the faeces continues at distal colon with the re-absorption of water, reducing their humidity content before their excretion through rectum.

The material accumulated in the caecum is mixed and fermented by the caecal microflora for several hours. Before the formation end expulsion of the soft faeces a reduction in the mobility of caecum and colon is observed. Caecal content is expulsed under the form of short clusters formed by 3-6 balls in 5 mm of diameter, that are covered by the mucus produced in proximal colon. At the moment of expulsion, the rabbit recognizes and ingests the soft faeces directly from the anus without chewing them.

The formation of hard and soft faeces and their expulsion are connected in time to the ingestion of the feed, thus presenting a circadian comportment (Carabaño and Piquer, 1998). The excretion of hard faeces happens few hours (6-8) after the ingestion of feed due to the rapid excretion of the fibrous constituents. Caecotrophy principally occurs in the diurnal hours (maximum at 12:00), but with a great variability (from 08:00 to 17:00) and sometimes with a two-phase rhythm. On the contrary, feed ingestion and hard faeces excretion occur mainly during the second half of the day and during the night.

After ingestion, soft faeces reach the stomach and remain there for 3-6 hours, still protected by the mucus. In that way proteins and nutritive substances are protected by the enzymatic degradation, while the development and production of microflora and volatile fatty acids continues. Once mucus is degraded, soft faeces leave the stomach and are digested in the small intestine. From a nutritional point of view, caecotrophy, together with fermentation and VFA absorption in caecum, represent a mechanism of recovering nutrients for the organism. The aspect and chemical composition of the two types of faeces are very different. Hard faeces are dry, while soft faeces are doughy. Soft faeces have a composition similar to caecal content and are richer in water, proteins and vitamins than hard faeces. Hard faeces contain almost double quantity of crude fibre and fibrous fractions than caecotrophes. Bacterial protein content in soft faeces varies from 30 to 60% of the total protein and the bacterial activity is also responsible for the high content in vitamins of the B complex. As the main origin of protein is microbial, proteins contained in soft faeces have an elevated biological value and represent 15 to 22% of the daily protein ingestion, regardless to the category (does or fatteners) and the physiological status of the animal. The contribution in essential aminoacids, such as lysine, sulfur aminoacids and threonine, by the soft faeces represent 10 to 20% of the total ingestion.

The result of fermentative activity of microflora is the production of VFAs and ammonia, as final products of the fermentation of carbohydrates and protein respectively. The VFA profile of rabbits is characteristic, with a predominance of acetic acid (C_2) (70-85% of total VFAs), followed by butyric acid (C_4) (8-20%), propionic acid (C_3) (3-10%) and caprilic acid (C_5) (0.5-1%). The proportions change during the day in relation to caecotrophy and to age of the rabbit in relation to the development of caecal microflora. Also feeding, by modifying the content in fibre and fermentable substances (starch, pectins, hemicelluloses etc), may affect the composition of caecal microflora and as a consequence the proportion of fermentation products. The VFAs produced in the caecum are in great part absorbed by the mucosa. Butyric acid is principally absorbed in colon and represents the fundamental substance for the development and protection of intestinal mucosa. Liver is the principal organ associated with the metabolism of propionic and butyric acid, while acetic acid is also available for the cellular energy metabolism.

Epizootic Rabbit Enteropathy and digestive disorders

The main health problem in rabbit production has always been represented by digestive disorders (Marlier *et al.*, 2003; Rosell, 2003). Recently, the Epizootic Rabbit Enteropathy (ERE) has greatly contributed to increase digestive disorders in rabbits. ERE first appeared in 1997 (Licois, 2004) and is still widely spread in rabbit farms in Europe (Xylouri and Fragkiadakis, 2006). The clinical symptoms of the disease are decreased feed consumption, presence of mucus and diarrhoea, high morbidity and mortality rates (Pérez de Rozas *et al.*, 2005; Licois *et al.*, 2006). Post mortem examination reveals stomach and small intestine filled with liquid and gas, caecal impaction and presence of mucus in the colon. 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; Xylouri *et al.*, 2008; Chamorro *et al.*, 2010). The first symptoms usually appear about ten days after weaning. The disease spreads very quickly and mortality occurs within the first two days in acute cases or within a period of 1-2 weeks. Morbidity may reach 100% and mortality even 60-70% (Pérez de Rozas *et al.*, 2005). The etiology of ERE has not been completed elucidated until now. The importance of *Clostridium perfringens* has been shown 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 where 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.

The main solution to ERE is the use of antibiotics, but the European rules on the use of auxinic antibiotics 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 not be applied in time to control economic losses (Xiccato *et al.*, 2008). In addition, the cost of therapy has an important impact in the rabbit production cost.

The intestinal barrier and the development of intestinal disorders

In presence of a pathogen, the population of saprophyte bacteria normally present in the digestive tract and the defensive mechanisms of the animal may prevent or reduce the development of such pathogen and the appearance of the disease. Such defensive mechanisms, like in other species (swine and poultry) including humans, are present in the rabbit and feeding strategies can be developed to favour the procedures of 'competitive exclusion' among the bacteria and the development of 'intestinal barrier' mechanisms (Carabaño *et al.*, 2005, 2006).

Competitive exclusion, which allows a non pathogen species to predominate over a pathogen one, can be realized in various ways: growth differences on a specific substrate; differences of the colonisation efficacy on the mucosa; the production of substances that inhibit the development of pathogens (short chain fatty acid, sulphuric acid, non-conjugated biliary acids and bacteriostatics) (Hampson *et al.*, 2001). Feeding can regulate the quantity of a substrate that may favour the development of a certain microorganism that in its turn may favour some mechanisms of competitive exclusion or directly inhibit the development of pathogens.

Once the pathogen is stabilized, then the intestinal barrier has the fundamental role of developing the defensive mechanisms, by restraining the colonization of the mucosa and the passage (translocation) of bacteria and toxins through it. The main pathogens of the gastrointestinal apparatus need to come in contact with the mucosa in order to express their pathogen activity. Numerous studies demonstrate the need of adherence on the mucosa of *E. coli* pathogen strains (Tsalie *et al.*, 2003). Other pathogens that belong to the *Clostridium* genera are not normally adherent, but express their virulence through the production of toxins (*C. spiroforme, perfringens, difficile, sporogenus,* etc). The rabbit death caused by ERE seems to be determined by a toxin (not yet classified) of a *C. perfringens* strain, which causes damages in the liver and kidneys (Peréz de Rozas *et al*., 2005). Though, before these toxins reach their target organs and perform their toxic function they must overcome the mucosa. The adhesion of the *C. perfringens* toxins on the membrane, such as an initial damage on it, seems necessary for their translocation (Mc Clane, 2001).

Intestinal barrier consists of multiple mechanisms that are developed in sequence. As shown in Figure 5 (Carabaño *et al.*, 2005), the barrier effect begins acting at lumen level through the mechanism of acidification and epithelium protection thanks to a cover of mucus that is excreted by the Goblet cells. Mucus protects the epithelium against mechanical, chemical or enzymatic damages and the adhesion of bacteria. Once this protection is overcome and the bacteria or toxins reach contact with the mucosa, the mucosa associated immune system (*Gut associated lymphoid tissue*, GALT) is activated, initially in a non-specific mode and then in a specific one. The non-specific activation is realized by the first contact with the antigen and determines transitory inflammatory processes; successively specific immunoglobins against the antigen are synthesized that normally give way to a tolerance of the organism against the antigen or, rarely, hyperactivity against itself (allergy). The maintenance of the structure and functionality of the intestinal mucosa, besides blocking the colonisation of pathogen bacteria, reinstate the proper functions of the digestive tract, meaning the digestion, absorbance, secretion and metabolism of nutrients. This explains why intestinal diseases are more frequent in young animals, which mucosa is still immature, and during the post-weaning period, given that weaning may provoke structural damage at intestinal mucosa level.

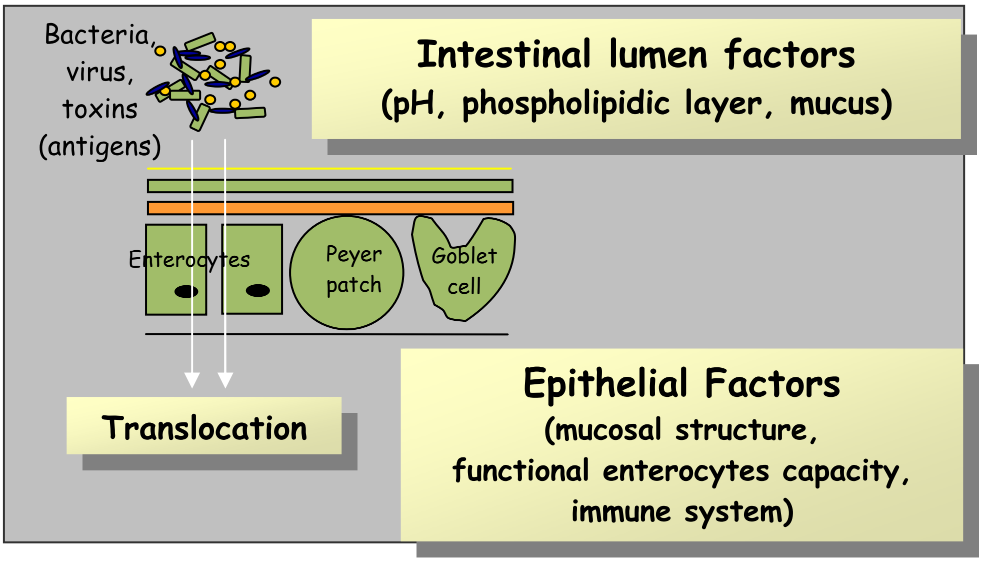


Figure 5. Intestinal barrier mechanisms that block the bacteria and toxin translocation (Carabaño *et al*., 2005)

The role of nutrition and the development of intestinal disorders

To control ERE occurrence and diffusion, various strategies have been proposed, such as decreasing the stressing factors that may create dysbiosis in rabbits, improving management systems (weaning, lactation system, etc), performing environmental control, modulating time and type of antibiotic treatments. At the same time, research into new feeding strategies or the use of feed additives capable of guaranteeing animal health has been carried out (Gidenne and García, 2006; Maertens *et al*., 2006).

Firstly, the diet must satisfy the nutritional requirements of rabbits, assure normal digestive physiology, thus favouring the establishment of protective intestinal microflora and limiting the growth of pathogens. Therefore, feeding strategies should be addressed to avoid damages to the mucosa structure and to favour the repair mechanisms of these damages, by supplying the necessary nutrients. Adequate characteristics of the mucosa not only improve the immune response, but also the efficiency of digestion and absorbance, by reducing the flux of nutrients in the caecum, which may favour the development of pathogens.

Dietary fibre, starch and protein play a key role in rabbit nutrition and a major function in the occurrence of digestive disorders. In particular, recently, a positive role has been recognized into digestible or soluble fibre supply in modulating intestinal microbiota and preventing the occurrence of digestive pathologies as well as into protein supply in terms of source and concentrations. On the other hand, for a long time, among different dietary nutrients, a negative role on digestive health has been attributed to starch. These topics are widely reviewed later on.

Fibre and fibre fractions

The term "dietary fibre" has been taken from human nutrition and extended to all mammals. Initially the definition of dietary fibre only referred to hemicelluloses, cellulose and lignin (Trowell, 1972); later it was extended to the structural framework of the plant cells that resists to hydrolysis by human digestive enzymes (Trowell, 1978), and actually, on the base of analytical progresses and new nutritional and physiological knowledge, it is going to be extended to include resistant starches as well as non-digestible oligosaccharides (Champ *et al.*, 2003). With regards to human nutrition, the Codex Committee on Nutrition and Foods for Special Dietary Purposes of the European Union is discussing the following definition of dietary fibre: "Dietary fibre means carbohydrate polymers with a degree of polymerization not lower than 3 which are neither digested nor absorbed in the small intestine. A degree of polymerization not lower than 3 is intended to exclude mono- and disaccharides" (García *et al.*, 2009).

Therefore, the definition of fibre includes rather different chemical constituents of vegetal origin with different effects and roles on digestive physiology and different nutritive values (Figure 6). As a consequence, 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 (Champ *et al.*, 2003; Gidenne, 2003; Mertens, 2003; De Vries and Rader, 2005; Uden *et al.*, 2005; García *et al.*, 2009). That is, according to García *et al.* (2009), "the quantitative analysis of the whole components of this fraction cannot be obtained by any analytical method or combination of methods".

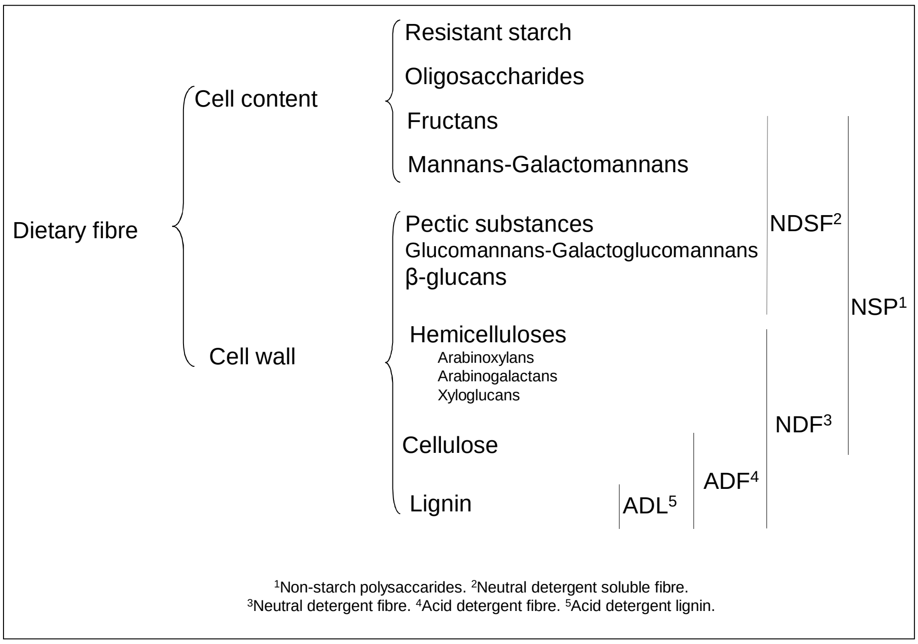


Figure 6 Major constituents of dietary fibre (Hall, 2003; García *et al.*, 2009).

Chemically, fibre is the structural part of vegetable cells and consists of the cell walls and is formed of cellulose microfibrils (the backbone) embedded in a matrix of lignins, hemicelluloses, pectins and proteins (Figure 7).

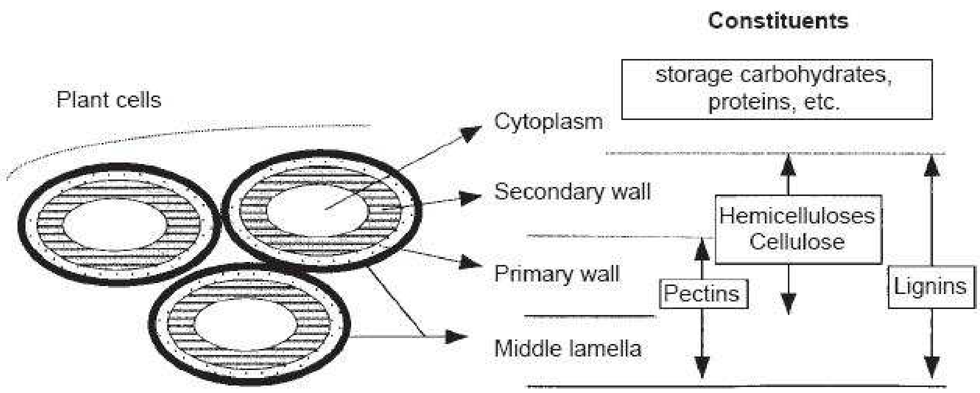


Figure 7. Schematic representation of plant cell walls and their main constituents (Gidenne, 2003)

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. Its concentration increases with the plant age. Cellulose is the major structural polysaccharide of the plant cell walls: a homopolymer formed from linear chains of β[1-4] linked D-glucose units. The degree of polymerization is usually around 8.000-10.000 units. Cellulose is soluble in strong acid solutions (i.e. 72% sulphuric acid) which partially hydrolyze it. 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) (Gidenne, 2003).

When considering physiological and nutritional aspects, first definitions of dietary fibre started from the consideration that dietary fibre was not digested at all in the whole intestinal tract of simple-stomached animals. Later, the extensive breakdown of most cell-wall polysaccharides in the large intestine of man and other animals was recognized and considered in the definitions (Champ *et al.,* 2003). In fact, the various fractions of fibre are more or less digestible and/or fermentable and have a different physiological role in humans and other animals, including rabbits. Lignin is indigestible even by bacteria, while cellulose may be hydrolyzed by intestinal bacteria to a different extent depending on the animal species considered. Pectins and hemicelluloses mainly constitute the so-called "digestible fibre" (Gidenne, 2003). Mertens (2003) defines insoluble dietary fibre as the indigestible (lignin) or slowly digesting organic matter of feeds (mostly hemicelluloses and cellulose) that occupies space in the gastrointestinal tract. This definition excludes soluble and rapidly fermenting polysaccharides of plant cell walls (fructans, gums and pectins) that do not occupy space in a liquid environment and are highly digestible.

In animal nutrition, dietary fibre is the sum of non-starch polysaccharides (NSP) and lignins which may be quantified by different analytical procedures (chemical-gravimetric, enzymatic-gravimetric and enzymatic-chemical) and separated into insoluble and soluble fibre (in aqueous solution) (Table 1) (Champ *et al.*, 2003).

When evaluating specifically feed for animal nutrition by the Weende method (AOAC, 1980), the "crude fibre" concentration is determined gravimetrically by means of acid and sequential alkaline hydrolyses. The residue includes most of the cellulose and lignin as well as cutine and suberine, so that crude fibre results to be composed by 30-100% cellulose, 16-90% lignin and 14-20% pentosanes, depending on the raw materials.

The sequential method of Goering and Van Soest (1970), modified by Robertson e Van Soest (1981), gives NDF, which measures insoluble dietary fibre content (Mertens, 2003) and contains the insoluble cell walls (hemicelluloses, cellulose, lignin and insoluble ashes) but also the pectins not soluble in water; ADF (Acid Detergent Fibre), which includes cellulose, lignin and insoluble ash; and ADL (Acid Detergent Lignin), which is represented by lignin.

Table 1. Main methods of dietary fibre analysis (from Champ *et al.*, 2003)

Reference	Name	Type	Measures	Main concerns
AOAC (1980)	Crude fibre (CF) or Weende method	Gravimetric	Most of the cellulose and lignin	Only adapted to forages
Van Soest and Wine (1967)	Van Soest method	Gravimetric	Most of the cellulose and acid insoluble hemicelluloses and lignin	Mainly adapted to forages
Prosky *et al.* (1985)	Total dietary fibre (TDF); AOAC 985.29	Enzymatic-gravimetric	Soluble and insoluble polysaccharides and lignin	Quantify only a portion of resistant starch. Inulin or polydextrose are not quantified
Englyst and Hudson (1987)	Englyst method	Enzymatic -chemical or GLC or HPLC	NSP	Lack of reproducibility
Theander *et al.* (1994)	Uppsala method	Enzymatic -chemical	Neutral and uronic residues and Klason lignin	Few users of the method
McCleary and Codd (1991)	AOAC 995.16; AACC 32-23	Enzymatic	B-glucans	
Englyst *et al.* (1992)	Resistant starch	Enzymatic	Resistant starch	Lack of reproducibility, but validated with in vivo data
McCleary and Monaghan (2002)	AOAC 2002-02; AACC 37.42	Enzymatic	Resistant starch	Consistent with *in vivo* data
Hoebregs (1997)	AOAC 977.08	Enzymatic and ion-exchange chromatography	Oligofructan, inulin, fructo-oligosaccharides	
Ouarne *et al.* (1999)		Ion-exchange chromatography	Oligofructan, inulin, fructo-oligosaccharides	
Craig *et al.* (2001)	AOAC 2000.11	HPLC	Polydextrose	

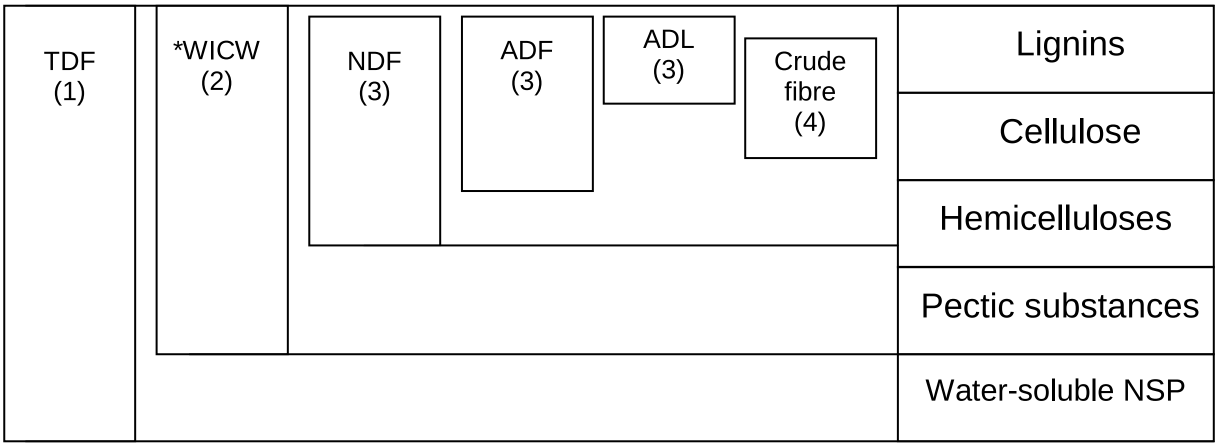


Figure 8. Gravimetric methods for the determination of dietary fibre and identification of the residue of analysis: (1) Lee *et al.*, 1992; Li (1995); (2) Carré and Brillouet (1989); (3) Van Soest *et al*. (1991); (4) according to the Weende technique (Hennenberg and Stohmann, 1864), (Gidenne, 2003) *WICW: Water Insoluble Cell Wall

The content of hemicelluloses is calculated by the difference between NDF and ADF, and the content of cellulose by the difference between ADL and ADL. NDF determination is highly variable among laboratories (Xiccato *et al.*, 1996; EGRAN, 2001). Udén *et al.* (2005) recommend the expression of ash-free NDF as well as the use of amylase and sodium sulphite to improve filtration and protein extraction, but also extraction of lignin and phenolic complexes. In fact, a limitation of NDF determination is that the protein content of NDF may be variable and that it may also contain some starch and pectins.

A gravimetric-enzymatic procedure, largely used in human nutrition, may be used to determine the total dietary fibre (TDF) and the insoluble dietary fibre (IDF) contents, 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. The insoluble fraction is composed by insoluble pectins, cellulose, hemicelluloses and lignin. The pectin content may be estimated by subtracting NDF to TDF, even if results is conditioned by the lack of correction for protein and ash content in NDF.

Based on the information above, a large consensus exists when referring to methods for evaluating the indigestible and less digestible fibre fractions for animals and, as a consequence, data are largely available on various feedstuff compositions which are easily comparable. Differently, when speaking about the most digestible fibre fractions, agreement is still lacking on the most correct definition, and there is still discussion whether it better referrs to soluble or digestible or fermentable fibre fractions, as well as on the chemical constituents to be definitively included in the definition and, as a consequence, on the analytical methods for their chemical determination (García *et al.*, 2009). Therefore, lacking routinely analytical methods for their determination, few data are available for the content of these fractions in the raw materials used in the feed animal industry.

With specific reference to the rabbit, this animal has a specific requirement for dietary fibre which relates to the exact role on its digestive physiology rather than to a generic nutrient or energy supply (Gidenne *et al*., 1998; Gidenne and García, 2006; Xiccato and Trocino, 2008). A well balanced diet for rabbits should guarantee enough fibre to stimulate intestinal peristalsis, maintain digestive transit, ensure caecotrophy and regulate intestinal microflora composition with the final aim of reducing the incidence of digestive troubles and diseases.

Indigestible fibre fractions are rapidly removed during the digestive process as the major constituents of hard faeces and have a recognized role in preventing digestive troubles and pathologies (Lebas, 1989; Blas e Gidenne, 1998; De Blas e Mateos, 1998). During the post weaning period, when young rabbits are highly susceptible to digestive troubles, a minimum ADL content of 5% and a minimum ADF supply of 16-17% are recommended (Gidenne and García, 2006) (Table 2).

Table 2. 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 (hemicellulose)	>12.0	>10.0	–	–
DgF[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, Univesidad Politécnica de Madrid.

Lower levels of indigestible 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). Higher levels of indigestible fibre over-stimulate peristalsis and digesta transit, reduce the time available for digestion and thus the apparent digestibility.

More recent studies outlined that, in young rabbits soon after weaning, caecum fermentative activity and microflora development are still incomplete, that is animals have a limited capacity of digesting high-fibrous diets rich in indigestible fractions which may favour digestive problems (Gidenne *et al.*, 2004a, 2004b; Gidenne and Licois, 2005). In 25-d weaned rabbits, fed diets containing 25, 30 and 35% NDF, mortality due to ERE, was lower with the intermediate NDF supply (Nicodemus *et al.*, 2004). Similarly, the reduction of dietary NDF from 36-38 to 30-32% NDF reduced mortality and improved performance and feed efficiency (Gutiérrez *et al.*, 2002; Feugier *et al.*, 2006). According to Gutiérrez *et al.* (2002), performance is improved and mortality is reduced in rabbits weaned at 25 days of age when fed a diet with 22.6% DM starch and 33.6% DM NDF, evidencing a limited capacity in digesting fibre in the period from 25 to 39 days of age. The same authors have evidenced a negative effect of the increase of NDF content from 33.6% to 40.6% DM in terms of lower digestibility of dry matter, starch, and crude protein, as well as lower growth performance and higher mortality (from 7.5% to 22.6%).

In fact, in young rabbits, 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 by promoting the proliferation of beneficial microbiota and improving the competitive exclusion with pathogens (Carabaño *et al.*, 2008). Gomez-Conde *et al.* (2007) found 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. Soler *et al.* (2003) observed a net reduction of mortality from 14 to less than 6% by increasing soluble fibre from 7 to 12%. By maintaining the ADF content constant and substituting starch and protein with digestible fibre (pectins and hemicelluloses) (Perez *et al*. 2000; Gidenne *et al*. 2001; Marguenda *et al.*, 2006) digestive problems were reduced and the sanitary status of the rabbits was improved, as a consequence of the positive effect of digestible fibre on the caecal fermentative activity (Gidenne and Bellier, 2000).

A positive effect on intestinal mucosa integrity of decreasing insoluble fibre (Gutiérrez *et al.*, 2002; Alvarez *et al.*, 2007) and increasing soluble fractions (García-Ruiz *et al.*, 1997; Carabaño *et al.*, 2008) has also been found.

According to Gidenne (2003) a DF/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. In other studies, though, even an increase of DF/ADF ratio equal to 1.4 with the reduction in starch content reduced the mortality (Xiccato *et al*., 2007, 2008).

On the base of the reviews (Carabaño *et al.*, 2008; García *et al.*, 2009) of the most recent studies on the role of dietary fibre on digestive health and performance, besides an optimal level of 30-32% NDF, the supply of 11-12% soluble fibre is recommended, as also indicated by Gidenne and García (2006) (Table 2).

<u>Starch</u>

Starch represents the main energy source in rabbit feeding: it is digested with high efficiency and its use is only conditioned by the possible appearance of digestive disturbances, especially in young animals (Gidenne, 1996; Blas and Gidenne, 1998).

Starch digestibility varies according to the physiological status of the animal and the source. Young animals do not totally digest this polysaccharide, since they lack the proper level of enzyme (amylase), which is reached around 5-6 weeks of age (Xylouri *et al*., 2008). Regarding the source, corn starch is significantly less digestible than barley (Blas *et al.*, 1990).

According to Cheeke (1987), an excessive content of dietary starch combined with a low level of fibre may result in an excessive flux of starch at caecal level; mainly in young animals which gut amylase activity is still limited. Starch at caecal level is fermented by amylolytic bacteria, followed by the production of VFAs and a rapid decrease of pH (5.0-5.5). In this context, in the presence of glucose deriving from starch digestion, *Clostridium spiroforme* produces a iota-like toxin, which causes enteritis and diaorrhea, and pathogen bacteria proliferate (Borriello and Carman, 1983).

There is a wide bibliography relative to the effects of starch over the performance of young rabbits and their health status (Blas and Gidenne, 1998). During the post-weaning, a starch content lower than 8% is recommended (Gidenne and Fortun-Lamothe, 2002). In their bibliographic review, Blas and Gidenne (1998) have come upon a direct relation between the starch level and mortality in only one study, however.

Generally, the optimal content of starch in diets for weaning rabbits ranges from 10 to 13% (Maertens, 1992; Gidenne and Fortun-Lamothe, 2002), reaching 17-20% in the last phases of fattening and reproducing does (Maertens, 1992; Xiccato, 1993), in order to satisfy the increased energy needs. According to Gidenne and García (2006), dietary starch for growing rabbits should be less than 20% (Table 2).

Apart from the role of fibre, it is also very important to consider the role of fibre to starch ratio, since in the formulation of the diets their concentrations are inversely proportional. The decrease in fibre to starch ratio results in a reduction of ileal passage of dry matter in the caecum. In that case a low fibrolytic bacterial activity in caecum is observed, associated to alteration in the fermentation pattern (high butyrate levels) (Gidenne *et al*., 2000; Nicodemus *et al*., 2003, 2004).

In the study mentioned above (Gutiérrez *et al.*, 2002), the low-fibre diets (NDF=33.6% DM), which improved performance and reduce mortality in youg rabbits weaned at 25 d, also contained an appreciable amount of starch (22.6% DM). Debray *et al*. (2002) observed higher feed intake (+13%) and litter weight at weaning (+6%) and lower mortality (0.8 *vs* 5.2%) from 25 to 32 d in young rabbits fed a diet with 17% starch compared to rabbits fed a diet with 14% starch. These results were also confirmed by Nicodemus *et al*. (2005) who observed, in the period from 21 until 25 d, higher feed intake (+39%) in suckling rabbits fed a diet with 19% starch compared to 10% starch along the first four lactations of rabbit does. No negative effect of dietary starch level on rabbit mortality was observed after weaning.

Fortun-Lamothe *et al*. (2005) proposed a diet with a similar level of NDF (30.6%) and lower starch (9.5%) replaced by fat (5.5%) as a compromise between doe and young nutrition in comparison with a diet lower in NDF (27.6%) and higher in starch (19.0%): the results of a large scale study (6 sites and about 550 does and 9000 rabbits) showed that the high-fat low-starch diet reduced the mortality of young rabbits without impairing their growth or the reproductive performances of does.

<u>Protein</u>

When speaking about nitrogen (N) requirements in rabbits, various reference units are used (De Blas and Mateos, 1998; Fraga, 1998; García-Ruiz *et al.*, 2005; Carabaño *et al.*, 2000, 2009). Crude protein (CP) and apparent digestible protein (DP) are the most commonly used units, for which both requirements and raw material composition are largely available (Villamide *et al.*, 1998; Maertens *et al.*, 2002). Really, rabbits have specific amino acids (aa) requirements and apparent faecal and true ileal digestible amino acids would be more reliable units. However, even if increasing information is given about the most diffused raw materials, digestible aa requirements and concentrations in feeds are scarcely known and even less information exists on ileal digestible aa (Carabaño *et al.*, 2009). In practice, due to the chemiostatic regulation of appetite in rabbits, nitrogen requirements are expressed in relation to dietary energy by the DP to DE ratio, which is directly correlated with body N retention and excretion.

Amino acids supply through caecotrophy has been for long time considered adequate to support essential aa requirements in rabbits (De Blas and Mateos, 1998). Really, in rabbits fed conventional diets, the contribution of soft faeces to total CP intake is only 15–18% (Fraga, 1998; Carabaño *et al.*, 2000), while few information is available for the different aa (Nicodemus *et al.*, 1999; Belenguer *et al.*, 2005; Abecia *et al.*, 2008). On the whole, literature on rabbit aa requirements is rather old and restricted to the most limiting aa in the diet (lysine, sulphur-containing aa, threonine, arginine) and the aa levels actually recommended are still those provided by Lebas (1989) and revised by De Blas and Mateos (1998). Recently, specific needs for certain essential and non essential aa (threonine, arginine, glutamate) have been hypothesised in order to optimize defence mechanisms of intestinal barrier against pathogens (Baylos *et al.*, 2008; Carabaño *et al.*, 2009; Chamorro *et al.*, 2010).

Besides optimizing productive performances, a correct dietary supply of protein and amino acids in growing rabbits would permit to improve gut health (Carabaño *et al.*, 2009) maximize nitrogen retention and reduce nitrogen excretion, which is of growing importance in view of controlling environmental pollution (Maertens *et al.*, 2005; Xiccato *et al.*, 2005, 2006b; Calvet *et al.*, 2008).

The needs in protein and amino acids depend on the age and the physiological status of the rabbit: they are higher in the first period of growth, considering also the needs for the maturation of the digestive apparatus, and lower in adult animals. The necessary level of dietary protein to satisfy the needs of the rabbit depends on the amino acid profile, the digestibility of the protein and the intake level, the later is directly correlated to the concentration of DE of the diet (Fraga, 1998). The need is about 15-16% of CP or 10.5-11% of DP and optimal DP to DE ratio between 10.5-11.0 g/MJ (Xiccato, 1993, 1996; Fraga, 1998; de Blas and Mateos, 1998; Xiccato and Trocino, 2010a). A minimum level of 0.54% of total sulphur-containing aa (0.40% of digestible aa) is required to obtain adequate productivity in growing rabbits (Taboada *et al.*, 1996). Recommended levels of lysine are 0.76–0.80% total lysine (0.60–0.64% digestible lysine) for maximum litter growth (Taboada *et al.*, 1994). Optimum supply is 0.64% total threonine and 0.44% digestible threonine (De Blas *et al.*, 1998).

Regarding the health status, the lack (<12%) or excess of CP (>18%) favour the presence of digestive disturbances and increase the mortality of the animals, by modifying the caecal fermentative activity and the composition of the microflora (Maertens and De Groote, 1988; Lebas, 1989; Carabaño *et al.*, 2008, 2009).

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%, but it depends on the intake of dry matter, the content and characteristics of dietary fibre and also by 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 that is fermented by microflora is converted to ammonia, which represents the major N source for the synthesis of bacterial protein. All microbial populations benefit from the protein content 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 fact, reducing dietary CP from 18 to 16% (in diets with the same ileal digestibility) significantly decreased the presence of *Clostridium perfringens* and mortality due to ERE (Table 3) (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).

Table 3. Effect of the level of protein in isofibrous diets (30% NDF) on pathogenic flora and mortality in 25-d weaned rabbits (Chamorro *et al*., 2007).

	18% CP	16% CP	Prob.
Ileal CP flow (g/d)	6.0	5.0	<0.05
Animals with *C. perfringens* (%)	47.2	18.0	<0.05
Mortality during fattening (%)	21.2	11.0	<0.05

When increasing ileal digestible protein, and thus decreasing the ileal flow of protein, by different sources of protein (sunflower meal, soybean meal, soybean and potato protein concentrate), a lower incidence of digestive problems occurred (Gutiérrez *et al.*, 2003; García-Ruiz *et al*., 2006; Chamorro *et al*., 2007). 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 and growth performance of early weaned rabbits.

Few data exist on the effect of the protein level and source on intestinal mucosa structure. In piglets, protein sources sometimes affect intestinal traits (Vente Spreeuwenberg *et al*., 2004a, 2004b). In rabbits, a positive effect on intestinal mucosa integrity was found when animal plasma replaced soybean meal in starter diets for early weaned rabbits (Gutiérrez *et al*., 2000), while the negative effect of the anti-nutritional factors present in legumes have been only hypothesized (Gutiérrez *et al*., 2003; Cano *et al*., 2004).

Beside optimizing performances and health of rabbits, the correct knowledge of protein and amino acid requirements play a key role in the control of N excretion, especially in rabbits for which around two thirds of ingested N is excreted (Maertens *et al*., 2005; Xiccato *et al.*, 2006b, 2007). In highly populated areas, vulnerable from hydrogeologic point of view, animal wastes represent a potential contaminant for water and soil. The European Directive

(91/676/EC), aiming to prevent or reduce the nitrate pollution of surface and underground water, asks to each member state reference values for N excretion of all livestock as well as the definition of feeding and management strategies to control environmental pollution.

Nitrogen excretion cannot be measured directly, because of the great loss of N (through volatile ammonia) from urine and faeces during waste stocking and treatment. According to the official methodology (ERM/AB-DLO, 1999), N excretion is quantified as the difference between N consumption and N retention in animal products, that is, for rabbits, body and foetal tissues and milk. Since N concentration in the body tissues of finishing rabbits is rather constant (0.29–0.32%) and the N of foetal tissues and milk are destined to be transferred into the body of fatteners, the farm N balance of rabbits can be calculated as the difference between the N input (dietary N) and the N output (body N of produced rabbits) in the farm.

Among the various factors that can affect N balance at farm level, N excretion is strictly dependant on dietary CP level (Maertens *et al*., 2005; Xiccato *et al*., 2005). In fattening rabbits, once the limiting aa requirements are satisfied by synthetic aa supplementation, dietary CP may be reduced below 17%, therefore decreasing N excretion without impairing productive performance (Maertens *et al*., 1997). Only below 13.8% CP, daily weight gain impairs (-9%), but N excretion is reduced by 38% (Figure 9).

When rabbits are fed a diet supplemented with the most limiting aa until slaughter at 63 days of age and 2.35 kg LW, decreasing dietary CP from 16.0 to 14.0% does not impair growth performance (García-Palomares *et al*., 2006b). When rabbits are slaughtered later (75–90 days) at a heavier LW (2.5–3.0 kg), feeding programs based on decreasing dietary CP would permit to better satisfy the higher protein requirements of the first growth period and to reduce excretion during fattening. In fact, in this latter period feed intake is higher and dietary N concentration can be reduced without consequences on performance and meat quality (Maertens *et al*., 1997; Maertens and Luzi, 1998; Trocino *et al*., 2000, 2001). Lowering dietary CP from 16.0 to 14.0% in the first period, from 32 to 56 days of age, reduces daily growth and body N retention (-6%) and N excretion at a similar extent (-7%) (Trocino *et al*., 2000). In the second period (56 to 77 days), a reduction of dietary CP from 15.4 to 14.3% decreases N excretion by 9% without impairing daily gain and body N retention. A further decrease of dietary CP until 13,1% permits to lower N excretion by 15% in comparison with the control diet, while reducing growth and N retention only by 3%.

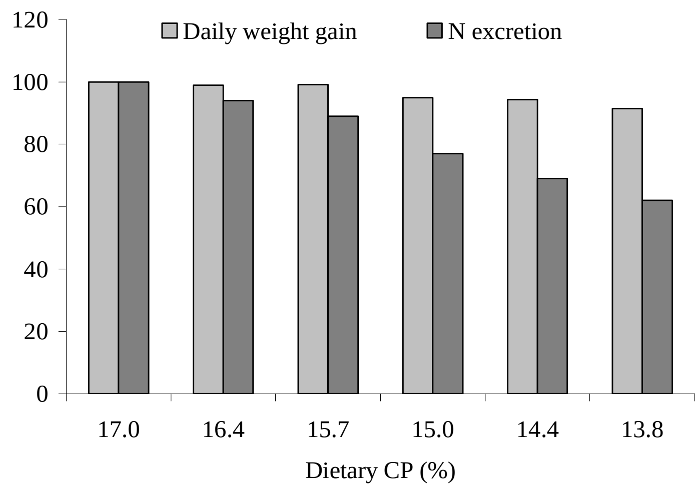


Figure 9. Daily weight gain and nitrogen excretion in rabbits (32 to 74 days of age) according to dietary CP concentration (17.0% CP = 100) (Maertens *et al*., 1997).

High-fibre low-starch diets with low DE concentration have been largely used in the last decade to reduce the risk of enteric diseases, like ERE (Gidenne, 2003; Gidenne and García, 2006). However, when lowering DE concentration, feed intake increases and, if dietary CP concentration remains unchanged, DP to DE ratio and N intake increase. Since growth rate is not modified and N retention remains constant, N excretion increases. As an example, when DE concentration decreases from 10.5 to 8.8 MJ/kg and dietary CP concentration is maintained at 15.0 with 70% digestibility, DP to DE ratio increases from 10 to 12 g /MJ. As shown in Figure 10, body N retention remains unchanged while daily N excretion (faecal + urinary) increases by 20%.

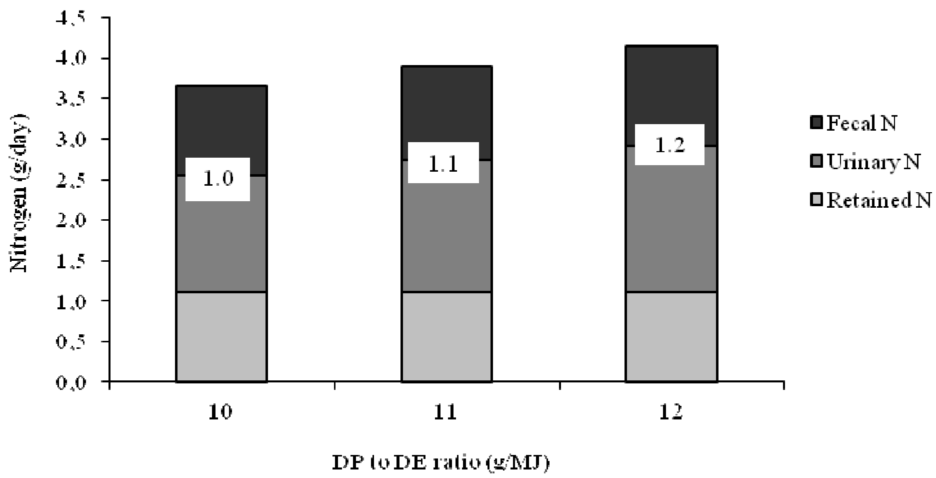


Figure 10. Daily N retention and excretion (faeces + urine) according to dietary DP to DE ratio (Xiccato *et al*., 2006b)

STATE OF ART AND GENERAL OBJECTIVES

The present PhD thesis was carried out at the Dipartimento di Scienze Animali, Università di Padova (UNIPD), Italy, supervised by Professor Gerolamo Xiccato and the Departamento de Producción Animal, Universidad Politécnica de Madrid, Spain, supervised by Professor Rosa Carabaño. The thesis is divided into four experiments that took place in the experimental facilities of the Padova University. Laboratory and histological analyses, statistical analysis and thesis preparation were performed partly in Italy and Spain. All experimental activity realized is part of a wider collaboration programme that the two Departments have shared for about 15 years, starting with the foundation of the European Group on Rabbit Nutrition (EGRAN) and continuing with national and European projects, such as ERAFE (European harmonization of rabbit feed evaluation, FAIR96) and COST 848 Multifaceted research in rabbit.

The experimental activities realized in the frame of the present PhD thesis followed the general objectives of defining rabbit nutritional needs during post-weaning and fattening with special regards to the different fibrous fractions, especially the most digestible (digestible fibre, pectins, hemicelluloses), the starch content in relation to fibre, as well as the dietary protein supply with the aim of *1*) improving rabbits intestinal conditions *2)* reducing the occurrence and seriousness of digestive system pathologies *3)* increasing feed efficiency and N retention, and *4)* guaranteeing high performance and final product quality.

Since 1996 the spread out of Epizootic Rabbit Enteropathy (ERE) in European rabbit breeding stock has remarkably increased mortality, losses and impaired feed conversion, because of the large number of sick animals. The lack of identification of an etiological agent and the attribution of a multifactorial character to it, as well as the limitations imposed by EU legislation on the use of antibiotics, have stimulated research on feeding and management strategies capable of preventing or at least limiting the impact of enteropathy. In particular, studies in the past few years intended to specify the nutritional requirements in rabbits during weaning and post-weaning, in relation to the possibility of reducing the damages caused by enteropathy.

Among the various nutrients, the most digestible fibre fractions (DF = pectins and hemicelluloses) seem to favour the health status of the animals, in addition to enhance caecal the fermentative activity and feed efficiency (Gidenne and García, 2006).

Dietary fibre and protein have an important role on feeding efficiency and the development of digestive system pathologies (Gidenne and García, 2006; Carabaño *et al*., 2008, 2009), while there is a certain discussion about the role of starch on caecal disorders (Blas and

Gidenne, 2010). Although sufficient information exists about the optimal levels of various nutrients on normal farm conditions (De Blas and Mateos, 1998), deeper information is needed since feeding should also improve animal enteric health status, optimize the feed conversion and reduce nitrogen excretion and pollution.

Regarding diet fibrous components, a low concentration of non-digestible fibre reduces intestinal transit and stimulates protein fermentation that increases the ammonia concentration and pH in caecum, so favouring the development of intestinal dysbiosis (Gidenne, 1996; Bennegadi *et al*., 2000). On the other hand, high non-digestible fibre content may stimulate excessively peristalsis, reducing feed digestibility. In young animals, which caecal activity is still developing, the administration of high-fibre diets, especially rich in non-digestible fractions, may create digestive problems (Gutiérrez *et al*., 2002; Gidenne *et al*., 2004a, 2004b; Nicodemus *et al*., 2004; Gidenne and Licois, 2005; Feugier *et al*., 2006).

In young rabbits, soluble fibre fractions are more fermentable (Marounek *et al*., 1995; Lavrencic, 2007) and may favour intestinal health status by promoting the proliferation of favourable microbe population and by improving the mechanisms of competitive exclusion (Carabaño *et al*., 2008). By increasing the soluble fibre content in starter weaning diets, *C. perfringens* and other opportunistic pathogens at caecal level were reduced as well as mortality caused by diarrhoea (Soler *et al.*, 2003; Gómez-Conde *et al*., 2007). By keeping a constant ADF content and replacing starch and protein by digestible fibre (Perez *et al.,* 2000; Gidenne *et al.*, 2001; Marguenda *et al*., 2006), digestive disorders decreased and rabbit health status improved, as a consequence of digestible fibre positive effect on caecal fermentative activity (Gidenne and Bellier, 2000). Reducing insoluble fibre content (Gutiérrez *et al.*, 2002; Alvarez *et al.*, 2007) and increasing the more soluble fractions (García-Ruiz *et al.*, 1997; Carabaño *et al.*, 2008) had also positive effects on intestinal mucosa integrity.

Little is yet known nowadays about the interaction between digestible fibre and protein content, although effects have been found over caecal fermentative activity with possible influence on health status and productive performance. Lack (<12%) or excess of protein (>18%) may favour digestive system disorders and mortality rate, modifying caecal fermentative activity and microflora composition (Maertens e De Groote, 1988; Lebas, 1989; Carabaño *et al*., 2008, 2009). Reducing protein level from 18 to 16% (in diets with the same ileal digestibility) significantly reduced the presence of *Clostridium perfringens* and mortality by epizootic enteropathy (Chamorro *et al*., 2007), while further reduction from 16 to 14% did not have any effect on mortality, though reducing anaerobic bacteria at ileum (García-Palomares *et al*., 2006a; Carabaño *et al*., 2009). The use of less digestible protein sources may also increase the nitrogen flux at caecal level favour the development of pathogens such as *E. coli* and *Clostridium spp.*, thus impairing animal health status and performance (Gutiérrez *et al.,* 2003; Chamorro *et al.,* 2005, 2007; Carabaño *et al.,* 2008, 2009). Given the late attention of the mass media and consumers' awareness on the use of not genetically modified raw materials, a certain interest exists in evaluating protein sources alternative to soybean, which are certainly not modified biotechnologically. Among these, sunflower meal is widely used in rabbit nutrition, usually combined with soybean meal. Diets based only on sunflower meal demands a careful evaluation of the aminoacidic equilibrium, however.

In this context, the present PhD thesis investigated wide variations in dietary digestible fibre and fibrous fractions, as well as the ratio between high-digestible or low-digestible fibre fractions and starch, or the source and level of dietary protein in young rabbit feeding during post weaning and fattening period. In particular, the objectives of the present thesis were pursued along the following four studies:

Experiment 1. *Digestible fibre level and substitution of soybean with sunflower meal in diets for growing rabbits*. The effect of the digestible fibre level (DF/ADF ratio from 1.0 to 1.2) and the protein source (soybean meal *vs*. sunflower meal) in diets were evaluated on growth performance, health status and slaughter results of growing rabbits.

Experiment 2. *Digestible fibre/starch ratio and protein level for feeding growing rabbits*. The trial aimed to study the effect of different DF/starch ratios (0.8, 1.5 and 2.8) and two crude protein levels (15 and 16%) in diets based on sunflower meal and without soybean protein on productive performance, health status, carcass and meat quality in growing rabbits.

Experiment 3. *Reducing dietary protein and increasing digestible fibre/starch ratio in diets for growing rabbits*. The trial aimed to evaluate the effect of protein level and DF/starch ratio with constant ADF level on health status, growth performance, caecal fermentative activity, intestinal mucosa characteristics, nitrogen equilibrium, slaughter, and carcass and meat quality traits in growing rabbits.

Experiment 4. *Starch and pectin levels in diets for growing rabbit: effects on health status, digestive physiology, growth performance and carcass and meat quality*. The trial aimed to assess the effect of feeding growing rabbits with various level of starch and digestible fibre composition, and in particular the combination of high and low starch (from 5 to 15%) and pectins (from 5 to 10%) supply, when hemicelluloses are maintained constant, on productive performance, health status, caecal traits and intestinal mucosa morphology, as well as on the slaughter results.

EXPERIMENT 1:
Digestible fibre level and substitution of soybean with sunflower meal in diets for growing rabbits

Material and methods

Rearing conditions

The trial was realized in the experimental farm of the University of Padova in the period November–December. Extraction fans and automatic heating system were used to control air circulation, temperature and humidity in the building. Maximum temperature was 27°C only during some days of the trial and minimum temperature reached 14°C (Figure 11). Average values of minimum and maximum temperature were 18 and 20°C, respectively.

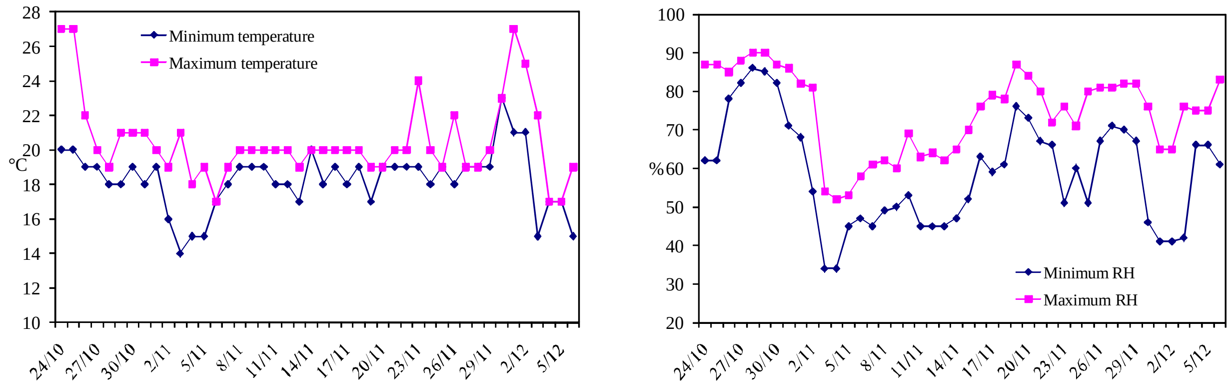


Figure 11. Daily values of minimum and maximum temperature and minimum and maximum relative humidity (RH) throughout the trial

Maximum relative humidity averaged 75%, with a minimum of 52% and a maximum of 90%. Minimum relative humidity averaged 59%, with a minimum of 34% and a maximum of 86%.

In the farm, both commercial cages for fattening (28 x 40 x 30 cm) and experimental cages for digestibility (25 x 40 x 30 cm) in galvanized iron net (Figure 12 and 13) were available. Both cages had 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 14).

Figure 12. Fattening cages with feeders

Figure 13. Experimental cages for *in vivo* digestibility trials

Figure 14. Detail of digestibility cages with removable feeders and baskets for the faeces collection

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

<u>Animals and experimental groups</u>

The animals were selected in a commercial farm. At 33 days of age, 216 rabbits of both genders from a hybrid line (Grimaud Frères, France) were selected from multiparous does (3-6 kindlings) 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 to reduce the transportation stress at minimum and at their arrival they presented an optimal health status. The rabbits were immediately were weighted (837±48 g LW), put in individual cages and identified using ear tags. The animals were divided in six experimental groups of 36 units, homogeneous in average weight and variability, and fed *ad* *libitum* with six diets formulated according to a 2 x 3 factorial arrangement, with two protein sources (soybean *vs*. sunflower meal) and three levels of DF/ADF ratio (1.0, 1.1 and 1.2), as shown in the following scheme:

<table>
<tr><td colspan="2"></td><td colspan="2">Protein source</td></tr>
<tr><td colspan="2"></td><td>Soybean (Soy)</td><td>Sunflower (Sun)</td></tr>
<tr><td rowspan="3">DF/ADF ratio</td><td>1.0 (DF1)</td><td>Diet Soy1</td><td>Diet Sun1</td></tr>
<tr><td>1.1 (DF2)</td><td>Diet Soy2</td><td>Diet Sun2</td></tr>
<tr><td>1.2 (DF3)</td><td>Diet Soy3</td><td>Diet Sun3</td></tr>
</table>

Four diets (Sun1, Sun3, Soy1, Soy3) were formulated by using soybean 46% CP (Soy) or sunflower 36% CP (Sun) as main protein source and by varying inclusion levels of fibre sources (mainly alfalfa meal and dried beet pulp) to obtain three DF/ADF ratios (Table 4). Dilution technique was used to obtain two more diets: Soy2 (0.5 Soy1 + 0.5 Soy3) and Sun2 (0.5 Sun1 + 0.5 Sun3). No antibiotic or drugs were given in feed or water, apart from coccidiostat (Cycostat, Robenidine 66%).

Rabbits were fed the experimental diets from 34 d of age until 5 days before slaughter. During the last days (71-76 d) experimental diets where replaced by a common commercial diet without coccidiostat.

Experimental diets

The formulation of the diets is presented in Table 4. All diets were in pellets with 3.5 mm of diameter and 1.0-1.1 cm of length. In all diets at least a minimum quantity of all raw materials was included, except of the two protein concentrates, in order to reduce the risks connected to the wrong evaluation of their chemical composition. Diets were supplemented with synthetic aminoacids, micro- and macro-minerals and vitamins to satisfy the nutritional needs of young rabbits (de Blas and Mateos, 1998). All diets were isoproteic (15.9% in average) (Table 5). The contents of crude fibre and various fibre fractions varied to acceptable ranges as to not modify the presumptions of the experimental trial. Only the ADF content of Soy diets resulted to be less variable than what expected from diet 1 to diet 3, and as a consequence, the variation interval of DF/ADF ratio resulted to be narrower than estimated. In both Soy and Sun diets, DF and pectins increased from DF1 diets to DF3 diets, because of beet pulp inclusion. The DF/starch ratio (from 2.3 to 2.6-2.9) and the pectins/starch ratio (from 0.6 to 1.0) increased by increasing the DF level, according to diet formulation.

Table 4. Ingredient composition (%) of experimental diets

Diet	Diet Soy1	Diet Soy3	Diet Sun1	Diet Sun3
DF/ADF ratio	1.0	1.2	1.0	1.2
Dehydrated alfalfa meal 17% CP	48.30	37.10	44.38	27.65
Wheat bran	28.00	13.00	30.00	20.00
Barley	4.00	10.00	3.00	7.00
Dried beet pulp	10.00	25.00	10.00	25.00
Soybean meal 46% CP	7.00	11.50	0.00	0.00
Sunflower meal 36% CP	0.00	0.00	10.00	17.50
Molasses	1.30	1.30	1.30	1.30
Calcium carbonate	0.00	0.00	0.00	0.10
Dicalcium phosphate	0.35	1.05	0.10	0.28
Sodium chloride	0.40	0.40	0.40	0.40
L-lysine	0.00	0.00	0.20	0.20
DL-methionine	0.08	0.08	0.05	0.00
Vitamine - mineral premix*	0.40	0.40	0.40	0.40
Choline	0.07	0.07	0.07	0.07
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.

Table 5. Chemical composition (as fed) of experimental diets

	Diet					
	Soy1	Soy2	Soy3	Sun1	Sun2	Sun3
Dry matter (DM), %	89.5	89.6	89.8	89.6	89.5	89.4
Crude protein (CP), %	16.0	16.2	15.9	15.6	15.7	15.9
Ether extract (EE), %	2.8	2.4	2.3	2.8	2.6	2.5
Crude fibre (CF), %	17.5	17.6	16.9	18.4	17.8	16.9
Ash, %	7.9	7.8	7.8	7.6	7.3	7.2
Total dietary fibre (TDF), %	43.5	44.2	45.0	44.0	44.7	45.1
NDF, %	38.0	36.7	36.5	38.0	37.2	36.7
Hemicelluloses (NDF-ADF), %	16.2	14.8	14.8	16.2	16.2	16.1
ADF, %	21.8	21.8	21.7	21.7	21.0	20.6
ADL, %	3.9	3.9	3.7	4.2	4.0	3.7
Digestible fibre (TDF-ADF)	21.7	22.4	23.3	22.3	23.7	24.5
Pectins (TDF-NDF), %	5.5	7.5	8.5	6.0	7.5	8.5
Starch, %	9.3	9.5	8.9	9.9	8.9	8.5
Gross energy (GE), MJ/kg	16.3	16.3	16.3	16.5	16.4	16.3
DF/ADF ratio	1.0	1.0	1.1	1.0	1.1	1.2
DF/starch ratio	2.3	2.4	2.6	2.3	2.7	2.9
Pectins/starch ratio	0.6	0.8	1.0	0.6	0.8	1.0

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 if in case of new symptoms, 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 apparent digestibility of nutrients and the digestible energy (DE) concentration of the diets were determined by *in vivo* digestibility trial on 60 growing rabbits (10 animals per diet) using 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 53 days of age with a 4-day collection period. Faeces were collected daily and stored in plastic bags at -18°C until analysis.

Caecal content and intestinal mucosa sampling

In order to measure the characteristics of the caecal content and the intestinal mucosa, 36 rabbits (6 per diet and representative of the experimental group for average live weight and variability) were slaughtered at 56 days of age by cervical dislocation. Then, the digestive apparatus was removed and weighed. The pH of the caecal content was immediately measured after the gastro-intestinal tract was removed. The caecal content was also removed, put into plastic bottles, diluted with 25% HPO_3 solution (10 ml of 25% ortho-meta-phosphoric acid) and stored at –18°C until chemical analysis. Moreover, intestinal tissues were sampled at the medium tract of jejunum for histological analyses.

Histological analyses

Intestinal tissues sampled at 56 d were fixed by using 4% para-formaldehyde in PBS, dehydrated, included in paraffin, cut in sections of 4 μm with a microtome (Leica RM2035, Germany) and dyed with haematoxylin-eosin. Villi height and crypt depth were measured on tissues by image analysis software (DP-soft, Olympus Optical Co GmbH, Hamburg Germany) (Figure 15).

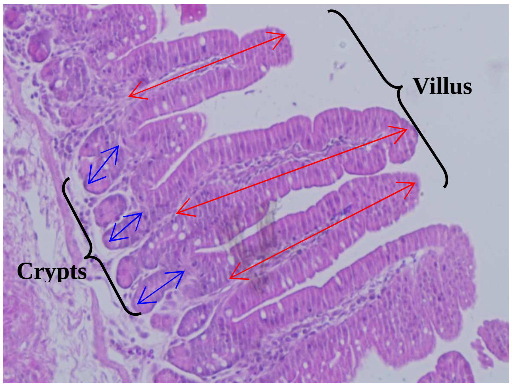


Figure 15. Section of intestinal tissue and measure of villi and crypt dimensions

Chemical analyses

All samples were ground at 1 mm of diameter with a grinder (mod. ZM 100, Retsch, Haan, Germany) and 1-mm diameter grid. The chemical analysis of faeces and feed samples were done according to A.O.A.C methods (2000), following the European harmonized procedures EGRAN (2001). The crude fibre content was determined by Weende method, while fibre fractions (NDF, ADF and ADL) were analyzed according to the method proposed by Goering and Van Soest (1970) and modified by Robertson and Van Soest (1981). Moreover, the content of Total Dietary Fibre (TDF) was determined through a gravimetric-enzymatic procedure that requires the treatment with α-amylase, protease and aminoglucosidase (Megazyme Int. Ireland Ltd., Wicklow, Ireland) (Methods AOAC 991.43, 32-07, 32-21, 985.29, 32-05). The content of digestible fibre (DF) was calculated as the difference between the TDF and ADF content, thus theoretically including, pectins and hemicelluloses.

The energy content of the samples was determined with adiabatic calorimetric bomb (Martillotti *et al.*, 1987). The level of starch was measured by HPLC (method 996.11 and 979.10, AOAC, 2000) after enzymatic treatment (Boehringer Mannheim Starch Determination, cat. no. 207748).

The pH of caecal content was measured using a pH-meter (HI 9025 C, Hanna Instruments, Padova, Italy) equipped with a combined Ingold electrode (406 M3). The thawed samples of caecal content were centrifuged for 10 min at 9000 *g*. Caecal ammonia-N was determined on the supernatant by 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 concentration was measured on the supernatant by 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) (JXR, Mega, Milano, Italy) using the method of Osl (1988).

Commercial slaughter, carcass dissection and meat quality analyses

At 76 d of age, 120 rabbits were selected (20 for each experimental group and representative in terms of average weight and variability) for commercial slaughter 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 10 rabbits per cage (20 rabbits/m^2) in a conditioned truck for about 60 minutes to a commercial slaughterhouse.

Slaughter began at 10.00 and ended at 11.30 a.m. approximately. Rabbits were individually weighed, then stunned by electro-anaesthesia and killed by jugulation. Carcasses were chilled at 4°C for 2.5 h 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 16). The lightness (L*), redness (a*) and yellowness (b*) (Rennere, 1982) were measured on the same muscles using a colorimeter Minolta Spectrophotometer CM-508 C (Minolta, Milano) (Figure 17), 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 to obtain the “reference carcass”. The right hind leg and the dissectible fat (scapular and perirenal fat) were separated. 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).

Statistical analyses

The data recorded were analyzed by ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC) and considering the effects of DF/ADF ratio and the protein source. The effects of gender was also included in the model to analyze variability of growth performance, slaughter results, carcass and meat traits. When necessary, the Bonferroni orthogonal test was used to compare means. Mortality, morbidity and sanitary risk were analyzed by the CATMOD procedure of SAS.

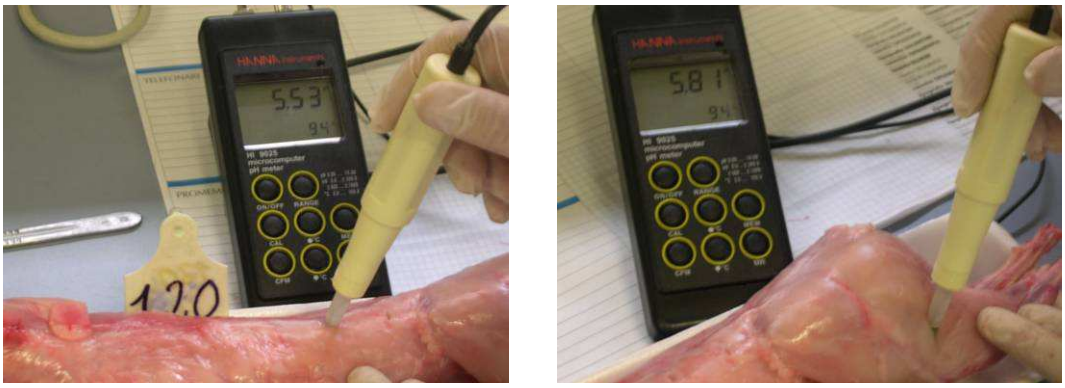


Figure 16. Measuring pH on m. *longissimus dorsi* and *biceps femoris*

Figure 17. Measuring colour on m. *longissimus dorsi* and *biceps femoris*

Results and discussion

<u>Digestibility and nutritive value of experimental diets</u>

The coefficients of digestibility are presented in Tables 6 and 7 as average of the main factors and interactions, respectively. The digestibility of dry matter, gross energy, crude fibre and fibrous fractions significantly increased with increasing DF/ADF ratio, due to the higher inclusion of beet pulp and the lower inclusion of alfalfa.

Undoubtedly, the digestibility and nutritive value of the diets increases with DF/ADF ratio, not only for the higher presence of digestible fibre constituents, but also for the increased digestibility (9-10 points) of all fibre fractions: ADF digestibility increased from 14.6 to 25.6%; hemicelluloses digestibility from 40.3 to 49.1%; pectins digestibility from 85.0 to 93.8% ($P<0.01$). This increment may be connected to the lower grade of lignification and complessity of cell walls and to the higher susceptibility of structural carbohydrates to the action of digestive enzymes, animal or bacterial ones.

Protein source had a more limited effect on the digestibility of nutrients. Crude protein digestibility tended to decrease ($P=0.06$) in the sunflower diets, consistently with the lower digestibility of sunflower rather than soybean protein (Maertens *et al.,* 2002). However, Gutiérrez *et al.* (2003) measured a higher ileal digestibility of protein and dry matter in diets based on sunflower meal (36% CP) rather than in diets based on soybean meal (48% CP), though without difference in faecal apparent digestibility. In the present trial, ether extract and hemicelluloses digestibility were more digestible (P<0.001) in sunflower diets than in soybean diets, while ADF and pectins resulted less digestible.

Table 6. Digestibility coefficients (%): effect of DF/ADF ratio and protein source

	DF/ADF ratio				Protein source			RSD[1]
	1.0	1.1	1.2	Prob.	Soybean	Sunflower	Prob.	
Rabbits, No.	19	19	17		26	29		
Dry matter	55.4[a]	58.3[b]	61.3[c]	<0.001	58.6	58.1	0.27	1.72
Crude protein	73.2	72.5	72.8	0.16	73.1	72.5	0.06	1.14
Ether extract	73.1[b]	68.4[a]	68.6[a]	<0.001	69.3	70.8	<0.001	1.30
Crude fibre	11.7[a]	18.7[b]	21.7[c]	<0.001	18.0	16.8	0.21	3.41
TDF	33.4[a]	40.4[b]	46.6[c]	<0.001	40.5	39.8	0.30	2.44
NDF	25.6[a]	30.2[b]	35.6[c]	<0.001	30.7	30.3	0.68	2.87
ADF	14.7[a]	20.2[b]	25.6[c]	<0.001	22.0	18.3	<0.001	3.27
Digestible fibre	51.9[a]	59.1[b]	65.0[c]	<0.001	58.4	59.0	0.18	1.68
Pectins	85.0[a]	90.7[b]	93.8[c]	<0.001	91.5	88.2	<0.001	0.39
Hemicelluloses	40.3[a]	43.8[b]	49.1[b]	<0.001	42.9	45.9	<0.001	2.31
Starch	96.8[b]	96.4[a]	96.4[a]	<0.001	96.4	96.7	<0.001	0.15
Gross energy	54.7[a]	57.5[b]	60.6[c]	<0.001	57.8	57.4	0.43	1.75

[1]Residual standard deviation

Table 7. Digestibility coefficients (%) and nutritive value (as fed) of experimental diets

	Diets						Prob.
	Soy1	Soy2	Soy3	Sun1	Sun2	Sun3	F x P[1]
Rabbits, No.	10	9	7	9	10	10	
Dry matter	55.5[a]	58.7[bc]	61.5[d]	55.2[a]	58.0[b]	61.0[cd]	0.93
Crude protein	72.8[ab]	73.4[b]	73.2[ab]	73.6[b]	71.6[a]	72.5[ab]	<0.01
Ether extract	72.8[c]	67.8[a]	67.4[a]	73.5[c]	69.1[ab]	69.8[b]	0.14
Crude fibre	11.8[a]	19.8[b]	22.3[b]	11.6[a]	17.6[b]	21.2[b]	0.68
TDF	33.8[a]	40.7[b]	47.0[c]	33.2[a]	40.1[b]	44.2[c]	0.99
NDF	26.2[ab]	30.1[bc]	35.7[d]	25.0[a]	30.4[c]	35.6[d]	0.70
ADF	16.5[ab]	21.9[cd]	27.7[e]	13.0[a]	18.5[bc]	23.6[de]	0.94
Digestible fibre	51.1[a]	59.0[b]	65.0[c]	52.8[a]	59.1[b]	65.0[c]	0.25
Pectins	86.1[b]	92.8[e]	95.6[f]	83.8[a]	88.6[c]	92.1[d]	<0.001
Hemicelluloses	39.3[a]	42.0[a]	47.4[bc]	41.2[a]	45.7[b]	50.9[c]	0.44
Starch	96.6[c]	96.0[a]	96.6[c]	97.0[d]	96.8[d]	96.3[b]	<0.001
Gross energy	54.7[a]	57.7[c]	61.0[d]	54.8[ab]	57.2[bc]	60.2[d]	0.76
Nutritive value:							
Digestible energy (DE), MJ/kg	8.90	9.39	9.93	9.05	9.38	9.79	
Digestible protein (DP), g/kg	116	119	116	115	112	115	
DP/ DE, g/MJ	13.1	12.7	11.7	12.7	12.0	11.8	

[1]Probability of the interaction DF/ADF (F) x protein source (P).

Significant interactions (Table 7) for crude protein, pectins and starch digestibility are attributable to minimal variations of analytical data over the composition of diets and faeces giving not perfectly linear responses to the values of digestibility, rather than to a digestive efficiency affected by the specific combination of DF and protein.

The DE concentration of diets was moderate and increased by DF/ADF ratio (from about 9-10 MJ/kg) regardless from the protein source (Table 7). The nutritive values fit well with the moderate starch content and variations of DF values, and follow the current recommendations for rabbit nutrition during post-weaning. The DP/DE ratio was consequently reduced, since DP content was relatively constant. Protein to energy ratio content was consistent with recommendations for weaning and post-weaning phase, but higher than those for fattening rabbits (10 g DP/MJ DE) (de Blas and Mateos, 1998; Carabaño *et al.*, 2000, 2009).

Health status and performance

During the trial, the sanitary problems were limited and not influenced by the feeding treatment (Tables 8 and 9): only 6 animals died, mostly between the third and forth week of the trial, of which two fed Soy1 diet, three Soy2 diet and one Sun3 diet. A seventh animal, belonging to Soy3 group, was sick for a long period and then discarded at the end of the trial since it weighted less than 2.2 kg, that is considered the minimum commercial weight for the Italian market.

Table 8. Mortality, morbidity and sanitary risk: effect of diet[1]

	Diet						Prob.
	Soy1	Soy2	Soy3	Sun1	Sun2	Sun3	
Mortality, %	6.7 (2)	10.0 (3)	- (0)	- (0)	- (0)	3.3 (1)	-[2]
Morbidity, %	13.3 (4)	23.3 (7)	10.0 (3)[3]	16.7 (5)	16.7 (5)	13.3 (4)	0.81
Sanitary risk, %	20.0 (6)	33.3 (10)	10.0 (3)	16.7 (5)	16.7 (5)	16.7 (5)	0.35

[1]In parenthesis is presented the number of dead or sick animals. [2]No statistical analysis was possible. [3]One sick animal discarded at the end of the trial because below commercial slaughter weight.

Table 9. Mortality, morbidity and sanitary risk: effect of DF/ADF ratio and protein level[1]

	DF to ADF ratio (F)				Protein source (P)			Interaction
	1.0	1.1	1.2	Prob.	Soybean	Sunflower	Prob.	F x P
Mortality, %	3.3 (2)	5.0 (3)	1.7 (1)	0.61	5.6 (5)	1.1 (1)	0.13	-[2]
Morbidity, %	15.0 (9)	20.0 (12)	11.7 (7)[3]	0.47	15.6 (14)[3]	15.6 (14)	0.89	0.70
Sanitary risk, %	18.3 (11)	25.0 (15)	13.4 (8)	0.32	21.2 (19)	16.7 (15)	0.65	0.32

[1]In parenthesis is presented the number of dead or sick animals. [2]No statistical analysis was possible. [3]One sick animal discarded at the end of the trial because below commercial slaughter weight.

All other animals fully expressed their growth potential of growth (Figures 18 and Table 10), reaching at 76 days of age a very high average live weight (about 3 kg). Daily growth rate decreased (40-45 g/d) only during the central phase of the trial, around the fourth week, because of the presence of a certain number of animals (10-23% according to the experimental group, P>0.10), that presented digestive disorders (diarrhoea, constipation, weight loss, decrease in feed intake).

In all cases, these symptoms lasted 5-8 days and were resolved spontaneously without the need of any therapeutic treatment. The animals regained their weight quickly during the final period of the trial, benefiting probably by the substitution of the experimental diets by a common coccidiostat-free feed during the final days of the trial that was likely more energetic. This hypothesis is confirmed by the better response in terms of growth rate, during the last week, in the animals fed the poorest diets (DF/ADF 1.0) (Figure 18a).

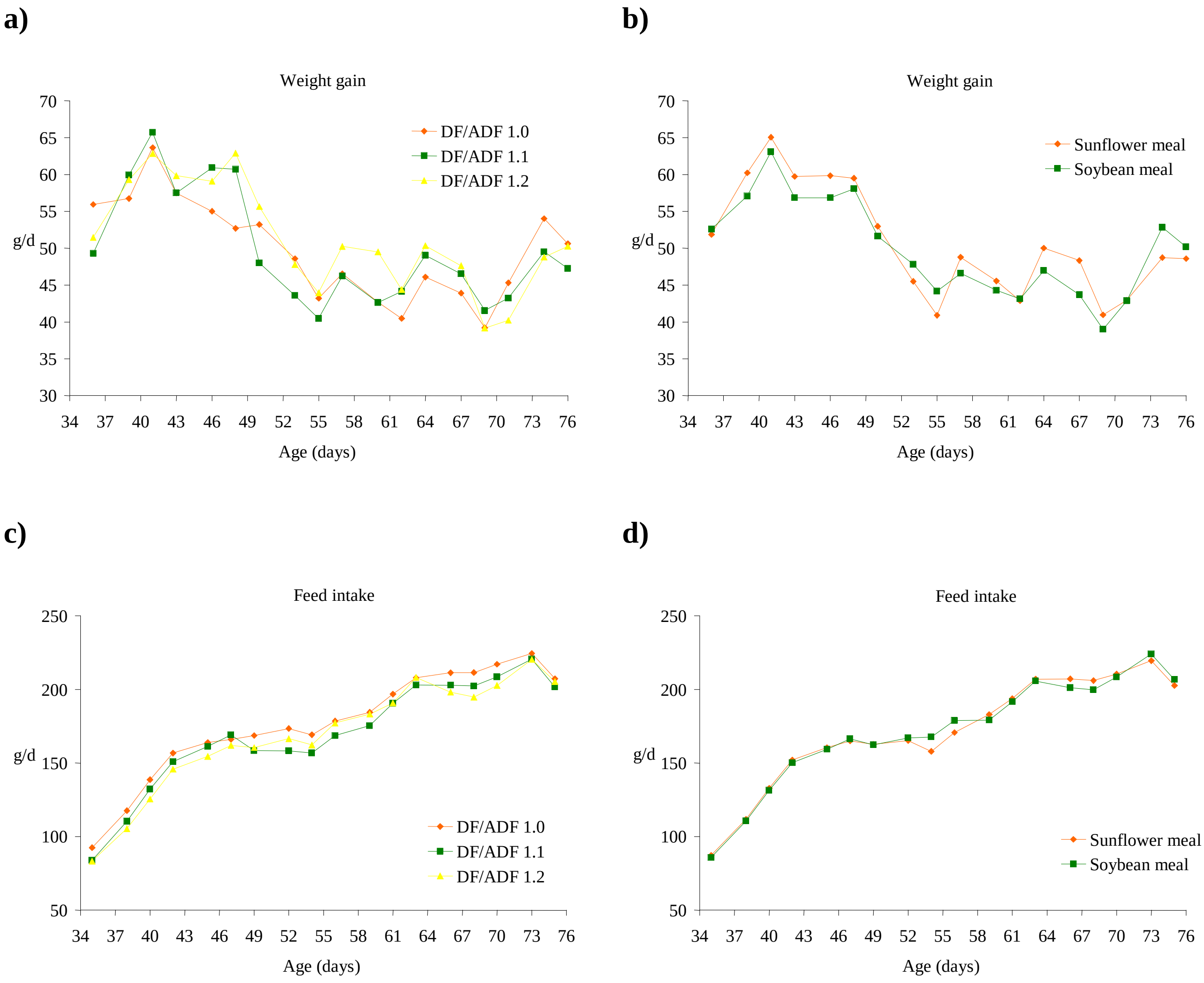


Figure 18. Effects of DF/ADF ratio (a, c) and protein source (b, d) on growth performance

By increasing DF/ADF ratio and the nutritive value of the diets, daily growth rate and live weight of rabbits did not vary, while feed consumption tended to decrease, mainly in the first period of the trial, from 34 to 55 days of age, and feed conversion linearly improved (from

3.55 to 3.42 and 3.30 in the whole trial; P<0.001) (Table 10). This result was quite expected and found by previous studies (Xiccato *et al.,* 2006a; Carraro *et al.,* 2007; Fragkiadakis *et al.,* 2007). Other studies have also shown a reduction in mortality by increasing digestible fibre fractions (Xiccato *et al.,* 2006a; Gómez-Conde *et al.,* 2007), which was not observed in the present trial due to the good health status of all animals. The absence of severe digestive disorders could be partially explained by the older weaning age (33 d) of the rabbits used in the present trial compared to the studies previously reported (25-30 d), that guarantees, according to the current opinion, a lower susceptibility to stress and digestive pathologies.

Growth performance was not affected by protein source, soybean or sunflower meal. Previous studies showed a modest or null effect of protein source, at similar protein contents, though significant decrease of mortality due to epizootic enteropathy was observed when increasing ileal protein digestibility (Gutiérrez *et al.,* 2002, 2003; Chamorro *et al.,* 2007).

Table 10. Performance from weaning to commercial slaughtering

	DF/ADF (F)			Protein Source (P)		Gender (G)		Probability				RSD[1]
	1.0	1.1	1.2	Soybean	Sunflower	Females	Males	F	P	G	F x P	
Rabbits, No.	58	57	58	84	89	95	78					
Live weight, g												
At 34 d	898	897	900	897	900	901	896	0.96	0.68	0.55	0.72	57
At 55 d	2032	2039	2080	2041	2060	2026	2075	0.40	0.56	0.13	0.17	206
At 76 d	2990	2996	3079	3008	3034	3007	3036	0.08	0.46	0.43	0.18	236
First period (34-55 d)												
Weight gain, g/d	54.0	54.4	56.2	54.5	55.2	53.6	56.1	0.38	0.61	0.07	0.17	9.0
Feed intake, g/d	149	143	141	144	144	142	147	0.08	0.95	0.14	0.27	19
Feed conversion	2.78[b]	2.67[ab]	2.57[a]	2.68	2.67	2.72	2.63	0.01	0.78	0.13	0.64	0.37
Second period (55-76 d)												
Weight gain, g/d	45.6	45.6	47.6	46.0	46.4	46.7	45.8	0.24	0.73	0.40	0.90	7.2
Feed intake, g/d	204	198	200	201	201	198	203	0.29	0.99	0.08	0.55	21
Feed conversion	4.63[b]	4.42[ab]	4.25[a]	4.48	4.37	4.35	4.50	0.03	0.29	0.17	0.89	0.73
Overall (34-76 d)												
Weight gain , g/d	49.8	50.0	51.9	50.3	50.8	50.1	51.0	0.07	0.49	0.31	0.18	5.2
Feed intake, g/d	176	170	171	173	173	170	175	0.10	0.98	0.05	0.28	17
Feed conversion	3.55[c]	3.42[b]	3.30[a]	3.44	3.41	3.40	3.44	<0.001	0.23	0.22	0.55	0.21

[1]Residual standard deviation

Limited differences were observed in growth performance of male and female rabbits, with higher growth rate during the first period (P=0.08) and higher consumption during the second one (P=0.07) in males than females. In the whole trial, only feed intake resulted statistically higher in male rabbits (175 *vs*. 170 g/d; P=0.05). Other studies reported a higher growth rate in females than in males, connected to a higher feed intake and development of the digestive apparatus. The absence of a clear gender effect on performance that was observed in the present trial and the difference with previous studies could be explained by the differences in the length of the growing period, besides the differences in precocity of the genetic types used in the various trials. Genetic types, selected for such a rapid development like in the present trial, probably do not express so early differences in growth between genders, but further studies are needed to confirm this hypothesis.

Caecal content and intestinal tissue characteristics

Despite of the differences in feed intake between the groups mentioned above, at 56 days of age, the filling of the principal compartments of the digestive apparatus, stomach and caecum was not influenced by the feeding treatment (Table 11). Considering that even the diets with the lowest DF/ADF ratio (1.0) contained a discrete quantity of beet pulp (10%) and that these diets had a higher feed intake, a further inclusion of beet pulp (up to 25%) in the diets with DF/ADF ratio of 1.2 did not modify the filling of the digestive tube, nor of the principal compartments, stomach and caecum. However, previous trials have shown that the inclusion of beet pulp in diets, at similar feed intake levels, increases the content and therefore the filling of gut, as a consequence of the slower feed transit (García *et al.*, 1992, 1993; 2000; Cobos *et al.*, 1995; Trocino *et al.*, 1999; Gidenne and Perez, 2000; Falcao e Cunha *et al.*, 2004).

Table 11. Caecal content and jejunum mucosa characteristics in rabbits at 56 d of age

	DF/ADF ratio				Protein source			RSD[1]
	1.0	1.1	1.2	Prob.	Soybean	Sunflower	Prob.	
Rabbits, No.	12	12	12		18	18		
Live weight, (LW) g	2122	2123	2061	0.46	2049	2155	0.03	139
Full stomach, % LW	7.2	6.8	6.5	0.14	6.9	6.7	0.37	0.8
Full caecum, % LW	8.4	8.7	8.4	0.55	8.3	8.7	0.13	0.7
Full gut, % LW	23.9	23.3	22.8	0.22	23.6	23.1	0.30	1.5
Caecal content characteristics:								
pH	6.19^{a}	6.14^{ab}	5.97^{b}	0.04	6.08	6.12	0.53	0.21
$N\text{-}NH_3$, mmol/l	4.27	5.12	5.69	0.38	5.57	4.49	0.20	2.47
Total VFAs, mmol/l	58.3	64.1	62.4	0.35	62.0	61.2	0.79	9.8
C_2, % mol. VFAs	81.6	81.4	82.7	0.44	82.2	81.9	0.68	2.22
C_3, % mol. VFAs	4.19	4.16	4.11	0.94	4.00	4.31	0.11	0.56
C_4, % mol. VFAs	13.6	13.4	12.6	0.50	13.2	13.2	0.92	2.15
C_5, % mol. VFAs	0.59	0.60	0.55	0.70	0.55	0.62	0.24	0.18
C_3/C_4 ratio	0.32	0.32	0.34	0.73	0.31	0.34	0.34	0.08
Jejunum mucosa characteristics:								
Villi height, µm	537	542	543	0.97	553	528	0.23	59
Crypts depth, µm	70	70	72	0.76	72	69	0.44	9
Villi/crypts ratio	7.78	7.95	7.66	0.87	7.83	7.77	0.90	1.34

[1]Residual standard deviation

Caecal fermentation activity, referred to as the quantity of volatile fatty acid and the ratio between various acids, was not influenced by feeding treatments, while pH of caecal content was significantly reduced by increasing the DF/ADF ratio (P=0.04). Such variation, even though not associated to an increase in the total VFA production, as found also by Carabaño *et al*. (1997), may be seen favourably in view of the maintenance of an equilibrium in the caecal bacterial population and fermentation. Previous studies have shown a favourable and significant increase of the caecal fermentative activity by increasing the dietary content of digestible and/or soluble fibre (García *et al.,* 2000; Falcao-e-Cunha *et al.,* 2004).

Neither caecal ammonia level was modified by diet, even if the increased ratio of DP/DE ratio (12-13 g/MJ) in all diets could have determined a high caecal flux of protein and an increase in the proteolytic fermentations with probable negative consequences on the intestinal health of rabbits. The low levels of caecal ammonia found in all rabbits (<5-6 mmol/l) demonstrate, in one hand, the high ileal digestibility of soybean and sunflower proteins (besides all other diet components), and in the other hand, the availability of fermentable carbohydrates capable of favouring the ammonia fixation into bacterial protein.

The structure of intestinal mucosa was not modified: since villi height and crypts depth did not change, the digestive functionality and the capacity of intestinal barrier to contrast the attack of pathogen bacteria were likely not altered by the dietary treatments.

Slaughter results and meat quality

No significant differences emerged in slaughter results between experimental groups (Table 12), confirming the little role of nutrition over carcass and meat quality, since the animals are fed balanced diets and there were no relevant differences in the final body weight.

Table 12. Results at commercial slaughter and carcass quality

	DF/ADF (F)			Protein source (P)		Gender (G)		Probability				RSD[1]
	1.0	1.1	1.2	Soybean	Sunflower	Females	Males	F	P	G	F x P	
Rabbits, No.	40	40	40	60	60	66	54					
Slaughter weight (SW), g[2]	2904	2910	2961	2914	2935	2923	2927	0.40	0.56	0.92	0.14	203
Transport losses, % LW[3]	3.3	3.4	3.1	3.3	3.3	3.3	3.3	0.33	0.86	0.95	0.40	0.9
Gut incidence, % SW[4]	19.0	18.7	18.7	18.7	19.0	19.1	18.6	0.69	0.27	0.08	0.51	1.5
Cold carcass (CC), g	1735	1745	1780	1748	1758	1744	1762	0.29	0.70	0.47	0.16	132
Cold dressing (SW), g	59.7	60.0	60.1	60.0	59.9	59.7	60.2	0.52	0.59	0.04	0.60	1.4
Head, %CC	7.5	7.5	7.5	7.5	7.5	7.4	7.6	0.98	0.77	0.21	0.64	0.5
Liver, %CC	6.4	6.2	6.3	6.4	6.1	6.2	6.4	0.54	0.10	0.23	0.09	1.0
Reference carcass (RC), g	1434	1448	1473	1445	1459	1448	1456	0.33	0.52	0.71	0.07	117
Dissectible fat, % RC	3.3	3.3	3.4	3.2	3.4	3.38	3.24	0.86	0.17	0.38	0.73	0.8
Hind leg, % RC	32.8	32.1	32.4	32.5	32.3	32.5	32.3	0.19	0.56	0.39	0.66	1.0
Muscle/Bone hind leg	6.12	6.22	6.29	6.20	6.23	6.18	6.24	0.49	0.77	0.64	0.15	0.42

[1]Residual standard deviation; [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.

This result has been often observed in previous studies and has been widely reported in literature (Xiccato, 1999; Hernández and Gondret, 2006; Hernández, 2008). Similarly, feeding treatments did not modify meat quality (Table 13).

Live weight at the slaughterhouse had an average value of 2.925 g, with losses during transportation around 3.2-3.3% of live weight at the farm. Cold dressing percentage, calculated as refrigerated carcass weight on live body weight at the slaughterhouse, resulted rather elevated (59.9% average). This result, higher than commercial values (56-57%), has a particular relevance when considering the higher intestinal filling of the animals that were fed *ad libitum* until the moment of the transportation.

Differently, in practical commercial conditions, feeding is suspended at least 6-8 hours before transportation. Such a high carcass yield is attributed both to the high conformation of the commercial hybrid used, and above all, to the elevated body weight at slaughter, positively correlated with dressing percentage (Parigi Bini *et al.,* 1992). Reference carcass presented on average 3.3% of dissectible fat, 32.4% of incidence of hind legs and muscles/bone ratio of the hind leg equal to 6.21. All these values are considered optimal compared to usual farm results.

Table 13. Meat quality: pH and colour of *longissimus lumborum* and *biceps femoris*

	DF/ADF (F)			Protein source (P)		Gender (G)		Probability				RSD[1]
	1.0	1.1	1.2	Soybean	Sunflower	Females	Males	F	P	G	F x P	
Rabbits, No.	40	40	40	60	60	66	54					
Longissimus lumborum:												
pH	5.58	5.59	5.57	5.58	5.59	5.59	5.57	0.58	0.67	0.27	0.99	0.09
L*	52.0	51.2	52.8	52.3	52.3	52.1	52.5	0.31	0.95	0.39	0.88	2.50
a*	-2.40	-2.53	-2.41	-2.50	-2.38	-2.58	-2.31	0.71	0.42	0.06	0.04	0.75
b*	0.06	-0.48	0.07	-0.13	-0.10	-0.47	0.24	0.37	0.94	0.06	0.09	1.96
C*	3.10	3.00	3.24	3.09	3.14	3.27	2.96	0.50	0.78	0.07	0.81	0.91
H*	-0.04	0.10	0.02	0.02	0.03	0.16	-0.11	0.56	0.95	0.02	0.19	0.61
Biceps femoris:												
pH	5.81	5.84	5.81	5.81	5.83	5.85	5.80	0.52	0.42	0.02	0.74	0.11
L*	50.7	50.4	51.5	50.7	51.0	49.9	51.8	0.16	0.57	<0.001	0.03	2.60
a*	-2.34	-2.36	-2.31	-2.38	-2.27	-2.47	-2.20	0.85	0.27	<0.001	0.18	0.40
b*	0.21	0.34	0.11	0.18	0.27	0.10	0.34	0.80	0.73	0.40	0.82	1.52
C*	2.69	2.84	2.70	2.71	2.77	2.86	2.63	0.41	0.54	0.03	<0.001	0.55
H*	-0.08	-0.13	-0.04	-0.07	-0.10	-0.03	-0.14	0.76	0.76	0.27	0.87	0.53

[1]Residual standard deviation.

As mentioned above, the absence of a significant effect of dietary DF on dressing percentage may be ascribed to the presence of a discrete content in beet pulp (10%) also in diets DF1. Differently, the dietary inclusion of beet pulp usually reduces the intestinal transit and increases the gut filling (Carabaño *et al.*, 1997; Hernández and Gondret, 2006). The null effect of the diet could be ascribed, at least in part, to the substitution of the experimental diets in the last

5 days of the trial with a common commercial coccidiostat-free diet, as requested by the existing law. In fact, both Margüenda *et al*. (2008) and Villena *et al*. (2008) found that the weight of the gastro intestinal tract at slaughter significantly changed according to NDF content of the diet given during the last week before slaughter.

According to the slaughter results, female rabbits tended to have (P=0.08) higher gut incidence and, as a consequence, a worse dressing percentage (P=0.04) in comparison to males, as also found previously (Lambertini *et al.*, 1990; Parigi Bini *et al.*, 1992; Petracci *et al.*, 1999; Trocino *et al.*, 2003; Lazzaroni *et al.*, 2009). Greater differences between genders were observed in terms of meat colour: on *longissimus lumborum*, H* index resulted higher in females (P<0.05); on *biceps femoris* were measured lower pH, lightness and redness and higher C* values in females. Other authors have observed a lower L* (Trocino *et al.*, 2003) and a lower coloration (Carrilho *et al.*, 2009) on the meat of females compared to males. In animals slaughtered at older age (>100 days), Lazzaroni *et al.* (2009) have measured a lower lightness, and higher values of redness and yellowness in females than males. Differently, Petracci *et al.* (1999) did not find any significant difference between gender in meat colour and other technological traits (drip and cooking losses).

EXPERIMENT 2:
Digestible fibre to starch ratio and protein level in growing rabbit feeding

Material and methods

Rearing conditions

The trial was realized in the experimental farm of the University of Padova in the period March-April. The characteristics of experimental facilities and cages have been detailed previously in the Experiment 1. Before the arrival of rabbits, the building was kept empty for a long period (sanitary vacuum) and then submitted to a disinfectant and fungicide treatment with enilconazole (Clinafarm, Janssen–Cilag Animal Health, Cologno Monzese, Italy).

Maximum temperature did not exceed 23°C and minimum temperature was not below 12°C (Figure 19). Higher temperature excursions between day and night were measured in the central period of the trial. In the whole period, minimum and maximum temperature averaged about 16°C and 21°C, respectively.

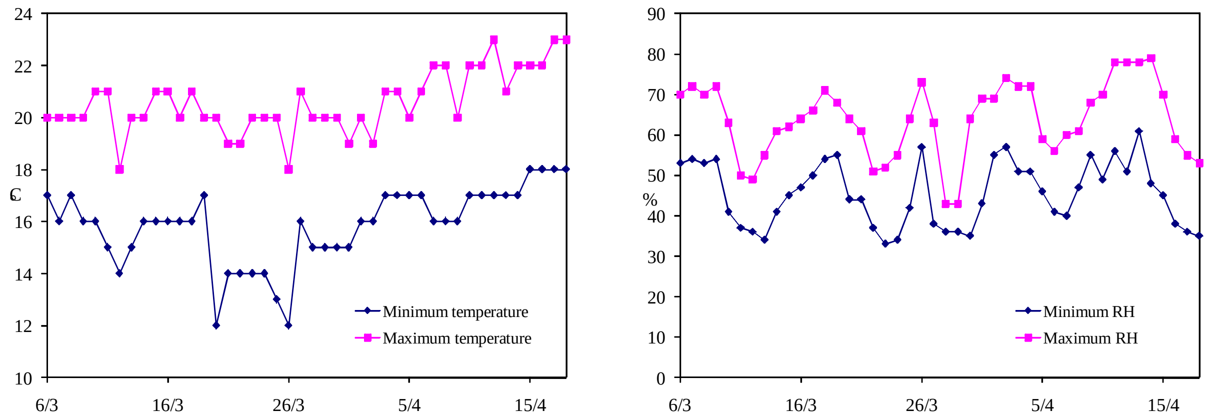


Figure 19. Daily values of minimum and maximum temperature and minimum and maximum relative humidity (RH) throughout the trial

Maximum relative humidity averaged at 64%, with a minimum of 49% and a maximum of 79%. Minimum relative humidity was on average 45%, with a minimum of 33% and a maximum of 61%.

Animals, experimental groups and recordings

The rabbits used in the trial were taken from a commercial farm with about 1,000 Grimaud hybrid does, submitted to a semi-intensive reproductive rhythm with a kindling-insemination interval of 18-19 days. At 32 days, 246 rabbits with homogeneous weight were selected from healthy litters of pluriparous does (2-5 births) and transferred to the experimental facilities, in collective cages at low density in a truck for animal transport. At their arrival, animals were healthy and weighed 782±53 g. Rabbits were controlled by sex, put into individual cages and marked using ear tags.

The animals were divided into six groups of 41 units, homogeneous for initial average weight and variability, and given six diets formulated with a constant ADF level (18%), but differing in protein levels (15 vs 16%) and DF/starch ratio (0.8, 1.5 and 2.8), according to a bi-factorial arrangement 2x3, as in the scheme below:

	Protein 15% (LP)	Protein 16% (HP)
DF/starch ratio 0.8 (R1)	Diet L1	Diet H1
DF/starch ratio 1.5 (R2)	Diet L2	Diet H2
DF/starch ratio 2.8 (R3)	Diet L3	Diet H3

The diets were given *ad libitum* from the beginning of the trial to 5 days before commercial slaughter (75 d), during which a unique coccidiostat-free commercial diet was administered to all animals. No antibiotics were used in their feed or in the drinking water.

Individual live weight and feed intake were 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. The *in vivo* digestibility trial was carried out on 60 animals from 52 to 56 d of age. At 55d d, 36 rabbits were slaughtered to sample caecal contents and mucosa of jejunum (intermediate tract) and ileum (final tract). Histological analysis and chemical analyses of experimental diets, faeces and caecal content 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 L1, L3, H1 and H3 were produced, while L2 and H2 diets were obtained at the experimental farm by mixing diets L1 and L3 (0.5+0.5) and the diets H1 and H3 (0.5+0.5), respectively. The diets did not contain soybean meal (Table 14). Higher protein level in HP diets was obtained by a higher inclusion (+14 to 15 points) of sunflower meal in comparison with LP diets. The increase of

DF/starch ratio from R1 to R3 diets was obtained by reducing alfalfa meal (-16 points) and barley (-29) and increasing beet pulp (+27) and wheat bran (+16) rates.

Table 14. Ingredient composition (%) of experimental diets

	Diet L1 DF/starch 0.8 CP 15%	Diet L3 DF/starch 2.8 CP 15%	Diet H1 DF/starch 0.8 CP 16%	Diet H3 DF/starch 2.8 CP 16%
Dehydrated alfalfa meal 17% CP	37.00	21.00	26.00	9.00
Wheat bran	10.00	26.60	3.50	19.40
Barley	34.30	5.00	37.80	9.00
Dried beet pulp	4.00	31.00	4.00	31.00
Sunflower meal 30% CP	10.00	0.00	19.00	10.00
Sunflower meal 36% CP	0.00	12.00	5.00	17.00
Soybean oil	1.00	1.00	1.00	1.00
Molasses	1.50	1.50	1.50	1.50
Calcium Carbonate	0.05	0.38	0.50	0.80
Dicalcium phosphate	0.65	0.20	0.40	0.10
Sodium chloride	0.40	0.40	0.40	0.40
L-lysine	0.42	0.30	0.33	0.23
DL-methionin	0.11	0.05	0.00	0.00
Vitamine-min. premix*	0.40	0.40	0.40	0.40
Choline	0.07	0.07	0.07	0.07
Coccidiostatic	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.

All diets contained at least a minimum quantity of the different raw materials, to reduce the risks connected to the wrong evaluation of their chemical composition, and were supplemented with synthetic amino-acids, macro- and micro-minerals and vitamins to satisfy the nutritional needs of rabbits (De Blas and Mateos, 1998).

Crude protein content in HP diets was higher than LP diets by one point as expected (Table 15). Crude fibre level increased by about one point from L1 to L3, and from H1 to H3 diets, while digestible fibre, mainly pectins rather than hemicelluloses, changed in a wider range. Starch content decreased from 20-22% in L1 and H1 diets to 16% in L2 and H2 diets and 9% in L3 and H3 diets.

The DF/starch ratio increased from L1 and H1 diets (0.8 on average) to L2 and H2 diets (1.3 on average) and to L3 and H3 diets (2.8 on average), as expected. The DF/ADF ratio changed less, increasing from L1 and H1 diets (0.9 on average) to L3 and H3 diets (1.2 on average). The starch/ADF ratio was more than halved from L1 and H1 diets (1.1 on average) to L3 and H3 diets (0.5 on average).

The L2 and H2 diets always showed intermediate chemical composition compared to L1-L3 and H1-H3 diets. Regardless from differences in the protein level, L1, L2 and L3 diets may be considered equivalent respectively to H1, H2 and H3 diets as what concerns digestible and structural carbohydrates.

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

	Diet L1	Diet L2	Diet L3	Diet H1	Diet H2	Diet H3
Dry matter, %	90.3	89.9	89.8	90.3	89.6	89.3
Crude protein, %	15.0	15.2	15.5	16.0	16.3	16.3
Ether extract, %	3.5	3.6	3.5	3.4	3.4	3.5
Crude fibre, %	16.4	16.8	17.4	16.6	17.6	18.2
Ash, %	6.8	6.6	6.5	6.3	6.3	6.2
Total dietary Fibre (TDF), %	37.4	40.6	44.8	35.7	41.0	48.5
NDF, %	33.1	34.8	35.7	32.9	34.0	36.8
ADF, %	19.3	20.1	20.5	19.4	19.7	21.8
ADL, %	4.4	4.1	4.1	4.7	4.6	4.8
Digestible fibre (TDF-ADF), %	18.0	20.5	24.2	16.3	21.2	26.7
Pectins (TDF-NDF), %	4.3	5.8	9.1	2.8	7.0	11.7
Hemicelluloses (NDF-ADF), %	13.8	14.7	15.1	13.5	14.3	15.0
Starch, %	21.5	16.3	9.3	20.2	15.8	8.8
DF/starch ratio	0.8	1.3	2.6	0.8	1.3	3.0
DF/ADF ratio	0.9	1.0	1.2	0.8	1.1	1.2
Starch/ADF ratio	1.1	0.8	0.5	1.0	0.8	0.4

Final slaughter, carcass dissection and meat quality analyses

At 75 d of age, the remaining rabbits (201 in total) were transported in cages (50 x 100 x 30 cm), putting 12 animals per cage (24 rabbits/m^2) to the slaughterhouse. Feed and water were available until loading (from 5:30 to 6:30). The transport lasted about 60 minutes.

Slaughter began at 8.30 and ended at 10.00 approximately. Rabbits were individually weighted, then stunned by electro-anaesthesia and killed by jugulation. Carcasses were chilled at 4°C for 2.5 h and then transported to the laboratories of the Department of Animal Science for dissection.

Slaughter procedure, carcass dissection and meat quality analyses were performed by using the procedures and methodologies detailed in the first experiment. Carcass dissection and meat quality analyses were done on 120 carcasses, 20 per experimental group representative for average live weight and variability.

Statistical analyses

The data recorded were analyzed by ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC) and considering the effects of DF/starch ratio and protein level. The effects of the gender was also included in the model to analyze variability of data of growth performance, slaughter results, carcass and meat traits. When necessary, the Bonferroni orthogonal test was used to compare means. Mortality, morbidity and sanitary risk were analyzed by the CATMOD procedure of SAS.

Results and discussion

Digestibility and nutritive value of experimental diets

Increasing dietary protein significantly improved dry matter digestibility (about +1 point) (Table 16), to be ascribed to the lower alfalfa meal inclusion rate in diets H compared to diets L. The digestibility of the other nutrients varied accordingly and depending on the protein level.

Table 16. Digestibility coefficients (%): effect of protein level and DF/starch ratio

	Protein level			DF/starch ratio				RSD[1]
	LP	HP	Prob.	R1	R2	R3	Prob.	
Rabbits, No.	30	30		20	20	20		
Dry matter	61.4	62.6	0.02	61.8	62.1	62.1	0.75	1.7
Crude protein	71.4	72.5	<0.001	72.5[b]	71.8[ab]	71.5[a]	0.07	1.3
Ether extract	79.8	81.1	<0.001	80.7[b]	80.9[b]	79.7[a]	<0.001	0.9
Crude fibre	22.5	25.9	<0.001	17.5[a]	24.8[b]	30.5[c]	<0.001	3.5
TDF	40.6	42.9	0.001	33.4[c]	42.2[b]	49.5[c]	<0.001	2.7
NDF	29.9	32.4	<0.01	26.3[a]	31.6[b]	35.5[c]	<0.001	3.2
ADF	21.3	22.7	0.13	16.7[a]	22.2[b]	27.1[c]	<0.001	3.6
Digestible fibre	59.1	62.1	<0.001	52.3[a]	61.4[b]	68.1[c]	<0.001	1.8
Pectins	101	98	<0.001	99.5[b]	99.8[c]	98.5[a]	<0.001	0.2
Hemicelluloses	41.8	46.2	<0.001	40.0[a]	44.7[b]	47.3[c]	<0.001	2.6
Starch	97.2	97.3	<0.001	97.6[a]	97.6[a]	96.6[b]	<0.001	0.1
Gross energy	62.0	63.1	0.02	62.3	62.7	62.7	0.75	1.7

[1]Residual standard deviation.

As what concerns the effect of DF/starch ratio, starch apparent digestibility significantly decreased (even if to a low extent in absolute value) when reducing dietary starch, while greatly increased fibre and fibrous fractions digestibility. Dry matter and gross energy digestibility did not change significantly, however. The energy (DE) content of diets did not change with DF/starch ratio (Table 17), since energy supplied by DF was similar to that provided by starch within constant crude fibre and NDF levels, as also observed by others (De Blas and Carabaño, 1996; Gidenne and Bellier, 2000). Since hemicelluloses did not change appreciably, this result may be largely ascribable to the complete digestibility of pectins (100%) and, to a lesser extent, to the general raise in digestibility of all fibrous fractions, also the less digestible ones (e.g. ADF). The DE content varied little depending on dietary protein and, therefore, DP/DE ratio was higher in diets at 16% CP than those at 15% CP. In both cases, however, protein supplies satisfy requirements of growing rabbits (De Blas and Mateos, 1998).

Table 17. Digestibility coefficients (%) and nutritive value (as fed) of diets

	Diet L1	Diet L2	Diet L3	Diet H1	Diet H2	Diet H3	Prob.[1]
Rabbits, No	10	10	10	10	10	10	
Dry matter	61.7	61.0	61.7	62.5	62.6	62.6	0.76
Crude protein	71.9ab	71.4ab	70.8a	73.1b	72.3ab	72.3ab	0.78
Ether extract	80.3b	80.7b	78.4a	81.0b	81.1b	81.1b	<0.001
Starch	97.5c	97.7c	96.4a	97.7c	97.6c	96.8b	<0.05
Crude fibre	17.0a	23.4b	27.2b	17.9a	26.1b	33.7c	<0.01
TDF	33.4a	41.5b	46.7c	33.3a	43.0b	52.3c	0.61
NDF	24.8a	31.0b	34.1bc	27.9a	32.3b	37.0c	0.21
ADF	16.0a	22.5b	25.5bc	17.4a	21.8b	28.8c	<0.001
Pectins	101d	105e	96b	98c	95a	101d	<0.001
Hemicelluloses	37.1a	42.6b	45.8bc	42.9b	46.8c	48.9c	0.25
Digestible fibre	52.2a	60.2b	64.8c	52.3a	62.7c	71.5d	<0.001
Gross energy	61.7	62.3	62.2	63.0	63.2	63.1	0.91
Digestible energy (DE), MJ/kg	10.26	10.38	10.37	10.53	10.55	10.52	
Digestible protein (DP), g/kg	108	109	110	117	118	117	
DP/DE, g/MJ	10.5	10.5	10.6	11.1	11.2	11.2	

[1]Probability of the interaction protein level x DF/starch ratio.

Health status and performance

During the trial, only nine animals died of which six fed diet H1, one diet H2, one diet L1 and one diet L3. Morbidity was moderate, although until the fourth week of the trial there were ill rabbits that generally recovered in few days. During the trial, both mortality and morbidity were low, even if a significant effect of dietary treatment was detected (Tables 18 and 19).

Mortality and sanitary risk tended (P=0.10-0.11) to increase in rabbits fed diets with the highest protein content. The administration of the diets with the lowest DF/starch ratio increased mortality (10 *vs*. 1.4 and 1.4%, P=0.04), but also reduced the number of ill rabbits that reached the end of the trial in comparison with the other diets, even if at a non-significant level, so that the sanitary risk associated to the diets was not different among the three groups.

Table 18. Mortality, morbidity and sanitary risk: effect of protein level and DF/starch ratio

	Protein level			DF/starch ratio			
	LP	HP	Prob.	R1	R2	R3	Prob.
Mortality[1], %	1.9	6.7	0.10	10.0^{B}	1.4^{A}	1.4^{A}	0.04
Morbidity, %	4.8	6.7	0.56	2.9	8.4	5.8	0.39
Sanitary risk, %	6.7	13.4	0.11	12.9	9.8	7.2	0.55

[1]DF/starch ratio R1 *vs*. R2+R3, P=0.01

The analysis of health condition according to the experimental diet (Table 19) evidenced significantly higher mortality in rabbits fed diet H1 (17.1%), with low DF/starch ratio and high protein level, compared to the other groups (1.5% on average) (P<0.001). Also sanitary risk was higher with diet H1 (20.0%) compared to the average value of the other diets (8.1%) (P=0.04).

Tabella 19. Mortality, morbility and sanitary risk: effect of diet

	Diet L1	Diet L2	Diet L3	Diet H1	Diet H2	Diet H3	Prob.[3]
Mortality[1], %	2.9	0.0	2.9	17.1	2.9	0.0	-
Morbility, %	2.9	8.6	2.9	2.9	8.6	8.6	0.71
Sanitary risk[2], %	5.8	8.6	5.8	20.0	11.5	8.6	0.40

[1]Diet H1 *vs.* H2+H3+L1+L2+L3, P<0.001; [2]Diet H1 *vs.* H2+H3+L1+L2+L3; P=0.04. [3]No statistical analysiswas possible.

When describing the rabbit health status in terms of daily morbidity, that is the number of ill animals present on each day of the trial, the first ill animals were observed about 10 days after the beginning of the trial (Figure 20). Ill rabbits were present for about 15 days, from 42-43 to 55-56 d of age, and then disappeared around 62-65 days. Finally, a small group of rabbit fell ill around slaughter. As what concerns the effect of experimental factors, the number of ill rabbits was higher with the higher protein diets (Figure 20).

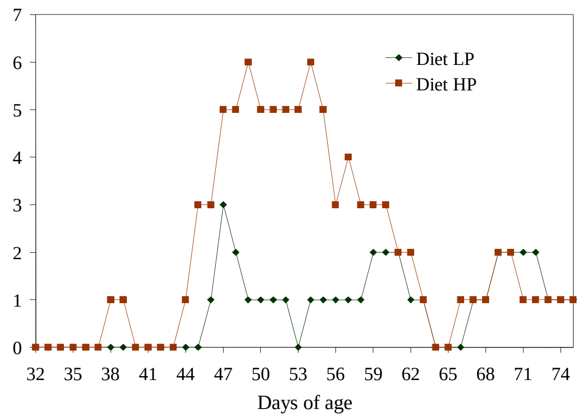


Figure 20. Effect of dietary protein level on the number of ill animals during the trial

The daily trend of health problems according to the DF/starch ratio was more confusing when represented graphically (Figure 21): from 44-45 to 53-54 days of age a peak of ill rabbits fed intermediate DF/starch ratio was found. In rabbits fed the other diets, the number of rabbits fell ill per day was on average lower, but the length of the illness appeared longer.

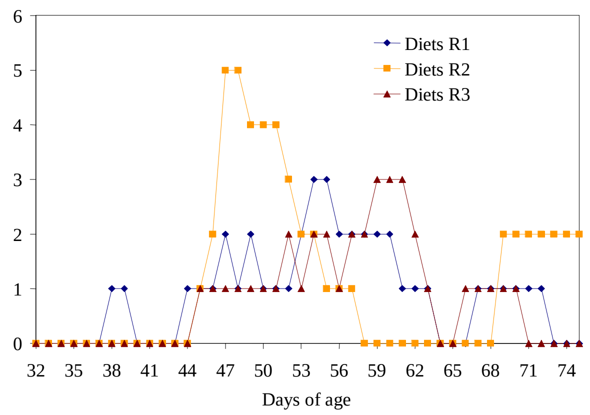


Figure 21. Effect of dietary DF/starch ratio on the number of ill animals during the trial

Growth performance was satisfying for the slaughter age and the genetic type used (Table 20 and Figures 22 and 23). No significant effect of experimental treatment was found on growth performance. Only gender significantly affected results, with females showing better growth and feed conversion in the second period of the trial compared to males.

Table 20. Performance from weaning to commercial slaughter

	Protein level			DF/starch ratio				Gender			RSD[1]
	LP	HP	Prob.	R1	R2	R3	Prob.	F	M	Prob.	
Rabbits, No	103	98		63	69	69		84	117		
Live weight, g											
At 33 d, g	828	830	0.84	827	830	829	0.94	828	830	0.78	56
At 54 d, g	1992	2010	0.53	1996	2006	2003	0.96	2008	1995	0.66	204
At 75 d, g	2746	2760	0.71	2731	2779	2745	0.60	2781	2725	0.15	265
First period (33-54 d)											
Weight gain, g/d	55.4	56.2	0.51	55.7	56.0	55.9	0.98	56.2	55.5	0.56	8.6
Feed intake, g/d	135	136	0.64	134	137	135	0.64	136	135	0.69	18
Feed conversion	2.44	2.45	0.79	2.42	2.47	2.44	0.27	2.44	2.45	0.78	0.20
Second period (54-75 d)											
Weight gain, g/d	35.9	35.7	0.84	35.2	36.8	35.4	0.31	36.8	34.8	0.04	6.9
Feed intake, g/d	156	154	0.67	152	158	155	0.35	155	155	0.85	21
Feed conversion	4.45	4.42	0.83	4.43	4.40	4.48	0.84	4.31	4.56	0.03	0.75
Overall (33-75 d)											
Weight gain, g/d	45.7	46.0	0.72	45.4	46.4	45.6	0.60	46.5	45.1	0.11	5.9
Feed intake, g/d	145	145	0.95	143	147	144	0.38	145	145	0.86	18
Feed conversion	3.18	3.16	0.61	3.16	3.19	3.17	0.82	3.13	3.21	0.03	0.25

[1]Residual standard deviation.

The restricted range of protein level that was tested, about one point of difference, and the rather high protein level, from 15 to 16%, may explain the absence of significant differences in growth depending on the protein level itself. With similar dietary protein and the aa supplementation used, in fact, protein needs were surely satisfied even in the first stages of growth, which are known to require more protein (Maertens *et al.*, 1997, 1998; Trocino *et al.*, 2000, 2001). With regards to the null or little effect of DF/starch ratio, the digestive utilization of fibre, especially pectins, rather than starch, permitted to maintain high the nutritive value of diets and, therefore, growth performance was comparable among the three groups of animals. As a last point, the absence of serious digestive problems during the trial contributed to minimize the differences of productive results.

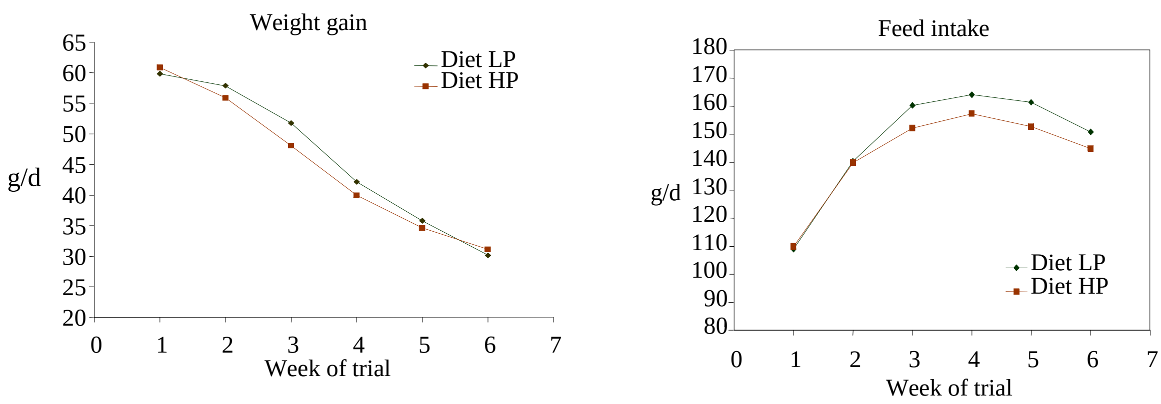


Figure 22. Effect of dietary protein level on performance during the trial

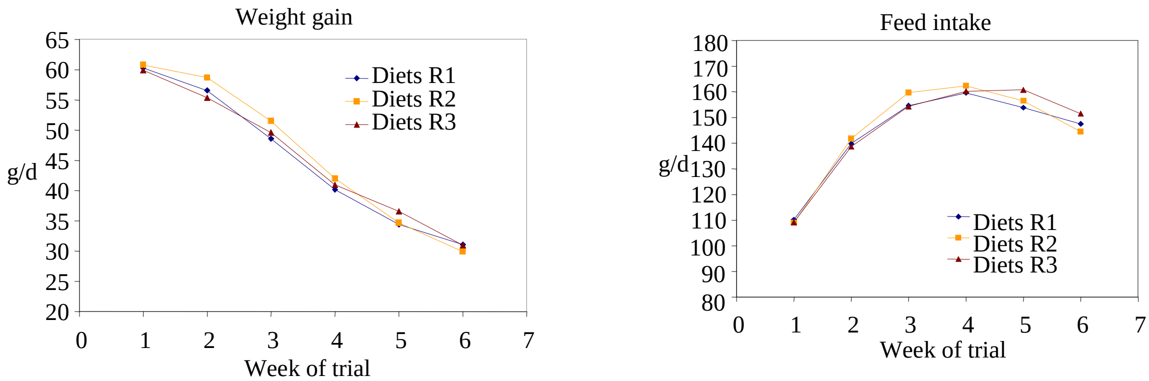


Figure 23. Effect of DF/starch ratio level on performance during the trial

<u>Caecal content and intestinal mucosa characteristics</u>

Live weight and gut filling at 55 d of age were not influenced by protein level or DF/starch ratio (Table 21). The protein level did not affect neither filling of single compartment or caecal fermentation activity. The limited range tested (15 *vs*. 16%) could explain this result.

Increasing DF/starch ratio increased the total amount of VFAs, even if at a non-significant level, and especially rose (P=0.02) the acetate proportion, main fermentation product, while reducing valerate incidence (P<0.01) usually associated with the activity of amylolitic bacteria and a high dietary starch. The fermentation pattern varied accordingly to the starch reduction and showed how increasing digestible fibre may sustain active and equilibrated caecal fermentations, guided towards acetate production.

Table 21. Caecal content and intestinal mucosa traits in rabbits at 55 d of age

	Protein level			DF/starch ratio				RSD[1]
	LP	HP	Prob.	R1	R2	R3	Prob.	
Rabbits, No.	18	18		12	12	12		
Live weight (LW), g	2032	2057	0.75	2046	2048	2040	0.99	232
Full gut, %LW	21.6	21.9	0.60	20.9	21.9	22.3	0.26	2.1
Full caecum, %LW	7.2	7.5	0.54	7.0	7.4	7.7	0.32	7.3
Full stomach, %LW	6.4	6.4	0.92	6.3	6.3	6.6	0.72	0.9
Caecal content characteristics								
pH	5.91	5.87	0.60	5.96	5.75	5.95	0.12	0.27
$N\text{-}NH_3$, mmol/l	7.17	7.64	0.72	9.07	6.93	6.20	0.18	3.8
Total VFAs, mmol/	63.1	68.4	0.26	60.3[a]	69.6[b]	67.2[ab]	0.24	13.7
C_2, % mol. VFAs	81.6	81.6	0.97	80.4[a]	81.1[ab]	83.3[ab]	0.02	2.5
C_3, % mol. VFAs	4.7	4.3	0.36	4.9	4.2	4.2	0.44	1.3
C_4, % mol. VFAs	13.0	13.5	0.59	13.9	14.1	11.7	0.05	2.5
C_5, % mol. VFAs	0.6	0.6	0.30	0.8[b]	0.6[ab]	0.5[a]	<0.01	0.2
C_6, % mol. VFAs	0.05	0.06	0.25	0.06	0.05	0.05	0.80	0.04
C_3/C_4 ratio	0.40	0.33	0.25	0.38	0.31	0.41	0.34	0.11
Jejunal mucosa characteristics								
Villi height, μm	686	678	0.84	708	704	635	0.22	113
Crypt depth, μm	103	97	0.13	99	100	100	0.98	11
Villi/crypts ratio	7.17	7.54	0.46	7.72	7.56	6.77	0.25	1.46
Ileal mucosa characteristics								
Villi height, μm	478	475	0.87	463	492	475	0.51	60
Crypt depth, μm	91	84	0.07	91	89	83	0.17	11
Villi/crypt ratio	5.38	5.89	0.15	5.54	5.73	6.65	0.90	1.04

[1]Residual standard deviation.

The histological analyses of the jejunal and ileal mucosa did not show any differences depending on the protein level, apart from a reduction in crypt depth when increasing dietary protein both at jejunum (P=0.07) and ileum (P=0.13) level (Table 21). However, when data of the two traits were analysed together (Table 22), both crypt depth (P=0.02) and villi/crypt ratio (P=0.11) changed. A lower crypt depth may be associated with a lower capacity of mucosa of repairing villi damages and therefore, indirectly, may explain the higher susceptibility to digestive disorders found with high-protein diets (1.9 *vs*. 6.7%; P=0.10) for LP and HP treatment, respectively. Also average values of villi/crypt ratio, even if not statistically different, were higher with diet H1 (7.14 *vs*. 6.36, average value of the other diets), which also showed the highest mortality.

Table 22. Characteristics of the intestinal mucosa: effect of dietary treatments and intestinal tract

	Protein level			DF/starch ratio				Intestinal tract			RSD[1]
	LP	HP	Prob.	R1	R2	R3	Prob.	Jejunum	Ileum	Prob.	
Rabbits, No.	36	36		24	24	24		36	36		
Villi height, µm	582	577	0.81	585	597	555	0.31	682	476	<0.001	89
Crypt depth, µm	97	91	0.02	95	94	92	0.46	100	88	<0.001	11
Villi/crypt ratio[2]	6.26	6.71	0.11	6.63	6.63	6.21	0.49	7.34	5.64	<0.001	1.25

[1]Residual standard deviation. [2]Average values: L1 = 6.12; L2 = 6.56; L3 = 6.12; H1 = 7.14; H2 = 6.70; H3 = 6.31; effect of the interaction Protein level x DF/starch ratio: P=0.40.

The DF/starch ratio did not modify any morphometric variable. While in swine a positive effect on mucosa development has been proved by including soluble fibre in weaning diets, in rabbits this effect has not yet been clearly evidenced (Gallois *et al.*, 2005; Gómez-Conde *et al.*, 2007; Xiccato *et al.*, 2008).

Slaughter results and meat quality

Experimental treatments only modified minor traits of carcasses and meat (Tables 23 and 24). The protein level did not affect slaughter results or meat quality, as pH or colour of main muscles, while increasing DF/starch ratio increased the gut incidence (from 17.6 to 18.3 and 18.4%, P<0.01) even without relevant effect on dressing percentage. A significant effect was also observed on *biceps femoris* traits with a reduction in pH and an increase in lightness (L*) and redness (a*).

Table 23. Results at commercial slaughter and carcass quality

	Protein level			DF/starch ratio				Gender			RSD[1]
	LP	HP	Prob.	R1	R2	R3	Prob.	Females	Males	Prob.	
Rabbits, No.	100	96		62	67	67		81	115		
Slaughter weight (SW), g[2]	2694	2710	0.68	2681	2729	2696	0.58	2731	2673	0.14	266
Transport losses, %LW[3]	1.9	1.9	0.83	2.0	1.9	1.9	0.66	1.9	1.9	0.77	0.9
Gut incidence, %SW[4]	18.2	18.1	0.62	17.6[a]	18.3[b]	18.4[b]	<0.01	18.6	17.6	<0.001	1.4
Cold carcass (CC), g	1641	1660	0.43	1640	1660	1652	0.80	1652	1649	0.92	165
Dressing percentage, % SW	60.9	61.3	0.12	61.2	60.8	61.3	0.21	60.5	61.7	<0.001	1.5
Head, %CC	7.8	7.7	0.94	7.8	7.8	7.7	0.34	7.6	7.8	0.02	0.5
Liver, %CC	5.0	4.9	0.06	4.8	5.0	5.0	0.24	4.9	5.0	0.16	0.6
Reference carcass (RC), g	1378	1397	0.34	1380	1394	1389	0.85	1392	1383	0.68	145
Reference carcass dissection:											
Dissectable fat, % RC	3.6	3.5	0.50	3.6	3.6	3.4	0.67	3.7	3.4	0.08	1.0
Hind leg, % RC	32.3	32.4	0.70	32.5	32.1	32.5	0.57	32.6	32.1	0.17	1.1
Muscle/bone ratio hind leg	5.97	5.93	0.68	5.82	6.12	5.92	0.11	5.86	6.04	0.16	0.44

[1]Residual standard deviation; [2]SW: live weight at the slaughterhouse immediately before slaughter; [3]LW: live weight at the farm; [4]Incidence of the full gastro-intestinal tract.

As what concerns animal gender, a higher gut incidence and a lower dressing percentage was measured in females than in males (P<0.001). Also fattening status, as dissectible fat of the reference carcass, tended to be higher in females (P=0.08), even if limited in amount (3.6% on average). The pH of *biceps femoris* and *longissimus lumborum* were higher in males than females, while *l. lumborum* lightness was lower and *b. femoris* redness was higher in males. Generally speaking, however, differences were in a narrow range even when significant, so that meat quality between genders did not change in an appreciable manner.

Table 24. Meat quality: pH and colour of *longissimus lumborum* and *biceps femoris*

	Protein level			DF/starch ratio				Gender			RSD[1]
	LP	HP	Prob.	R1	R2	R3	Prob.	Females	Males	Prob.	
Rabbits, No	60	60		40	40	40		51	69		
Longissimus lumborum:											
pH	5.60	5.60	0.86	5.63^b	5.60^{ab}	5.58^a	0.07	5.57	5.63	0.001	0.10
L*	50.7	51.2	0.36	50.8	51.2	50.8	0.82	51.8	50.1	<0.01	2.95
a*	-2.24	-2.21	0.80	-2.39	-2.16	-2.12	0.14	-2.27	-2.17	0.43	0.64
b*	1.14	1.07	0.85	0.35	1.45	1.51	0.03	1.08	1.13	0.90	2.18
C*	3.28	3.13	0.41	3.11	3.20	3.31	0.71	3.20	3.22	0.94	1.04
H*	-0.35	-0.38	0.76	-0.15	-0.46	-0.48	0.05	-0.34	-0.39	0.71	0.66
Biceps femoris:											
pH	5.80	5.80	0.99	5.83^b	5.80^{ab}	5.77^a	<0.01	5.78	5.82	0.03	0.09
L*	50.4	50.2	0.59	49.4^a	50.3^{ab}	51.1^b	<0.01	50.2	50.4	0.67	2.13
a*	-1.99	-1.82	0.12	-2.10^a	-1.91^{ab}	-1.69^b	<0.01	-2.08	-1.73	<0.01	0.58
b*	0.56	0.67	0.62	0.49	0.56	0.80	0.49	0.76	0.47	0.22	1.25
C*	2.35	2.35	0.97	2.59^b	2.23^a	2.23^a	<0.01	2.47	2.23	0.03	0.57
H*	-0.26	-0.27	0.97	-0.18	-0.30	-0.32	0.54	-0.31	-0.22	0.44	0.60

[1]Residual standard deviation.

As previously discussed, also for carcass and meat traits, the absence of a significant effect of dietary protein may be explained by the rather high protein level, which in diets supplemented for most limiting aa permitted to maximize growth performance and, therefore, to produce high quality carcasses. Only below 13% dietary CP, Maertens *et al*. (1997) evidenced a worsening of slaughter results. Moreover, slaughter results and carcass and meat traits in rabbits hardly change with the feeding regime, when nutritional requirements are satisfied (Ouyahon, 1998; Xiccato, 1999). Therefore, since diets had similar nutritive value, a uniformity of carcasses was reasonably expected in rabbits fed diets also with very different DF/starch ratio. Only a great change in fibre content, especially non-digestible fibre, could have impaired dressing percentage due to a higher gut incidence (Parigi Bini *et al.*, 1994).

EXPERIMENT 3:

Reducing dietary protein and increasing digestible fibre/starch ratio in diets for growing rabbits

Material and methods

Rearing conditions

The trial was realized in the experimental farm of the University of Padova in the period March-May. The characteristics of experimental facilities and cages have been detailed previously. Before arrival of rabbits, the building was kept empty for a long period and then submitted to a disinfectant and fungicide treatment.

Maximum temperature did not exceed 24°C and minimum temperature was not below 15°C. In the whole period, minimum and maximum temperature averaged about 17 and 20°C, respectively. Maximum relative humidity averaged at 69%, with a minimum of 51% and a maximum of 84%. Minimum relative humidity was on average 45%, with a minimum of 32% and a maximum of 77%.

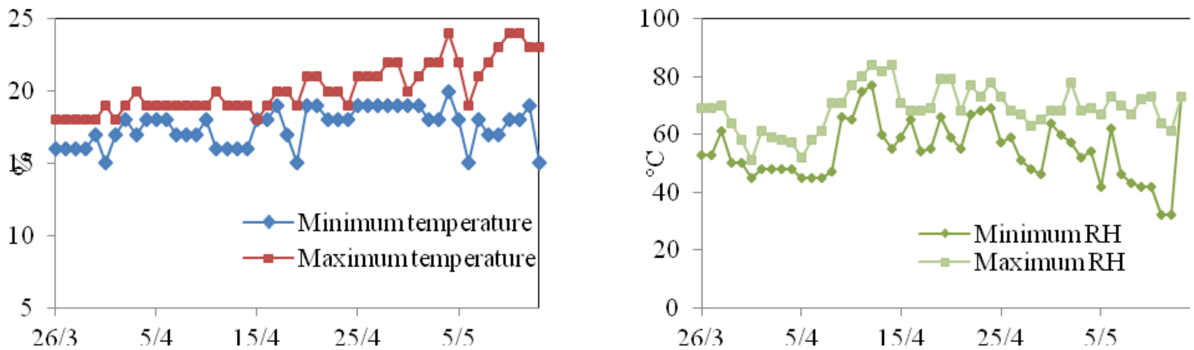


Figure 24. Daily values of minimum and maximum temperature and minimum and maximum relative humidity (RH) throughout the trial

Animals, experimental groups and recordings

At 29 days, 282 Hyla hybrid rabbits were selected in a commercial farm from healthy litters of pluriparous does (2-5 kindlings) with homogeneous weight and transferred to the experimental facilities in collective cages at low density. On their arrival, animals were healthy and weighed 596±58 g. Rabbits were controlled by gender, put into individual cages and marked using ear tags.

The animals were divided into six groups of 47 ones, homogeneous for initial average live weight and variability, and given six diets with similar content of ADF (18%) and differing in crude protein level and DF/starch ratio, according to a bi-factorial arrangement 2x3, as in the scheme below:

		Crude protein (CP) level		
		CP 14.0% (LP)	CP 15.5% (MP)	CP 17.0% (HP)
DF/starch ratio	DF18/St18 (R1)	Diet L1	Diet M1	Diet H1
	DF25/St13 (R2)	Diet L2	Diet M2	Diet H2

The diets were given *ad libitum* from the beginning of the trial until 5 days before commercial slaughter. Form 73 d of age to slaughter (78 d), a unique coccidiostat-free commercial diet was administered to all animals. 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 for the first experiment.

Two *in vivo* digestibility trials were performed on 60 animals (10 per diet) from 35 to 39 d and from 56 to 60 d of age; at 38 d caecal content and jejunum mucosa were sampled from 36 rabbits (6 animals per group). Digestibility trials, caecal and gut mucosa sampling, histometric analysis and chemical analyses of experimental diets, faeces and caecal content were performed according to the methodologies previously detailed for the first experiment.

Experimental diets

All diets were pelleted with 3.5 mm of diameter and 1.0-1.1 cm of length. The diets L1, L2, H1 and H2 were produced, while M1 and M2 diets were obtained at the experimental farm by mixing diets L1 and H1 (0.5+0.5) and the diets L2 and H2 (0.5+0.5), respectively.

The ingredient inclusion rates and the chemical composition of experimental diets are presented in Tables 25 and 26.

Protein content increased from LP to HP diets by reducing alfalfa and increasing soybean and sunflower meal. The L1 diet contained only alfalfa as protein source, while the others contained also soybean and sunflower meal, in various rates. Starch level was modified by varying wheat bran and barley proportions. Digestible fibre content of R1 and R2 diets was maintained constant by using different levels of wheat bran and dried beet pulp. To increase digestible fibre content from R1 to R2 diets, alfalfa was reduced (-15 and -22 points) and dried beet pulp increased (+30 and +25 points). The diets were integrated with aa, macro- and micro-minerals and vitamins to satisfy the nutritional requirements of growing rabbits (De Blas and Mateos, 1998).

Table 25. Ingredient composition (%) of experimental diets

	Diet L1 CP 14% DF 18% Starch 18%	**Diet H1** CP 17% DF18% Starch 18%	**Diet L2** CP 14% DF 25% Starch 13%	**Diet H2** CP 17% DF 25% Starch 13%
Dehydrated alfalfa meal 17% CP	44.00	31.00	22.00	15.40
Wheat bran	35.20	12.00	18.25	5.50
Barley	16.00	27.00	16.00	20.20
Dried beet pulp	0.00	8.00	30.00	33.00
Soybean meal 48% CP	0.00	4.00	4.00	9.00
Sunflower meal 35% CP	0.00	14.25	5.00	13.50
Soybean oil	1.50	0.50	1.00	0.00
Molasses	1.50	1.50	1.50	1.50
Calcium carbonate	0.15	0.13	0.00	0.00
Dicalcium phosphate	0.10	0.55	0.90	0.93
Sodium chloride	0.40	0.40	0.40	0.40
L-lysine (liquid)	0.36	0.10	0.20	0.00
DL-methionine	0.12	0.00	0.11	0.00
DL-threonine	0.10	0.00	0.07	0.00
Vitamin-mineral premix*	0.40	0.40	0.40	0.40
Choline	0.07	0.07	0.07	0.07
Coccidiostatic	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.

Table 26. Chemical composition (as fed) of experimental diets

	Diet					
	L1	M1	H1	L2	M2	H2
Dry matter, %	89.0	89.1	89.2	88.9	88.6	89.0
Crude protein, %	13.7	15.1	16.9	14.1	15.7	18.0
Ether extract, %	4.0	3.4	2.6	3.0	2.6	1.9
Crude fibre, %	14.3	13.9	13.7	13.1	13.0	12.9
Ash, %	8.1	7.9	7.6	7.6	7.4	7.4
TDF, %	37.7	36.8	36.8	42.3	41.6	40.4
NDF, %	34.7	33.3	31.7	32.3	31.0	29.3
ADF, %	17.5	17.3	16.8	16.8	16.6	16.0
Hemicelluloses (NDF-ADF), %	17.2	16.0	14.9	15.4	14.5	13.3
ADL, %	3.3	3.2	3.1	2.5	2.5	2.5
Digestible fibre (TDF-ADF)	20.2	19.5	20.1	25.5	25.0	24.4
Pectins (TDF-NDF), %	3.0	3.5	5.2	10.1	10.6	11.1
Starch, %	18.0	17.7	17.6	14.0	12.7	12.3
Gross energy, MJ/kg	16.52	16.30	16.30	16.10	15.92	15.82
DF/starch ratio	1.1	1.1	1.1	1.8	2.0	2.0
DF/ADF ratio	1.2	1.1	1.2	1.5	1.5	1.5

Crude protein content in LP and HP diets corresponded to the expected values (about 14% in LP and 17% in HP diets). Only in diet H2, the protein level was one point higher than expected. The MP diets had an intermediate chemical composition compared to LP and HP diets.

Digestible fibre content in R1 diets resulted slightly higher than what expected (20 *vs.* 18%), while in accordance in R2 diets (25%). Considering the difficulty in determining the digestible fibre content and mainly the absence of an updated database of the raw materials used, the obtained result may be considered satisfactory: the differences in DF content between R1 and R2 diets were sufficiently high, even if the variation interval was a bit narrower than expected.

On average, hemicelluloses content was reduced (-1.6 points) from R1 to R2 diets, while pectins content increased about 6.7 points. ADF concentrations were somewhat lower than what calculated and decreased from LP to HP diets and from R1 to R2 diets (-0.5 to -2.0 points). Similarly, starch content decreased from LP to HP diets and from R1 to R2 diets. Therefore, the difference in DF/starch ratio was only slightly lower than the expected (1.1 *vs.* 1.9 rather than 1.0 *vs.* 2.0 for R1 and R2 respectively).

Commercial slaughter, carcass dissection and meat quality analyses

At 78 d of age, 120 rabbits were selected (20 per each group of diets and representative in terms on average weight and variability) for commercial slaughter and dissection. Feed and water were available until loading (from 5:30 to 6:30). The animals were weighted at the experimental farm before loading and were transported in cages (50 x 100 x 30 cm, 20 rabbits/m^2) for about 60 minutes to a commercial slaughterhouse. Slaughter began at 9.00 and ended at 11.00 approximately. Rabbits were individually weighted, stunned by electro-anaesthesia and killed by jugulation. Carcasses were chilled at 4°C for 2 h 30 min and then transported to the laboratories of the Department of Animal Science for dissection. Slaughter procedure, carcass dissection and meat quality analyses were performed by using the procedures and methodologies detailed in the first experiment.

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)

The same data were statistically analysed in order to evaluate the effect of dietary factors on N retention, intake and excretion.

Statistical analysis

The data recorded were analyzed by ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC) and considering the effects of protein level, DF/starch ratio as well as their interaction. The effects of the gender was also included in the model to analyze variability of data of growth performance, slaughter results, carcass and meat traits. When necessary, the Bonferroni orthogonal test was used to compare means.

The digestibility coefficients of nutrients measured in the two digestibility trials were analyzed by a three-way ANOVA using the GLM procedure and considering the effects of animal age, protein level, DF/starch ratio as well as their interaction.

Results and discussion

Digestibility and nutritive value of experimental diets

The effects of experimental factors on diet apparent digestibility are presented in Table 27, while the digestibility coefficients of experimental diets (results of the interaction between protein level and DF/starch ratio) are reported in Table 28. Generally speaking, all diets are out of usual commercial standards and rather extreme in formulation and diet composition and, within the tested interval, in dry matter and energy digestibility as well as digestive utilization of fibre and fibrous fractions.

Table 27. Digestibility coefficients (%): effect of protein level, DF/starch ratio and animal age

	Protein level (P)			DF/starch ratio (R)		Age during digestibility trial (A)		Probability		
	LP	MP	HP	R1	R2	35	56	P	R	A
Rabbits, No.	44	46	44	67	67	66	68			
Dry matter	61.4[a]	63.3[b]	66.1[c]	60.4	66.8	63.4	63.8	<0.001	<0.001	0.24
Crude protein	76.1[a]	77.3[b]	79.1[c]	77.4	77.6	79.3	75.7	<0.001	0.66	<0.001
Ether extract	79.9[a]	77.5[b]	72.6[c]	80.0	73.3	76.8	76.5	<0.001	<0.001	0.38
Crude fibre	15.0[a]	19.3[b]	23.6[c]	10.9	27.6	16.7	21.9	<0.001	<0.001	<0.001
TDF	38.3[a]	41.8[b]	46.5[c]	31.5	52.9	41.7	42.8	<0.001	<0.001	0.02
NDF	25.1[a]	27.3[b]	30.0[c]	20.3	34.6	26.0	28.9	<0.001	<0.001	<0.001
ADF	11.8[a]	15.7[b]	18.0[c]	7.4	22.9	13.3	17.0	<0.001	<0.001	<0.001
Hemicelluloses	39.0[a]	40.1[a]	44.0[b]	34.0	48.0	39.9	42.1	<0.001	<0.001	<0.001
Digestible fibre	58.3[a]	61.5[b]	67.6[c]	52.3	72.6	62.8	62.1	<0.001	<0.001	0.04
Starch	97.5[b]	97.0[a]	96.9[c]	97.6	96.7	96.4	97.8	<0.001	<0.001	<0.001
Gross energy	61.9[a]	63.6[b]	66.2[c]	60.8	66.9	64.2	63.6	<0.001	<0.001	0.07

Increasing the protein level significantly increased nutrients digestibility, and thus the nutritive value, but ether extract and starch digestive utilization. The partial replacement of alfalfa by soybean and sunflower meal and the increase in barley and beet pulp maintained a constant DF level and decreased the less digestible fibrous fractions, thus reducing the feed transit and increasing the digestibility. Lower ether extract digestibility is explained by the lower inclusion of vegetable oils, characterized by a higher digestibility compared to the fat coming from vegetal raw materials (Xiccato, 2010). The slight but significant reduction of starch digestibility with increasing dietary protein is correlated with the lower starch level, as also found previously, in high-protein diets. When looking at dietary sources of starch (barley and wheat bran), both increasing dietary protein and DF/starch ratio are associated with an increasing proportion of starch coming from barley rather than from wheat bran. The lower starch digestibility could be therefore ascribed to a lower digestibility of barley starch compared to wheat bran starch. However, these differences in digestibility have not been found by other authors (Blas and Gidenne, 1998). The presence of starch coming from other raw material (e.g. beet pulp, alfalfa meal, sunflower meal) in few quantity but relatively higher with low starch concentration, as well as the presence of analytical artefacts, could be taken into account to explain the different starch digestibility with decreasing dietary starch.

Table 28. Digestibility coefficients (%) and nutritive value of experimental diets

	Diet						Prob.[1]	RSD[2]
	L1	M1	H1	L2	M2	H2		
Rabbits, No.	21	23	21	21	23	23		
Dry matter	57.5[a]	60.2[b]	63.4[c]	65.2[cd]	66.3[d]	68.8[e]	0.02	1.9
Crude protein	76.3[a]	77.2[ab]	78.7[bc]	75.9[a]	77.4[ac]	79.3[c]	0.25	1.6
Ether extract	82.8[e]	80.5[d]	76.7[c]	77.0[c]	74.4[b]	68.5[a]	<0.01	1.7
Crude fibre	6.5[a]	11.3[b]	15.0[b]	23.5[c]	27.3[cd]	32.2[d]	0.79	4.6
TDF	26.0[a]	30.8[b]	37.7[c]	50.6[d]	52.7[de]	55.3[e]	<0.001	2.9
NDF	17.5[a]	19.8[a]	23.6[b]	32.7[c]	34.7[c]	36.5[c]	0.32	3.9
ADF	2.6[a]	8.6[b]	11.1[b]	21.0[c]	22.7[c]	25.0[c]	0.03	4.5
Hemicelluloses	32.6[a]	31.9[a]	37.6[b]	45.5[c]	48.2[cd]	50.3[d]	0.04	3.7
Digestible fibre	46.3[a]	50.6[b]	60.0[c]	70.2[d]	72.4[e]	75.2[f]	<0.001	2.0
Starch	97.8[b]	97.6[b]	97.3[b]	97.2[b]	96.3[a]	96.5[ab]	0.04	0.7
Gross energy	58.0[a]	60.6[b]	63.8[c]	65.7[d]	66.5[d]	68.5[e]	<0.01	1.9
Nutritive value								
Digestible energy, MJ/kg	9.6	9.9	10.4	10.6	10.6	10.8		
Digestible protein, g/kg	104	117	133	107	122	143		
DP/DE ratio, g/MJ	10.9	11.8	12.8	10.1	11.5	13.2		

[1]Significance of interaction; [2]Residual standard deviation.

As far as concerns the effect of dietary fibre, nutrient digestive efficiency significantly improved by increasing DF/starch ratio. Protein digestibility did not change, while ether extract digestibility decreased. Within R1 diets (Table 27), dry matter digestibility significantly increased by increasing crude protein content. The reduction of alfalfa, the increase of barley and beet pulp, at constant DF content, may explain the increase in digestibility due to the slower feed transit caused by the reduction of less digestible fibre (mainly hemicelluloses) and the increase of pectins content (see Table 26). Dry matter digestibility in diets L2 and M2 increased compared to R1 diets, though it was not significantly different to diet H1. Only the further reduction of alfalfa and wheat bran permitted a significant increase of dry matter digestibility, up to 70%. A similar trend was observed for gross energy digestibility, while digestibility of the other nutrients followed the patterns described above.

The effect of the age of animals during the digestibility trial was considered and corrected using the method of Parigi Bini *et al.* (1991) that is considering the faecal excretion of one day related to the intake of the previous day. In young, just weaned, rabbits feed intake is not yet stable and increases a lot from one day to the following compared to faeces excretion, so that feed intake is superior to excretion, feed transit is low and apparent digestibility is higher compared to older animals. Feed intake correction according to Parigi Bini *et al*. (1991) allows the correction of this deviation.

Dry matter and gross energy digestibility did not change with age. Digestive utilization of crude protein significantly decreased (from 79.2 to 75.7%, P<0.001), while digestibility of starch (from 96.4 to 97.9%, P<0.001), crude fibre (from 16.7 to 22.2%, P<0.001) and of fibrous fractions (TDF, NDF, ADF, hemicelluloses) increased, except DF. These results are in accordance with other studies that agree about the limited capacity of young rabbits to utilize dietary starch and fibrous fractions (Gutiérrez *et al*., 2002).

On average, digestible energy content was close to recommendations for post-weaning and fattening rabbits (9.6-10.4 MJ/kg) for R1 diets while closer to diets for reproducing animals (>10.5 MJ/kg) for R2 diets (De Blas and Mateos, 1998; Xiccato and Trocino, 2008). Digestible protein content and DP/DE ratio in LP and MP diets remained within recommended limits for growing and fattening rabbits, while were higher in HP diets.

<u>Growth performance, nitrogen balance and health status</u>

Growth results during the trial are presented in Table 29 and Figure 25 in function of protein level, DF/starch ratio, gender and interactions.

In the first period of growth (29-50 d of age), reducing the dietary protein below 15.5% (LP diets) significantly reduced (P<0.001) daily weight gain (from 53.1 and 55.1 g/d in MP and HP diets to 49.3 g/d in LP diets) and live weight at 50 d of age (1712 and 1755 *vs*. 1631 g), without affecting feed intake. In the second period (50-78 d), growth performance was not influenced by protein level. In the overall period, rabbits fed LP diets showed lower growth performance than those fed HP diets; while those fed MP diets did not differed significantly from the other groups. Feed conversion varied according to the growth rate and significantly improved in the post-weaning and whole period by increasing protein, while was not influenced in the second period.

Table 29. Growth performance from weaning to slaughter

	Protein level (P)			DF/starch (R)		Gender (G)		Probability				RSD[1]
	LP	MP	HP	R1	R2	Females	Males	P	R	G	P x R	
Rabbits, No.	78	77	76	116	115	128	103					
Live weight, g												
A 29 d	597	596	598	596	597	594	599	0.99	0.86	0.46	0.95	60
A 50 d	1631[a]	1712[b]	1755[b]	1678	1720	1694	1704	<0.001	0.02	0.57	0.57	137
A 78 d	2790[a]	2840[ab]	2908[b]	2826	2867	2845	2847	<0.01	0.14	0.95	0.72	207
First period (29-50 d)												
Weight gain, g/d	49.3[a]	53.1[b]	55.1[b]	51.5	53.4	52.4	52.6	<0.001	<0.01	0.76	0.35	5.2
Feed intake, g/d	108	111	110	114	105	109	110	0.32	<0.001	0.57	0.79	12
Feed conversion	2.21[c]	2.09[b]	2.00[a]	2.22	1.98	2.10	2.10	<0.001	<0.001	0.77	<0.01	0.14
Second period (50-78 d)												
Weight gain, g/d	41.4	40.3	41.2	41.0	40.9	41.1	40.8	0.38	0.96	0.65	0.84	5.1
Feed intake, g/d	159	157	158	163	153	157	159	0.80	<0.001	0.25	0.97	17
Feed conversion	3.87	3.95	3.87	4.03	3.76	3.85	3.94	0.49	<0.001	0.11	0.59	0.46
Overall (29-78 d)												
Weight gain, g/d	44.8[a]	45.8[ab]	47.1[b]	45.5	46.3	45.9	45.9	<0.001	0.12	0.87	0.67	3.9
Feed intake, g/d	137	137	138	142	133	136	138	0.99	<0.001	0.28	0.91	13
Feed conversion	3.07[b]	3.01[b]	2.92[a]	3.13	2.87	2.98	3.02	<0.001	<0.001	0.07	0.22	0.21

[1]Residual standard deviation.

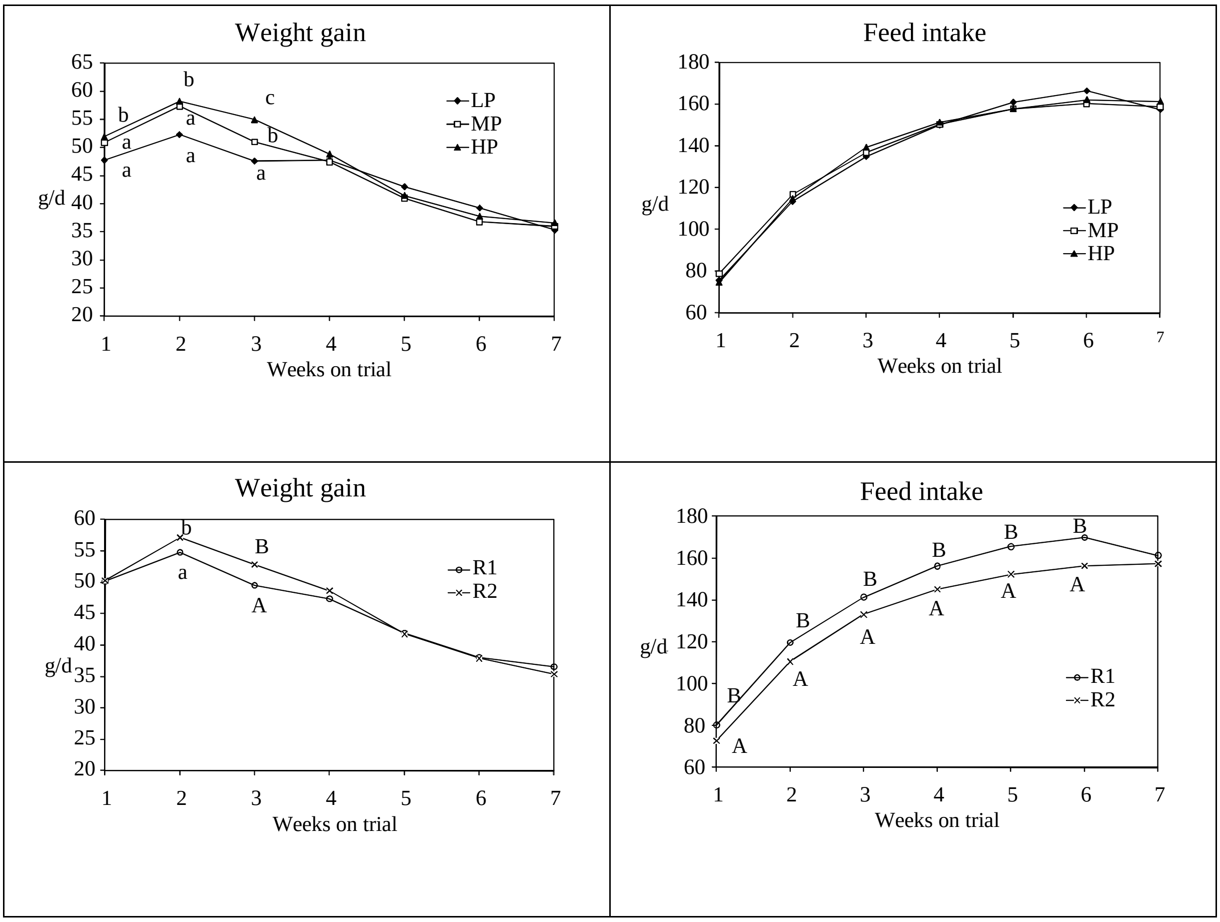


Figure 25. Weekly growth performance according to protein level (LP, MP, HP) and DF/starch ratio (R1, R2)

Both protein level and DF/starch ratio significantly influenced N balance both in the first and second period of growth (Tables 30 and 31). Increasing dietary protein, despite stimulating N retention, as a consequence of the higher growth rate in the high-protein groups, total N excretion increased from 80.4 to 95.0 and 115.0 g, corresponding to 1.64, 1.94 and 2.35 g N excreted per day. When referring to HP diets (=100), N excretion decreased by 17 and 30% by reducing the protein level in MP and LP diets, while retained nitrogen diminished only by 3 and 6%, respectively (Figure 26a). Excretion reduction was stronger in the first rather than in the second period of growth (Figure 26a), even if by only a few points, since the animals fed low protein diets penalized in the beginning, showed a compensatory growth in the second period thus favouring overall N efficiency.

Table 30. Nitrogen excretion in rabbits fed the experimental diets

	Diet					
	L1	M1	H1	L2	M2	H2
Live weight at 29 d, g	597	593	597	596	599	598
Live weight at 50 d, g	1597	1693	1741	1665	1728	1766
Live weight at 78 d, g	2758	2717	2901	2822	2864	2913
Body N at 29 d, g	17.2	17.1	17.2	17.2	17.3	17.3
Body N at 50 d, g	47.6	50.6	52.1	49.7	51.7	52.9
Body N at 78 d, g	85.2	87.1	90.0	87.3	88.7	90.4
Dietary CP, %	13.7	15.1	16.9	14.1	15.7	18.0
First period (29-50 d)						
Feed intake, g/d	113	116	113	103	106	106
N intake, g	51.8	58.6	64.2	49.1	56.1	64.3
N retention, g	30.4	33.5	34.9	32.5	34.4	35.6
N excretion, g	21.5	25.1	29.3	16.6	21.7	28.7
N excretion, g/d	1.02	1.20	1.40	0.79	1.03	1.36
Second period (51-78 d)						
Feed intake, g/d	164	162	163	153	151	153
N intake, g	100.8	109.8	123.5	97.0	106.5	123.7
N retention, g	37.6	36.5	37.9	37.6	37.0	37.5
N excretion, g	63.3	73.3	85.6	59.3	69.5	86.2
N excretion, g	2.26	2.62	3.06	2.12	2.48	3.08
Overall (29-78 d)						
Feed intake, g/d	142	142	142	132	132	133
N intake, g	152.6	168.5	187.8	146.1	162.6	188.0
N retention, g	67.9	70.0	72.8	70.1	71.4	73.1
N excretion, g	84.7	98.4	114.9	75.9	91.2	114.9
N excretion, g/d	1.73	2.01	2.36	1.55	1.86	2.34

Table 31. Nitrogen excretion: effect of protein level and DF/starch ratio

	Protein level (P)				DF/starch ratio (R)			Prob.	RSD[1]
	LP	MP	HP	Prob.	R1	R2	Prob.	P x R	
Rabbits. No.	78	77	76		116	115			
Body N at 29 d, g	17.2	17.2	17.2	0.99	17.2	17.2	0.87	0.95	1.76
Body N at 50 d, g	48.7[a]	51.2[b]	52.5[b]	<0.001	50.1	51.4	0.02	0.58	4.31
Body N at 78 d, g	86.2[a]	87.9[ab]	90.2[b]	<0.01	87.4	88.8	0.14	0.72	6.95
First period (29-50 d)									
Feed intake, g/d	108	111	110	0.32	114	105	<0.001	0.79	12
N intake, g	50.5[a]	57.4[b]	64.3[c]	<0.001	58.3	56.5	0.03	0.30	6.07
N retention, g	31.4[a]	34.0[b]	35.3[b]	<0.001	32.9	34.2	<0.01	0.37	3.44
N excretion, g	19.1[a]	23.4[b]	29.0[c]	<0.001	25.3	22.3	<0.001	<0.01	3.82
N excretion, g/d	0.91	1.12	1.38	<0.001	1.21	1.06	<0.001	<0.01	0.18
Second period (51-78 d)									
Feed intake, g/d	159	157	158	0.80	163	153	<0.001	0.97	17
N intake, g	98.9[a]	108.3[b]	123.7[c]	<0.001	111.5	109.1	0.12	0.54	11.9
N retention, g	37.6	36.7	37.7	0.43	37.3	37.4	0.92	0.84	4.83
N excretion, g	61.4[a]	71.6[b]	86.0[c]	<0.001	74.2	71.8	0.06	0.30	10.1
N excretion, g/d	2.19[a]	2.56[b]	3.07[c]	<0.001	2.65	2.56	0.06	0.30	0.36
Overall (29-78 d)									
Feed intake, g/d	137	137	138	0.99	142	133	<0.001	0.91	13
N intake, g	149.4[a]	165.7[b]	188.0[c]	<0.001	169.8	165.6	0.04	0.35	15.7
N retention, g	69.0[a]	70.7[ab]	72.9[b]	0.001	70.2	71.6	0.12	0.67	6.47
N excretion, g	80.4[a]	95.0[b]	115.0[c]	<0.001	99.6	94.1	<0.001	0.07	12.0
N excretion, g/d	1.64[a]	1.94[b]	2.35[c]	<0.001	2.03	1.92	<0.001	0.07	0.24

[1]Residual standard deviation

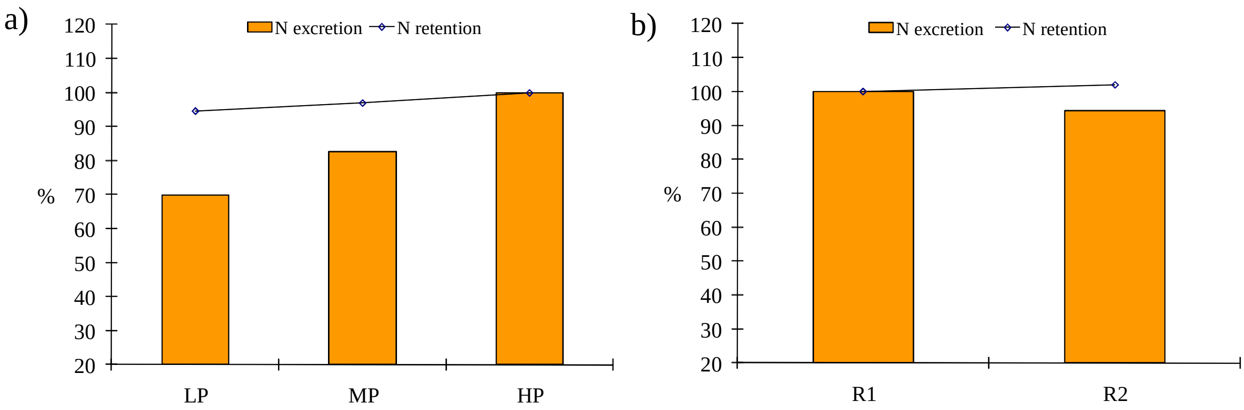


Figure 26. Nitrogen balance in growing rabbits from weaning (29 d) to commercial slaughter (78 d) in relation to a) protein level (HP=100) and b) DF/starch ratio (R1=100)

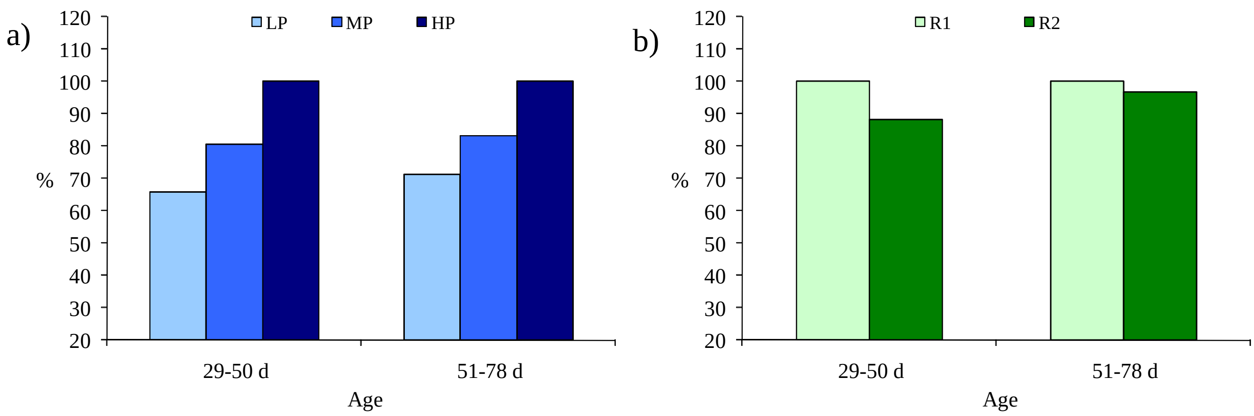


Figure 27. Nitrogen excretion in growing rabbits during post-weaning (29-50 d) and fattening (51-78 d) in relation to a) protein level (HP=100) and b) DF/starch ratio (R1=100)

The effect of digestible fibre level was clear since the first weeks of the trial (Table 29). During post-weaning period, daily growth rate was significantly stimulated, feed intake reduced and as a consequence feed conversion improved by increasing DF/starch ratio. During the last four weeks, from 50 days of age until slaughter, growth was similar in R1 and R2 groups, but the effects on feed intake and conversion were evident and significant. As a consequence, higher DF/starch ratio increased animals body weight at 50 days of age, even if the differences were no longer significant at the end of the trial, reducing feed intake and conversion (lower than 2.9) in the whole experimental period. Increasing DF/starch ratio also reduced N excretion by 5.5%, but without affecting body retention that improved by 2% (Figure 26b). The effect on N excretion reduction was more evident during the first (-12%) rather that the second period of growth (-3%) (Figure 27b).

The positive effect of substituting starch by DF during the post-weaning period on growth rate may be explained by the limited capacity of young animals to use low-digestible fibre and their capability to utilize the high-digestible fibre (mainly pectins) as energy source. The change in digestibility has affected all fibrous fractions, including the less digestible ones (hemicelluloses and celluloses) and so compensated more than proportionally the decrease in starch content. This result was also found in other studies (Gutiérrez *et al.*, 2002). The improvement of feed conversion by increasing DF may be related to the slower intestinal transit, the increase of digestibility and nutritive value of the diets and has also been found in previous studies by the Padova team (Xiccato *et al.*, 2006a, 2008; Carraro *et al.*, 2007; Fragkiadakis *et al.*, 2007).

Health status was satisfactory and not influenced by feeding treatments. During the trial, 15 animals died of which one fed diet L1, three fed diet M1, three fed diet H1, three fed diet L2, two fed diet M2 and three fed diet H2. Deaths were not only caused by ERE, but also due to respiratory problems. The presence of *Staphylococcus aureus* was ascertained, creating abscesses on rabbits. All live rabbits by the end of the trial had a commercially acceptable body weight (>2.400 g). In previous studies, mortality caused by ERE significantly decreased (Gutiérrez *et al*., 2002, 2003; Chamorro *et al*., 2007) by reducing protein level and increasing ileal protein digestibility, due to the improvement of the intestinal microbial population (Carabaño *et al*., 2009), as also happens by increasing digestible fibre (Xiccato *et al*., 2006a; Gómez-Conde *et al*., 2007).

Caecal content and intestinal mucosa characteristics

Caecal content and jejunum mucosa characteristics in 38 days-old rabbits are presented in Table 32. By increasing the protein content from 14 to 17.0%, VFA production increased and caecal pH decreased. Higher inclusion of barley and dried beet pulps, at constant DF content, in diets with higher protein content may explain the more intense caecal fermentative activity. On the other hand, the absence of significant variation of ammonia concentration, by increasing dietary protein, may be considered as an index of balanced caecal environment and fermentations, where a higher supply of not digested protein at ileal level can stimulate rather than impair bacterial fermentative activity.

Table 32. Caecal content and intestinal mucosa characteristics in rabbits at 38 d of age

	Protein level (P)				DF/starch ratio (R)			Prob.	RSD[1]
	LP	MP	HP	Prob.	R1	R2	Prob.	P x R	
Rabbits, No.	12	12	12		18	18			
Live weight (LW), g	1035	1054	1076	0.62	1053	1058	0.85	0.62	102
Full stomach, % LW	9.0	9.1	8.7	0.59	8.8	9.0	0.65	0.66	1.1
Full caecum, % LW	7.9	8.5	8.1	0.45	7.8	8.5	0.08	0.90	1.1
Full digestive tract, % LW	26.9	27.5	26.6	0.44	26.5	27.5	0.11	0.52	1.7
Caecal content traits:									
pH	5.92	5.71	5.75	0.05	5.87	5.71	0.03	0.12	0.22
$N\text{-}NH_3$, mmol/l	3.51	3.85	3.21	0.74	4.7	2.3	<0.001	0.69	1.99
Total VFAs, mmol/l	61.9[a]	73.7[ab]	78.0[b]	0.01	64.0	78.4	<0.01	0.20	13.0
C_2, % mol. VFAs	86.8	88.0	87.0	0.58	87.4	87.6	0.84	0.35	2.9
C_3, % mol. VFAs	4.0	3.6	4.4	0.26	3.7	4.2	0.25	0.36	1.2
C_4, % mol. VFAs	8.6	8.1	7.6	0.51	8.5	7.9	0.47	0.72	2.7
C_5, % mol. VFAs	0.3	0.3	0.3	0.44	0.3	0.3	0.61	0.09	0.12
C_3/C_4	0.47	0.49	0.69	0.16	0.50	0.60	0.33	0.96	0.3
Jejunum mucosa characteristics									
Villi height, µm	497	570	530	0.20	554	511	0.20	0.59	97
Crypt depth, µm	82	83	86	0.74	87	81	0.22	0.90	13
Villi/crypt ratio	6.37	7.04	6.48	0.16	6.67	6.60	0.82	0.35	0.90

[1]Residual standard deviation

By increasing DF and reducing starch, intestinal transit got slower and gut incidence tended to increase, mainly due to caecal filling. Caecal fermentative activity increased as indicated by VFA production (from 64.0 to 78.4 mmol/l; P<0.001), therefore reducing pH (from 5.87 to 5.71) and ammonia concentration (from 4.7 to 2.3 mmol/l; P<0.001) of caecal content. In previous studies, the caecal fermentative activity increased by increasing digestible and/or soluble fibre content (García *et al*., 2000; Falcao e Cunha *et al*., 2004). The decreased production in ammonia may be explained by the reduction of alfalfa and the increase in soybean and sunflower meal which protein is characterized by a better ileal digestibility and thus a lower passage into the caecum. The higher ileal digestibility of crude protein (86-93%) from soybean and sunflower meal compared to alfalfa hay (73-74%) has been demonstrated (Carabaño *et al*., 2009).

Jejunum mucosa characteristics were not influenced by either dietary protein or digestible fibre level, having a similar villi height and crypt depth. As a consequence, in the present conditions, a feeding effect on mucosal functionality has not been demonstrated.

Slaughter results and meat quality

Results of commercial slaughter at 78 days of age and carcass traits are presented in Table 33. As widely known, feeding treatment effect is associated with limited variations of body weight (Xiccato, 1999; Hernandez and Gondret, 2006; Hernandez, 2008).

Table 33. Results at commercial slaughter and carcass quality

	Protein level (P)			DF/starch (R)		Gender (G)		Probability				RSD[1]
	LP	MP	HP	R1	R2	Females	Males	P	R	G	P x R	
Rabbits. No.	40	40	40	60	60	61	59					
Slaughter weight (SW), g[2]	2729[a]	2788[ab]	2840[b]	2763	2808	2793	2779	0.04	0.20	0.71	0.71	193
Transport losses, %LW[3]	2.1	2.0	2.3	2.2	2.1	2.1	2.2	0.22	0.83	0.65	0.13	0.8
Gut incidence, %SW[4]	19.0	18.3	18.4	18.5	18.7	18.9	18.2	0.03	0.63	<0.01	0.86	1.2
Cold carcass, (CC) g	1640[a]	1687[ab]	1726[b]	1671	1699	1677	1692	<0.01	0.21	0.50	0.78	123
Dressing percentage, SW	60.1	60.5	60.8	60.5	60.5	60.0	60.9	0.09	0.95	<0.001	0.96	1.3
Head, %CC	7.7	7.7	7.7	7.7	7.7	7.6	7.8	0.69	0.65	0.02	0.50	0.45
Liver, %CC	5.3	5.2	5.3	5.3	5.3	5.3	5.3	0.71	0.78	0.83	0.90	0.9
Reference carcass (RC), g	1374[a]	1417[ab]	1447[b]	1401	1424	1409	1415	0.01	0.23	0.77	0.74	107
Reference carcass dissection:												
Dissectible fat, %RC	3.3	3.1	3.3	3.4	3.1	3.3	3.1	0.47	0.06	0.34	0.77	0.9
Hind legs, %RC	32.6	33.2	32.8	32.9	32.9	33.0	32.8	0.23	0.88	0.51	0.31	1.1
Muscle/bone ratio hind leg	5.69	5.91	5.95	5.71	5.99	5.94	5.76	0.33	0.07	0.25	0.53	0.58

[1]Residual standard deviation; [2]SW: live weight at the slaughterhouse immediately before slaughter; [3]LW: live weight at the farm; [4]Incidence of the full gastro-intestinal tract.

Protein effect on animals live weight was evident also on slaughter weight and, as a consequence, on all variables correlated to body weight. Increasing dietary protein content significantly increased live weight (P=0.04) and carcass weight (P=0.01) and tended (P=0.09) to improve slaughter dressing percentage. Other carcass and meat characteristics were not affected, (Tables 33 and 34).

Table 34. Meat quality: pH and colour of *longissimus lumborum* and *biceps femoris*

	Protein level` (P)			DF/starch ratio (R)		Gender (G)		Probability				RSD[1]
	LP	MP	HP	R1	R2	Females	Males	P	R	G	P x R	
Rabbits, No.	40	40	40	60	60	61	59					
Longissimus lumborum:												
pH	5.52	5.52	5.52	5.53	5.51	5.51	5.54	0.89	0.04	<0.01	0.97	0.06
L*	55.0	55.3	55.3	54.8	55.6	55.6	54.7	0.90	0.15	0.09	0.33	3.0
a*	-1.96	-1.90	-1.74	-2.00	-1.74	-1.98	-1.76	0.52	0.12	0.73	0.18	0.89
b*	2.54	2.52	3.33	2.56	3.03	2.73	2.86	0.16	0.23	0.75	0.37	2.17
C*	3.63	3.66	4.20	3.74	3.92	3.76	3.91	0.15	0.49	0.57	0.27	1.46
H*	-0.63	-0.67	-0.76	-0.70	-0.68	-0.70	-0.67	0.66	0.83	0.82	0.23	0.66
Biceps femoris												
pH	5.74	5.76	5.74	5.76	5.73	5.73	5.77	0.81	0.07	0.02	0.99	0.10
L*	50.7	50.2	50.5	50.0	50.9	50.8	50.1	0.45	0.01	0.08	0.35	1.9
a*	-2.63	-2.79	-2.75	-2.72	-2.72	-2.79	-2.66	0.24	0.99	0.10	0.40	0.43
b*	2.97	3.04	3.06	3.03	3.01	2.99	3.06	0.95	0.93	0.77	0.95	1.34
C*	4.11	4.23	4.22	4.19	4.18	4.21	4.17	0.82	0.95	0.81	0.74	0.96
H*	-0.80	-0.78	-0.80	-0.80	-0.79	-0.77	-0.81	0.97	0.79	0.45	0.88	0.26

[1]Residual standard deviation

As for growth performance, DF/starch ratio slightly affected slaughter and carcass traits, with small variations (P<0.10) of dissectible fat and hind leg muscle/bone ratio (Table 33). The pH of the two main muscles and lightness of the *b. femoris* varied in a limited range (from 50.0 to 50.9), though at a statistically significant level, in relation to dietary fibre (Table 34).

Females showed higher gut incidence than males and, as a consequence, a lower dressing percentage, as previously found (Lambertini *et al*., 1990; Parigi Bini *et al*., 1992; Petracci *et al*., 1999; Trocino *et al*., 2003: Lazzaroni *et al*., 2009). Females had also lower head incidence on cold carcass (Table 33), lower pH of *l. lumborum* and *b. femoris* pH as well as higher lightness. Other authors observed darker (Trocino *et al*., 2003) and more coloured meat (Carrilho *et al*., 2009) in rabbit females than in males. In animals slaughtered at an older age (>100 days), Lazzaroni *et al*. (2009) measured lower lightness values, and higher red and yellow values in females rather than males. Differently, Petracci *et al*. (1999) did not observe any significant differences between genders in meat colour or technological traits (drip and cooking losses).

EXPERIMENT 4:

Starch and pectin levels in diets for growing rabbits: effects on health status, digestive physiology, growth performance and carcass and meat quality

Material and methods

Rearing conditions

The trial was realized in the experimental farm of the University of Padova from March to April. The characteristics of experimental facilities and cages have been detailed previously. Before the arrival of rabbits, the building was kept empty for a long period (sanitary vacuum) and then submitted to a disinfectant and fungicide treatment.

Maximum temperature did not exceed 25°C and minimum temperature did not go below 15°C (Figure 28). During the whole period, the average values of minimum and maximum temperature were 18°C and 21°C, respectively. Maximum relative humidity averaged around 69%, with minimum 36% and maximum 86%. Minimum relative humidity was on average 55%, with minimum 32% and maximum 75% (Figure 28).

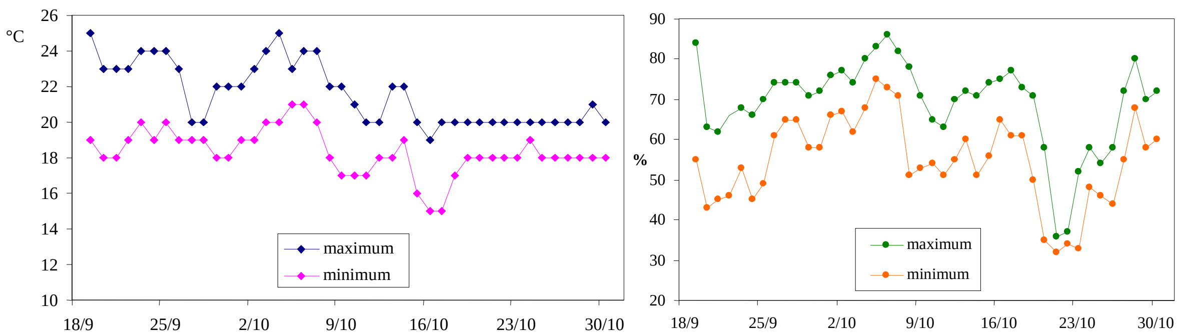


Figure 28. Daily values of minimum and maximum temperature and minimum and maximum relative humidity (RH) throughout the trial

Animals, experimental groups and recordings

Two hundred and forty rabbits of 33 d of age were selected in a commercial farm from Grimaud hybrid does without clinical signs of diseases. The animals were transported to the Department facilities in an air-conditioned truck, inside 20 collective cages, at low density (12 rabbits per cage) to reduce the transport stress to minimum. On their arrival, the rabbits presented optimal health status and average weight of 827±26 g. They were controlled by gender, marked using ear tags and put in individual cages. Then rabbits divided into six experimental groups of 40 units each and fed ad libitum from weaning until slaughter six different diets formulated according to a bi-factorial arrangement 2 x 3, with three levels of starch (St1, St2, St3) and two levels of pectins (LPc and HPc), as shown in the following chart:

	Pectins 5% (LPc)	Pectins 10% (HPc)
Starch 5% (St1)	Diet L1	Diet H1
Starch 10% (St2)	Diet L2	Diet H2
Starch 15% (St3)	Diet L3	Diet H3

The trial started the day after the arrival of rabbits on 34 d of age and the experimental diets were given *ad libitum* from the beginning of the trial to 5 days before commercial slaughter. From 70 d of age to slaughter (75 d), a unique coccidiostatic-free commercial diet was administered to all animals. Individual live weight and feed intake were recorded three times a week. Health status was controlled daily to detect the occurrence of diseases, especially digestive problems. The *in vivo* digestibility trial (60 animals from 52 to 56 d of age), the sampling of caecal content and ileum and jejunum mucosa (36 rabbits at 51 d), histological analysis and chemical analyses of experimental diets, faeces and caecal content were performed according to the methodologies previously detailed.

Experimental diets

Six diets were used. Diets L1, L3, H1 and H3 were produced (Table 35), while diets L2 and H2 were obtained by mixing in equal parts diets L1 and L3 and diets H1 and H3, respectively. Starch level increased from 5% to 10 to 15% in St1, St2 and St3 diets by increasing barley inclusion rate. Pectins rose from 5% to 10% in LPc and HPc diets by increasing levels of beet pulp at the expenses of alfalfa meal. Inclusion rates of wheat bran, soybean and sunflower meals were modulated to maintain dietary protein at a constant level (15.5-16.0%).

All diets were integrated with synthetic amino acids (lysine, threonine, sulphuric amino acids), macro- and micro-minerals and vitamins to satisfy the nutritional requirements of growing rabbits (De Blas and Mateos, 1998).

Dietary crude protein varied between 15.5 and 15.9% (Table 36). Ether extract changed with soybean oil supplementation and was higher in LPc diets compared to HPc diets.

Table 35. Ingredient composition (%) of experimental diets

	Diet L1 Starch 5% Pectins 5%	Diet L3 Starch 15% Pectins 5%	Diet H1 Starch 5% Pectins 10%	Diet H3 Starch 15% Pectins 10%
Dehydrated alfalfa meal 17% CP	72.20	35.00	35.00	0.00
Wheat bran	13.00	18.80	17.00	0.00
Barley	0.00	20.00	0.00	30.00
Dried beet pulp	5.00	6.00	33.00	34.10
Soybean meal 44% CP	0.00	0.00	10.40	5.00
Sunflower meal 30% CP	4.00	15.50	0.00	26.50
Soybean oil	2.00	1.00	1.00	0.00
Molasses	1.50	1.50	1.50	1.50
Calcium carbonate	0.00	0.83	0.00	1.18
Dicalcium phosphate	0.98	0.10	1.05	0.65
Sodium chloride	0.40	0.40	0.40	0.40
L-lysine (liquid)	0.25	0.30	0.00	0.20
DL-methionine	0.10	0.00	0.08	0.00
Vitamin-mineral supplement*	0.40	0.40	0.40	0.40
Choline	0.07	0.07	0.07	0.07
Coccidiostatic	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.

Table 36. Chemical composition (as fed) of experimental diets

	Diet					
	L1	L2	L3	H1	H2	H3
Dry matter, %	91.6	91.4	90.9	91.1	91.1	90.4
Crude protein, %	15.7	15.5	15.7	15.8	15.9	15.8
Ether extract, %	3.9	3.7	3.6	2.9	2.5	2.0
Crude fibre, %	20.0	17.9	16.8	16.4	15.4	14.3
Ash, %	10.0	9.0	8.1	8.1	7.4	6.7
TDF, %	47.2	44.3	42.4	48.3	46.4	43.2
NDF, %	40.0	38.7	35.6	36.8	35.5	31.8
ADF, %	25.3	23.7	20.5	20.8	19.7	17.4
Hemicelluloses (NDF-ADF), %	14.7	15.0	15.1	16.0	15.8	14.4
ADL, %	5.0	5.0	4.3	3.4	3.1	2.9
Digestible fibre (TDF-ADF)	21.9	20.6	21.9	27.5	26.6	25.8
Pectins (TDF-NDF), %	7.2	5.6	6.9	11.4	10.8	11.4
Starch, %	6.1	10.5	15.1	5.8	10.1	14.2
Gross energy, MJ/kg	16.5	16.5	16.6	16.2	16.2	16.1
DF/starch ratio	3.6	2.0	1.5	4.8	2.6	1.8
DF/ADF ratio	0.9	0.9	1.1	1.3	1.4	1.5
Pectins/starch ratio	1.2	0.5	0.5	2.0	1.1	0.8

Crude fibre concentration was higher in LPc than HPc diets and decreased from diets St1 to St3, respectively. Total dietary fibre (TDF) was on average lower in LPc (44.6%) than in HPc diets (46.0%) and decreased from diets St1 to diets St3. The concentration of NDF and ADF changed accordingly to TDF level. Pectins concentration, calculated as the difference between

TDF and NDF content, varied accordingly to what expected in formulation (from 6.5 to 11.2 rather than from 5 to 10%). Dietary starch averaged 5.9% in diets St1, 10.3% in diets St2 and 14.6% in diets St3. Pectins/starch ratio ranged from a minimum of 0.46 (diet L3) to a maximum value of 1.97 (diet H1).

Commercial slaughter, carcass dissection and meat quality analyses

At 75 d of age, 120 rabbits were selected (20 for each group of diets and representative in terms of average weight and variability) for commercial slaughter and dissection. Feed and water were available until loading (from 5:30 to 6:30 a.m.). The animals were weighted at the experimental farm before loading and then transported in cages (50 x 100 x 30 cm, 20 rabbits/m^2) for about 60 minutes to a commercial slaughterhouse. Slaughter began at 9.00 and ended at 11.00 a.m. approximately. Rabbits were individually weighted, stunned by electro-anaesthesia and killed by jugulation. Carcasses were chilled at 4°C for 2 h 30 min and then transported to the laboratories of the Department of Animal Science 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 recorded were analyzed by ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC) and considering the effects of the starch and pectin levels as well as their interaction. The effects of the gender was also included in the model for data of growth performance, slaughter results, carcass and meat traits. The Bonferroni test was used to compare means.

Results and discussion

Digestibility and nutritive value of experimental diets

Nutrient digestibility and nutritive value changed greatly among diets and increased with increasing levels of both starch and pectins (Table 37). The diets L3 (5% pectin, starch 15%) and H1 (10% pectin, starch 5%), characterized by extreme values of pectin/starch ratio (0.5 vs. 2.0), showed very similar digestibility and nutritive values (DE: 9.5 MJ/kg): with the same inclusion rate of alfalfa meal, replacing barley meal with beet pulp maintained diet digestibility at high level. The starch coming from barley in L3 diet was replaced in diet H1 by digestible fibre (especially pectins) provided by beet pulp.

The digestibility of crude fibre and fibre fractions changed depending on the dietary pectin level: within LPc diets, digestibility of crude fibre changed in a restricted range (from

7.2% to 9.6%, P>0.05), while significant differences were found for fibre fractions; within HPc diets, digestibility increased from diet H1 to diet H3 for crude fibre (23.5 to 30.3%), NDF (34.3 to 45.1%), ADF (25.7 to 32.1%) and hemicelluloses (45.4 to 60.8%), without significant differences for pectins digestibility. The nutritive value of diets increased with starch, pectins and digestible fibre level from diet L1 to diet H3 (DE: 8.11 to 10.85 MJ/kg) and, consequently, DP/DE ratio decreased from diet L1 to diet H3 (13.8 to 10.8 g/MJ), even if always remained above the minimum values recommended for growing rabbits (De Blas and Mateos, 1998).

Table 37. Digestibility coefficients (%) and nutritive value (as fed) of experimental diets

	Diet						Prob.
	L1	L2	L3	H1	H2	H3	
Rabbits, No.	10	10	10	10	10	10	
Dry matter, %	49.9^{a}	54.5^{b}	57.5^{c}	59.5^{c}	63.8^{d}	68.3^{e}	<0.001
Crude protein, %	71.2^{a}	72.7^{abc}	74.0^{c}	71.5^{ab}	73.2^{bc}	73.7^{c}	<0.001
Ether extract, %	78.5^{c}	79.8^{cd}	81.4^{d}	73.1^{a}	73.9^{a}	75.9^{b}	<0.001
Crude fibre, %	7.6^{a}	7.2^{a}	9.6^{a}	23.5^{b}	25.4^{bc}	30.3^{c}	<0.001
NDF, %	19.7^{a}	25.4^{c}	24.3^{bc}	34.3^{d}	41.8^{e}	45.1^{f}	<0.001
ADF, %	13.4^{a}	19.3^{b}	15.7^{ab}	25.7^{c}	29.7^{cd}	32.1^{d}	<0.001
DF, %	47.2^{ab}	45.4^{a}	50.1^{b}	62.2^{c}	68.4^{d}	71.9^{e}	<0.001
Pectins, %	80.7^{b}	73.4^{a}	81.2^{b}	85.7^{d}	85.2^{cd}	85.9^{d}	<0.001
Hemicelluloses, %	30.8^{a}	35.0^{ab}	36.0^{b}	45.4^{c}	56.9^{de}	60.8^{e}	<0.001
Starch, %	95.8^{a}	97.2^{b}	97.8^{cd}	96.0^{a}	98.0^{d}	98.8^{e}	<0.001
Gross energy, %	49.2^{a}	53.6^{b}	56.9^{c}	58.2^{c}	62.8^{d}	67.5^{e}	<0.001
Nutritive value							
Digestible Energy, MJ/kg	8.11	8.84	9.45	9.47	10.20	10.85	
Digestible protein, g/kg	11.2	11.3	11.6	11.3	11.7	11.7	
DP/DE, g/MJ	13.8	12.8	12.3	11.9	11.5	10.8	

When the apparent digestibility of diets is expressed in function of the variability factors considered (Table 38), the level of starch and pectins affected always significantly digestive efficiency.

As mentioned above, increasing dietary starch improved dry matter and nutrient digestibility (from 54.7% of St1 diets to 62.9% of St3 diets). The reduction of alfalfa inclusion rate and its substitution with more digestible raw materials, like barley and sunflowers, can easily explain this result. The inclusion of a large quantity of beet pulp, more than 30%, replacing alfalfa meal, in high-pectins diets, accounts for the higher digestibility of crude fibre (8.1 to 26.4% from LPc to HPc diets) and all fibre fractions of these latter diets. Only ether extract digestibility significantly diminished because of the lower supplementation in HPc diets with soybean oil, of which digestibility is higher compared to fat contained in vegetable raw materials used in diet formulation.

Table 38. Digestibility coefficients (%): effect of starch and pectins level

	Starch level				Pectins level			RSD[1]
	St1	St2	St3	Prob.	LPc	HPc	Prob.	
Rabbits, No.	20	20	20		30	30		
Dry matter	54.7[a]	59.2[b]	62.9[c]	<0.001	54.0	63.8	<0.001	1.7
Crude protein	71.4[a]	73.0[b]	73.9[b]	<0.001	72.7	72.9	<0.001	1.4
Ether extract	75.8[a]	76.8[b]	78.6[c]	<0.001	79.9	74.3	<0.001	1.3
Crude fibre	15.5[a]	16.3[a]	20.0[b]	<0.001	8.1	26.4	<0.001	3.7
TDF	37.7[a]	41.7[b]	44.6[c]	<0.001	31.3	51.4	<0.001	2.6
NDF	27.0[a]	33.6[b]	34.7[b]	<0.001	23.1	40.4	<0.001	3.1
ADF	19.5[a]	24.5[b]	23.5[b]	<0.001	16.1	29.1	<0.001	3.4
DF	54.7[a]	56.9[a]	61.0[b]	<0.001	47.5	67.5	<0.001	2.1
Pectins	83.2[b]	79.3[a]	83.5[b]	<0.001	78.4	85.6	<0.001	2.3
Hemicelluloses	38.1[a]	45.9[b]	48.4[b]	<0.001	33.9	54.4	<0.001	3.1
Starch	95.9[a]	97.6[b]	98.2[c]	<0.001	96.9	97.6	<0.001	0.3
Gross energy	53.7[a]	58.2[b]	62.2[c]	<0.001	53.2	62.8	<0.001	1.8

[1]Residual standard deviation

Health status and performance

During the trial there were no health problems caused by the dietary treatment or specific diseases: only two animals died at different times of the experimental period. All animals showed the patterns of growth and consumption correspondent to the genetic type used (Figures 29 and 30) until 75 days of age and an average slaughter weight of about 2,700 g, as requested by the North-Italian market.

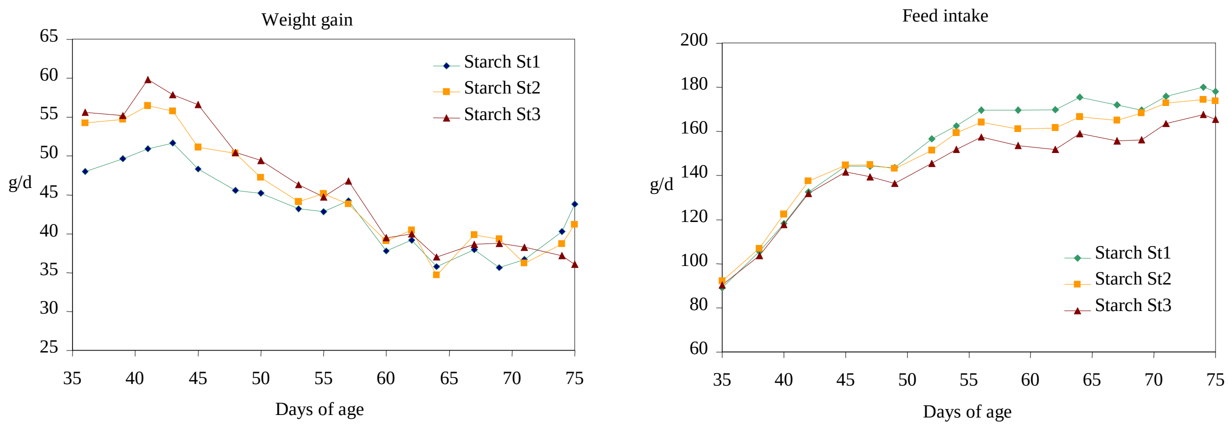


Figure 29. Performance according to dietary starch level

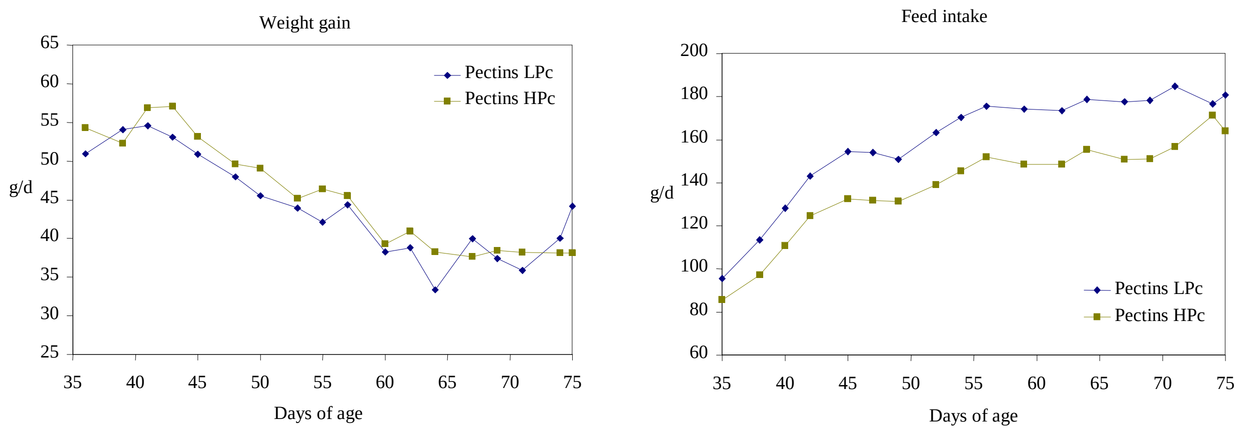


Figure 30. Performance according to dietary pectins level

Growth performance during post-weaning (34 to 55 days of age), fattening (55 days to slaughter) and the whole trial are reported in Table 39. Increasing dietary starch significantly increased animal weight at 75 d and daily growth and reduced feed intake with a corresponding improvement in feed conversion (3.52 to 3.31 and 3.13, P<0.001) in the whole period. While during post-weaning daily weight gain was higher (P<0.001) and feed intake tended to be lower with high-starch diets (P=0.06), in the second period daily growth was similar, but feed intake was significantly reduced (172 to 159 g/d; P<0.001) likely because of a better functioning of the chemiostatic regulation of appetite.

Table 39. Performance from weaning to commercial slaughter

	Starch (St)			Pectins (Pc)		Gender (G)		Probability				RSD[1]
	St1	St2	St3	LPc	HPc	Female	Male	St	Pc	G	St x Pc	
Rabbits, n.	69	70	69	103	105							
Live weight, g												
34 d	880	883	882	882	882	884	880	0.72	0.94	0.31	0.40	28.4
55 d	1870^{a}	1957^{b}	2000^{b}	1919	1966	1968	1917	0.001	0.01	0.01	0.54	120
75 d	2663^{a}	2758^{b}	2780^{b}	2707	2760	2782	2685	0.001	0.05	0.001	0.83	193
First period (34-55 d)												
Weight gain, g/d	47.1^{a}	51.1^{b}	53.2^{b}	49.4	51.6	51.6	49.4	0.001	0.01	0.01	0.69	5.3
Feed intake, g/d	133^{ab}	134^{b}	130^{a}	142	123	136	129	0.06	0.001	0.001	0.40	11.3
Feed conversion	2.84^{c}	2.64^{b}	2.45^{a}	2.89	2.39	2.65	2.64	0.001	0.001	0.62	0.58	0.18
Second period (55-75 d)												
Weight gain, g/d	39.6	40.0	39.0	39.4	39.7	40.7	38.4	0.56	0.76	0.01	0.98	5.70
Feed intake, g/d	172^{b}	167^{b}	159^{a}	176	156	168	164	0.001	0.001	0.07	0.75	17.2
Feed conversion	4.40^{b}	4.21^{ab}	4.11^{a}	451	3.97	4.15	4.32	0.001	0.001	0.01	0.62	0.44
Overall (34-75 d)												
Weight gain, g/d	43.5^{a}	45.7^{b}	46.3^{b}	44.5	45.8	46.3	44.0	0.001	0.05	0.001	0.90	4.5
Feed intake, g/d	152^{b}	151^{b}	144^{a}	159	139	152	146	0.001	0.001	0.01	0.63	12.7
Feed conversion	3.52^{c}	3.31^{b}	3.13^{a}	3.59	3.05	3.30	3.34	0.001	0.001	0.11	0.79	0.19

[1]Residual standard deviation

Dietary pectins level significantly affected growth performance: increasing concentrations stimulated daily weight gain (especially during post-weaning), reduced feed intake and improved feed conversion (3.59 vs. 3.05; P<0.001). Final live weight was higher in animals fed diets containing 10% pectins compared to those fed with diets with 5% pectins, both at 55 days of age and at the end of the trial.

The effect of gender was immediately apparent in the post-weaning period, with higher live weight, growth rate and feed intake in females compared to males, while feed conversion was slightly affected (P=0.11).

Caecal content and intestinal mucosa characteristics

Table 40 shows the effect of experimental factors on the characteristics of the caecal contents and intestinal tissue in rabbits slaughtered at 51 days of age. Live weight of animals significantly increased with dietary starch and, conversely, stomach, caecum and gut incidence decreased. The differences in animal live weight and the low fibre level of high-starch diets may explain this result. Several studies have measured decreasing incidence of slaughter losses with increasing live weight as well as with low-fibre diets (Xiccato, 1999, Hernández and Gondret, 2006).

Table 40. Caecal traits and mucosa characteristics in rabbits at 51 d of age

	Starch level (St)				Pectins level (Pc)			Prob.	RSD
	St1	St2	St3	Prob.	LPc	HPc	Prob.	St x Pc	
Rabbits, No.	12	12	12		18	18			
Live weight (LW), g	1701[a]	1781[ab]	1814[b]	0.02	1742	1789	0.16	0.83	98
Full stomach, % LW	8.2[b]	7.6[ab]	6.9[a]	<0.01	7.6	7.5	0.62	0.56	0.9
Full caecum, % LW	7.9	7.7	7.2	0.11	7.5	7.7	0.40	0.07	0.84
Full digestive tract, % LW	24.6[b]	23.7[b]	22.0[a]	0.001	23.5	23.4	0.83	0.93	1.60
Caecal content traits:									
pH	5.78	5.79	5.80	0.97	5.88	5.70	0.02	0.13	0.22
$N\text{-}NH_3$, mmol/l	7.20[b]	5.51[ab]	4.93[a]	0.03	6.11	5.66	0.52	0.16	2.10
Total VFAs, mmol/l	74.4	81.6	79.0	0.54	73.8	82.8	0.10	0.72	15.9
C_2, % mol. VFAs	84.1	85.5	85.8	0.12	84.7	85.5	0.26	0.56	2.0
C_3, % mol. VFAs	4.0[b]	3.5[a]	3.3[a]	<0.01	3.61	3.59	0.85	0.86	0.48
C_4, % mol. VFAs	11.5	10.6	10.5	0.48	11.2	10.5	0.34	0.54	2.07
C_5, % mol. VFAs	0.41	0.38	0.37	0.43	0.43	0.35	0.01	0.90	0.09
C_3/C_4	0.35	0.34	0.35	0.95	0.33	0.37	0.37	0.47	0.11
Jejunum mucosa:									
Villi height, µm[1]	728	632	682	0.11	709	655	0.12	0.86	105
Crypt depth, µm[2]	116	102	112	0.08	114	106	0.15	0.52	15.9
Villi height/crypt depth	6.60	6.53	6.36	0.89	6.59	6.41	0.67	0.43	1.23
Ileum mucosa:									
Villi height, µm[3]	491	466	527	0.09	494	496	0.95	0.41	64.4
Crypt depth, µm	72.1	70.6	72.7	0.92	71.8	71.8	0.99	0.81	12.8
Villi height/crypt depth	7.28	7.22	7.66	0.78	7.41	7.37	0.94	0.93	1.66

[1]Linear component of variance, L=0.26; quadratic component, Q=0.07; [2]L=0.11, Q=0.11; [3]L=0.03, Q=0.79

Caecal fermentation activity changed little with dietary treatment: N-ammonia production was higher in the caecum of animals fed low-starch diets, likely because of the higher protein intake (DP/DE: 12.9, 12.2 and 11.6 g/MJ respectively for the diets with 5, 10, and 15% starch) (Carabaño *et al*., 2009). Total VFA production did not change, while the increase of acetate (P=0.12) and the reduction of propionate (P<0.01) with increasing dietary starch were unexpected. High dietary starch would have been expected to increase VFA production, because of a larger availability of fermentable substrate, and to reduce acetate proportion in presence of increased butyrate proportion, as a result of a high activity of fermentative amylolytic bacteria (Gidenne and García, 2006). The chemical characteristics of the diets may explain the result obtained in this experiment: 1) even the highest level of starch used (about 16%, average value of diets L1 and H1) was compatible with the digestive capacity of the animal at the age considered (51 d) and did not produce a significant flow of undigested starch at caecum; 2) besides, diets had a similar DF content (24.7, 23.6 and 23.8% for diets with 5, 10 and 15% starch) to ensure a comparable fermentative activity and more oriented towards acetate production rather than propionate and butyrate.

The increase in the pectins level reduced pH (P=0.02), but had little effect on propionate and butyrate proportions. Only total VFA production tended to increase with the administration of diets containing 10% pectins (73.8 *vs*. 82.8 mmol/l; P=0.10) and percentage of valerate decreased (0.43 *vs*. 0.35%; P<0.01).

The results of the histology of jejunum and ileum mucosa revealed no significant differences in the villi and crypts development. The height of the villi at both jejunum and ileum tended to be lower (P=0.11 and 0.09) in rabbits fed diets containing levels of starch (about 10%) recommended for the post-weaning than in those fed diets containing less (5%) or higher (15%) concentrations. On the other hand, the villi height was found to be lower in those dietary treatments formulated for health maintenance.

Slaughter results and meat quality

The effect of dietary starch on slaughter results mainly depended on differences in animal live weight (Table 41). Heavier rabbits, that received the diets St2 and St3, showed higher slaughter and carcass weights and dressing percentage, lower incidence of slaughter losses and greater incidence of dissectible fat (P<0.01). The effect of increasing dietary pectins was fair and the higher slaughter weight (2628 *vs*. 2687 g; P<0.05) was not associated to higher slaughter dressing percentage. Gut incidence in rabbits fed HPc diets was significantly higher (18.5 *vs*. 19.0%, P<0.05) than in rabbit fed LPc diets. The higher degree of gut filling and the slower intestinal transit of digesta associated with increased dietary pectins (from beet pulp) account for this result (Carabaño *et al*., 1997; Hernández and Gondret, 2006).

Table 41. Results at commercial slaughter and carcass quality

	Starch (St)			Pectins (Pc)		Gender (G)		Probability				RSD[1]
	St1	St2	St3	LPc	HPc	Female	Male	St	Pc	G	St x Pc	
Rabbits. No.	40	40	40	60	60	57	63					
Slaughter weight (SW), g[2]	2598^{a}	2674^{ab}	2700^{b}	2628	2687	2689	2625	0.02	0.04	0.04	0.64	158
Transport losses, %LW[3]	2.9	2.7	2.9	2.9	2.7	3.0	2.7	0.51	0.29	0.06	0.52	0.9
Gut incidence, %SW[4]	19.5^{b}	18.5^{b}	18.1^{a}	18.5	19.0	18.8	18.6	0.001	0.05	0.36	0.39	1.4
Cold carcass weight (CC), g	1542^{a}	1609^{b}	1638^{b}	1582	1611	1607	1586	0.001	0.10	0.23	0.71	95.5
Cold dressing percentage, %SW	59.4^{a}	60.2^{b}	60.7^{b}	60.2	60.0	59.8	60.4	0.001	0.34	0.03	0.92	1.4
Dissection cold carcass:												
Head, %CC	8.1	7.9	7.8	7.9	7.9	7.7	8.1	0.03	0.86	0.001	0.28	0.5
Liver, %CC	5.6^{b}	5.4^{ab}	5.0^{a}	5.7	5.0	5.3	5.4	0.001	0.001	0.29	0.81	0.7
Reference carcass (RC), g	1280^{a}	1344^{b}	1375^{b}	1314	1352	1347	1318	0.001	0.02	0.07	0.59	85
Dissection of reference carcass:												
Total dissectible fat, %RC	2.8^{a}	3.3^{b}	3.2^{ab}	3.1	3.1	3.2	3.0	0.01	0.48	0.14	0.62	0.8
Hind legs, %RC	33.1	33.3	31.9	33.1	32.4	32.9	32.6	0.10	0.20	0.65	0.43	2.2
Muscle/bone ratio of hind leg	5.86	6.25	6.01	6.06	6.02	6.07	6.02	0.19	0.81	0.80	0.58	0.66

[1]Residual standard deviation; [2]SW: live weight at the slaughterhouse immediately before slaughter; [3]LW: live weight at the farm; [4]Incidence of the full gastro-intestinal tract.

The effects of dietary treatments were limited on meat quality, in terms of pH, lightness, redness and yellowness measured on the *longissimus lumborum* and *biceps femoris* muscles 24 hours after slaughter (Table 42).

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

	Starch level (St)			Pectins level (Pc)		Gender (G)		Probability				RSD
	St1	St2	St3	LPc	HPc	Female	Male	St	Pc	G	St x Pc	
Rabbits, No.	40	40	40	60	60	57	63					
Longissimus lumborum:												
pH	5.61	5.61	5.63	5.60	5.64	5.61	5.63	0.40	0.001	0.13	0.06	0.08
L*	50.6	50.2	50.0	50.5	50.0	50.7	49.9	0.56	0.30	0.08	0.89	2.31
a*	-2.19	-2.0	-1.99	-2.00	-2.15	-2.11	-2.04	0.59	0.38	0.65	0.81	0.89
b*	0.41	-0.02	0.05	0.19	0.11	0.10	0.2	0.62	0.83	0.79	0.35	2.06
C*	2.82	3.00	2.70	2.80	2.88	3.02	2.66	0.47	0.68	0.08	0.89	1.10
H*	-0.13	-0.06	0.14	-0.01	-0.02	0.06	-0.09	0.18	0.97	0.25	0.94	0.67
Biceps femoris:												
pH	-5.81	5.84	5.85	5.81	5.86	5.83	5.84	0.15	0.01	0.86	0.04	0.09
L*	51.3	50.6	50.3	51.0	50.5	50.6	50.9	0.08	0.12	0.37	0.73	1.88
a*	-1.86	-1.97	-1.81	-1.84	-1.92	-2.01	-1.75	0.34	0.43	0.01	0.15	0.49
b*	0.19	-0.26	0.25	0.19	-0.06	0.03	0.09	0.10	0.24	0.79	0.91	1.14
C*	2.24	2.27	2.06	2.21	2.16	2.27	2.12	0.16	0.58	0.13	0.45	0.53
H*	-0.10	0.06	-0.12	-0.10	-0.07	-0.03	-0.07	0.30	0.34	0.69	0.92	0.54

Few significant differences were found and small in absolute value, with a substantial uniformity in the colour of the meat. Finally, gender affected slaughter weight, higher in females, while slaughter yield was higher in males due to the lower incidence of slaughtering losses, thus confirming the effect of gender on carcass traits, especially when rather praecox genetic types are used and animals are slaughtered above 2.5 kg (Lambertini *et al.*, 1990; Parigi Bini *et al.*, 1992).

GENERAL DISCUSSION

The present chapter presents a general discussion on the results obtained in the four experiments and focuses on the effects of the nutritional factors investigated (digestible fibre, starch and protein) in feeding of growing rabbits.

Role of digestible fibre and starch

Due to their nutritional role on digestive physiology, fibre and starch supply are usually considered together when discussing about rabbit nutrition and feeding. In most cases, when formulating diets, the concentration of the two nutrients, fibre and starch, are negatively correlated. Therefore, also the present discussion will try to join and consider the effect of both nutrients.

Regarding fibre, the present thesis focused on the supply of the different fibrous fractions, especially the most digestible ones (hemicelluloses, pectins), as well as the relationships with less digestible fibre (ADF) and starch content.

In all experiments, where increasing DF to ADF ratio at similar starch level were obtained by replacing alfalfa meal with beet pulp, the **digestibility** of dry matter, gross energy, crude fibre and fibrous fractions significantly increased. Apparent faecal digestibility of more digestible and soluble fibrous fractions (like those contained in beet pulp) may indeed reach 60-70%, while that of insoluble fractions (NDF), main constituent of alfalfa fibre, ranges from 15 to 30% (Carabaño *et al.*, 2001; García *et al.*, 2009).

Undoubtedly, the digestibility and nutritive value of the diets increased with DF/ADF ratio, not only for the higher presence of digestible fibre constituents, but also for the increased digestibility (9-10 points) of all fibre fractions, which may be ascribed to the lower grade of lignification and complexity of cell walls and to the higher susceptibility of structural carbohydrates to the action of digestive enzymes, animal or bacterial ones.

Differently, when DF replaced starch (increasing DF/starch ratio from 0.8 until 2.8) with limited changes in ADF level (DF/ADF ratio from 0.9 to 1.2) (Experiments 1, 2), dry matter and gross energy digestibility and, therefore, the diet nutritive value did not change, since energy supplied by DF was similar to that provided by starch in agreement with others (De Blas and Carabaño, 1996; Gidenne and Bellier, 2000). In fact, with the same inclusion rate of alfalfa meal, replacing barley meal with beet pulp (up to 30-31%) maintained diet digestibility at high level. The increasing ratio between DF and starch significantly decreased starch apparent digestibility, while greatly increased fibre and fibrous fractions digestibility, as already found by other authors (De Blas and Carabaño, 1996; Gidenne and Bellier, 2000; Xiccato *et al*., 2006a; Fragkiadakis *et al*., 2007). In the Experiments 3 and 4, the nutritive value of diets increased by increasing DF/starch ratio from 1.1-1.7 to 1.9-4.2. The contemporary increase in DF/ADF ratio (from 0.9-1.0 to 1.4-1.5) and the very low beet pulp inclusion rate in the diets with the lowest DF/starch ratio (even 0% in Experiment 3 towards 33-34% maximum) could account for this result. In the Experiment 4, the diets characterized by extreme values of pectins/starch ratio (5% pectin, starch 15%=0.5 and 10% pectin, starch 5%=2), showed very similar digestibility and nutritive values: with the same inclusion rate of alfalfa meal, replacing barley meal with dried beet pulp maintained diet digestibility at high level. The diet with the highest DF and starch content also showed the highest nutritive value.

Changes of **growth performance** are a direct consequence of the nutritive value of diets. Due to the chemiostatic regulation of appetite, increasing the DF/ADF ratio and, therefore, the nutritive value of diets, feed consumption decreased (Gidenne and Lebas, 2005; Xiccato and Trocino, 2010b), especially in the first period of the trial and feed conversion linearly improved, even without differences in final live weight and daily weight gain (Experiment 1). The improvement of feed conversion with increasing DF content may be related to a slower intestinal transit and the increase of digestibility and nutritive value of the diets, and has also been found in other studies (Xiccato *et al*., 2006a, 2008; Carraro *et al*., 2007; Fragkiadakis *et al*., 2007).

In Experiment 2, increasing the DF/starch ratio barely affected on growth performance. Also García *et al*. (1993) observed that diets based on beet pulp did not impair growth performance when substituting starch-raw materials because of their similar nutritive and energy value. In Experiment 3, the effect of DF/starch ratio was evident since the first weeks of the trial. During post-weaning, daily growth rate was significantly stimulated and feed intake reduced; as a consequence feed conversion improved with increasing DF/starch ratio. During the last weeks before slaughter, growth was generally similar among groups, but the effects on feed intake and conversion remained significant even when the whole experimental period was considered. Similarly, in Experiment 4, increasing dietary pectins from 5 to 10% (with DF/starch ratio raising from 2.4 to 3.1) significantly stimulated daily weight gain (especially during post-weaning) and increased final live weight, reduced feed intake and improved feed conversion. Increasing DF/starch ratio also reduced N excretion, without effecting body N retention especially during the first rather that the second period of growth (Experiment 3).

The positive effect of substituting starch by DF during the post-weaning period on growth rate may be explained by the limited capacity of young animals to use low-digestible fibre and their capability to utilize the high-digestible fibre (mainly pectins). The change in digestibility affected all fibrous fractions, including the less digestible ones (hemicelluloses and celluloses) and so compensated more than proportionally the decrease in starch content. This result was also found by others (Gutierrez *et al.,* 2002).

Increasing dietary **starch** increased final live weight and growth rate and reduced feed intake with a corresponding improvement in feed conversion (Experiment 4), this was evident especially in the second period of growth when the chemiostatic regulation of appetite is working better.

Equilibrium of **fermentations at caecum** is considered a key indicator of possible effects of nutrients and levels on rabbit digestive health. In Experiment 1, increasing DF/ADF ratio within the tested range (from 1.0 to 1.2) did not modify VFA production, even if caecal pH was significantly reduced. This variation, even though not associated with an increase in the total VFA production as found also by Carabaño *et al*. (1997), may be seen favourably in view of the maintenance of an equilibrium in the caecal bacterial population and fermentation.

Increasing DF and reducing starch may slow down intestinal transit and increase gut incidence, mainly due to a higher caecal repletion. As a consequence, fermentations were stimulated, total production of VFAs increased as well as the acetate proportion. Differently, valerate incidence usually associated with the activity of amylolitic bacteria, decreased as well as pH and ammonia concentration (Experiments 2 and 3). Increasing dietary pectins concentration reduced caecal pH, tended to increase total VFAs and reduced valerate proportion (Experiment 4). Therefore, the fermentation pattern varied according to DF increase and starch reduction and shows how increasing DF may sustain active and equilibrated caecal fermentations, oriented towards acetate production.

Previous studies have shown a favourable and significant increase of the caecal fermentative activity by increasing the dietary content of digestible and/or soluble fibre (García *et al.*, 2000; Falcao-e-Cunha *et al.,* 2004). The limited levels of caecal ammonia found in all experiments and the trend to a reduction with DF/starch ratio suggest that DF supplies suitable carbohydrates capable of favouring the ammonia fixation into bacterial protein.

In all experiments of the present thesis, great attention and an important part of work were devoted to the evaluation of the conditions of **intestinal mucosa** as a means to evaluate rabbit health status and/or susceptibility to illness. In fact, after weaning, the change from a milk based feeding to a solid food can negatively influence intestinal mucosa traits by reducing the villi height and crypt proliferation and, therefore, the animal absorbing capacity (Van Dijk *et al.,* 2002). In this way, the efficiency of intestinal barrier may be decreased, creating the conditions for the attack of pathogenic bacteria and the passage of toxins produced in the intestine. While in pigs a positive effect of the inclusion of soluble fibre in diets has been proven on the development of the intestinal mucosa at weaning (Vente-Spreeuwenberg *et al*., 1997), in rabbits this was not yet fully proven (Gallois *et al*., 2005; Gómez-Conde *et al.*, 2007; Xiccato *et al*., 2008). Previous studies showed that the increase in most soluble fibrous fractions (from 7 to 12%), rather than the decrease in NDF content (from 36-38 to 30-32%), may favour the integrity of intestinal mucosa, reduce mortality and improve growth performance in young rabbits (Gutiérrez *et al.*, 2002; Feugier *et al.*, 2006; Álvarez *et al.*, 2007; Gómez-Conde *et al.*, 2007).

In the present thesis, however, increasing DF/ADF ratio (Experiment 1), DF/starch ratio (Experiments 2, 3 and 4) and pectin concentration (Experiment 4) did not produce remarkable alterations on intestinal mucosa structure, both at ileum and jejunum level. Only in Experiment 4, the height of the villi at both ileum and jejunum tended to be lower (P=0.09 and 0.10) in rabbits fed diets containing levels of starch (about 10%) recommended for the post-weaning than in those fed diets containing lower (5%) or higher (15%) levels than suggested. The relative uniformity in caecal equilibrium and the structure of intestinal mucosa observed was accompanied by a relatively good health status in all trials performed. In fact, this good condition did not permit to assess correctly the effect of the dietary treatments on the health status in rabbits or on the possibility of controlling the diffusion and reducing the damage of ERE.

Increasing dietary DF/ADF ratio (Experiment 1), DF/starch ratio and pectins level (Experiments 3 and 4) did not significantly affect rabbit **health status**. Only in Experiment 2, even though mortality and morbidity were low, a significant effect of dietary treatment was detected: the lowest DF/starch ratio diets provoked acute ERE and increased mortality, though affected animals recovered faster, so the sanitary risk did not differ among the three groups. When analysing the health condition according to the experimental diet, the highest mortality and sanitary risk were associated to the diet with lowest DF/starch ratio and the highest protein level in comparison with other groups.

In the present thesis, the absence of severe digestive disorders could be partially explained by the older weaning age (33 d, on average) of the rabbits used in the present trial compared to the studies previously performed (25-30 d) and later mentioned, that should guarantee, according to the current opinion, a lower susceptibility to stress and digestive pathologies. Also the good sanitary conditions of the commercial farms from which rabbits were taken as well as those of the experimental facilities could have contributed to maintain the rabbits in good health conditions, whatever the diet. In less favourable health conditions of animals, previous studies showed a positive effect of replacing starch or protein with digestible fibre in iso-ADF diets (Perez *et al.,* 2000; Soler *et al.,* 2004) or increasing DF/ADF ratio (Gómez-Conde *et al.,* 2004; 2007; Xiccato *et al.,* 2006a; Carraro, 2006) on mortality and morbidity due to ERE and other digestive disorders.

In the four trials, the feeding treatment hardly affected **slaughter results** and **carcass characteristics** or **meat traits**, confirming the minor role of nutrition over carcass and meat quality, since the animals were fed *ad libitum* balanced diets and there were no relevant differences in the final body weight (Xiccato, 1999; Hernández and Gondret, 2006; Hernández, 2008; Xiccato and Trocino, 2010b). Only the administration of diets with different levels of fat, from different sources, could change appreciably the carcass and meat characteristics (Hernández, 2008).

Great changes in low-digestible fibre could have impaired dressing percentage due to a higher gut incidence (Parigi Bini *et al.,* 1994), but, as for growth performance, increasing DF/ADF ratio slightly affected slaughter and carcass traits (Experiment 1). A uniformity of carcasses was reasonably expected in rabbits fed diets with very different DF/starch ratio, but similar nutritive values, as in Experiments 2, 3 and 4, during which rabbits showed increasing gut incidence with increasing DF/starch ratio, but similar dressing percentages. The higher degree of gut filling and the slower intestinal transit of digesta associated with increased dietary pectins (from beet pulp) accounted for this result (Carabaño *et al*., 1997; Hernández and Gondret, 2006. In Experiment 1, in fact, the absence of a significant effect of dietary DF on dressing percentage may be ascribed to the presence of a discrete content in beet pulp (10%) also in the reference diets.

Within fibre level, the effect of dietary starch on slaughter results mainly depended on differences in live weight, as in Experiment 4: heavier rabbits, that received the high-starch diets, showed higher slaughter and carcass weights and dressing percentage, lower incidence of slaughter losses and greater incidence of dissectible fat. Differently, the effects of dietary treatments were always scarce on meat quality, in terms of pH, lightness, redness and yellowness measured on the *longissimus lumborum* and *biceps femoris* muscles 24 hours after slaughter.

Role of protein

Both sources and level of protein may modify protein efficiency of utilization, productive results as well as caecal fermentation equilibrium (Carabaño *et al*., 2008; 2009; Xiccato and Trocino, 2010a).

The results of Experiment 1 concern **protein sources** and outline the possibility of fully replacing soybean meal, traditionally used in rabbit feeding, with sunflower meal. Clearly, when fed the sunflower diets, rabbits showed lower crude protein digestibility, consistently with the lower CP digestibility in sunflower meal than in soybean meal (Maertens *et al.,* 2002). The pattern of caecal fermentations did not change; health status, growth performance and slaughter results were not affected. Other studies showed a small or null effect of protein source, at similar protein contents, even though significant decrease of mortality due to epizootic rabbit enteropathy was observed when increasing ileal protein digestibility (Gutiérrez *et al.,* 2003; Chamorro *et al.,* 2007). This result was attributed to a reduction of nitrogen flux at caecal level and a positive regulation of the intestinal bacterial population (Carabaño *et al.,* 2009). Gutiérrez *et al.* (2003) measured a higher ileal digestibility of protein and dry matter in diets based on sunflower meal (36% CP) rather than in diets based on soybean meal (48% CP).

Starting from data available in pigs (Vente Spreeuwenberg *et al*., 2004a, 2004b), a somewhat effect of protein source on intestinal mucosa of young rabbits could have be expected, which was not the case of the present trial. A positive effect on the mucosa integrity has been observed in early-weaned rabbits fed diets containing animal plasma rather than soybean (Gutiérrez *et al*., 2000), while negative effects of legumes anti-nutritional factors have been only hypothesized (Gutiérrez *et al*., 2003; Cano *et al*., 2004). On the other hand, no effects of the protein source have been previously measured over the intestinal mucosa structure of rabbits, even in the presence of variations in the protein ileal flux and the composition of caecal bacterial population (Chamorro *et al.,* 2007).

The increase of dietary **protein concentration** (from 15 to 16% in Experiments 2 and from 14 to 17% in Experiment 3) resulted in a higher nutrient and protein **digestibility** because of the partial replacement of alfalfa protein by soybean and sunflower protein. Moreover, the increase in barley and beet pulp maintained a constant digestible fibre level and decreased the less digestible fibrous fractions, thus reducing the feed transit and increasing the diet digestibility.

Reducing dietary protein below 15% impaired daily weight gain and live weight immediately after post-weaning (first three weeks after weaning). This negative effect was still evident at the end of the trial, when considering the whole growth, even if during the last weeks before slaughter dietary protein may be reduced until 14% without negative consequences on **performance** (Experiment 3). These results confirm previous observation showing that protein content in commercial diets for weaning and growth is higher than animal requirements (Maertens *et al.,* 1997; Trocino *et al.,* 2000, 2001; García-Palomares *et al.,* 2006a; Eiben *et al.,* 2008). Recommended protein level for growing rabbits is from 15 to 16% of CP, that corresponds to 10.5-11.0% DP, for a DP/DE from 10.5 to 11.0 g/MJ (De Blas and Mateos, 1998). In fact, it was observed that decreasing dietary protein from 17 to 14% substantially reduced (-30%) total N excretion (from 2.35 to 1.64 g N excreted per day), even with some negative consequences on N retention, especially in the first period of growth. According to Maertens *et al.* (1997), protein content in fattening rabbits may be reduced below 17%, once requirements are satisfied by amino acids supplementation, without negative consequences on performance, but with a reduction of N excretion; below 13.8% CP daily growth is rather impaired (-9%) but N excretion greatly decreases (-38%).

In rabbits slaughtered at 63 days of age and 2.35 kg of live weight, typical conditions of the Spanish market, the reduction in dietary protein from 16 to 14% did not influence growth performance (García-Palomares *et al.*, 2006b). In animals slaughtered later (75-90 d) and at heavier weight (2.5-3.0 kg), as requested by the Italian market, decreasing protein levels from the first to the second period permitted to better satisfy protein needs during the first growth phase and reduced **N excretion** during the second one. In fact, during the latter period, feed intake is higher and dietary protein may be reduced without negative consequences on performance and meat quality (Maertens *et al.*, 1997; Maertens and Luzi, 1998; Trocino *et al.*, 2000, 2001).

As discussed above, on the whole, **health status** was high in all trials and ERE did not appear. Therefore considerations on the effect of the dietary treatment on health status are rather hard to be presented. However, mortality and sanitary risk were found to increase in rabbits fed diets with high protein content and low DF/starch ratio (Experiment 2). In fact, a dietary protein excess could impair animal health, altering caecal equilibrium and promoting protein utilization for energy production, increasing N-ammonia and pH, so favouring the development of pathogen bacterial populations (Lebas *et al.*, 1998). In previous studies, mortality caused by ERE significantly decreased (Gutiérrez *et al*., 2003; Chamorro *et al*., 2007) by reducing protein level and increasing ileal protein digestibility, due to the maintenance of the equilibrium in the intestinal microbial population (Carabaño *et al*., 2009), as also happens by increasing digestible fibre (Xiccato *et al*., 2006a; Gómez-Conde *et al*., 2007). The effects of a low DF/starch ratio diet (see discussion above) may be negatively accentuated in case of high protein dietary supply.

One-point difference in protein between diets (from 15 to 16%) was not sufficient to significantly affect **caecal fermentative activity** (Experiment 2), while when dietary protein raised from 14 to 17%, caecal fermentation was stimulated (Experiment 3). The contemporary higher inclusion of barley and beet pulp, at constant DF content, may explain the more intense caecal fermentative activity in rabbits fed diets with higher protein content as well as the balanced caecal environment and fermentations, where the protein not digested at ileal level can stimulate rather than impair caecal fermentation.

Similarly to what reported above for the protein source, also the protein level played a minor role on the characteristics of **intestinal mucosa** (Experiment 2). The lower crypt depth found with increasing dietary protein may be associated with a lower capacity of the mucosa of repairing villi damages and therefore, indirectly, may explain the higher susceptibility to digestive disorders found in rabbits fed high-protein diets.

Finally, increasing dietary protein may affect **slaughter results** only when growth performance and final live weights are affected too, that is at the lowest protein concentrations (14-15%) (Experiments 2 and 3). Also for carcass and meat traits, the weak effect of dietary protein may be explained by the sufficiently high protein levels tested, which in diets supplemented for most limiting aa permitted to maximize growth performance and, therefore, to reach high standards for carcass and meat quality.

MAIN CONCLUSIONS AND IMPLICATIONS

The experimental activities performed in the present trial permit to draw some major conclusions in relation to the general objectives stated in the initial chapters, that is defining rabbit nutritional needs during post-weaning and fattening periods with special regards to the most digestible fibre fractions (pectins, hemicelluloses), the starch content in relation to fibre, as well as the dietary protein supply with different aims:

***1*) improving rabbits intestinal conditions**. Changes in main nutrients (DF, ADF, starch and protein) within the tested intervals may affect the intestinal condition of rabbits and caecum equilibrium: increasing **DF** at the expenses of **starch** or increasing **protein** in diets with appreciable DF supply stimulate caecal fermentation, limit ammonia production and influence caecal pH to a favourable extent for caecal bacterial population. Starch, DF and ADF levels did not affect the morphology and integrity of intestinal mucosa in rabbits after weaning and, therefore, the immune response of the animals and their susceptibility to digestive disorders. On the other hand, there is some evidence that high protein levels associated with low DF/starch supply may negatively influence intestinal mucosa functionality.

***2)* reducing the incidence and severity of digestive pathologies**. Despite the wide variations in the inclusion rate of the different raw materials (alfalfa meal, barley, soybean and sunflower meal, dried beet pulp) and the variations in the chemical composition of experimental diets, there were no effects on animal health. The non-occurrence of enteric pathologies could also be attributed to the chemical characteristics of experimental diets, consistent with current recommendations (ADF>17-18%), suitable to control the spread of digestive disorders. Therefore, we are not able to evaluate the effect and possible influence of the tested dietary treatments on the health conditions of a commercial farm stroked by ERE. However, there is evidence that low **DF/starch** ratio (≤1) increases mortality and reduces the number of ill rabbits that can recover and reach the end of the trial. This trend is accentuated if a low DF/starch ratio (<1) in the diet is associated to high dietary **protein** (>16%), so diets with high DF/starch ratio (1-1.5) and moderate protein level (about 15%) are recommended. By substituting starch with DF, high dietary energy level is maintained and combined with a low flux of undigested protein at caecal level; it may reduce the sanitary risk.

***3)* increasing feed efficiency and N retention**. The increase of dietary **DF** at the expenses of less digestible fibre fractions or of **starch** (at constant ADF level) improves feed conversion, by reducing feed intake, and allows an appreciable reduction of nitrogen excretion. This latter result is of great importance in view of improving the environmental sustainability of rabbit farms. Starch and digestible fibre may be considered alternative in rabbit feeding and, potentially, additive: the association of high starch and pectin level could results in even better results on feed conversion and profitability.

Given the increasing costs of raw materials used for animal feeding in the last years, up to 100% for some raw materials (e.g. soybean meal), the reduction of feeding costs and feed conversion and the improvement of slaughter results become primary targets for the containment of production costs.

4) **guaranteeing high performance and final product quality**. Increasing **DF/ADF** ratio undoubtedly improves diet nutritive value and their efficiency of utilization for growth. On the other hand, increasing **DF/starch** ratio does not modify the nutritive value and the energy utilization of diets and, in most cases, neither growth performance nor slaughter results. Growth rate may be rather stimulated in some cases, especially during the first weeks of growth when young rabbits show a high utilization of both DF and starch even if their digestive capacity is not yet fully efficient. The economic importance of this result rests upon the statement that farm feed efficiency may be optimized and the rabbit production costs reduced only by feeding high-energy diets rather than high-fibre regimes.

To maximize growth performance, dietary crude **protein** should not be reduced below 15.5% in the first weeks of growth since the subsequent compensatory growth that rabbits may show in the fattening period before slaughter does not permit to recover the weight loss. Therefore, 14-15% crude protein seems sufficient to satisfy the nutritional needs of growing rabbits, to increase N efficiency and limit its excretion even in diets based exclusively on sunflower protein, integrated with the required aa. Dietary protein has to be controlled both in the first and especially in the second period of growth, during which protein requirements are lower,.

Changes in DF, starch or protein produce significant differences in slaughter results only when capable of modifying slaughter live weight and, therefore, all carcass traits directly correlated to live weight. Meat traits, pH and colour, of main muscles never change with the dietary treatment to an extent which may be appreciable at markets or consumers.

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