UNIVERSIDAD POLITÉCNICA DE MADRID

ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRONÓMICA,
ALIMENTARIA Y DE BIOSISTEMAS
DEPARTAMENTO DE PRODUCCIÓN AGRARIA

**NUTRITIONAL STRATEGIES TO
IMPROVE CARCASS YIELD AND
CARCASS COMPOSITION IN
FATTENING RABBITS**

PhD THESIS

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Agricultural Engineer

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ABBREVIATIONS

Abbreviation / Abreviatura	ENGLISH	ESPAÑOL
A	Arbocel®	Arbocel®
ADF	Acid detergent fibre	Fibra ácido detergente
ADFI	Average daily feed intake	Consumo medio diario
ADG	Average daily gain	Ganancia media diaria
ADL	Acid detergent lignin	Lignina ácido detergente
AIC	Akaike information criterion	Criterio de información de Akaike
AJDU	Apple juice depectinising Activity	Actividad pectinasa en zumo de manzana
AL	<i>Ad libitum</i>	<i>Ad libitum</i>
ATTD	Apparent total tract digestibility	Digestibilidad total aparente
B	Basal	Basal
BIA	Bioelectrical impedance technique	Técnica de impedancia bioeléctrica
BIC	Bayesian information criterion	Criterio de información bayesiano
BGU	B-glucanase Unit	Unidad β -glucanasa
BW	Body weight	Peso vivo
BWS	Body weight before slaughter	Peso vivo al sacrificio
CP	Crude protein	Proteína bruta
CCW	Cold carcass weight	Peso canal fría
CMC	Carboxymethyl cellulose	Carboximetilcelulosa
d	Day / days	Día / días
D2O	Deuterium oxide	Óxido de deuterio
DE	Digestible energy	Energía digestible
DEi	Digestible energy intake	Consumo de energía digestible
DM	Dry matter	Materia seca
DN	Digestible nitrogen	Nitrógeno digestible
DNi	Digestible nitrogen intake	Consumo de nitrógeno digestible
DoP	Dressing out percentage	Rendimiento a la canal
DP	Digestible protein	Proteína digestible
DWG	Daily weight gain	Ganancia media diaria

Abbreviation / Abreviatura	ENGLISH	ESPAÑOL
EE	Ether extract	Extracto etéreo
ELW	Empty live weight	Peso vivo vacío
ER	Energy retained	Energía retenida
ERE	Epizootic rabbit enteropathy	Enteropatía epizootica del conejo
Exp.	Experiment	Experimento
FAU	Fungal alpha-amylase unit	Unidad alfa-amilasa fúngica
FCR	Feed conversion ratio	Índice de conversión
FI	Feed intake	Consumo de pienso
G	Defatted grape seed meal	Granilla de uva
GE	Gross energy	Energía bruta
GEi	Gross energy intake	Consumo de energía bruta
GLM	General linear model	Modelo lineal general
HCW	Hot carcass weight	Peso canal caliente
HUT	Haemoglobin unit tyrosine base	Unidades de hemoglobina en base de tirosina
kg	Kilogram	Kilogramo
kHz	Kilohertz	Kilohercios
kJ	Kilo Joules	Kilojulios
LW	Live weight	Peso vivo
MJ	Mega Joules	Megajulios
MRI	Magnetic resonance imaging	Imagen por resonancia magnética
N	Nitrogen	Nitrógeno
Ni	Nitrogen intake	Consumo de nitrógeno
NDF	Neutral detergent fibre	Fibra neutro detergente
NIRS	Near-infrared spectroscopy	Espectroscopía del infrarrojo cercano
NR	Nitrogen retained	Nitrógeno retenido
OM	Organic matter	Materia orgánica
<i>P</i>	Probability	Probabilidad
<i>ppm</i>	Parts per million	Partes por millón

Abbreviation / Abreviatura	ENGLISH	ESPAÑOL
PRC	Poultry Research Centre	Centro de investigación de avicultura
R&D	Research & Development	Investigación y desarrollo
SD	Standard deviation	Desviación estándar
SEM	Standard error of the mean	Error estándar de la media
SLW	Slaughter live weight	Peso vivo al sacrificio
TOBEC	Total Body Electrical Conductivity	Conductividad eléctrica total
UPV	Polytechnical University of Valencia	Universidad Politécnica de Valencia
VFA	Volatile Fatty acids	Ácidos grasos volátiles
XU	Xylanase unit	Unidad xilanasa

DISCLAIMER

Some results of this PhD Thesis are already published:

- Experiment 4: Crespo, R., Alfonso, C., Saiz del Barrio, A., García Ruiz, A.I., Marco, M. and Nicodemus, N. (2020). Effect of feed restriction on performance, carcass yield and nitrogen and energy balance in growing rabbits. *Livest. Sci.*, 241: 104278.

SUMMARY

SUMMARY

The main objective of this PhD was to study the effect of different nutritional strategies on carcass traits. Secondary, it was also researched the effect of those strategies on growth performance, carcass chemical composition and nitrogen and energy balance in growing rabbits. For this purpose, four experiments were conducted. In experiment 1 was studied the effect of supplementation of a multi-enzyme complex at different doses on a standard diet on performance parameters, carcass traits, nutrient digestibility and nitrogen and energy balance in growing rabbits. In the experiment 2, same multi-enzyme complex than in experiment 1 was used, but two different diets were formulated, with different DP, DE and NDF content. Experiment 3 studied the effect of three levels and two sources of lignin. And, in the last one, experiment 4, feeding restriction on growth performance, carcass traits, nutrient digestibility and nitrogen and energy balance in growing rabbits was studied. All the feeds supplied to the rabbits were non-medicated.

In experiment 1, the purpose was to compare the inclusion of a fungal multi-enzyme complex (activity of 100 XU/g pentosanase, 40 CMC/g cellulase, 30 FAU/g amylase, 100 XU/g xylanase, 700 HUT/g protease, 4000 AJDU/g pectinase and 200 BGU/g β -glucanase) in the feed at different doses (0, 100, 200, 400 and 800 ppm), on productive parameters, carcass traits and nitrogen and energy balance in growing rabbits. A total of 225 rabbits, weaned at 34 d of age, were used. They were housed in individual cages and randomly assigned to five treatments. A basal feed was formulated (CP: 17.6%; starch: 17.4%, NDF: 35.9%, DE: 2797 kcal/DM kg) and supplemented with different doses of the enzyme complex. Feed intake and animal weight (from 34 to 48 d of age and from 49 to 60 d) were controlled. In 11 rabbits per treatment, body composition was estimated by Bioelectrical Impedance technique (BIA) at 34 and 60 d of age to estimate the nitrogen balance. At the end of the trial, 24 rabbits per treatment were slaughtered and the carcass,

the entire digestive tract, the full and empty stomach, and the full caecum were weighted. Between days 53 and 57 of life, a digestibility trial was done (for DM, OM, CP, EE, NDF, GE; n=11), except for the 100-ppm dose. During the first period (34-48 d) enzyme supplementation linearly improved the feed conversion ratio (FCR) ($P<0.05$), without affecting feed intake (DFI) or growth (DWG). In the second period (49-60 d), both FCR and DWG varied quadratically ($P<0.05$) with enzyme supplementation, being the optimum level for both: 400 ppm/kg. The digestibility of DM, OM, EE and GE decreased linearly ($P<0.05$) and the weight of the digestive tract (% BW) increased linearly ($P<0.05$) with the increase of enzyme complex dose, without affecting the carcass yield, which was 57.8%, on average. Enzymes had no effect on nitrogen and energy balance, except on energy losses in faeces ($P<0.05$) which increased with the dose due to the worse GE digestibility. Carcass composition was neither affected ($P>0.05$) by the level of the enzyme complex supplementation.

In experiment 2, the objective was to compare the effect of supplementation with the same multi-enzyme complex in two feeds with different levels of digestible energy (DE), digestible protein (DP) and NDF on growth performance, carcass characteristics and nitrogen and energy balance in growing rabbits. Three doses (0, 100 and 200 ppm) of the same enzyme complex were added to two different basal diets (Diet A: CP: 17.6% DM; starch: 17.4% DM; NDF 35.9% DM, DE: 2797 kcal/kg DM and Diet B: CP: 15.8% DM, starch: 17.5% DM, NDF: 37.4% DM, DE: 2585 kcal/kg DM). Therefore, a total of 270 rabbits were weaned at 34 d and housed in individual cages. The parameters measured were the same as in the previous experiment. The digestibility trial (between days 53 and 57 of age) was performed on both diets (A and B) but only to the 0 and 200 ppm doses. The addition of enzymes did not affect the digestibility, or any other productive parameters studied in the growing period. Diet B decreased ($P<0.01$) the digestibility of

DM, OM, NDF and GE *vs.* diet A (5.98, 5.66, 4.91 and 22.59%, respectively). Digestible nitrogen (DNi) and DE intake were lower for diet B than diet A (10% and 7% respectively, $P<0.01$). The lower DN intake of animals fed with diet B led to a lower DN in carcass (4%; $P<0.05$) and a reduction of the nitrogen excreted in urine (19.4%; $P<0.05$) and in faeces (6.3%; $P<0.05$). Digestible energy intake of diet B was lower ($P<0.05$) compared with diet A (0.998 *vs.* 0.947 MJ/kg BW^{0.75}.d. Accordingly, animals of diet B had lower energy retention efficacy, both in whole body (0.389 *vs.* 0.360 kJ/kg BW^{0.75}.d) and in carcass (0.231 *vs.* 0.211 kJ/kg BW^{0.75}.d). Due to the lower energy ingestion and efficacy, energy excreted in faeces was higher (0.660 *vs.* 0.705 MJ/kg BW^{0.75}.d; $P<0.05$) in animals of diet B, whereas energy excreted in skin and viscera was higher in diet A (0.157 *vs.* 0.148 MJ/kg BW^{0.75}.d). The lower retention of N and energy of diet B, resulted in a lower weight at 48 d of age (1650 g *vs.* 1622 g, $P<0.05$), due to a lower DWG in the first period (58.2 *vs.* 60.2; $P<0.05$), although second fattening period and final weight did not differ significantly. Feed intake during the whole growing period was 2.92% higher for diet B, so FCR increased (2.52 *vs.* 2.63, $P<0.05$). The type of diet did not affect carcass yield, although the rabbits of diet B had heavier digestive tract and full and empty stomach ($P<0.05$). Carcass chemical composition of animals fed diet A showed a higher EE and energy content ($P<0.05$), whereas diet B had higher water, CP, and ash content ($P<0.05$).

In the third trial, the aim was to study the effect of including different levels of acid detergent lignin (ADL) (Low (L): 5% DM, Medium (M): 6% DM and High (H): 7% DM) and different sources of lignin (Arbocel® (A) *vs.* defatted grape seed meal (G)) on performance, carcass characteristics and nitrogen and energy balance in growing rabbits. A total of 936 rabbits were used, 216 were weaned at 34 d of age and housed in individual cages, and the remaining 720 were weaned at 35 d of age and housed in collective cages (5 rabbits/cage). Five different feeds were formulated: one control diet for both sources

(AL and GL: DE: 2939 kcal/kg DM, CP: 18.4% DM, NDF: 36.4% DM; ADF: 21.3% DM; ADL: 4.78% DM; Starch: 15.5% DM), two diets with an average level for each source (AM (DE: 2825 kcal/kg DM, CP: 17.7% DM, NDF: 37.8% DM; ADF: 23.8% DM; ADL: 5.87% DM; Starch: 15.1% DM) and GM (DE: 2909 kcal/kg DM, CP: 17.9% DM, NDF: 36.2% DM; ADF: 21.8% DM; ADL: 6.03% DM; Starch: 15.6% DM)) and other two diets with a high level for each source AH (DE: 2848 kcal/kg DM; CP: 18.1% DM, NDF: 37.7% DM; ADF: 23.8% DM; ADL: 6.23% DM; Starch: 14.5% DM) and GH (DE: 2908 kcal/kg DM, CP: 17.6% DM, NDF: 35.4% DM; ADF: 22.0% DM; ADL: 6.72% DM; Starch: 15.9% DM)). In rabbits housed in collective cages, weight and intake were measured at the beginning and at the end of the growing period (35 and 61 d of age), feed conversion ratio (FCR) was calculated for that period, and mortality was recorded daily. For rabbits housed in individual cages average daily intake and weight were controlled in two periods from 34-48 d, and 49-60 d of life, as well as the FCR and daily mortality during growing period. In 11 rabbits per treatment, body composition was estimated by Bioelectrical Impedance technique at 34 and 60 d of age to estimate the nitrogen and energy balance. At the end of the trial, 24 rabbits per treatment were slaughtered and the carcass weight, the entire digestive tract, the full and the empty stomach and full caecum were measured. Between days 53 and 57 of life a digestibility trial was done (DM, OM, CP, EE, NDF and GE, n=11). For rabbits in collective cages, the highest level of lignin affected the FCR compared with the other two levels (2.65 on average vs. 2.69 g/g, $P<0.05$) without affecting daily feed intake (139 g/d) or growth (52.2 g/d). The digestive tract weight (%BW) decreased with medium and high levels of lignin, compared with the control one (17.4 on average vs. 17.9%; $P=0.05$) but carcass yield was not affected (58.2% on average). The apparent feed digestibility of DM, OM and GE was higher with the lowest level of ADL compared to the average of the other two: DM (58.3

vs. 56.8%), OM (60.2 vs. 58.8%), and GE (60.2 vs. 58.9%). Apparent digestibility of CP for the medium level was lower than the low level (70.1 vs. 72.6%, $P=0.05$) while the diet with the highest level of ADL showed an intermediate value (71.0%). EE digestibility increased with level inclusion of ADL (84.7, 85.8, and 88.0%, respectively). The animals fed high levels of ADL consumed less DN than low and medium levels (1.901 vs. 1.992 g NDi/kg $BW^{0.75}$.d, on average, $P<0.05$), and tended to improve the retention efficiency (0.348 vs. 0.338%, on average, $P=0.07$), so the N retained in all lignin levels was similar (0.669 g NR carcass/kg $BW^{0.75}$.d). N excretion in urine was lower for M and H lignin levels (average of 0.894 vs. 1.003 g N/kg $BW^{0.75}$.d). Otherwise the energy losses in faeces increased in these two diets (+5%) compared with the low level of ADL. Arbocel® increased FCR during the growing period in individual cages (2.56 vs. 2.52 g/g, $P<0.05$), as well as in group cages (+1.42%, $P<0.05$). The Arbocel® OM digestibility was worse than defatted grape seed meal (56.9 vs. 57.7%, $P<0.05$). The carcass chemical composition of the animals was not affected neither by the source nor by the level of feed ADL.

The aim of the experiment 4 was to study the effect of different feeding restriction strategies in growing rabbits. A total of 600 rabbits were weaned at 34 d, caged in groups (5 animals/cage; 24 cages/treatment) and randomly assigned to the 5 treatments. Control group (AL) was fed *ad libitum* and the others four were restricted 80 and 70% of theoretical *ad libitum* intake (R80 and R70, respectively), during the whole fattening period or until 48 d (R80AL and R70AL, respectively). Only one diet was formulated (CP: 159 g/kg DM; NDF: 324 g/kg DM; DE: 2689 kcal/kg DM). At the end of the fattening period, 24 rabbits per treatment were slaughtered and the weight of carcasses and digestive organs were measured. A digestibility trial was done in individual metabolic cages between 53 and 57 d (12 rabbits/treatment). Feed restriction impaired ($P<0.001$)

the feed conversion ratio (1.93 vs. 1.82 g/g, on average) and daily weight gain (42.4 vs. 60.3 g, on average) from 34 to 48 d. However, from 48 to 61 d there was a compensatory growth in rabbits fed *ad libitum* (R80AL and R70AL) once they finished the restriction period (61.1 vs. 46.4 g/d, on average). On the whole fattening period, the compensatory growth led to a better feed conversion ratio (2.25 vs. 2.33 g/g; $P<0.001$), and rabbits fed R80AL finished the fattening period with a similar final body weight than those fed the control diet. Internal organs increased ($P<0.05$) their size, when rabbits were fed *ad libitum* after the restriction period, especially in those restricted at 70% in the first phase. Average mortality was 12.8% and it was not affected by treatments. Protein digestibility in treatments R80 and R70 tended to be higher than those restricted until 48 d. The digestibility of the other constituents was not affected by any of the restriction strategies. The nitrogen and ashes concentration in carcass increased, whereas fat and energy content decreased in whole fattening period of restricted groups ($P<0.001$). Efficacy of nitrogen retention tended to increase ($P=0.074$) and the nitrogen and energy excretion through the faeces, and urine+heat losses decreased ($P<0.001$) in restricted animals.

From the results of these 4 experiments, it can be concluded that in growing rabbits the supplementation with this multi-enzyme complex did not improve digestibility, nitrogen balance or reduce the weight of the digestive tract, and had no effect on the carcass yield. The use of enzymes improved the feed conversion ratio in the first period, so its use would be interesting in animals after weaning. Under the conditions of this study, the addition of enzymes in a lower nutritional value feed, neither improved the feed efficiency, the N balance, nor the carcass yield. Including ADL levels above 5% in isoenergetic diets decreased gastrointestinal weight, although this effect was not reflected in carcass yield. Arbocel® got worse FCR without affecting the growth. Restriction until

48 d could be a useful tool to reduce the environmental impact of the farms without an impairment of growth performance and carcass yield in the whole fattening period.

In non-enteropathy conditions, none of the strategies studied had an effect on mortality.

RESUMEN

RESUMEN

La presente Tesis Doctoral se compone de cuatro experimentos cuyo objetivo es el estudio de los efectos de diferentes estrategias nutricionales sobre, principalmente, el rendimiento y las características de la canal. Y, secundariamente, estudiar cómo afectan dichas estrategias a la composición química de las canales y, al balance nitrogenado y energético de conejos en crecimiento. En el experimento 1 se estudió el efecto de la suplementación con un complejo enzimático a diferentes dosis sobre los parámetros productivos, el rendimiento y las características de la canal, la digestibilidad de los nutrientes y el balance del nitrógeno y la energía. En el experimento 2 se utilizó el mismo complejo enzimático que en el experimento 1 pero, en dos piensos diferentes, que variaban en su contenido en ED, PD y FND. El experimento 3 consistió en estudiar el efecto de tres niveles y dos fuentes de LAD. Y, por último, en el experimento 4, se estudió el efecto de diferentes niveles de restricción alimentaria sobre los parámetros productivos, el rendimiento y las características de la canal, la digestibilidad de los nutrientes y el balance del nitrógeno y energía en conejos de cebo. Ninguno de los piensos que se suministraron a los animales estaba medicado.

En el experimento 1, el objetivo fue comparar la inclusión de un complejo enzimático de origen fúngico (actividad de 100 XU/g pentosanasa, 40 CMC/g de celulasa, 30 FAU/g amilasa, 100 XU/g de xilanasa, 700 HUT/g de proteasa, 4000 AJDU/g de pectinasa y 200 BGU/g de β -glucanasa) en el pienso de cebo a distintas dosis (0, 100, 200, 400 y 800 ppm), sobre los parámetros productivos, las características de la canal y el balance nitrogenado y energético de los gazapos en crecimiento. Se utilizaron 225 gazapos destetados a los 34 d de edad, alojados en jaulas individuales y asignados aleatoriamente en cinco tratamientos. Se formuló un pienso basal (PB: 17,6% MS; Almidón: 17,4% MS; FND: 35,9% MS; EB: 4.319 kcal/kg MS), al que se suplementó

con las distintas dosis del complejo enzimático. Se controló el consumo y el peso de los animales desde los 34 a los 48 d de edad y desde los 49 a los 60 d. En 11 gazapos por tratamiento, se estimó la composición corporal mediante la técnica de Impedancia Bioeléctrica a los 34 y 60 d de edad para estimar el balance de nitrógeno y de energía. Al final del cebo, 24 gazapos por tratamiento fueron sacrificados y se midió el peso de la canal, de todo el tracto digestivo, del estómago lleno, del estómago vacío y del ciego lleno. Entre los días 53 y 57 de vida, se realizó una prueba de digestibilidad (MS, MO, PB, EE, FND, EB; n=11), excepto para la dosis de 100 ppm. En el primer periodo del cebo (34-48 d), la suplementación enzimática mejoró linealmente ($P<0,05$) el índice de conversión (IC), sin afectar el consumo (CMD) o el crecimiento (GMD). En el segundo periodo (49-60 d), tanto el índice de conversión (IC) como la GMD variaron cuadráticamente ($P<0,05$) con la suplementación enzimática, siendo el nivel óptimo para ambos: 400 ppm/kg. La digestibilidad de la MS, MO, EB y EE empeoraron linealmente ($P<0,05$) y el peso del aparato digestivo (%PV) aumentó linealmente ($P<0,05$) al incrementar la dosis de inclusión del complejo enzimático, sin afectar al rendimiento a la canal, que fue de media 57,8%. Las enzimas no tuvieron efecto sobre el balance de nitrógeno ni de energía, excepto para la excreción de energía en heces ($P<0,05$) que incrementó con la dosis, por la peor digestibilidad de la EB. Ni la composición corporal de los gazapos in vivo a los 60 d de edad, ni la composición de las canales se vieron afectadas ($P>0,05$) por el nivel de suplementación del complejo enzimático. En el experimento 2: el objetivo fue comparar el efecto de la suplementación del complejo enzimático en dos piensos con distintos niveles de energía digestible (ED), de proteína digestible (PD) y de FND sobre los rendimientos productivos, las características de la canal y el balance del nitrógeno y energético de gazapos en crecimiento. Para ello, se utilizó el mismo complejo enzimático a tres dosis (0, 100 y 200 ppm) y se formularon dos

piensos basales distintos (Pienso A: PB: 17,6% MS; almidón: 17,4% MS; FND: 35,9% MS; ED: 2.797 kcal/kg MS y Pienso B: PB: 15,8% MS; almidón: 17,5% MS; FND: 37,4% MS; ED: 2.585 kcal/kg MS). Se utilizaron un total de 270 gazapos destetados a 34 d, que fueron alojados en jaulas individuales. Se controlaron los mismos parámetros que en la prueba anterior. La prueba de digestibilidad (entre los días 53 y 57 de vida), se realizó en los dos piensos (A y B) pero sólo para las dosis 0 y 200 ppm. La adición de enzimas no afectó a la digestibilidad ni a ninguno de los parámetros productivos estudiados durante el cebo. El pienso B empeoró ($P<0,01$) la digestibilidad de la MS, MO, FND y EB con respecto al pienso A (un 5,98; 5,66; 4,91 y 22,59%, respectivamente). El consumo de nitrógeno digestible (NDi) y ED fueron menores para el pienso B que para el A (un 10% y 7%, respectivamente; $P<0,01$). Este menor consumo de ND en los animales del pienso B, dio lugar a que, a pesar de que presentaron una mayor eficacia de retención del ND con respecto a los del pienso A (43 vs. 40%; $P<0,05$), la retención de ND en la canal fuera un 4% menor ($P<0,05$) y la excreción de nitrógeno en la orina ($P<0,05$) y en las heces ($P<0,05$) fueran también un 19,4 y un 6,3%, respectivamente, más bajas. El consumo de ED (0,998 vs. 0,947 MJ/kg $PV^{0.75}$.d; $P<0,05$), la eficiencia de retención de la energía, tanto corporal (0,389 vs. 0,360 kJ/kg $PV^{0.75}$.d), como en la canal (0,231 vs. 0,211 kJ/kg $PV^{0.75}$.d) fueron menores en el pienso B que en el A. debido a la menor ingestión de energía y a la menor eficiencia de retención, la energía en heces fue mayor (0,660 vs. 0,705 MJ/kg $PV^{0.75}$.d; $P<0,05$) en los animales que ingirieron el pienso B, mientras que la energía excretada en piel y vísceras fue mayor en el pienso A (0,157 vs. 0,148 MJ/kg $PV^{0.75}$.d). La menor retención de N y de EB del pienso B, dio lugar a un menor peso a 48 d de edad de los gazapos (1650 g vs. 1622 g; $P<0,05$), debido a una menor GMD en el primer periodo de cebo (58,2 vs. 60,2; $P<0,05$), aunque en el segundo periodo de cebo y en el peso final no hubo diferencias significativas. El consumo de pienso durante el cebo

fue un 2,92% mayor en el pienso B, por lo que el IC empeoró (2,52 vs. 2,63; $P<0,05$). El tipo de pienso no afectó al rendimiento canal, aunque los gazapos del pienso B tuvieron mayor peso del aparato digestivo y del estómago lleno y vacío ($P<0,05$). La composición química de la canal se vio afectada por el tipo de pienso ($P<0,05$). El pienso A presentó un mayor contenido de EE y de energía ($P<0,05$), mientras que el pienso B tuvo mayor contenido en agua, PB y cenizas ($P<0,05$).

En el tercer ensayo el objetivo fue estudiar el efecto de la inclusión de distintos niveles de lignina ácido detergente (LAD) (Bajo (B): 5% MS; Medio (M): 6% MS y Alto (A): 7% MS) y de distintas fuentes de lignina (Arbocel® (A) vs. granilla desengrasada de uva (G)) sobre los parámetros productivos, las características de la canal y el balance de nitrógeno y energético de gazapos en crecimiento. Para ello se utilizaron 936 gazapos, 216 fueron destetados a los 34 d de edad y alojados en jaulas individuales, y los 720 restantes se destetaron a los 35 d de edad, y se alojaron en jaulas polivalentes (5 gazapos/jaula). Se formularon cinco piensos distintos: un pienso control para ambas fuentes (AB y GB: ED: 2939 kcal/kg MS; PB: 18,4% MS; FND: 36,4% MS; FAD: 21,3% MS; LAD: 4,78% MS; Almidón: 15,5% MS), dos piensos con un nivel medio para cada fuente (AM (ED: 2825 kcal/kg MS; PB: 17,7% MS; FND: 37,8% MS; FAD: 23,8% MS; LAD: 5,87% MS; Almidón: 15,1% MS) y GM (ED: 2909 kcal/kg MS; PB: 17,9% MS; FND: 36,2% MS; FAD: 21,8% MS; LAD: 6,03% MS; Almidón: 15,6% MS)) y otros dos piensos con un nivel alto para cada fuente (AA (ED: 2848 kcal/kg MS; PB: 18,1% MS; FND: 37,7% MS; FAD: 23,8% MS; LAD: 6,23% MS; Almidón: 14,5% MS) y GA (ED: 2908 kcal/kg MS; PB: 17,6% MS; FND: 35,4% MS; FAD: 22,0% MS; LAD: 6,72% MS; Almidón: 15,9% MS)). En el cebo colectivo, se controló el peso y el consumo de cada jaula al inicio y al final del cebo (35 y 61 d de edad), y el índice de conversión y la mortalidad diaria. En el cebo individual se controló el consumo medio diario y el peso en

dos periodos, de 34 a 48 d y de 49 a 60 d de vida, así como el índice de conversión y la mortalidad diaria durante el cebo. En 11 gazapos por tratamiento, se estimó la composición corporal mediante la técnica de Impedancia Bioeléctrica a los 34 y 60 d de edad para estimar el balance de nitrógeno y de energía. Al final del cebo, 24 gazapos por tratamiento fueron sacrificados y se midió el peso de la canal, de todo el tracto digestivo, del estómago lleno, del estómago vacío y del ciego lleno. Entre los días 53 y 57 de vida, se realizó una prueba de digestibilidad (MS, MO, PB, EE, FND y EB; n=11). En el cebo colectivo el nivel más alto de lignina afectó al IC, respecto a los otros dos niveles (2,65 de media vs. 2,69 g/g, $P<0,05$), sin que variase la ingesta (139 g/d) ni el crecimiento (52,2 g/d). En el cebo individual el peso del aparato digestivo (%PV) disminuyó con los niveles medio y alto de lignina, con respecto al control (17,4 de media vs. 17,9%; $P=0,05$) sin afectar al rendimiento a la canal (58,2 % de media). La digestibilidad aparente de la MS, MO y EB del pienso con el nivel más bajo de LAD fue mayor con respecto a la media de los otros dos: MS (58,3 vs. 56,8%), MO (60,2 vs 58,8%), EB (60,2 vs. 58,9%). La digestibilidad aparente de la PB del nivel medio fue menor que la del nivel más bajo (70,1 vs. 72,6%; $P = 0,05$) y el pienso con el nivel alto de LAD presentó un valor intermedio (71,0%). La digestibilidad del EE aumentó con el nivel de inclusión de LAD (84,7; 85,8; 88,0%, respectivamente). Los animales del nivel alto de LAD, consumieron menos ND que los de los niveles medio y bajo (1,901 vs. 1,992 g NDi/kg PV^{0,75}.d, de media; $P<0,05$), y tendieron a mejorar la eficiencia de retención (0,348 vs. 0,338%, de media; $P<0,05$), por lo que el N retenido en todos los niveles de LAD fue similar (0,669 g N/kg PV^{0,75}.d). La excreción de N en orina fue menor para los dos niveles más altos de lignina (0.894 de media vs. 1,003 g N/ kg PV^{0,75}.d). Por el contrario, la excreción de energía en heces en estos dos piensos aumentó (+5%), con respecto al nivel de LAD más bajo. La inclusión de Arbocel® como fuente de LAD empeoró el IC en el cebo individual (2,56 vs. 2,52 g/g;

P<0,05), así como en el colectivo (+1,42%, P<0,05). El Arbocel® empeoró la digestibilidad de MO, respecto a la granilla desengrasada de uva (56,9 vs. 57,7%; P<0,05). La composición química de la canal no se vio afectada ni por la fuente ni por el nivel de LAD del pienso.

El objetivo en el experimento 4 fue estudiar el efecto de diferentes estrategias de restricción en los conejos durante el cebo. Se utilizaron un total de 600 gazapos, destetados a 34 d, alojados en grupos (5 animales por jaula; 24 jaulas/tratamiento) y asignados aleatoriamente en uno de los cinco tratamientos: grupo control (AL), consumo *ad libitum*, y los otros cuatro tratamientos que fueron restringidos un 80 y 70% del consumo teórico *ad libitum* (R80 y R70, respectivamente), durante todo el cebo o solo hasta los 48 d de vida (R80AL y R70AL, respectivamente). Se formuló un solo pienso (PB: 159 g/kg MS; FND: 324 g/kg MS; ED: 2.689 kcal/kg MS). Al final del cebo, se sacrificaron 24 conejos por tratamiento y se midieron los pesos de la canal y de los órganos digestivos. La prueba de digestibilidad se realizó en jaulas metabólicas individuales entre los 53 y 57 d de vida (12 gazapos/tratamiento). La restricción alimentaria empeoró ($P < 0,001$) el índice de conversión (1,93 vs. 1,82 g/g, de media) y la ganancia media diaria (42,4 vs. 60,3 g, de media) de 34 a 48 d de vida. Sin embargo, de 48 a 61 d de edad hubo un crecimiento compensatorio en los gazapos realimentados a *ad libitum* (R80AL y R70AL) cuando terminó el periodo de restricción (61,1 vs. 46,4 g/d, de media). Durante todo el cebo, el crecimiento compensatorio condujo a una mejora del índice de conversión (2,25 vs. 2,33 g/g; $P < 0,001$), y los conejos del tratamiento R80AL terminaron el cebo con un peso vivo similar que el grupo control. Los órganos internos aumentaron ($P < 0,05$) su tamaño cuando los gazapos se realimentaron *ad libitum*, sobre todo en aquellos restringidos al 70% durante la primera fase. La mortalidad media fue del 12,8% y, los diferentes tratamientos no tuvieron ningún efecto. La digestibilidad de la

proteína en los tratamientos R80 y R70 tendió a aumentar en los animales restringidos hasta los 48 d. La digestibilidad del resto de nutrientes no se vio afectada por ninguna de las estrategias de restricción. La concentración de nitrógeno y de cenizas en la canal aumentó, mientras que el contenido en energía y grasa disminuyó en todo el cebo en los grupos restringidos ($P<0.001$). La eficacia de retención del nitrógeno tendió a aumentar ($P=0.074$) y la excreción de nitrógeno en heces y las pérdidas por orina + calor disminuyeron ($P<0.001$) en los animales restringidos.

De los resultados de estos cuatro ensayos se puede concluir que el suministro del complejo enzimático en los piensos de gazapos en crecimiento no mejoró la digestibilidad, la excreción de nitrógeno al medio, ni redujo el peso del aparato digestivo, aunque no tuvo un efecto negativo sobre el rendimiento de la canal. La utilización de enzimas mejoró el índice de conversión en la primera etapa del cebo, por lo que su uso sería recomendable en animales jóvenes tras el destete. En las condiciones de este estudio, la adición de enzimas a un pienso menos digestible tampoco mejoró la eficacia de utilización de los nutrientes, ni tuvieron efecto sobre rendimiento de la canal. La inclusión de niveles de LAD por encima de un 5% en piensos isoenergéticos, disminuyó el peso del aparato digestivo, aunque este efecto no se vio reflejado en una mejora del rendimiento de la canal. El Arbocel® empeoró el IC sin afectar al crecimiento. La restricción hasta los 48 d podría ser una herramienta útil para reducir el impacto ambiental de las granjas, sin perjudicar los rendimientos productivos ni el rendimiento de la canal en todo el periodo de cebo.

En condiciones donde no hay incidencia de enteropatía ninguna de las estrategias estudiadas tuvo efecto sobre la mortalidad.

INTRODUCTION

I. INTRODUCTION

The goal of every meat production system is to get a carcass and final product of quality with the final purpose to optimize not only the zootechnical parameters, but also the economic benefit. Meat quality refers to its nutritional, sensory, technological, and sanitary characteristics (Dalle Zotte, 2002); and carcass quality to the carcass yield, ratio meat/bone or the proportion of certain muscle with higher commercial value (Blasco *et al.*, 1982)

In this sense, previous studies in other species, like cattle (Berg and Butterfield, 1979) proposed as a logical aim in the production the increased of the total muscle mass, based in the fact that in a same point of the growing curve, it seems, not to be differences in the distribution of muscle masses among dairy and beef bovine breeds. The difference in the muscle masses distribution is based in the precocity of the lines. On the other hand, Richmond and Berg (1971) in swine and Fourie (1965) in sheep reached similar conclusions, setting as main target in production the increase of the total meat in the carcass. In rabbits, like in other species, the most effective way to improve this parameter is by the genetics (Hernández *et al.*, 2004; Pascual and Pla, 2007), due to the high cost that normally would supposes to increase the muscle mass by nutrition. It could be reached by increasing the energy and protein feed supply, what could compromise the profitability of the farms, due to the price increase of the raw materials in the last years.

Moreover, it has been demonstrated that the inclusion of high protein levels in rabbit's feed increases the nitrogen emission to the environment and causes digestive pathologies. In addition, in Spain it is applied the European Regulation about the contamination of the environment (Regulation EC N° 1069/2009); therefore, the demand to decrease the residues produced in the farms due to their potential contaminant effect in

the environment has been increased (Royal Decree 1528/2012, 8th of November), imposing ever-increasing sanctions to the farms that do not comply with these regulations.

On the other hand, the awareness of the consumers about the environment has been increasing and demand the minimum environmental impact. Thus, for the nutritional point of view is interesting to look for strategies that improve the performance and carcass quality keeping or reducing the residues produced. At the same time the consumer is looking more for a healthy diet demanding more lean meat and good quality fat intake.

However, nutrition can play a role in carcass yield and composition. Some strategies have been already tested, like different feed formulations and feed supplements. For example, the use of exogenous enzymes in feed for rabbits could have an effect on the carcass due to its capacity to improve the nutrients digestibility, reduce emissions to the environment and decrease the digestive content (Falçao-e-Cunha *et al.*, 2007). Another way to improve this parameter would be the inclusion of high levels fibre that increases the passage rate of the digesta (Gidenne *et al.*, 2001; de Blas *et al.*, 1999) and the emptying of the digestive tract (Nicodemus *et al.*, 1999; García *et al.*, 2002a). Therefore, the increase of the carcass yield, by reducing the content and/or size of the digestive tract, would suppose a lower environmental impact due to the reduction of the wastes that must be disposal, and due to the better productivity in the slaughterhouse.

LITERATURE REVIEW

II. LITERATURE REVIEW

2.1. Carcass quality for the different links of the food chain

The concept of carcass quality differs among each sector and step of the meat production industry (Ouhayaun, 1998). The variety of interests makes difficult to give a unique definition of quality valid for all the food chain levels and sometimes even impossible to satisfy every target: sensorial, dietary, or technological. Following there is a short description of the quality concept for each step of the food chain: farmer, slaughterhouse or industry, butcher or retailer and customer.

Farmer:

For the farmer, the main interest is to optimize the growth and performance parameters of the animals. Its main objective is to produce saleable animals in the less time possible at the lowest cost, complying at the same time with the weight, formation, and fat cover demanded by the industry. In practice, the variability in the carcass fat cover and weight can be responsible, largely, of its value.

The farmer has to take into account also the demand of the final consumer and get carcasses that fit with that demand. However, usually what is commercially most valuable as the highest quality is not the most profitable, either because the cost increase considerably or because the productive potential is lost (Consigli, 2001), making it necessary to find an optimal balance.

Slaughterhouse or industry:

The industry requires carcasses with the maximum proportion of muscle, the minimum of bone and a proper amount of fat, according to market demand.

Its priority is to acquire animals with a good carcass yield, good profiles, and presence of certain level of fat, mainly fat cover. The fat and its distribution are an aspect of maximum interest in the sale of the carcasses (Berg and Butterfield, 1979), by its own importance and because of the tight relation with the muscle.

Butcher (retailer):

The butcher looks for satisfy the customer's demand, but this, in turn, depends on the carcasses that the slaughterhouse has or sells in each moment. In consequence, they must consider these factors taking into consideration the size of the pieces and the amount of fat (ratio muscle/bone and muscle/fat). Obviously, the retailer centres its priority in the carcasses with high proportion of premium pieces, that have higher demand and are, therefore, easily sold at a higher price.

Consumer:

The consumer generally demands a meat with high content of muscle and some fat. They also consider, largely, the colour and tenderness as acceptance parameters. However, the demands of the consumer are many and diverse, and could be summarized as follow:

- Appearance: meat and fat colour, shape, and weight of the piece.
- Composition: proportion of meat and fat in the pieces and the residues left in the plate.
- Organoleptic characteristics: tenderness, flavour, juiciness, and satisfaction aroused when is eaten.

2.2. Rabbit commercial carcass in Spain

The best economic moment to slaughter the animals is when they reach a live weight (LW) between 2 and 2.8 kg, that are reached at the age of 8 to 10 weeks of life, respectively, with a carcass yield that vary between 54 to 61%. Since the accumulation of fat starts from the 2.5 kg LW, the demands of the consumer are met, because they prefer tender and lean meats. In the Mediterranean countries, where the rabbit meat consumption is higher, the slaughtered weights vary, being Spain the country with the lowest weight (Table 1).

Table 1. Average carcass weights demanded in Spain, France, and Italy.

COUNTRY	CARCASS WEIGHT (kg)*	CARCASS WEIGHT (kg)**
Spain	1.0-1.2	1.2
France	1.4-1.6	1.4
Italy	1.4-1.6	1.5

*Camps (1993). **FAO (2013)

Carcass is defined as the remains of a dead animal after bled and skinned. Another particularity of Spain is that the carcass is normally sold with head, kidneys, and liver (Blasco *et al.*, 1993). The carcass weight demanded by the market are 1-1.2 kg, therefore, the rabbits are slaughtered with two months of age and around 2 kg of LW. The average carcass weight is around 60%, with a fat content lower than 5% and less than 20% of bones (Ouhayoun, 1998).

Actually, carcasses are presented entire or cut. The typical cutting consists in seven cuts: the two hind legs, two front legs, the loin (premium pieces) and two thorax portions (second category pieces). However, the processing of the rabbit meat is lapping behind in comparison with other species (Petracci *et al.*, 2012).

2.2.1. Carcass yield

The carcass yield is one of the most used technical parameters to evaluate the carcass quality and one of the most important, economically, for the slaughterhouse.

According to the rules of the World Rabbit Scientific Association collected by Blasco *et al.* (1993) and modify by Blasco and Ouhayoun (1996) to unify the definitions of some of the parameters used on researches about rabbits, carcass yield is defined as:

- Hot Carcass Weight (HCW): Weight of the carcass 15-30 min after slaughter.
- Chilled Carcass Weight (CCW): Weight of the hot carcass after chilling for 24h between 0- 4°C.
- Standardized Live Weight (LW): Liveweight of rabbits at the end of the experimental period before fasting, digestive tract content and urinary bladder must be included.
- Slaughter Live Weight (SLW): Live weight of the rabbit before the slaughter.
- Empty Live Weight (ELW): SLW without digestive tract.

The differences between LW and SLW are due to losses caused by fasting, droppings and dehydration during transport and vary from 2 up to 3.5% (Villena *et al.*, 2008; Mazzone *et al.*, 2010; Margüenda *et al.*, 2012), and can be even up to 4.5%, when the transport to the slaughterhouse is longer than 8 hours (Trocino *et al.*, 2003).

Depending on the weights uses there can be different definitions of carcass yield:

- Dressing out Percentage (DoP): ratio, expressed in percentage, between the chilled carcass weight and the live weigh of the animal: $CCW/LW \times 100$.
- Slaughterhouse Yield: $CCW/SLW \times 100$.
- Real Yield: $CCW/ELW \times 100$.

Despite the most common is the dressing out percentage, it is important to indicate which one is used. Even if the farmers are paid according to the animals LW , being the DoP of technical and economic importance mainly for the slaughterhouses, there is a tendency to pay in the future to the farmer according to the carcass quality and DoP (Such, 1981). Nowadays, rabbit carcass yield varies between 50 and 65% of LW. This wide variation is due, partly, to the criteria used for its definition (dressing out percentage, slaughterhouse or real yield), but there are also other factors that can be intrinsic, specific of each animal (genetic, breed, slaughter age (from 8 to 12 weeks), slaughtered weight) or extrinsic, like the environment of the animal and its management (environmental conditions, feeding during the fattening phase, fasting before slaughtered) (Such, 1981, Ouhayoun, 1991a, Parigi Bini *et al.*, 1992; Bianospino *et al.*, 2006; and Metzger *et al.*, 2006). In Figure 1 is shown the typical commercial cutting of a 2.3 kg LW rabbit. The digestive tract is approximately the 20 – 21% of the LW, which represents the 60% of the total wastes produced in the slaughterhouse (Margüenda *et al.*, 2012).

Due to the European legislation about Transmissible Spongiform Encephalopathy the wastes produced in the slaughterhouses are consider hazardous material and must be disposed, normally by incineration, what results in an added cost (Margüenda *et al.*, 2012).

Figure 1. Commercial cutting of a 2.3 kg live weight rabbit (Ouhayoun, 1991a).

2.2.2. Factors influencing on the carcass yield

2.2.2.1 Carcass presentation

The diversity of carcasses presentations is one of the most important causes of the variation in the DoP. For example, in Spain, the head is part of the carcass, while in other countries not. Therefore, is not consider for the DoP calculation.

2.2.2.2. Intrinsic (genetic, sex and age)

In this group are considered the parameters that depends on the animal. Basically, genetic, sex and age.

2.2.2.2.1 *Genetic*

Carcass characteristics are basically influenced by the adult weight and by the maturity of the rabbits at slaughter (Pla *et al.*, 1996; Piles *et al.*, 2000, Dalle Zotte, 2002, Metzger *et al.*, 2006). If rabbits are sacrificed at the same age, the breeds that reach before the adult weight have a lower carcass yield that those that have a later maturity (Such,

1981, Lukefahr *et al.*, 1982; Maertens, 1992; Pla, 1996; Pla *et al.*, 1996; Dalle Zotte and Ouhayoun, 1998). This is partially due to the positive allometry presented by the growth of the intestinal tract (Cantier *et al.*, 1969; Deltoro and López, 1985) in relation to the total body weight, that makes, proportionally, higher the digestive tract weight regarding the LW in the animals with faster growth than in the slower ones (Ouhayoun, 1998). On the other hand, Hernández *et al.* (2004) observed that when genetically different animals are sacrificed at the same maturity status, at different ages, there are no differences on the DoP due to the genetic improvement. Therefore, if the target were only to obtain the maximum carcass yield, it would be interesting to slaughter the animals with a higher weight in genetically similar breeds. However, the heavier the animals, the higher the proportion of fat and lower the ratio muscle/bone (Hernández *et al.*, 2004). If a rabbit reaches its adult weight at 4 kg, the adiposity increases from 2100 g and, the ratio muscle/bone will tend to decrease from 2450 g of LW, whereby the optimal slaughter weight would be around 2300 g LW (that gives carcasses of 1300 g).

In Spain rabbits are sacrificed with less than 55% the adult weight, what improve the feed conversion ratio (FCR) and produce carcasses with less fat content but worse dressing out percentage. Carcass weights over 1430 g have a 6% higher DoP (Ouhayoun, 1991a).

According to Metzger *et al.* (2006) in order to increase the meat content, the genetic selection in late growth breeds should be done selecting by both growth rate and carcass traits at an earlier age.

2.2.2.2.2. Sex

In most of the species males have higher growth potential than females and reach earlier the adult weight. Males carcass weight is also heavier, and its carcass composition

is leaner than the females (Lazzaroni *et al.*, 2009). However, these differences are not relevant in the rabbit (Bernardini *et al.*, 1995) because they are slaughtered much earlier than they reach the puberty, period when it starts to show up more the differences. In this sense, Trocino *et al.* (2003) observed that females had lower carcass yield and higher losses during transport until the slaughterhouse than males, although in this study rabbits were slaughtered with more than 2.5 kg LW, much higher than in Spain.

2.2.2.2.3. Age

The age is a factor with high importance on the DoP. Many authors (Parigi Bini *et al.*, 1992; Piles *et al.*, 2000; Dalle Zotte, 2002 and Gondret *et al.*, 2002) have confirmed that the older, the higher the carcass yield up to an optimum and then decreases. The age is link to the live weight of the animal, because the older the animal, the heavier it is. Therefore, all these works concluded that is convenient to do comparison among breeds or genetic lines at a same slaughter age to avoid differences attributed to the somatic maturity. Nevertheless, despite the age improves the DoP impairs the feed conversion and the carcasses are fatter (Fernández and Fraga, 1996), consequently it is important to find a compromise among all these variables to optimize the economic profit.

2.2.2.3. Extrinsic (management, environment, feeding)

These factors refer to the environment where the animals are and the factors that do not depend on the animal itself.

2.2.2.3.1. Management

Among these factors are those related to the type of housing, the density during the fattening period, and the conditions before slaughter, such as fasting and transport. Some studies have shown that group housing in cages during the fattening period with more

space and straw litter worse the DoP compared to conventional cages (Dal Bosco *et al.*, 2002; Metzger *et al.*, 2003; Lazaroni *et al.*, 2009). Housing systems on the ground with access to the outside (D'Agata *et al.*, 2009) also reduced the DoP, as well as extensive versus intensive breeding systems (Rymkiewicz and Lewczuk, 1999; Bielański, 2000). However, in other studies no effect of the housing system on DoP have been found (Daszkiewicz *et al.*, 2012). The density can also affect the DoP but high densities seem to have a more negative influence on productive parameters than on DoP (Aubret and Duperray, 1992; Xiccato *et al.*, 1999; Paci *et al.*, 2013). According to Martínez and Fernández Carmona (1981) the group creates a microclimate that dampens the variations of the environment but can be counterproductive in hot periods. However, even under conditions of heat stress Villalobos *et al.* (2008) did not observe negative effects of a higher density on DoP.

Fasting prior slaughter also affects the DoP. During the first 6 hours of fasting, the animal weight losses are mainly due to the emptying of the digestive system, while during the next 6 hours there is also moisture and nutrients losses from the body tissues. In several studies (Masoero *et al.*, 1992; Szendro y Kustos, 1992; Trocino *et al.*, 2003; Margüenda *et al.*, 2008 and 2009) have been observed that the animals fasted before slaughter presented a lower weight of the digestive system and, therefore, greater carcass yield.

2.2.2.3.2. Environment

The time of the year, the room temperature, the hygienic-sanitary conditions, etc. are parameters that affect the growth of the animals and therefore the DoP. In rabbits, the increase in environmental temperature above the thermoneutrality values reduces feed consumption (Martínez and Fernández Carmona, 1981; Cervera and Fernández

Carmona, 2010) and consequently, also reduces the growth rate. This causes a lower slaughter weight, which is more evident in the summer, but sometimes improves DoP due to a lower proportion of skin, intestines and waste (Chiericato *et al.*, 1993 and 1996).

2.2.2.3.3. Feeding

The improvement of DoP through feeding is based on two strategies, the first would be through an increase in the carcass weight due to a greater deposition of protein and / or fat, and the second would consist in achieving a reduction in the relative weight of the digestive system. Carcass weight increased through a greater amount of protein and energy intake does not have much interest since the protein is expensive and in addition, the excess nitrogen is polluting (Maertens *et al.*, 1997), and causes digestive disorders (Carabaño *et al.*, 2009). An overconsumption of energy would also lead to more fatty carcasses, which are less valued by consumers. Therefore, below are some of the strategies that can be used to decrease the relative weight of the digestive system:

2.2.2.3.3.1. Decrease the feed consumption:

When the feed energy concentration is higher than 9-9.5 MJ/kg, the rabbits regulate their intake by the energy content. Below this energy value there is a physical regulation, not metabolic (Xiccato and Trocino, 2010). One way to increase the feed energy level is adding fat. In some studies it has been observed that a low or moderate level of fat inclusion (2-6%) increases the DoP (Raimondi *et al.*, 1974; Castellini and Battaglini, 1992), but in most of the papers it has not been found effect of fat addition on DoP (Partridge *et al.*, 1986; Santomá *et al.*, 1987; Fernández and Fraga, 1996).

Another alternative to reduce the consumption would be to use a DP/DE ratio (g digestible protein/MJ digestible energy) below the recommendations (10 g DP/MJ DE;

De Blas and Mateos, 2010), although this alternative would be convenient at the end of the fattening phase in order not to adversely affect the growth of the animals. In swine, strategies of this type have already been studied, replacing conventional feed with barley at the end of the fattening period to improve the carcass yield and obtain fatter carcasses (Garitano *et al.*, 2012).

a) Feed restriction:

Feed restriction could be considered more a management strategy than feed strategy, however, the target is to decrease the feed consumption of the animals. The information of its impact on the carcass yield is scarce and the not always positive (Birolo *et al.*, 2016; Kdunsen *et al.*, 2017).

This strategy has been implemented in the farms mainly to reduce the mortality and morbidity caused by ERE and to decrease the preventive usage of antibiotics. It is commonly used in some countries, like France or Italy, with good success in reduction of mortality and morbidity and for improving feed conversion, especially due to the compensatory growth when the animals are fed *ad libitum* again (Gidenne *et al.*, 2012; Birolo *et al.*, 2016).

Several post-weaning restriction strategies have been studied. Varying the length and period of application (1 to 5 weeks), the intensity (from 90% to 40% of the voluntary intake) or the method used (quantitative restriction, water restriction, limited access time to the feeder). Some authors (Di Meo *et al.*, 2007; Tumová *et al.*, 2007; Gidenne *et al.*, 2009a and b) have observed an improvement on nutrient digestibility when restricting the voluntary feed intake. However, these results are not consistent (Gidenne *et al.*, 2012).

2.2.2.3.3.2. *Increase the digestive transit*

Fibre plays an important role in the regulation of digestive transit (Fraga *et al.*, 1991; Gidenne and Pérez, 1994; García *et al.*, 1999), control of intestinal microbiota and maintenance of mucosal integrity (De Blas *et al.*, 1999; Gidenne, 2003; Gidenne and García, 2006).

a) Definition of fibre:

The European Commission define the fibre for humans in the regulation 1169/2011/EU that was applied on 13th of December 2014, as ‘carbohydrate polymers with three or more monomeric units (degree of polymerization ≥ 3), which are neither digested nor absorbed in the human small intestine, belonging to the following categories: a) edible carbohydrate polymers naturally occurring in the food as consumed; b) edible carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and, which have a beneficial physiological effect demonstrated by generally accepted scientific evidence; c) edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence’.

Dietary fibres are defined as polysaccharides and associated substances resistant to mammal enzyme digestion and absorption, that can be partially or totally fermented in the gut (Gidenne, 2015). Cham *et al.* (2003) provided a concise synopsis of various views regarding the classification of dietary fibres. The overall tendency is towards an extension of the definition by including resistant starches as well as non-digestible oligosaccharides, and it was recently revisited by De Vries *et al.* (2010) to develop an official enzymatic-gravimetric method that recovers all fibre components of the feed.

Fibre is one of the main components in the commercial diets for rabbits in intensive rearing. These feeds usually include a third part of forage and fibrous by-products (De Blas *et al.*, 1999). The different fractions of the fibre are one of the most important nutritional factors for the digestive physiology in the rabbit (De Blas *et al.*, 1999), and the usage of the sequential method developed by Van Soest *et al.* (1991) (NDF, ADF and residue in ADL), officially recognized by AFNOR (1997), has improved the fibre recommendations in fibre to reduce digestive disorders in young rabbits for fattening (Gidenne, 2000; García-Ruiz *et al.*, 2006). In addition, this method has the advantage to estimate different fractions of the fibre with only one sample (Gidenne, 2000).

b) Lignin:

The lignin is a cross-linked polymer of aromatic subunits formed by the union of various phenylpropanoid acids and alcohols (coumaryl, coniferyl and sinapyl). The random linkage of these radicals creates a complex and branched three-dimensional structure with a high molecular weight. This structure serves as a matrix for other polysaccharides compounds, providing rigidity and resistance to the cell wall against compression to external agents, like microorganisms, preventing the penetration of destructive enzymes into the cell wall. It also provides hydrophobic properties to the cell wall making them impermeable to water (Whetten and Sederoff, 1995).

Lignin is the most limiting factor in terms of availability of the plant cell wall compounds for the animal digestion (Van Soest, 1994). It is covalently bound to the hemicellulose (Van Soest, 1994) and, therefore, the level of lignification of the NDF negatively influences the hemicellulose digestibility (Gidenne *et al.*, 2010a). It is considered almost undegradable, however, has been observed positive values in the lignin

digestibility that might be caused more by its solubilization than its hydrolysis. Some sources of lignin are:

b.1) Defatted grape seed meal:

A by-product from the wine industry and is formed by the seeds of the grape. The seeds are compound by a tegument or cover very lignified and by albumen rich in lipids. The oil has a high commercial value and, therefore, is usually extracted with hexane. The resulting product is called defatted grape seed meal (1-2 % of ether extract) and content approximately a 50% of crude fibre and a high content of ADL (42%) (FEDNA, 2019). This material is normally sold as meal and presents the advantage that is sterilized in the oil extraction process. Sometimes is market complete and mixed with other wine by-products (skin and stalk) being all together the grape marc. In a study done by García *et al.* (2002a) observed that the inclusion of a 15% of defatted grape seed meal promoted feed ingestion, improved DWG and the specific activity of the disaccharidases in the small intestine of growing rabbits, without alter the caecum fermentation.

b.2) Arbocel®:

A lignocellulose derived from wood by-products with a high lignin content. In a trial performed by Krieg *et al.* (2008), where the ratio lignin-cellulose was increased with a 3% Arbocel®, suggested that this increase could reduce fibre digestibility and decrease the microbial fermentation rate.

The fibre level influences the weight of the cecum content, and therefore the weight of the digestive system. Figure 2 shows how the feed NDF level influences quadratically the cecum content weight in growing rabbits (de Blas *et al.*, 1999). The minimum weight of the cecum content is obtained for a 39.5 NDF (% DM) and values

above or below this optimum produce an increase in the cecum content weight. However, the same level of NDF can lead to different results, depending on the type of fibre: and if it is more or less lignified (Fraga *et al.*, 1991; Nicodemus *et al.*, 1999; García *et al.*, 1999), more or less fermentable (Gómez-Conde *et al.*, 2009, Trocino *et al.*, 2011; Martínez-Vallespín *et al.*, 2013) or larger or smaller particle size (Gidenne, 1993; Nicodemus *et al.*, 2006; Romero *et al.*, 2011).

Figure 2. Effect of the neutro detergent fibre (NDF) on the weight of the cecum content (CCW; %LW) (De Blas *et al.*, 1999).

In terms of DoP, decreasing levels of fibre and its corresponding raising level of starch increase the caecum content of the rabbits (Dehalle, 1981; De Blas *et al.*, 1986; García *et al.*, 1993), a sign of the longer retention time of the digesta in the caecum. While in high fibre diets higher stomach content was found (De Blas *et al.*, 1986; Parigi Bini *et al.*, 1994), due basically to the higher ingestion level. These variations could be considered to modify the DoP (Xiccato, 1999) that, in general, is impaired by a higher digestive tract weight. Some studies have observed an increased DoP with increasing levels of NDF of the feed (Fabre *et al.*, 2006; Liangzhan *et al.* 2017); provided always at a level close to the optimum that minimizes the weight of the cecum content. However, an excess of NDF could worsen the DoP as have been observed by Magüenda *et al.* (2006 and 2012); Villena *et al.* (2008) and Trocino *et al.* (2011).

Not only the fibre level, but the more fermentable and less particle size fibres support an accumulation of digesta in the cecum because they cause a longer time of ileum-rectal retention (Gidenne, 1993; García *et al.*, 1999; Nicodemus *et al.*, 2006). Consequently, they can worsen the DoP (García *et al.*, 1993), although moderate inclusions of this type of fibres seem to have no negative effects on the DoP (Trocino *et al.*, 2011; Margüenda *et al.*, 2012). In fact, currently it is recommended the inclusion of moderate levels of soluble fibre (11-12%; Martínez-Vallespín *et al.*, 2011; Trocino *et al.*, 2013; Delgado *et al.*, 2018) in order to avoid the use of antibiotics and prevent the epizootic rabbit enteropathy (ERE).

On the contrary, more lignified fibres accelerate the digestive transit (Pérez *et al.*, 1994; Gidenne *et al.*, 2001) and reduce the weight of the cecal content (Nicodemus *et al.*, 1999; García *et al.*, 2002b; Caîsin *et al.*, 2020). Figure 3 shows how an increase in the ADL concentration of the feed, linearly decreases the cecal content weight, so it could also improve the DoP.

In addition, several studies indicated that all the factors that increase the retention time (decreased levels of fibre, reduction of feed particle size) contribute to disrupt the caecum microbial activity and encourage the enteritis (Gidenne *et al.*, 2010b). In this sense, Nicodemus *et al.* (1999) recommended a minimal ADL concentration of around 4.1% in growing rabbits to ensure the growth performance and health of the animals. It has been observed that a high or low level of ADL in diet can negatively affect the passage rate and change caecal fermentation (pH and VFA concentrations) (García *et al.*, 1999, 2000 and 2002b; Gidenne *et al.*, 2001). However, the usage of raw materials rich in lignin in other areas of animal nutrition is increasing the prices (Colin *et al.*, 2007). In this context, products rich in fibre like grape and wood by-products are interesting as a supply of lignin and cellulose for animal nutrition after being crushed and dried.

Figure 3. Effect of acid detergent fibre (ADL) level on the average retention time of the digesta (ART) in caecum and on the cecum content weight (CCW) (García *et al.*, 2002b).

2.2.2.3.3. Increase the digestibility

An improvement of nutrients digestibility promotes its absorption, reduces the flow of fermentable elements to the caecum and therefore, could reduce the relative weight of the digestive system and the cecum content, improving the carcass yield, decreasing the wastes at the slaughterhouse and in consequence, decreasing the cost of the disposal removal and reducing the environmental contamination (Margüenda *et al.*, 2012). A strategy to improve digestibility is to use different additives such as enzymes, organic acids, prebiotics, probiotics (Falcao-e-Cunha *et al.*, 2007). Studies with the use of enzymes in cuniculture are scarce and in many cases results are contradictory.

Enzymes are molecules of protein nature that catalyse chemical reactions in determined conditions of pH and temperature, making a chemical reaction that is energetically possible, but slow, although it takes lower time than without the presence of the enzyme (Grisham *et al.*, 1999). Enzymes are produced by all the living organisms and many of them participate in the digestion; the enzymes that act on the digestive process can be:

- Endogenous: produced by the animal in different parts of the digestive tract and in variable quantity depending on the age and the animal species.
- Exogenous: coming from the diet. The enzymes supplemented in the feeds usually have bacterial or mould origin (Biovet, 2008). Once the exogenous enzymes are in the digestive tract are digested like the other proteins. Therefore, they do not leave residues in the faeces or the urine and it is not required a withdrawal period of the animals fed with one or more enzymes (Rojas Méndez, 2011). Consequently, the acceptance is good by the European consumers (Biovet, 2008). Enzymes can be found individually or in cocktails of different enzymes. These enzymes are produced from bacteria or moulds by liquid fermentation and later purified normally in the individual enzyme, that can be sell individually or mixed and market in a cocktail of enzymes. This kind products must be registered as a feed additive in the European Union and requires a high investment of resources by the companies, but on the other hand, it warranties its safety and effect. However, in the market are appearing enzyme complex products that are based on the fermentation of one filamentous mould strain on a solid substrate (corn, DDGS, etc.) producing a wide range of enzymes that have enzymatic activity to improve digestibility in the animals. In addition, it can improve the digestibility of the raw materials used as a substrate and it allows the use of coproducts from the industry, like DDGS. Another advantage of this products is that in the European Union are classified as a raw material and they do not require the extensive registration process like a feed additive.

In some studies, enzymatic supplementation improves mortality, digestibility of some nutrients and / or productive traits (Gutiérrez *et al.*, 2002; Cachaldora *et al.*, 2004;

Eiben *et al.*, 2004; García-Ruiz *et al.*, 2006; El-Kelawy and El-Shafey, 2017). However, in other studies (Sequeira *et al.*, 2000, García-Palomares *et al.*, 2006; Falcao-e-Cunha *et al.*, 2007) no improvements were observed with the addition of enzymes on any of these parameters. On the other hand, the works that study the effect of the dietary enzymatic supplementation on the carcass yield are even scarcer, and with equal disparity of results (Shanmuganathan *et al.*, 2004; Abaza and Omara, 2011; Abd El-Aziz *et al.* 2019). The lack of clearly positive effects and variability of results could be due that the cecum of the rabbits at 4 weeks of age contents most of the total activity of the pectinases (0.43), amylases (0.45), lactases (0.57), xylanases (0.65), cellulases (0.69), β -glucosidases (0.70) and ureases (0.80) present in the digestive tract of the rabbit, and these values increase with the age (Marounek *et al.*, 1995). It could be also due to the peculiar digestive physiology of the rabbit, specially the caecotrophy, because is possible to find microbial enzymes along the whole digestive tract (Marounek *et al.*, 1995). These lack of consistency of enzymes could be in addition, partially explain by the different experimental protocols and hygienic conditions (Falcao-e-Cunha *et al.* 2004).

Anyhow, this would not exclude the interest of enzyme supplementation, but probably, it would restricted to certain phases of the rabbits' life; or the supplementation with only the less digestible feeds, because in this diets is where it can be observed an impairment in carcass yield (Margüenda *et al.*, 2012), performance and nitrogen retention (García *et al.*, 1993). In these cases, the use of exogenous enzymes could improve the nutrient utilization efficacy and retention and in consequence, the productivity. It could allow the use of other kind of raw materials, cheaper and / or that do not compete with the human food, generally of lower quality, and where enzyme supplementation could have similar performance.

In addition, the concern about the contamination of the soil (eutrophication, accumulation of salts and heavy metals), the water (contamination with nitrates) and the air (contaminant gases, aerosol formation, odours) caused, among others, by the nitrogen produced by the livestock animals is increasing. Even more in some countries of the European Union where it is becoming a reality, with regulations to control the nitrogen (N) production of the farms. For example, the Netherlands and Belgium (Maertens *et al.*, 2005). In fact, there is a European Regulation for the disposal production in the farms (Regulation EC N° 1069/2009), that is applied in Spain by the Royal Law 1528/2012, of 8th of November. In rabbits, approximately 2/3 of N ingested is excreted (Carabaño *et al.*, 2009), and therefore, an improved digestibility of the feed could decrease this N excretion.

2.2.2.3.3.4. Usage of other feed additives

There are certain feed additives that have a positive effect on intestinal health and / or on the animal's immune system, causing better nutrient absorption and better growth. For example, trace elements, the papers that study the effect of the supplementation with trace elements on the DoP are scarce. In this sense, Ebeid *et al.* (2012), observed an improvement of the DoP with the addition of organic selenium.

Other feed additives, like combinations of herbs, essential oils or probiotics could also have an improvement on carcass yield (Abdel-Wareth *et al.*, 2018; Fathi *et al.*, 2017), under challenge situations. However, results are contradictory and in other works results are not conclusive and have not observed an improvement of the carcass yield (Dalle Zotte *et al.*, 2016; Ayyat *et al.*, 2018)

2.3. Chemical composition of the rabbit meat

Nowadays the rabbit carcasses that are mostly found in the Spanish market come from selected breeds with an average live weight between 2-2.2 kg, which provide very homogeneous carcasses of totally lean meat that has an excellent flavour and texture (Dalle Zote, 2002). The quality of rabbit meat can be assessed by its physical, chemical, and sensorial composition, which are the most valued characteristics by the final consumer, although they are rarely considered by breeders, slaughterhouses and sellers (Xiccato, 1999).

Compared with other domestic species, it has a low content of fat, calories, sodium and a high proportion of protein (Sunki *et al.*, 1978; Ouhayoun *et al.*, 1985) rich in essential amino acids (Hernández and Dalle Zotte, 2010). In addition, the digestibility of its protein is high, with a good biological value (Gilka, 1975; Ouhayoun, 1991b, Hernández and Dalle Zotte, 2010).

Meat is mainly composed of water, protein, and fat, although it also has small amounts of other substances, such as non-protein nitrogen, carbohydrates, and minerals.

In domestic rabbits, the main component of meat is water (69-73%), followed by protein (18.5-21%), including non-protein nitrogenous substances. Fat, along with water, is the meat component that presents the most variations (6.5-10.5%); while ashes is the least variable fraction, representing about 1% (Cobos, 1993). Finally, rabbit meat, like those of other mammals, is a poor source of carbohydrates. The normal glycogen content of mammalian muscle ranges from 0.5-1% (Price and Schweigert, 1976) in the live animal, but this is a labile component that descends rapidly after a short period of intense muscular activity that accompanies the death of the animal, as well as the irreversible

anaerobic glycolysis that occurs in the muscle when death occurs and oxygen supply fails (Cobos, 1993).

2.3.1. Factors that influence the chemical composition

In the same way as in the carcass yield, the chemical composition depends on several factors that can be intrinsic and extrinsic.

2.3.1.1. Intrinsic factors

2.3.1.1.1. Age and slaughter weight

As the animal grows, the chemical composition of the animals undergoes variations (Dalle Zotte, 2002). The percentage of fat in the rabbit carcass changes from an initial phase of rapid decline (up to 5-6 weeks of age) to a second phase of moderate growth (Deltoro and López, 1986).

Most of the authors (Rao *et al.*, 1978; García *et al.*, 1992a, 1993; Saiz, 2016; Saiz *et al.*, 2017) pointed to an increase in fat content and a decrease in the amount of moisture in the meat of rabbits with the weight of the animal.

Therefore, slaughter age and weight have a marked impact on tenderness, fatness and nutritional value of meat and fat (Fernández Martínez, 1993). Young rabbits have meat with a higher percentage of moisture and protein and lower fat. When the reproductive age arrives, its meat is much more tasteless, compact, and greasy. According to Parigi-Bini *et al.* (1992) for the same breed, age seems to be more important than weight in terms of the variability of the fat content of the carcasses.

If the reproductive age is not reached, the slaughter weight does not seem to affect the ash content of the meat, nor does the protein content vary, and the levels of sodium and cholesterol are still low (Fernández Martínez, 1993). Rao *et al.* (1978) did not observe

either changes in the protein content of meat with a greater slaughter weight. However, Ouhayoun *et al.* (1974) indicated an increase in the protein content of meat with a greater slaughter weight, although the influence of weight is much less than that observed in fat and moisture.

Weaning age has no appreciable effect on the final body composition of rabbits (Rao *et al.*, 1978; Fraga *et al.*, 1983).

2.3.1.1.2. Breed

It is another of the factors that most influence the composition of meat. Ciruzzi *et al.* (1973), obtained higher values of moisture and protein in rabbits of the New Zealand White breed than in Californians, who nevertheless had more fat. The effect of the breed has also been studied by Ouhayoun (1980) and Deltoro *et al.* (1988b). These authors suggested that the order of precocity for fat deposition in the main breeds used in intensive farms is, from major to minor, Californian, New Zealand, and Flemish Giant.

However, rabbits raised for meat, in practice, are commercial hybrids derived from selection programs based on three crosses, with adult weights between 4 and 5 kg. Its weight at commercial slaughter ages (from 9 to 13 weeks) means that the composition of the carcass in the final product does not show differences (Ouhayoun, 1998). According to previous results (Lambertini *et al.*, 1996; Hernández *et al.*, 1998), the differences between genetic lines in terms of meat quality are negligible and it seems that the composition of rabbit meat is usually quite constant.

2.3.1.1.3. Sex

Fraga *et al.* (1983) observed that males of the Spanish Giant rabbit contained 1% more nitrogen and 0.8% less fat than females of the same breed. Raimondi *et al.* (1974)

also observed fatter and less moisture in female meat. Similarly, Deltoro and López (1986) observed that males had a significantly lower fat content in the carcass than females. However, other authors (Costantini and Bosi, 1968; Ciruzzi *et al.*, 1973; Fraga *et al.*, 1978) did not find significant differences due to sex, which seems to be because most rabbits are marketed before reproductive age.

2.3.1.1.4. Carcass part

Although the rabbit carcass is quite homogeneous, its chemical composition has slight modifications, depending on the area analysed. Thus, the posterior third of the carcass is richer in moisture and protein and poorer in fat than the front one, while the loin has intermediate values (Ciruzzi *et al.*, 1973; Granat *et al.*, 1977). That is, the parts that have rapid growth are richer in protein and moisture than those of slow growth, confirming the observations made by Abdel-Naby (1979).

2.3.1.2. Extrinsic factors

2.3.1.2.1. Feeding

The chemical composition of the feed also influences the chemical composition of the meat, being one of the most important factors (Ouhayoun, 1998; Hernández and Dalle Zotte, 2010).

Fibre has a particularly important role because of its relationship with the digestive transit, together with the energy content and with the DP/DE ratio. When the feeds have a high fibre content cause a decrease in consumption, growth, slaughter weight and carcass yield, but the carcasses usually have a lower fat content (García *et al.*, 1993; Hernández and Dalle Zotte, 2010). In this sense, Fraga *et al.* (1983) pointed out that an DE / DP ratio of 22.8 is the one that provides the lowest fat content and a greater amount

of body protein in the rabbit. This value is close to that indicated by De Blas *et al.* (1981) to obtain a maximum growth rate (23.5 kcal DE / g DP). If the DE/DP ratio is increased, the fat content in the carcass tends to increase (Fraga *et al.*, 1983; Dalle Zotte *et al.*, 1997) and significantly reduce the protein, water, and ash content (Pérez, 1978). In other studies, feeds with a different DE/DP ratio during the fattening phase did not modify the carcass characteristics or the chemical composition of the meat (Xiccato *et al.*, 1993). Only when the modifications are extreme in the DE/DP ratio is it when the effect on body composition is detected (Xiccato, 1999).

In addition to an adequate DP/DE ratio, feed must ensure a correct supply of essential amino acids. In rabbits the most limiting are methionine, lysine, and threonine. A deficit in these amino acids has more effect on the productive parameters, than on the carcass characteristics. In fact, DoP and body composition are influenced by the decrease in average daily gain (DWG) and consumption. It has not yet been shown if there is an effect on carcass characteristics when the amount of amino acids is changed without modifying the DWG (Xiccato, 1999). Some studies have shown that methionine supplementation improves DoP and increases the proportion of premium pieces (hind and front legs, loins, and kidneys) (Taboada *et al.*, 1996) and increases the meat / bone ratio (Berchiche *et al.*, 1995).

The addition of fat to the fattening feed implies an increase in the fat content of the carcass (Cobos *et al.*, 1993; Fernández and Fraga, 1996). The same effect has been observed when feeding the rabbits only during the post-weaning (45-55 d of age) with a feed with added fat (Dalle Zotte *et al.*, 1997; Xiccato *et al.*, 1998).

Feed restriction have also shown some effects the chemical composition of the carcass, like less fat content and increasing of protein content in the restricted animals (Gidenne *et al.*, 2012; Chodová and Tumová, 2013).

2.3.1.2.2. *Environment*

The season of the year has a clear influence on the growth rate of rabbits (Blasco *et al.*, 1983; Ouhayoun, 1984). This effect is indirect, since the environmental temperature influences on the growth rate, through the ingestion of feed. When the temperature increases, the intake decreases (Cervera and Fernández Carmona, 2010). However, in other species a direct effect of the season has also been indicated when the energy metabolism (Close and Stanier, 1984) or the photoperiod is modified (Schanbacher *et al.*, 1982; Tucker *et al.*, 1984). Deltoro *et al.* (1988a), in California and New Zealand rabbits slaughtered between 1 and 20 weeks of age, observed that only significant differences in the chemical composition of meat appeared in the first three weeks of life (the meat of rabbits born in summer had a lower fat content and higher water and protein) and between 13 and 16 weeks of age (the meat of winter rabbits had higher fat content and lower water and protein). Other researchers (Chiericato *et al.*, 1996) observed a paler meat with a higher proportion of saturated fatty acids at temperatures above thermo neutrality.

2.4. Measurement of chemical composition

There are several methods to measure and/or estimate the chemical composition of rabbits. The reference method used is comparative sacrifice, but this method is expensive and avoid following the evolution of body composition over time in the same animal. For this reason, other types of in vivo methods, called indirect ones, have been studied and developed that would prevent animal sacrifice, such as near-infrared spectroscopy (NIRS), especially in breeding rabbits (Masonero *et al.*, 1992), the technique of dilution

with deuterium oxide (D₂O) (Fekete and Brown, 1992), magnetic resonance imaging (MRI) (Szendrő *et al.*, 1992; Köver *et al.*, 1996 and 1998), or Total Body Electrical Conductivity (TOBEC) (Fortun-Lamothe *et al.*, 2002). However, these methods are still expensive in order to be considered as alternative methods to the comparative sacrifice.

The Bioelectric Impedance (BIA) method is another *in vivo* method of simpler and cheaper application than the previous ones, which has already been evaluated to estimate body composition in other species such as lambs (Berg and Marchello, 1994), calves (Marchello *et al.*, 1992 and 1999), pigs (Swantek *et al.*, 1992, 1999; Marchello *et al.*, 1999), Iberian pigs (Daza *et al.*, 2006) and even in humans (Lukaski *et al.*, 1985a and b). The BIA measures the reduction in voltage that occurs in a body when it is crossed by an alternating current of 425 μ A intensity and at a frequency of 50 KHz. This reduction depends on the conductivity of the body, its geometry, and its volume, in addition to the intensity and frequency of the current. It has been proven that the conductivity of an animal depends on its body composition. Thus, intra and extracellular fluids, electrolytes and minerals act as conductors and comprise a large percentage of lean tissue in the body. The cell membranes behave like capacitors resisting a current until a threshold is reached before allowing the passage of it. The fat acts as an insulator and resists the passage of the applied current. Due to the reduced conductivity of fatty tissue, in comparison to the other compounds of the organism, an animal with a higher fat content has a higher impedance value than an animal with more lean content (Swantek *et al.*, 1999). This is because the current is carried by the body through water and its fluids. In other species it has been described that fat-free tissues have a higher water content, and the electrical signal passes more easily (Marchello and Slanger, 1994).

Recently this technique has already been validated and used with good results to predict *in vivo* the body reserves of breeding rabbits in different physiological states

(Nicodemus *et al.*, 2009; Pereda *et al.* 2010; Rebollar *et al.*, 2011). It has also been used and validated to estimate the body composition of growing rabbits between 25 and 77 d of age (Saiz *et al.*, 2017) and to estimate the chemical composition of carcasses from 35 to 63 d old (Saiz, 2016). The interest of using this technique in growing animals is that it allows us to estimate the retention and efficiency utilization of energy and protein.

OBJETIVES

III. OBJECTIVES

The general objective of this PhD Thesis was to investigate the capacity of different nutritional strategies (exogenous enzyme supplementation, source and level of ADL and feed restriction) to improve carcass yield, carcass composition and reduce nitrogen excretion in growing rabbits.

The specific objectives of this PhD Thesis are:

- Study the effect of the supplementation with different doses of a multi-enzyme complex in two types of diets on growth performance, carcass traits, nutrient digestibility and nitrogen and energy balance in growing rabbits.
- Investigate the result of the dietary level and source of lignin on growth performance, carcass traits, nutrient digestibility and nitrogen and energy balance in growing rabbits.
- Research the effect of different feed restriction levels on growth performance, carcass traits, nutrient digestibility and nitrogen and energy balance in growing rabbits.

MATERIALS AND METHODS

IV. MATERIALS AND METHODS

4.1. Experimental design

The trials for this PhD were conducted in the Poultry and Rabbit Research Centre (PRC) of Trouw Nutrition R&D in Toledo (Spain) in collaboration with the Polytechnic University in Madrid (UPM).

All the experimental procedures were approved by the Ethics Committee of Trouw Nutrition R&D Poultry Research Centre. And the animals were handled in accordance with the Spanish guidelines for experimental animal protection (Spanish Royal Decree 53/2013; BOE, 2013).

In all experiments the rabbit's breed used was New Zealand x Californian, from the maternal line V x paternal line R from UPV, Valencia Spain. Environmental conditions were controlled: 12h of light and 12 of darkness, lateral extraction with dynamic ventilation and the temperature ranged between 20 and 24°C. All cages had one manual feeder and nipple drinking system. All diets were antibiotic free. Feed and water were supplied *ad libitum*, except in some of the treatments of experiment 4.

The size of the individual cages used in experiments 1, 2 and 3 were 25 x 44 x 30 cm. The dimensions of the collective cages in experiments 3 and 4 were 39 x 100 x 30 cm. In the collective cages, 5 rabbits per cage were allocated.

4.1.1. Experiment 1: Effect of an enzyme complex on the productive performance, slaughter traits and nitrogen and energy balance in growing rabbits.

A commercial enzymatic complex was supplemented in the feed: 0, 100, 200, 400 and 800 ppm to evaluate the on top effect of different doses in rabbit diets. The enzymatic

complex used was a coproduct derived from the fermentation on solid state of *Aspergillus niger* on a solid substrate which have residual enzymatic activity (Table 2).

Table 2. Enzymatic activity of the compound used (according to the producer)

Enzyme	Enzymatic activity
Amylase	30 FAU/g
Pentosenase	100 XU/g
Celulase	40 CMC/g
Protease	700 HUT/g
Xylanase	100 XU/g
Pectinase	4,000 AJDU/g
β -glucanase	200 BGU/g

FAU= Fungal Alpha-Amylase unit; CMC= Carboxymethyl cellulose; HUT= Hemoglobin Unit Tyrosine base; XU= Xylanase Unit; AJDU= Apple Juice Depectinising Activity; BGU= β -glucanase Unit.

A total of 225 crossbred mixed-sex rabbits weaned at 34 d of age, with an average body weight (BW) of 818 ± 104 g (mean $\pm$ standard deviation (SD)), were housed in individual cages.

Animals were blocked by litter and randomly assigned to one of the five treatments (45 animals/treatment: Basal feed (B); B+100 ppm of the enzymatic complex; B+200 ppm; B+400 ppm; B+800 ppm. Feed was formulated following de Blas and Mateos (2010) recommendations (Table 3). Feed and water were supplied *ad libitum*.

Feed intake and weight of the rabbits was measured in two periods (at weaning (34 d), 48 and 60 d) from 34 to 48 d and from 49 to 60 d of age. Daily feed intake, daily weight gain and feed conversion ratio were calculated by period and for the whole fattening cycle. Mortality was recorded daily.

Table 3. Ingredients and analysed chemical composition of the experimental feed (*Experiment 1*).

Ingredients composition, g/kg as fed	Experimental Feed
Alfalfa	250
Barley	233
Wheat bran	200
Sunflower meal	105
Sugar beet pulp	80.0
Wheat straw	50.0
Soybean meal	17.1
Wheat	15.7
Soybean oil	15.0
Sepiolite	13.1
Calcium Carbonate	10.8
Sodium Chloride	6.00
Mineral and Vitamin premix*	3.00
L-Lysine	1.30
L-Threonine	0.60
Analysed chemical composition, g/kg DM	
Dry matter	901
Ash	80.3
Crude Protein	176
Neutral Detergent Fibre**	359
Acid Detergent Fibre **	215
Acid Detergent Lignin**	44.3
Starch (enzymatic)	174
Ether Extract	34.2
Gross Energy, kcal/kg DM	4791
Digestible Energy, kcal/DM***	2797
DP/DE***	11.0

*Provided by Trouw Nutrition. Mineral and vitamin composition (per kg of complete diet): Calcium: 0.44 g/kg; Sodium: 0.33 g/kg; Magnesium (MgO): 0.24 g; Manganese (Mn₂O₃): 20 mg/kg; Zinc (ZnO): 60 mg/kg; Copper (CuSO₄·5H₂O): 18 mg/kg; Iodo (KI): 1.10 mg/kg; Cobalt (CoCO₃·H₂O): 0.49 mg/kg; Selenium (Na₂SeO₃): 0.05 mg/kg; Iron (FeCO₃): 78 mg/kg; Vitamin A: 10,000 IU; Vitamin D₃ : 1,080 IU; Vitamin E (dl-alfa-tocopherol acetate): 36 IU; Vitamin K: 1 mg; Vitamin B₁: 2 mg; Vitamin B₂: 6 mg; Vitamin B₆: 2 mg; Vitamin B₁₂: 10 mcg/kg; Niacine: 50 mg; Calcium pantothenate: 20 mg; Pantothenic acid: 18.40 mg; Folic acid: 5 mg; Biotin: 60 mcg; Choline chloride: 260 mg; Sepiolite: 0.38 g; Butylhydroxyanisol: 0.12 mg; Butylhydroxytoluene: 1.32 mg; Etoxiquine: 0.19 mg; Diclazuril 0.5 g/100 g (Clinacox 0.5 % Premix®): 1mg. ** Values corrected for ash. *** Determined *in vivo* by a digestibility trial.

4.1.2. Experiment 2: Effect of an enzyme complex in a protein and energy reduced diet on productive performance, slaughter traits and nitrogen and energy balance in growing rabbits.

The target of this second trial was to evaluate the capacity of the same enzyme complex to compensate a reduction in digestible protein (DP) and energy (DE).

Therefore, two experimental feeds were formulated to differ in their protein (176 vs. 158 g DP/kg DM) and energy (2797 vs. 2585 kcal DE/kg DM). Control feed was the same than in the previous trial. Each feed was supplemented with three different doses of the enzymatic complex: 0, 100 and 200 ppm.

A total of 270 crossbred mixed-sex rabbits weaned at 34 d of age, with a weaning BW of 808 ± 107 g (mean $\pm$ SD), were randomly allocated to the different treatments and housed in individual cages.

Animals were blocked by litter and randomly assigned to one of the six treatments (36 animals/treatment: diet A +0 ppm enzymatic supplementation; diet A +100 ppm; diet A +200 ppm; diet B +0 ppm; diet B +100 ppm and diet B +200 ppm). Control diet, diet A, was formulated following de Blas and Mateos (2010) recommendations (2797 kcal DE/kg DM; 176 g CP/kg DM and 359 g NDF/kg DM). Diet B was formulated to have lower DP and DE and higher NDF (2585 kcal DE/kg DM; 158 g CP/kg DM and 374 g NDF/kg DM; Table 4).

Feed intake and weight of the rabbits was measured in two periods (at weaning (34 d), 48 and 60 d) from 34 to 48 d and from 49 to 60 d of age. Daily feed intake, daily weight gain and feed conversion were calculated by period and for the whole fattening cycle. Mortality was recorded daily.

Table 4. Ingredients and analysed chemical composition of the experimental feeds (*Experiment 2*).

Ingredients composition, g/kg as fed	Diet A	Diet B
Alfalfa	250	181
Barley	233	146
Wheat bran	200	108
Sunflower meal	105	130
Sugar beet pulp	80.0	73.8
Wheat straw	50.0	150
Soybean meal	17.1	6.90
Wheat	15.7	145
Soybean oil	15.0	15.0
Sepiolite	13.1	20.0
Calcium Carbonate	10.8	13.1
Sodium Chloride	6.00	6.00
Mineral and Vitamin premix*	3.00	3.00
L-Lysine	1.30	2.00
L-Threonine	0.60	0.70
Analysed chemical composition, g/kg DM		
Dry matter	901	909
Ash	80.3	86.3
Crude Protein	176	158
Neutral Detergent Fibre**	359	374
Acid Detergent Fibre **	215	224
Acid Detergent Lignin**	44.3	45.8
Starch (enzymatic)	174	175
Ether Extract	34.2	30.0
Gross Energy, kcal/kg DM	4791	4672
Digestible Energy, kcal/DM***	2797	2585
DP/DE***	11.0	10.9

*Provided by Trouw Nutrition. Mineral and vitamin composition (per kg of complete diet): Calcium: 0.44 g/kg; Sodium: 0.33 g/kg; Magnesium (MgO): 0.24 g; Manganese (Mn₂O₃): 20 mg/kg; Zinc (ZnO): 60 mg/kg; Copper (CuSO₄·5H₂O): 18 mg/kg; Iodo (KI): 1.10 mg/kg; Cobalt (CoCO₃·H₂O): 0.49 mg/kg; Selenium (Na₂SeO₃): 0.05 mg/kg; Iron (FeCO₃): 78 mg/kg; Vitamin A: 10,000 IU; Vitamin D₃: 1,080 IU; Vitamin E (dl-alfa-tocopherol acetate): 36 IU; Vitamin K: 1 mg; Vitamin B₁: 2 mg; Vitamin B₂: 6 mg; Vitamin B₆: 2 mg; Vitamin B₁₂: 10 mcg/kg; Niacine: 50 mg; Calcium pantothenate: 20 mg; Pantothenic acid: 18.40 mg; Folic acid: 5 mg; Biotin: 60 mcg; Choline chloride: 260 mg; Sepiolite: 0.38 g; Butylhydroxyanisol: 0.12 mg; Butylhydroxytoluene: 1.32 mg; Etoxiquine: 0.19 mg; Diclazuril 0.5 g/100 g (Clinacox 0.5 % Premix®): 1mg. ** Values corrected for ash. *** Determined *in vivo* by a digestibility trial.

4.1.3. Experiment 3: Effect of different lignin levels and sources on productive performance, slaughter traits and nitrogen and energy balance in growing rabbits.

For the aim of this trial, five different treatments in a 3 x 2 factorial arrangement, with three different levels of acid detergent lignin (4.5, 5.5 and 6.5% DM) and two sources of lignin (Arbocel® or defatted grape seed meal) were used.

A total of 936 crossbred mixed-sex rabbits were used. Of all of them, 216 were weaned at 34 d, BW of 915 ± 85 g (mean $\pm$ SD) and housed in individual cages until the 60 d of age. The remaining 720 animals were weaned at 35 d of age, BW of 808 ± 107 g (mean $\pm$ SD) and housed in collective cages until 61 d.

Diets were formulated to have same energy (2886 ± 47 kcal DE/kg DM), protein (179 ± 3 g CP/kg DM) and fibre (367 ± 10 g NDF/kg DM) levels differing in lignin content (3.5, 4.5 and 5.5%) and lignin source. Feeds were formulated following de Blas and Mateos (2010) recommendations. Feed and water were supplied *ad libitum*.

Animals were blocked by litter and randomly assigned to one of the five diets (36 animals/treatment: low lignin and defatted grape seed meal (LG); low lignin and Arbocel® (LA); medium lignin and defatted grape seed meal (MG); medium lignin and Arbocel® (MA); high lignin and defatted grape seed meal (HG); high lignin and Arbocel® (HA).

Tables 5 and 6 show the chemical composition of the different lignin sources used. Whereas in Table 7 is shown the feeds ingredients and chemical composition.

In the individually housed rabbits, feed intake and weight of the rabbits was measured in two periods (at weaning (34 d), 48 and 60 d) from 34 to 48 d and from 49 to

60 d of age. In the rabbits housed collectively, weight and feed intake were measured at the beginning (35 d) and at the end (61 d) of the fattening period, in only one period from 35 to 61 d of age. Daily feed intake, daily weight gain and feed conversion ratio were calculated by period and for the whole fattening cycle. Mortality was recorded daily.

Table 5. Chemical composition of the defatted grape seed meal, comparison of different analyses (*Experiment 3*).

	García <i>et al.</i> (2002)	Gidenne (2003)	Own Analysis	FEDNA (2019)*
<i>Chemical composition:</i>				
Dry matter	90.0	-	86.0	91.8
Ash	5.84	-	3.07	2.90
Ether Extract	3.60	-	1.40	11.1
Neutral Detergent Fibre	80.6	73	65.2	67.0
Acid Detergent Fibre	72.0	65	57.1	57.0
Acid Detergent Lignin	59.0	55	44.8	42.9
Acid Detergent Cutin	46.0	-	-	-
Crude Fibre	46.3	44.1	42.8	41.4
Crude Protein	11.0	9.9	9.98	9.30
Insoluble Pectines in water	-	20	-	-
Gross Energy, kcal/kg DM	4899	-	-	-

* Non-Defatted Grape Pips.

Table 6. Chemical composition of Arbocel® (%), comparison of different analysis (*Experiment 3*).

	Colin <i>et al.</i> (2007)	Own Analysis
Protein	0.80	-
Ether Extract	0.10	-
Crude Fibre	67.6	69.4
Crude Ashes	0.30	-
Starch	0.00	-
Neutral Detergent Fibre	84.8	86.6
Acid Detergent Fibre	70.8	73.2
Acid Detergent Lignin	23.9	27.7

Table 7. Ingredients and analysed chemical composition of the experimental feeds (*Experiment 3*).

Ingredients composition, g/kg as fed	LG & LA	MG	HG	MA	HA
Wheat bran	320	320	320	276	265
Alfalfa	306	313	320	320	285
Wheat	150	150	150	150	151
Sunflower meal	62.4	79.3	96.2	124	89.6
Wheat straw	60.0	30.0	-	38.7	-
Soybean meal	47.4	28.5	9.60	11.5	57.8
Soybean oil	21.0	21.6	22.2	22.1	24.9
Sepiolite	16.8	18.4	20.0	20.0	20.0
Salt	6.00	6.00	6.00	6.00	6.00
Calcium Carbonate	4.40	6.20	8.00	7.00	8.00
Mineral and Vitamin premix*	3.00	3.00	3.00	3.00	3.00
Monocalcium Phosphate	1.70	2.40	3.00	0.60	6.00
L-Lysine, monohydrochloride	0.50	0.80	1.20	1.20	0.20
L-Treonine	0.40	0.50	0.70	0.60	0.30
Arbocel®	-	-	-	20.0	83.0
Defatted grape seeds meal	-	20.1	40.2	-	-
Analysed chemical composition, g/kg DM					
Dry matter, g/kg	890	889	895	898	897
Ash	99.9	102	104	104	102
Crude Protein	184	179	176	177	181
Neutral Detergent Fibre**	364	362	354	378	377
Acid Detergent Fibre**	213	218	220	238	238
Acid Detergent Lignin**	47.8	60.3	67.2	58.7	62.3
Starch (enzymatic)	155	156	159	151	145
Ether Extract	34.0	40.5	40.5	38.5	40.5
Gross Energy, kcal/kg	4873	4920	4875	4848	4873
Digestible Energy, kcal/kg***	2939	2909	2908	2825	2848
DP/DE***	10.9	10.6	10.2	10.5	11.0
Hemicellulose (NDF – ADL)	13.4	12.7	12.0	12.5	12.5
Cellulose (ADF – ADL)	13.4	12.8	12.3	14.6	14.4
Ratio ADL/Cellulose	0.32	0.42	0.49	0.36	0.39

LA: Low dose of Arbocel; LG: Low dose of defatted grape seed meal; MA: Medium dose of Arbocel; MG: Medium dose of defatted grape seed meal; HA: High dose Arbocel; HG: High dose defatted grape seed meal.

* Provided by Trouw Nutrition España (Tres Cantos, Spain). Mineral and vitamin composition (per kg of final feed): Calcium: 0.44 g; Sodium: 0.33g; Magnesium (MgO): 0.24 g; Manganese (Mn₂O₃): 20 mg; Zinc (ZnO): 60 mg; Copper (CuSO₄·5H₂O): 18 mg; Iodo (KI): 1.10 mg; Cobalt (CoCO₃·H₂O): 0.49 mg; Selenium (Na₂SeO₃): 0.05 mg; Iron (FeCO₃): 78 mg; Vitamin A: 10,000 IU; Vitamin D₃: 1080 IU; Vitamin E (dl-alfa-tocopherol acetate): 36 IU; Vitamin K: 1 mg; Vitamin B₁: 2 mg; Vitamin B₂: 6 mg; Vitamin B₆: 2 mg; Vitamin B₁₂: 12 mcg; Niacine: 50 mg; Calcium pantothenate: 20 mg, Pantothenic acid: 18.4 mg; Folic acid: 5 g; Biotine: 60 mcg; Choline Chloride: 260 mg; Sepiolite: 0.38; Butodroxianisol: 0.12 mg; Butohidroxitolueno: 1.32 mg; Etoxiquine: 0.19 mg; Diclazuril 0.5 g/100 g (Clinacox 0.5% Premix©): 1 mg. ** Values corrected for ash. *** Determined *in vivo* by a digestibility trial.

4.1.4. Experiment 4: Effect of feed restriction on growth performance, carcass yield and nitrogen and energy balance in growing rabbits.

A total of 600 rabbits, weaned at 34 d weighing 660 ± 64.3 g (mean $\pm$ SD), were assigned at random to the five experimental treatments (120 rabbits/treatment, resulting 24 replicates per treatment). Each treatment was applied with different levels of feed restriction. In the control group rabbits were fed *ad libitum* during the whole fattening period (34 – 61 d of age). In the other four treatments, the animals were restricted by 80 or 70% of the theoretical *ad libitum* consumption during the whole fattening period (R80 and R70 respectively), or restricted by 80 or 70% only until the 48th d of life and then fed *ad libitum* until 61 d of age (R80AL and R70AL, respectively).

All rabbits received the same feed without antibiotics (2,450 kcal DE/kg; 16% CP and 34% NDF; Table 8) during the whole experimental period. Feed intake and weight of the rabbits were measured in two periods, from 34 to 48 d and from 49 to 61 d of age. The feed conversion ratio (FCR) was calculated as well as for these two periods. Mortality was recorded daily.

Table 8. Ingredients and analysed chemical composition of the experimental feed (Experiment 4).

Ingredients composition, g/kg as fed	Experimental feed
Wheat bran	237
Barley	226
Alfalfa	192
Wheat straw	107
Sunflower meal	98.3
Soybean meal	45.8
Sugar beet pulp	39.4
Soybean oil	16.5
Sepiolite	13.4
Calcium Carbonate	13.1
Sodium Chloride	6.00
Mineral and vitamin premix*	3.00
Monocalcium phosphate	1.70
L-Lysine	0.60
DL-Methionine	0.20
Analysed chemical composition, g/kg DM	
Dry matter, g/kg	911
Ash	96.8
Crude protein	159
Neutral Detergent Fibre**	324
Acid Detergent Fibre**	189
Acid Detergent Lignin**	44.0
Starch (enzymatic)	131
Ether extract	30.0
Gross Energy (GE), kcal/kg DM	4668
Digestible Energy, kcal/kg DM***	2754

* Provided by Trouw Nutrition España (Tres Cantos, Spain). Mineral and vitamin composition (per kg of final feed): Calcium: 0.44 g; Sodium: 0.33g; Magnesium (MgO): 0.24 g; Manganese (Mn₂O₃): 20 mg; Zinc (ZnO): 60 mg; Copper (CuSO₄·5H₂O): 18 mg; Iodo (KI): 1.10 mg; Cobalt (CoCO₃·H₂O): 0.49 mg; Selenium (Na₂SeO₃): 0.05 mg; Iron (FeCO₃): 78 mg; Vitamin A: 10,000 IU; Vitamin D₃: 1080 IU; Vitamin E (dl-alfa-tocopherol acetate): 36 IU; Vitamin K: 1 mg; Vitamin B₁: 2 mg; Vitamin B₂: 6 mg; Vitamin B₆: 2 mg; Vitamin B₁₂: 12 mcg; Niacine: 50 mg; Calcium pantothenate: 20 mg; Pantothenic acid: 18.4 mg; Folic acid: 5 g; Biotine: 60 mcg; Choline Chloride: 260 mg; Sepiolite: 0.38; Butodroxianisol: 0.12 mg; Butohidroxitolueno: 1.32 mg; Etoxiquine: 0.19 mg; Diclazuril 0.5 g/100 g (Clinacox 0.5% Premix©): 1 mg.

** Values corrected for ash. *** Determined *in vivo* by a digestibility trial.

4.2. Apparent total-tract digestibility

The apparent total-tract digestibility (ATTD) of dry matter (DM), organic matter (OM), gross energy (GE), crude protein (CP), neutral detergent fibre (NDF) and ether extract (EE) were determined following the indications of the European Reference Method (Perez *et al.*, 1995). Feed intake and faeces were recorded from 53 to 57 d of age.

In experiments 1, 2 and 3 digestibility analysis were done in 11 rabbits per treatment. While in experiment 4 were used 12 rabbits per treatment. In Experiment 4, at 53 d of age, 60 animals were transferred to another facility, with same environmental conditions, and randomly housed in the individual cages. Transfer was done on day 50 to have an adaptation period of three d. The rest of experiments were housed already in the individual cages for the digestibility trial during the whole fattening period.

4.3. Slaughter traits

In order to evaluate the slaughter traits, at the end of each trial, 62 d of age, 24 animals per treatment were selected based on average body weight, and without fasting. Average body weight in each experiment were: 2355 ± 93 ; 2616 ± 86 ; 2316 ± 112 ; 1993 ± 155 g, respectively.

Rabbits were electrically stunned and immediately slaughtered and bled from 8:00 to 12:00 h (according to the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, (Directive 2010/63/EU). After that, skin, genitals, urinary bladder, gastrointestinal tract, and the distal part of the legs were removed. Body weight before the slaughter (BWS) was recorded and the carcass were weighted after cooled at 5 °C–10 °C (CCW), including the head, liver, kidneys and the organs located in the thorax and neck (lungs, oesophagus, trachea, thymus and heart). The full gastrointestinal tract (included the stomach, caecum and intestinal content and the urogenital tract with the empty urinary bladder), liver (excluding gall bladder), and the full and empty stomach, and full caecum were also weighted. The carcass assessment was done according to the recommendations of Blasco and Ouhayoun (1993). The dressing out percentage was calculated as: $CCW \times 100 / BWS$.

4.4. Carcass chemical composition and nitrogen and energy balances

In vivo whole body and carcass chemical composition (water, protein, ash and fat) and energy content were estimated by the bioelectric impedance method (BIA) in 15 animals/treatment using the multiple regression equations developed by Saiz (2016) and Saiz *et al.* (2017). Resistance and reactance were measured in rabbits with a body composition analyzer (Model Quantum II, RJL 149 Systems, Detroit, MI, USA) always in the same animal. Measures were taken twice per animal at 34 and 60 d of age and at 34 and 61 d in Experiment 4. Animals were blocked by litter.

Carcass nitrogen and energy content estimated by BIA method and the ATTD were used to obtain the nitrogen and energy retention in the carcass (NR carcass and ER carcass, respectively) between 34 and 60 d of age in 11 rabbits/treatments except in Experiment 4 that was estimated between 34 and 61 d of age in 12 animals/treatment. In the same way, estimated values for the total body nitrogen and energy content were used to obtain the nitrogen and energy retention in the whole body (NR whole body and ER whole body, respectively) at 34 and 60 d of age or 34 and 61 d in Experiment 4. Values were expressed per kg BW^{0.75} and d (BW was calculated as the average of the final and the initial BW).

Moreover, nitrogen and GE intake (Ni and GEi, respectively) and digestible nitrogen and energy intake (DNi and DEi, respectively) were recorded to calculate the overall N and GE carcass retention efficiency as: NR carcass/DNi and ER carcass/DEi, both expressed as %. The overall N and GE in whole body retention efficiency were estimated as: NR whole body/DNi and ER whole body/DEi, both expressed as %.

Total N and GE losses as skin and viscera, faeces or heat production and urine were calculated as follows:

- N lost as skin and viscera ($\text{g/kg BW}^{0.75}$ and d) = $(\text{g NR whole body} - \text{g NR carcass})/\text{kg BW}^{0.75}$ and d.
- N excreted as faeces ($\text{g/kg BW}^{0.75}$ and d) = $(\text{Ni} - \text{DNi})/\text{kg BW}^{0.75}$ and d.
- N excreted as urine ($\text{g/kg BW}^{0.75}$ and d) = $(\text{DNi} - \text{NR whole body})/\text{kg BW}^{0.75}$ and d.
- Energy lost as skin and viscera ($\text{MJ/kg BW}^{0.75}$ and d) = $(\text{MJ ER whole body} - \text{MJ ER carcass})/\text{kg BW}^{0.75}$ and d.
- Energy excreted as faeces ($\text{MJ/kg BW}^{0.75}$ and d) = $(\text{GEi} - \text{DEi})/\text{kg BW}^{0.75}$.d.
- Energy lost as urine and heat production ($\text{MJ/kg BW}^{0.75}$ and d) = $(\text{DEi} - \text{ER whole body})/\text{kg BW}^{0.75}$ and d.

4.5. Chemical analysis

All chemical analysis, both feed and faeces, were done twice. Procedures of the AOAC (2005) were used to determine DM (method 934.01), ash (method 942.05), CP (method 968.06 $\text{N} \times 6.25$; FP-528 LECO®, St. Joseph, MI, USA), ether extract (method 920.39) and starch amyloglucosidase- α -amylase method; method 996.11). Dietary NDF was determined using the filter bag system (Ankom Technology, New York) according to Maertens *et al.* (2002), and a thermo-stable amylase without any sodium sulphite added. It was corrected for its own ash. Dietary acid detergent fibre and acid detergent lignin were analyzed according to Goering and Van Soest (1970). Gross energy was measured by adiabatic bomb calorimeter (Model 6100, Parr Instrument Company, Moline, IL, USA).

4.6. Statistical analysis

In Experiment 1 growth traits, digestibility, slaughter traits, chemical body and carcass composition and nitrogen and energy balance were analysed using a covariance

analysis studying the linear and quadratic effects of the dose by including it as a covariate. The experimental unit was the cage. Initial weight at weaning (34 d) was used as a covariable for the growth traits, slaughter traits and slaughter weight. Carcass chemical composition at 34 d was used as a covariate of each constituent for the analysis of the carcass chemical composition at the end of each experiment. Statistical analyses were done using the GLM procedure of SAS vs 9.2 (SAS Institute Inc., 2002).

In Experiment 2 productive performance, body and carcass chemical composition and slaughter traits were analysed as a randomized 3x2 factorial model. Whereas digestibility and nitrogen and energy balance were analysed using a 2x2 factorial model (two diets x two doses (0 and 200 ppm)). The experimental unit was the cage. Initial weight at weaning (34 d) was used as a covariate for the growth traits, slaughter traits and slaughter weight, and carcass chemical composition at 34 d was used as a covariate of each constituent for the analysis of the carcass chemical composition at the end of each experiment. Statistical analyses were done using the GLM procedure of SAS vs 9.2 (SAS Institute Inc., 2002). A Tukey test was used for means comparisons.

In Experiment 3 all parameters and results were analysed as a randomized 3x2 model. The experimental unit was the cage. Initial weight at weaning (34 d) was used as a covariate for the growth traits, slaughter traits and slaughter weight, and carcass chemical composition at 34 d was used as a covariable of each constituent for the analysis of the carcass chemical composition at the end of each experiment. Statistical analyses were done using the GLM procedure of SAS vs 9.2 (SAS Institute Inc., 2002). A Tukey test was used for means comparisons. Mortality of collective cages were analysed using a Poisson model, with the GENMOD procedure and considering a Poisson distribution.

In Experiment 4, growth traits in the whole fattening period, digestibility, slaughter traits and chemical body and carcass composition were analysed as a randomized model, with the level of feed restriction as main effect of variation. Growth traits were analysed as a repeated measurement model considering the phase of fattening (34–48 d and 48–61 d) as time effect, the treatment, the interaction treatment $\times$ phase of fattening, and the weaning weight as a covariate. We compared four different model structures (compound symmetry and auto-regressive order one, both considering homo and heterogeneous variances). The best fit (considering BIC or AIC) was for a compound symmetry and heterogeneous variance. Growth traits were also analysed as a factorial arrangement (excluding control group) phase of fattening $\times$ treatment. The experimental unit for growth traits was the cage. For the analysis of the chemical composition, the initial value (at 34 d) of each constituent was used as a covariate. Statistical analyses were done using the MIXED procedure of SAS vs 9.2 (SAS Institute Inc., 2002). A Tukey test was used for means comparisons because of the interest to compare control diet with the different restriction strategies and the latter among them.

RESULTS

V. RESULTS

5.1. Experiment 1: Effect of an enzyme complex on the productive performance, slaughter traits and nitrogen and energy balance in growing rabbits.

5.1.1. Growth performance

During the first fattening phase (34 to 48 d of live), the enzymatic supplementation improved linearly ($P<0.05$) the feed conversion ratio (FCR), without affecting the average daily feed intake (ADFI) neither the average daily weight gain (DWG) (Table 9).

In the second phase (from 49 to 60 d), there was a quadratic variation for both FCR and DWG ($P<0.05$) with an optimal dose in both parameters of 400 ppm.

However, during the whole fattening phase these effects are lost or diluted, as only a quadratic tendency in FCR was observed, being numerically optimal for 400 ppm. Mortality was extremely low (1.77%). Only two rabbits from treatment B and another two from B+ 200 ppm died in whole fattening period.

5.1.2. Slaughter traits

Enzymes did not affect carcass yield (57.8 % on average; Table 10). There was only a significant effect ($P<0.05$) in the digestive tract weight, relative to the live weight of the animal, that increased linearly with the enzyme supplementation.

5.1.3. Apparent total-tract digestibility (ATTD)

Dry matter, organic matter, gross energy, and ether extract digestibility decreased linearly ($P<0.05$) with the multienzyme complex inclusion (Table 11). However, dry

matter intake, crude protein, and neutral detergent fibre digestibility were not affected by the enzyme supplementation ($P>0.05$).

5.1.4. Carcass chemical composition and nitrogen and energy balances

Carcass chemical composition was not affected by the enzymatic complex supplementation (Table 12).

Any of the parameters evaluated for the nitrogen balance were affected ($P>0.05$) by the treatments (Table 13), except energy balance that showed a significant ($P<0.05$) linear decrease on digestible energy intake and an increase in the energy excreted in the faeces.

Table 9. Effect of enzyme dose supplementation (100, 200, 400 and 800 ppm) on zootechnical parameters in rabbits from 34 to 60 d of age. (*Experiment 1*).

Item	Treatments					SEM*	p-Value	
	Basal (B)	B+ 100ppm	B+ 200ppm	B+ 400ppm	B+ 800ppm		Linear	Quadratic
1st phase: from 34 to 48 d of life								
Weight at 34 d, g	823	809	832	798	821	12.2	0.91	0.27
Weight at 48 d, g	1680	1657	1645	1666	1679	11.5	0.30	0.10
Daily weight gain, g	61.6	60.0	59.1	60.6	61.5	0.82	0.30	0.10
Daily feed intake, g	125	120	121	121	122	1.36	0.84	0.06
Feed conversion ratio, g/g	2.03	2.01	2.04	2.00	1.99	0.01	<0.05	0.83
2nd phase: from 49 to 60 d of life								
Weight at 60 d, g	2295	2287	2264	2315	2300	15.9	0.30	0.86
Daily weight gain, g	51.3	52.5	51.6	54.1	51.7	0.69	0.59	<0.05
Daily feed intake, g	164	167	166	166	168	1.71	0.17	0.95
Feed conversion ratio, g/g	3.21	3.19	3.23	3.08	3.26	0.03	0.41	<0.05
Whole phase: from 34 to 60 d of life								
Daily weight gain, g	56.8	56.5	55.6	57.6	57.0	0.61	0.30	0.86
Daily feed intake, g	143	142	141	142	143	1.34	0.48	0.31
Feed conversion ratio, g/g	2.52	2.51	2.54	2.46	2.52	0.02	0.58	0.07

*SEM: Standard Error of Means (n = 45 animals/treatment).

Table 10. Effect of enzyme dose supplementation (100, 200, 400 and 800 ppm) on slaughter traits at 62 d. (Experiment 1)

Item	Treatments					SEM*	<i>p</i> -Value	
	Basal (B)	B+ 100ppm	B+ 200ppm	B+ 400ppm	B+ 800ppm		<i>Linear</i>	<i>Quadratic</i>
Body weight at slaughter, g	2372	2321	2347	2373	2364	16.7	0.43	0.87
Cold carcass weight, g	1280	1235	1256	1268	1265	11.3	0.70	0.45
Carcass yield, %	58.2	57.4	57.9	57.6	57.8	0.29	0.69	0.35
Digestive tract weight, % BW [#]	17.4	17.8	17.6	17.9	18.2	0.25	<0.05	0.64
Liver weight, % BW	4.17	4.19	4.40	4.18	4.25	0.11	0.82	0.62
Full stomach weight, % BW	4.25	4.62	4.21	4.55	4.58	0.14	0.17	0.83
Empty stomach weight, % BW	0.89	0.96	0.94	0.94	0.95	0.02	0.13	0.17
Full caecal weight, % BW	6.55	6.67	6.92	6.66	6.87	0.17	0.31	0.69

*SEM: Standard Error of Means (n = 24 animals/treatment).

[#] Body weight.**Table 11.** Effect of enzyme dose supplementation (100, 200, 400 and 800 ppm) on apparent total-tract digestibility (ATTD) from 53 to 57 d of age. (Experiment 1)

Item	Treatments				SEM*	<i>p</i> -Value	
	Basal (B)	B+ 200ppm	B+ 400ppm	B+ 800ppm		<i>Linear</i>	<i>Quadratic</i>
Dry matter intake, g/d	150	154	155	151	5.10	0.90	0.38
Digestibility Coefficients, %							
Dry Matter	58.4	57.3	56.8	56.6	0.48	<0.05	0.19
Organic Matter	60.3	58.9	58.9	58.4	0.50	<0.05	0.26
Crude Protein	69.3	67.1	68.0	67.5	0.80	0.22	0.35
Gross Energy	59.9	58.5	57.1	56.8	0.47	<0.001	0.03
Neutral Detergent Fibre	19.2	20.8	20.0	19.1	1.11	0.69	0.36
Ether Extract	86.6	86.4	85.6	84.7	0.45	<0.05	0.88

* SEM: Standard Error of Means (n = 11 animals/treatment).

Table 12. Effect of enzyme dose supplementation (100, 200, 400 and 800 ppm) on carcass chemical composition at 34 and 60 d of age. (Experiment 1)

Item	Treatments					SEM*	p-Value	
	Basal (B)	B+ 100ppm	B+ 200ppm	B+ 400ppm	B+ 800ppm		Linear	Quadratic
Carcass composition at 34 d of age, % DM								
Body weight, g	798	836	808	781	803	32.0	0.67	0.71
Water, %	71.1	71.3	71.2	71.4	71.2	0.15	0.79	0.35
Crude Protein	62.7	63.2	62.8	63.2	63.0	0.47	0.73	0.56
Ash	16.1	16.0	16.2	16.1	16.1	0.22	0.90	0.80
Ether extract	19.5	22.0	19.6	19.1	19.4	1.26	0.42	0.76
Gross Energy, kJ/100 g DM	2114	2124	2109	2109	2114	13.7	0.80	0.71
Carcass composition at 60 d of life, % DM								
Body weight, g	2292	2294	2277	2261	2286	62.7	0.90	0.71
Water, %	66.8	66.7	67.1	66.7	66.6	0.31	0.57	0.73
Crude Protein	55.3	54.8	55.8	54.9	55.4	0.70	0.88	0.86
Ash	12.6	12.5	12.7	12.5	12.5	0.36	0.91	0.99
Ether extract	29.2	29.8	28.5	29.7	29.5	0.69	0.77	0.94
Gross Energy, kJ/100 g DM	2446	2451	2434	2453	2445	26.4	0.97	0.97

* SEM: Standard Error of Means (n = 15 animals/treatment).

Table 13. Effect of enzyme dose supplementation (100, 200, 400 and 800 ppm) on nitrogen and energy balance from 34 to 60 d. (Experiment 1).

Item	Treatments				SEM*	<i>p</i> -Value	
	Basal (B)	B+ 200ppm	B+ 400ppm	B+ 800ppm		<i>Linear</i>	<i>Quadratic</i>
Nitrogen intake[#] [g/kg BW^{0.75}.d]:							
Ni	2.590	2.619	2.634	2.617	0.042	0.699	0.519
DNi	1.792	1.746	1.798	1.760	0.031	0.716	0.929
Nitrogen retained[#] [g/kg BW^{0.75}.d]¹:							
NR body	1.103	1.093	1.104	1.094	0.019	0.818	0.957
NR carcass	0.535	0.541	0.537	0.529	0.010	0.571	0.591
Nitrogen efficiency							
NR body/DNi	0.617	0.626	0.614	0.624	0.013	0.816	0.894
NR carcass/DNi	0.300	0.310	0.299	0.301	0.007	0.776	0.775
<i>Nitrogen losses</i> [g/kg BW^{0.75}.d]							
Skin and Viscera	0.567	0.553	0.567	0.564	0.010	0.845	0.759
Faeces	0.799	0.873	0.836	0.856	0.003	0.218	0.264
Urine	0.688	0.654	0.695	0.667	0.032	0.847	0.929
Energy intake[#], MJ/kg BW^{0.75}.d							
GEi	1.671	1.693	1.684	1.688	0.027	0.763	0.766
DEi	1.004	0.992	0.964	0.956	0.016	<0.05	0.506
Energy retained[#], kJ/kg BW^{0.75}.d²							
ER body	394	385	396	391	11.718	0.969	0.980
ER carcass	233	229	233	232	7.490	0.995	0.899
Energy efficiency							
ER body/Dei	0.394	0.385	0.396	0.391	0.012	0.961	0.989
ER carcass/DEi	0.233	0.229	0.233	0.232	0.007	0.990	0.907
Energy losses, MJ/kg BW^{0.75}.d							
Skin and viscera	0.159	0.155	0.163	0.160	0.005	0.695	0.774
Faeces	0.644	0.675	0.691	0.706	0.012	<0.05	0.197
Urine + heat production	0.609	0.606	0.567	0.567	0.018	0.065	0.541

** Standard Error of Means (n = 11 animals/treatment). #Ni: nitrogen intake. DNi: digestible nitrogen intake. NR: nitrogen retained. GEi: gross energy intake. DEi: digestible energy intake. ER: energy retained. Skin and viscera (g/kg BW^{0.75} and d): (g N retained in body – g retained in carcass)/kg BW^{0.75} and d. Faeces (g/kg BW^{0.75} and d): (Total N intake -DNi)/kg BW^{0.75} and d. Urine (g/ kg BW^{0.75} and d): (DNi – N Retained in carcass – N excreted in skin and viscera)/kg BW^{0.75} and d. Skin and viscera (MJ/kg BW^{0.75} and d): (MJ gross energy retained in – MJ gross energy retained in carcass)/kg BW^{0.75} and d. Faeces (MJ/kg BW^{0.75} and d): (Gross Energy intake -DEi)/kg BW^{0.75} and d. Urine + heat production (MJ/ kg BW^{0.75} and d): (DEi – Gross energy retained in carcass – GE excreted in skin and viscera)/kg BW^{0.75} and d.

5.2. Experiment 2: Effect of an enzyme complex in a protein and energy reduced diet on productive performance, slaughter traits and nitrogen and energy balance in growing rabbits.

5.2.1. Growth performance

Enzyme dose did not influence on any of the zootechnical parameters studied ($P>0.05$; Table 14). Any interaction between diet and dose was observed ($P>0.05$). However, diet had a significant effect ($P<0.05$) on live weight at 48 d (1650 vs. 1622 g on average, for diet A and B, respectively) and tended ($P=0.095$) to reduce live weight at 60 d in the rabbits fed the diet B, with lower DP/DE. There was a significant effect of live weight at weaning (34 d), therefore, initial live weight was used as a covariable for the analyses of productive performance.

In B diet feed intake tended ($P=0.057$) to be higher in the first phase, whereas in the second phase (49 to 60 d) and during the whole fattening (34 to 60 d) it was +3.74 % and +2.92 significantly ($P<0.05$) higher than in diet A.

In diet A DWG tended to be higher (+3.4%, $P<0.05$) in the first phase (34 to 48 d). However, during the second phase this effect disappear and there was not observed any significance ($P>0.05$) between diets (52.05 g/d on average). In the whole phase (34 to 60 d) DWG in diet A tended ($P=0.095$) to be higher.

Mortality was low (1.85 % on average) and no effect of diet, enzyme dose or interaction was observed.

5.2.2. Slaughter traits

Enzyme supplementation did not show any significant effect on the slaughter traits studied. No interaction between diet and enzyme dose was observed.

Animals fed diet A showed higher body weight at slaughter (2337 *vs.* 2295 g and cold carcass weight (1250 *vs.* 1225 g) ($P < 0.05$; Table 15) than rabbits of the diet B. On the contrary, digestive tract weight, full and empty stomach weights were higher in diet B. Neither carcass yield nor liver nor full caecal weights were affected by any of the treatments.

5.2.3. Apparent total-tract digestibility (ATTD)

Enzyme supplementation had no effect ($P > 0.05$) on the digestibility of any of the nutrients studied (Table 16).

Diet did not show any significant ($P > 0.05$) effect in DM and OM intakes, CP, ash and EE digestibility. However, DM, OM, NDF and GE digestibility was impaired in diet B (-5.98; -5.53; -24.5 and -4.90%, respectively, $P < 0.05$).

Table 14. Effect of enzyme dose supplementation (0, 100, 200 ppm) and type of diet on zootechnical parameters in rabbits from 34 to 60 d of age. (Experiment 2)

(Experiment 2)																	
Item	Diet		Enzyme dose, ppm			Diet A			Diet B			SEM*		Diet x Enz	p-Value		
	A	B	0	100	200	0	100	200	0	100	200	Diet	Enz		Diet	Enz.	Diet x Enz
1 st phase: from 34 to 48 d																	
Weight at 34 d, g	821	791	814	797	808	825	810	828	803	784	788	7.12	8.73	12.3	<0.05	0.37	0.74
Weight at 48 d, g	1650	1622	1642	1644	1624	1669	1643	1640	1614	1645	1608	6.87	8.35	11.8	<0.05	0.18	0.05
Daily weight gain, g	60.2	58.2	59.6	59.7	58.3	61.5	59.6	59.4	57.6	59.8	57.1	0.49	0.60	0.84	<0.05	0.18	0.05
Daily feed intake, g	121	124	124	123	121	124	120	120	124	126	122	0.88	1.07	1.51	0.057	0.11	0.08
Feed conversion ratio, g/g	2.02	2.13	2.09	2.06	2.08	2.02	2.01	2.03	2.16	2.11	2.13	0.01	0.01	0.02	<0.001	0.19	0.47
2 nd phase: from 49 to 60 d																	
Weight at 60 d, g	2272	2250	2270	2268	2244	2285	2270	2262	2256	2267	2227	9.40	11.4	16.1	0.095	0.21	0.57
Daily weight gain, g	51.8	52.3	52.4	52.0	51.7	51.3	52.2	51.9	53.5	51.8	51.6	0.45	0.54	0.76	0.434	0.69	0.17
Daily feed intake, g	165	171	167	170	167	164	166	166	171	174	169	1.16	1.41	1.99	<0.05	0.34	0.38
Feed conversion ratio, g/g	3.20	3.29	3.21	3.28	3.25	3.20	3.19	3.22	3.22	3.37	3.29	0.02	0.03	0.04	<0.05	0.21	0.10
Whole phase: from 34 to 60 d																	
Daily weight gain, g	56.3	55.5	56.2	56.2	55.2	56.8	56.2	55.9	55.7	56.1	54.6	0.36	0.44	0.62	0.095	0.21	0.57
Daily feed intake, g	142	146	144	144	142	142	141	141	146	148	143	0.86	1.05	1.48	<0.05	0.32	0.23
Feed conversion ratio, g/g	2.52	2.63	2.56	2.57	2.58	2.51	2.51	2.53	2.62	2.64	2.63	0.01	0.01	0.02	<0.001	0.70	0.60

* Standard Error of Means (n = 45 animals/treatment).

Table 15. Effect of enzyme dose supplementation (0, 100, 200 ppm) and type of diet on slaughter traits at 62 d. (*Experiment 2*)

Item	Diet		Enzyme dose, ppm			Diet A			Diet B			SEM*			<i>p</i> -Value		
	A	B	0	100	200	0	100	200	0	100	200	Diet	Enz.	Diet x Enz.	Diet	Enz.	Diet x Enz.
Body weight at slaughter, g	2337	2295	2336	2311	2301	2362	2313	2337	2310	2310	2266	10.9	13.3	18.8	<0.05	0.17	0.18
Cold Carcass weight, g	1250	1225	1250	1231	1232	1273	1229	1249	1227	1232	1215	6.99	8.5	12.0	<0.05	0.19	0.11
Carcass yield, %	57.8	57.7	57.8	57.5	57.8	58.1	57.3	57.8	57.5	57.6	57.9	0.16	0.20	0.28	0.66	0.32	0.31
Digestive tract weight, %BW [#]	17.7	18.6	18.1	18.1	18.2	17.4	17.9	17.7	18.7	18.4	18.7	0.14	0.18	0.25	<0.001	0.89	0.22
Liver weight, % BW	4.26	4.29	4.29	4.23	4.31	4.18	4.21	4.40	4.41	4.25	4.21	0.07	0.08	0.12	0.77	0.78	0.21
Full stomach weight, %BW	4.37	4.95	4.65	4.74	4.59	4.26	4.64	4.21	5.04	4.84	4.96	0.08	0.10	0.13	<0.001	0.52	0.06
Empty stomach weight, %BW	0.93	0.97	0.93	0.96	0.96	0.89	0.96	0.95	0.97	0.96	0.97	0.01	0.01	0.02	<0.05	0.10	0.06
Full caecal weight, %BW	6.77	6.91	6.74	6.74	7.04	6.61	6.71	6.98	6.87	6.76	7.10	0.10	0.12	0.17	0.30	0.11	0.81

*Standard Error of Means (n = 24 animals/treatment).

[#] BW: Body Weight.

Table 16. Effect of enzyme dose supplementation (0, 200 ppm) and type of diet on apparent total-tract digestibility (ATTD) from 53 to 57 d. (*Experiment 2*)

Item	<u>Diet</u>		<u>Enzyme dose, ppm</u>		<u>Diet A</u>		<u>Diet B</u>		<u>SEM*</u>			<u>p-Value</u>		
	A	B	0	200	0	200	0	200	Diet	Enz.	Diet x Enz	Diet	Enz.	Diet x Enz
Dry matter intake, g/d	152	155	153	154	150	154	156	153	2.98	2.98	4.21	0.49	0.89	0.38
Digestibility coefficients, %														
Dry matter	57.8	54.4	56.1	58.4	58.4	57.3	53.9	54.9	0.35	0.35	0.50	<0.001	0.96	0.06
Organic matter	59.6	56.3	58.2	57.7	60.3	58.9	56.0	56.5	0.36	0.36	0.50	<0.001	0.38	0.07
Crude protein	68.2	67.7	68.1	67.8	69.3	67.1	67.0	68.5	0.67	0.68	0.95	0.61	0.75	0.06
Gross energy	59.2	56.3	57.9	57.6	59.9	58.5	55.9	56.7	0.51	0.51	0.72	<0.05	0.71	0.14
Neutral detergent fibre	20.0	15.1	17.0	18.1	19.2	20.8	14.8	15.4	0.83	0.83	1.18	<0.05	0.36	0.64
Ether extract	86.5	85.6	85.9	86.2	86.6	86.4	85.1	86.0	0.43	0.43	0.60	0.13	0.58	0.42

*Standard Error of Means (n = 11 animals/treatment).

BW: Body Weight.

5.2.4. Carcass chemical composition and nitrogen and energy balances

Carcass chemical composition were not affected ($P>0.05$) by enzyme complex supplementation. No interaction was neither observed between diet and enzyme supplementation (Table 17).

However, chemical composition was affected by the diet. There were initial significant ($P<0.05$) differences of diet on weaning weight (34 d), crude protein and ether extract and a tendency ($P=0.06$) on ash content. Crude protein (62.3 *vs.* 63.8 %DM) was higher in B than in A diet, while weaning weight (814 *vs.* 752 g), ash (16.3 *vs.* 16.1 %DM; $P=0.06$) and ether extract (19.5 *vs.* 19.0 %DM) were lower in B with respect to A.

Each initial parameter was used as a covariable for the statistically analysis of that value at 60 d of age. At the end of the trial all parameters were significantly ($P<0.05$) affected by the diet. Diet A showed higher body weight (2246 *vs.* 2181 g), ether extract (29.1 *vs.* 27.8 %DM) and gross energy (2440 *vs.* 2386 kJ/100 g DM) and a decreased in water (66.9 *vs.* 67.6 %), crude protein (55.5 *vs.* 56.6 %DM) and ash (12.6 *vs.* 13.4 %DM) than diet B.

Regarding nitrogen balance there was significant effects on nitrogen intake (Table 18), lower consumption was observed in diet B (Ni: 2.604 *vs.* 2.388 g/kg BW^{0.75}.d; $P<0.001$; DNi: 1.769 *vs.* 1.605 g/kg BW^{0.75}.d; $P<0.001$) than in diet A. Same effect was observed in the nitrogen retained, both in live body (1.098 *vs.* 1.065 g/kg BW^{0.75}.d; $P=0.07$) and carcass (0.538 *vs.* 0.514 g/kg BW^{0.75}.d; $P<0.05$). However, the opposite effect was observed in nitrogen efficiency being higher in diet B (efficiency in carcass: 0.305 *vs.* 0.323; $P<0.05$). Due to the differences in the nitrogen intake and the efficiency, nitrogen excretion in faeces (0.836 *vs.*

0.783 g/kg BW^{0.75}.d; P<0.05) and urine (0.671 vs. 0.51 g/kg BW^{0.75}.d; P<0.05) were lower in diet B.

Enzyme supplementation decreased the nitrogen content in skin and viscera (0.565 vs. 0.544 g/kg BW^{0.75}.d; P<0.05).

There was an interaction between diet and enzyme dose for nitrogen excreted in faeces. Enzyme supplementation in diet A increases the nitrogen excretion (0.799 vs. 0.873 g/kg BW^{0.75}.d) whereas in diet B the enzyme supplementation decreased its excretion via faeces (0.827 vs. 0.738 g/kg BW^{0.75}.d).

Regarding the energy balance (Table 19), enzyme supplementation did not have any significant effect on energy balance.

Digestible energy intake of diet B was lower (P<0.05) compared with diet A (0.998 vs 0.947 MJ/kg BW^{0.75}.d. Accordingly, animals of diet B had lower energy retention efficacy, both in whole body (0.389 vs. 0.360 kJ/kg BW^{0.75}.d) and in carcass (0.231 vs. 0.211 kJ/kg BW^{0.75}.d). Due to the lower energy ingestion and efficacy, energy excreted in faeces was higher (0.660 vs. 0.705 MJ/kg BW^{0.75}.d; P<0.05) in animals of diet B, whereas energy excreted in skin and viscera was higher in diet A (0.157 vs. 0.148 MJ/kg BW^{0.75}.d).

Table 17. Effect of enzyme dose supplementation (0, 100, 200 ppm) and type of diet on carcass chemical composition at 34 and 60 d. (Experiment 2)

Item	<u>Diet</u>		<u>Enzyme dose, ppm</u>			<u>Diet A</u>			<u>Diet B</u>			<u>SEM**</u>			<u>p-Value</u>		
	A	B	0	100	200	0	100	200	0	100	200	Diet	Enz.	Diet x Enz.	Diet	Enz.	Diet x Enz.
Carcass composition at 34 d of age, % DM																	
Body weight, g	814	752	779	792	778	798	836	808	759	748	748	17.8	21.8	30.9	<0.05	0.87	0.73
Water, %	71.3	71.3	71.3	71.3	71.3	71.2	71.4	71.3	71.3	71.3	71.3	0.06	0.08	0.11	0.95	0.77	0.83
Crude protein	62.9	63.8	63.3	63.3	63.4	62.8	63.0	62.8	63.8	63.5	64.0	0.20	0.24	0.35	<0.05	0.89	0.65
Ash	16.3	16.1	16.2	16.1	16.3	16.2	16.2	16.4	16.2	16.0	16.2	0.06	0.07	0.11	0.06	0.49	0.78
Ether extract	19.5	19.0	19.3	19.2	19.2	19.5	19.4	19.5	19.0	19.0	18.9	0.08	0.10	0.14	<0.05	0.87	0.89
Gross Energy, kJ/100 g DM	2101	2107	2104	2108	2100	2103	2103	2097	2104	2112	2104	3.04	3.71	5.26	0.19	0.38	0.74
Carcass composition at 60 d of age, % DM																	
Body weight, g	2246	2181	2245	2212	2183	2284	2223	2230	2206	2201	2136	18.8	22.3	31.9	<0.05	0.17	0.48
Water, %	66.9	67.6	67.2	67.1	67.5	66.8	66.7	67.1	67.6	67.4	67.9	0.14	0.18	0.25	<0.05	0.23	0.95
Crude protein	55.5	56.6	55.9	55.7	56.5	55.5	54.8	56.0	56.3	56.5	57.1	0.33	0.39	0.56	<0.05	0.29	0.72
Ash	12.6	13.4	12.9	13.0	13.1	12.5	12.5	12.7	13.3	13.4	13.6	0.16	0.19	0.27	<0.05	0.70	0.97
Ether extract	29.1	27.8	28.7	28.8	27.8	29.2	29.5	28.5	28.1	28.1	27.2	0.26	0.32	0.46	<0.05	0.16	0.94
Gross Energy, kJ/100 g DM	2440	2386	2422	2412	2404	2447	2440	2432	2397	2383	2376	8.75	1.7	15.16	<0.05	0.64	0.98

** Standard Error of Means (n = 15 animals/treatment).

Table 18. Effect of enzyme dose supplementation (0, 200 ppm) and type of diet on nitrogen balance from 34 to 60 d. (*Experiment 2*)

Item	<u>Diet</u>		<u>Enzyme dose,</u> <u>ppm</u>		<u>Diet A</u>		<u>Diet B</u>		<u>SEM*</u>			<u>p-Value</u>		
	A	B	0	200	0	200	0	200	Diet	Enz.	Diet x Enz	Diet	Enz.	Diet x Enz
<u>Nitrogen intake[#], g/kg BW^{0.75}.d</u>														
Ni	2.604	2.388	2.519	2.474	2.590	2.619	2.448	2.329	0.031	0.031	0.044	<0.001	0.31	0.10
DNi	1.769	1.605	1.706	1.668	1.792	1.746	1.620	1.590	0.031	0.023	0.032	<0.001	0.24	0.81
<u>Nitrogen retained[#], g/kg BW^{0.75}.d¹</u>														
NR body	1.098	1.065	1.093	1.069	1.103	1.093	1.083	1.046	0.013	0.013	0.018	0.07	0.20	0.46
NR carcass	0.538	0.514	0.528	0.524	0.535	0.541	0.521	0.507	0.008	0.008	0.011	<0.05	0.71	0.36
<u>Nitrogen efficiency</u>														
NR body/DNi	0.622	0.664	0.643	0.643	0.617	0.626	0.668	0.660	0.010	0.010	0.014	<0.05	0.96	0.52
NR carcass/DNi	0.305	0.323	0.312	0.316	0.300	0.310	0.323	0.322	0.005	0.005	0.008	<0.05	0.55	0.47
<u>Nitrogen losses, g/kg BW^{0.75}.d</u>														
Skin and viscera	0.560	0.549	0.565	0.544	0.567	0.553	0.562	0.535	0.007	0.007	0.009	0.23	<0.05	0.50
Faeces	0.836	0.783	0.813	0.805	0.799 ^{ab}	0.873 ^a	0.827 ^a	0.738 ^b	0.016	0.016	0.023	<0.05	0.74	<0.05
Urine	0.671	0.541	0.613	0.599	0.688	0.654	0.538	0.544	0.022	0.022	0.031	<0.05	0.66	0.53

** Standard Error of Means (n = 11 animals/treatment).

^{a,b} Values within a row with different superscripts differ significantly at P < 0.05.

Ni: nitrogen intake. DNi: digestible nitrogen intake. NR: nitrogen retained. Skin and viscera (g/kg BW^{0.75} and d): (g N retained in body – g retained in carcass)/kg BW^{0.75} and d. Faeces (g/kg BW^{0.75} and d): (Total N intake – DNi)/kg BW^{0.75} and d. Urine (g/ kg BW^{0.75} and d): (DNi – N Retained in carcass – N excreted in skin and viscera)/kg BW^{0.75} and d.

Table 19. Effect of enzyme dose supplementation (0, 200 ppm) and type of diet on energy balance from 34 to 60 d. (*Experiment 2*)

Item	Diet		Enzyme dose, ppm		Diet A		Diet B		SEM*			<i>p</i> -Value		
	A	B	0	200	0	200	0	200	Diet	Enz.	Diet x Enz	Diet	Enz.	Diet x Enz
Energy intake[#], MJ/kg BW^{0.75}.d														
GEi	1.682	1.678	1.686	1.674	1.671	1.693	1.700	1.656	0.021	0.021	0.030	0.90	0.71	0.28
DEi	0.998	0.947	0.978	0.967	1.004	0.992	0.951	0.942	0.012	0.012	0.017	<0.05	0.54	0.92
Energy retained[#], kJ/kg BW^{0.75}.d¹														
ER body	389	360	383	365	394	385	372	347	6.948	6.948	9.824	<0.05	0.09	0.40
ER carcass	231	211	226	217	233	229	218	204	4.621	4.621	6.534	<0.05	0.18	0.47
Energy efficiency														
ER body/DEi	0.389	0.360	0.383	0.366	0.394	0.385	0.372	0.347	0.007	0.007	0.010	<0.05	0.09	0.40
ER carcass/DEi	0.231	0.211	0.226	0.217	0.233	0.229	0.218	0.204	0.005	0.005	0.007	<0.05	0.18	0.47
Energy excreted, MJ/kg BW^{0.75}.d														
Skin and viscera	0.157	0.148	0.157	0.149	0.159	0.155	0.154	0.142	0.003	0.003	0.005	<0.05	0.09	0.36
Faeces	0.660	0.705	0.683	0.681	0.644 ^b	0.675 ^{ab}	0.722 ^a	0.687 ^{ab}	0.011	0.011	0.016	<0.05	0.91	<0.05
Urine + heat production	0.608	0.588	0.594	0.601	0.609	0.606	0.579	0.596	0.012	0.012	0.018	0.26	0.69	0.58

** Standard Error of Means (n = 11). ^{a,b} Values within a row with different superscripts differ significantly at P < 0.05.

GEi: gross energy intake. DEi: digestible energy intake. ER: energy retained. Skin and viscera (MJ/kg BW^{0.75} and d): (MJ gross energy retained in – MJ gross energy retained in carcass)/kg BW^{0.75} and d. Faeces (MJ/kg BW^{0.75} and d): (Gross Energy intake – DEi)/kg BW^{0.75} and d. Urine + heat production (MJ/kg BW^{0.75} and d): (DEi – Gross energy retained in carcass – GE excreted in skin and viscera)/kg BW^{0.75} and d.

5.3. Experiment 3: Effect of different lignin levels and sources on productive performance, slaughter traits, and nitrogen and energy balance in growing rabbits.

5.3.1. Growth performance

In the individual housed rabbits, lignin level did not affect ($P > 0.1$) on any of the studied parameters, there was neither an interaction ($P > 0.05$) between the source and level of lignin (Table 20). However, in treatment HA was observed a tendency to impair FCR during the first period and the whole fattening phase ($P=0.06$ and $P=0.08$; respectively). This interaction could indicate an effect of the source that is supported by the impairment of FCR with Arbocel® during the second fattening phases (3.33 vs. 3.23 g/g; $P<0.05$) and in the global period (2.56 vs. 2.52; $P<0.05$), and by the tendency to increase the feed intake during the first phase (129 vs. 127 g; $P=0.07$) without effect neither on the next period nor on the global one. The growth was similar independently of the source for all the phases.

In the collective housed animals (Table 21), there were a significant effect ($P<0.05$) of the source, the level, and an interaction between them on FCR. Like observed in the individual fattening, the rabbits fed with the highest level of Arbocel® worsen their FCR (+1,42 %, $P<0.05$) however, no effect was observed in the defatted grape seed meal. The highest level of lignin impaired the FCR compared with the other two levels (2.70 vs. 2.66 g/g, $P<0.05$). Defatted grape seed meal improved FCR compared with Arbocel® (2.65 vs. 2.69; $P<0.05$). Despite the effect on FCR, no differences were observed ($P>0.05$) neither on the feed intake (139 g/d) nor on growth (52.3 g/d), consequently final weight was similar.

The mortality was extremely low, 0.56% in the individual cages and 2% in the group ones, and there were no differences ($P>0.05$) among treatments.

5.3.2. Slaughter traits

Body weight at slaughter and carcass weight were significantly influenced by the level of ADL ($P<0.05$; Table 22). Rabbits feed the M level of ADL showed the lowest weight for both variables with respect to the other two levels.

Higher lignin levels tended to decrease total digestive tract weight (L: 17.9% *vs.* average M&H: 17.45%; $P=0.052$). This tendency could be related with the significance decrease ($P<0.05$) observed in full caecal weigh with the increase of lignin level.

Despite the impact on digestive tract weigh, carcass yield was not influenced neither by the level nor by the source of lignin.

5.3.3. Apparent total-tract digestibility (ATTD)

Arbocel® presented a lower digestibility for OM (58.9 *vs.* 59.7 %; $P<0.05$) than defatted grape seed meal (Table 23). Digestibility of DM, OM and GE were higher in the low ADL level treatments than the other two: DM (58.3 *vs.* 56.75%), MO (60.2 *vs.* 58.8 %), GE (60.2 *vs.* 58.9 %). CP digestibility was lower in medium lignin level than L level (70.1 *vs.* 72.6%; $P<0.05$), while H level had an intermediate value (71.0%). EE digestibility increased with inclusion of ADL level (85.0, 86.1 and 88.2 %, respectively).

There was an interaction between source and level for EE digestibility because the digestibility in L and M levels with Arbocel® were similar (85.3 %, on average) but H level was significantly higher (89.4 %), however, when defatted grape seed meal source is used, M and H lignin level were similar (86.7 %, on average) but L level had low digestibility (84.6 %).

Table 20. Effect of lignin source and level on zootechnical parameters in rabbits from 34 to 60 d of age, breed in individual cages. (*Experiment 3*)

	<u>Source (S)</u>		<u>Level (L)**</u>			<u>Arbocel® (A)</u>			<u>Defatted Grape Seed Meal (G)</u>			<u>SEM*</u>			<u>P-value</u>		
	A	G	Low (L)	Medium (M)	High (H)	L	M	H	L	M	H	S	L	SxL	S	L	SxL
1st phase: from 34 to 48 d																	
Weight at 34 d, g	922	909	922	907	918	925	909	931	919	905	904	7.25	8.81	12.3	0.21	0.46	0.56
Weight at 48 d, g	1807	1796	1803	1795	1806	1804	1809	1807	1803	1781	1804	6.25	7.59	10.6	0.20	0.53	0.35
Daily weight gain, g	63.4	62.6	63.1	62.5	63.3	63.2	63.5	63.4	63.1	61.5	63.2	0.45	0.54	0.76	0.21	0.53	0.35
Daily feed intake, g	129	127	128	128	130	128	129	132	127	126	128	0.84	1.02	1.43	0.07	0.13	0.46
Feed conversion ratio, g/g	2.05	2.04	2.02	2.05	2.06	2.02	2.03	2.08	2.02	2.06	2.03	0.01	0.01	0.02	0.64	0.12	0.06
2nd phase: from 49 to 60 d																	
Weight at 60 d, g	2405	2406	2407	2396	2414	2407	2406	2402	2407	2386	2426	9.79	11.9	16.6	0.90	0.53	0.38
Daily weight gain, g	49.8	50.9	50.3	50.1	50.7	50.3	49.7	49.5	50.4	50.5	51.8	0.50	0.62	0.86	0.13	0.78	0.41
Daily feed intake, g	165	163	164	163	166	164	165	166	164	160	166	1.18	1.44	2.01	0.28	0.22	0.37
Feed conversion ratio, g/g	3.33	3.23	3.28	3.27	3.29	3.28	3.34	3.37	3.28	3.19	3.21	0.02	0.03	0.04	<0.05	0.87	0.11
Whole phase: from 34 to 60 d																	
Daily weight gain, g	57.1	57.2	57.2	56.8	57.5	57.2	57.2	57.0	57.2	56.4	57.9	0.38	0.46	0.64	0.90	0.53	0.38
Daily feed intake, g	146	144	144	144	147	144	146	148	144	142	145	0.90	1.09	1.53	0.12	0.13	0.51
Feed conversion ratio, g/g	2.56	2.52	2.52	2.54	2.55	2.52	2.55	2.59	2.52	2.52	2.51	0.01	0.01	0.02	<0.05	0.28	0.08

*SEM: Standard Error of Means (n = 36 animals/treatment).

** Low: 3.5% ADL; Medium: 4.5% ADL; High: 5.5% ADL.

Table 21. Effect of lignin source and level on zootechnical parameters in rabbits from 35 to 61 d of age, breed in collective cages. (Experiment 3)

	<u>Source (S)</u>		<u>Level (L)**</u>			<u>Arbocel® (A)</u>			<u>Defatted Grape Seed Meal (G)</u>			<u>SEM*</u>			<u>P-value</u>		
	A	G	Low (L)	Medium (M)	High (H)	L	M	H	L	M	H	S	L	SxL	S	L	SxL
Weight at 35 d, g	832	854	842	836	851	839	821	838	845	851	865	9.22	11.3	16.0	0.10	0.61	0.72
Weight at 60 d, g	2199	2204	2202	2201	2200	2200	2202	2194	2205	2201	2205	6.73	8.21	11.6	0.60	0.97	0.85
Daily weight gain, g	52.1	52.3	52.3	52.2	52.2	52.2	52.3	52.0	52.4	52.2	52.4	0.26	0.32	0.45	0.60	0.98	0.85
Daily feed intake, g	140	139	138	139	141	138	141	142	139	138	140	0.66	0.80	1.14	0.13	0.11	0.13
Feed conversion ratio, g/g	2.69	2.65	2.65 ^b	2.67 ^b	2.70 ^a	2.64 ^a	2.70 ^{bc}	2.73 ^c	2.65 ^a	2.64 ^a	2.66 ^{ab}	0.01	0.01	0.01	<0.05	<0.05	<0.05
Mortality, %	1.39	2.22	0.83	2.92	1.67	0.83	1.67	1.67	0.83	4.17	1.67	-	-	-	0.63	0.36	0.73

*SEM: Standard Error of Means (n = 24 cages/treatment).

^{a-c} Values within a row with different superscripts differ significantly at P<0.05.

** Low: 3.5% ADL; Medium: 4.5% ADL; High: 5.5% ADL).

Table 22. Effect of lignin source and level on slaughter traits at 62 d. (*Experiment 3*)

	<u>Source (S)</u>		<u>Level (L)**</u>			<u>Arbocel® (A)</u>			<u>Defatted Grape Seed Meal (G)</u>			<u>SEM*</u>			<u>P-value</u>		
	A	G	Low (L)	Medium (M)	High (H)	L	M	H	L	M	H	S	L	SxL	S	L	SxL
Body weight at slaughter, g	2513	2515	2521 ^a	2486 ^b	2536 ^a	2515	2471	2554	2527	2502	2518	9.79	11.2	16.9	0.87	<0.05	0.11
Cold carcass weight, g	1462	1465	1471 ^a	1447 ^b	1473 ^a	1466	1437	1482	1475	1457	1464	6.15	7.02	10.6	0.69	<0.05	0.15
Carcass Yield, %	58.2	58.2	58.3	58.2	58.1	58.3	58.1	58.1	58.4	58.2	58.1	0.12	0.14	0.21	0.61	0.49	1.00
Digestive tract weight, %BW [#]	17.7	17.5	17.9 ^a	17.4 ^b	175 ^b	17.9	17.3	17.7	17.8	17.6	17.2	0.11	0.14	0.19	0.37	0.05	0.12
Full stomach weight, %BW	4.16	4.12	4.23	4.20	3.99	4.26	4.07	4.14	4.20	4.33	3.83	0.09	0.11	0.15	0.77	0.18	0.14
Empty stomach weight, %BW	0.93	0.93	0.93	0.94	0.91	0.93	0.95	0.90	0.93	0.92	0.92	0.01	0.01	0.02	0.89	0.20	0.25
Full caecal weight, %BW	6.38	6.21	6.63 ^a	6.16 ^b	6.09 ^b	6.66	6.34	6.13	6.60	5.98	6.04	0.08	0.10	0.14	0.14	<0.05	0.49

*SEM: Standard Error of Means (n = 24 animals/treatment).

** Low: 3.5% ADL; Medium: 4.5% ADL; High: 5.5% ADL).

BW: Body Weight.

^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 23. Effect of lignin source and level on apparent total-tract digestibility (ATTD) from 53 to 57 d. (*Experiment 3*)

	<u>Source (S)</u>		<u>Level (L)**</u>			<u>Arbocel® (A)</u>			<u>Defatted Grape Seed Meal (G)</u>			<u>SEM*</u>			<u>P-value</u>		
	A	G	Low (L)	Medium (M)	High (H)	L	M	H	L	M	H	S	L	SxL	S	L	SxL
Dry matter intake, g/d	164	164	165	164	163	163	165	165	166	164	162	2.49	3.06	4.31	0.98	0.95	0.77
Digestibility coefficients, %																	
Dry matter	56.9	57.7	58.3 ^a	56.8 ^b	56.7 ^b	58.4	56.3	56.0	58.2	57.3	57.5	0.27	0.33	0.47	<0.05	<0.05	0.22
Organic matter	58.8	59.7	60.2 ^a	58.7 ^b	58.9 ^b	60.3	58.2	58.0	60.1	59.3	59.7	0.26	0.32	0.45	<0.05	<0.05	0.11
Crude protein	71.3	71.2	72.6	70.1	71.0	72.6	69.4	71.9	72.5	70.9	70.2	0.56	0.69	0.97	0.94	0.05	0.23
Gross energy	59.0	59.7	60.2 ^a	58.8 ^b	59.0 ^b	60.3	58.4	58.4	60.2	59.2	59.6	0.28	0.34	0.48	0.09	<0.05	0.38
Neutral detergent fibre	18.9	18.4	19.6	18.5	17.7	19.9	19.1	17.5	19.3	17.9	18.0	0.55	0.68	0.96	0.55	0.16	0.68
Ether extract	86.3	86.0	84.7 ^b	85.8 ^b	88.0 ^a	84.7 ^d	85.1 ^{cd}	89.2 ^a	84.6 ^d	86.6 ^{bc}	86.8 ^b	0.32	0.41	0.58	0.48	<0.001	<0.05

*SEM: Standard Error of Means (n = 11 animals/treatment).

** Low: 3.5% ADL; Medium: 4.5% ADL; High: 5.5% ADL).

^{a-d} Values within a row with different superscripts differ significantly at $P < 0.05$.

5.3.4. Carcass chemical composition and nitrogen and energy balances

Carcass chemical composition was not affected by the enzymatic complex supplementation (Table 24).

Any of the parameters evaluated for the nitrogen balance were affected ($P>0,05$) by the treatments (Table 25), except energy balance that showed a significant ($P<0.05$) linear decrease on digestible energy intake and an increase in the energy excreted in the faeces (Table 26).

Table 24. Effect of lignin source and level on carcass chemical composition at 34 and 60 d. (*Experiment 3*)

	<u>Source (S)</u>		<u>Level (L)**</u>			<u>Arbocel® (A)</u>			<u>Defatted Grape Seed Meal (G)</u>			<u>SEM*</u>			<u>P-value</u>		
	A	G	Low (L)	Medium (M)	High (H)	L	M	H	L	M	H	S	L	SxL	S	L	SxL
Carcass composition at 34 d of age, % DM																	
Body weight, g	942	931	952	919	938	971	914	940	933	923	937	17.3	21.2	29.9	0.66	0.54	0.74
Water, %	71.9	71.8	71.9	71.9	71.8	71.9	72.0	71.9	71.9	71.8	71.8	0.07	0.08	0.12	0.42	0.77	0.76
Crude Protein	60.3	60.0	60.4	60.2	59.9	60.4	60.5	60.1	60.4	59.8	59.7	0.28	0.34	0.48	0.36	0.57	0.77
Ash	17.3	17.4	17.4	17.4	17.4	17.4	17.3	17.3	17.4	17.4	17.4	0.12	0.15	0.21	0.53	1.00	0.97
Ether extract	18.2	18.5	17.8	18.5	18.7	17.6	18.3	18.7	18.0	18.7	18.8	0.26	0.32	0.45	0.36	0.11	0.91
Gross Energy, kJ/100 g DM	1867	1874	1864	1872	1875	1861	1864	1876	1868	1880	1874	6.11	7.48	10.6	0.43	0.60	0.70
Carcass composition at 60 d of age, % DM																	
Body weight, g	2426	2407	2440	2384	2425	2446	2403	2427	2434	2364	2422	19.5	23.9	33.7	0.49	0.23	0.86
Water, %	65.5	65.6	65.4	65.8	65.6	65.2	65.8	65.6	65.5	65.8	65.6	0.14	0.17	0.24	0.55	0.23	0.75
Crude Protein	50.2	50.8	50.0	50.9	50.5	49.4	50.8	50.4	50.6	51.1	50.7	0.44	0.54	0.76	0.37	0.49	0.81
Ash	13.5	13.2	13.4	13.4	13.2	18.0	18.0	18.0	18.1	18.2	17.8	0.11	0.13	0.14	0.09	0.53	0.35
Ether extract	35.3	35.6	35.9	34.9	35.5	36.4	34.7	34.8	35.4	35.1	36.3	0.49	0.61	0.85	0.68	0.51	0.35
Gross Energy, kJ/100 g DM	2236	2254	2258	2235	2243	2267	2220	2221	2249	2249	2266	11.0	13.5	19.0	0.25	0.47	0.24

*SEM: Standard Error of Means (n = 15 animals/treatment).

** Low: 3.5% ADL; Medium: 4.5% ADL; High: 5.5% ADL).

Table 25. Effect of lignin source and level on nitrogen and balance from 34 to 60 d. (*Experiment 3*)

	Source (S)		Level (L)**			Arbocel®			Defatted Grape Seed Meal (G)			SEM*			P-value		
	A	G	Low (L)	Medium (M)	High (H)	L	M	H	L	M	H	S	L	SxL	S	L	SxL
Nitrogen intake[#], g/kg BW^{0.75}.d[#]																	
Ni	2.776	2.729	2.805	2.747	2.706	2.786	2.784	2.758	2.823	2.710	2.653	0.029	0.035	0.050	0.255	0.139	0.319
DNi	1.990	1.934	2.025 ^a	1.959 ^{ab}	1.901 ^b	2.013	1.995	1.961	2.038	1.923	1.840	0.025	0.031	0.044	0.125	<0.05	0.236
Nitrogen retained[#], g/kg BW^{0.75}.d[#]																	
NR body	1.038	1.026	1.022	1.052	1.022	1.003	1.078	1.034	1.041	1.027	1.010	0.018	0.022	0.030	0.623	0.541	0.327
NR carcass	0.666	0.671	0.666	0.680	0.660	0.656	0.676	0.667	0.676	0.683	0.653	0.012	0.015	0.020	0.788	0.626	0.714
Nitrogen efficiency[#]																	
NR body/DNi	0.523	0.532	0.506 ^b	0.537 ^a	0.539 ^a	0.500	0.541	0.528	0.512	0.533	0.550	0.007	0.008	0.012	0.370	<0.05	0.459
NR carcass/DNi	0.335	0.348	0.328	0.347	0.348	0.326	0.339	0.340	0.331	0.355	0.357	0.005	0.007	0.009	0.108	0.070	0.791
Nitrogen losses, g/kg BW^{0.75}.d																	
Skin and viscera	0.374	0.356	0.359	0.372	0.363	0.350	0.401	0.370	0.368	0.343	0.357	0.011	0.014	0.020	0.273	0.788	0.167
Faeces	0.786	0.794	0.777	0.788	0.806	0.770	0.790	0.799	0.783	0.787	0.813	0.017	0.021	0.029	0.733	0.590	0.945
Urine	0.952	0.908	1.003 ^a	0.909 ^b	0.878 ^b	1.010	0.920	0.927	0.996	0.898	0.830	0.020	0.024	0.034	0.123	<0.05	0.423

** Low: 3.5% ADL; Medium: 4.5% ADL; High: 5.5% ADL).

^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$.

[#]Ni: nitrogen intake. DNi: digestible nitrogen intake. NR: nitrogen retained. Skin and viscera (g/kg BW^{0.75} and d): (g N retained in body – g retained in carcass)/g BW^{0.75} and d. Faeces (g/kg BW^{0.75} and d): (Total N intake -DNi)/kg BW^{0.75} and d. Urine (g/ kg BW^{0.75} and d): (DNi – N Retained in carcass – N excreted in skin and viscera)/kg BW^{0.75} and d.

Table 26. Effect of lignin source and level on energy balance from 34 to 60 d. (*Experiment 3*)

	Source (S)		Level (L)**			Arbocel®			Defatted Grape Seed Meal (G)			SEM*			P-value		
						L	M	H	L	M	H	S	L	SxL	S	L	SxL
	A	G	Low (L)	Medium (M)	High (H)												
Energy intake[#], MJ/kg BW^{0.75}.d																	
GEi	1.784	1.769	1.768	1.800	1.762	1.756	1.823	1.774	1.780	1.777	1.750	0.019	0.900	0.032	0.563	0.476	0.533
DEi	1.055	1.053	1.071	1.053	1.039	1.066	1.060	1.039	1.076	1.045	1.038	0.014	0.535	0.024	0.924	0.409	0.876
Energy retained[#], kJ/kg BW^{0.75}.d																	
ER body	398.8	396.4	396.9	403.1	392.9	387.7	410.7	397.9	406.1	395.4	387.8	7.969	9.763	13.76	0.835	0.768	0.419
ER carcass	236.5	239.5	240.7	239.2	234.1	236.6	236.7	236.1	244.8	241.6	232.1	4.679	5.733	8.081	0.649	0.696	0.740
Energy efficiency																	
ER body/DEi	0.399	0.397	0.398	0.403	0.393	0.389	0.411	0.397	0.408	0.395	0.388	0.008	0.010	0.014	0.873	0.748	0.393
ER carcass/DEi	0.238	0.240	0.242	0.239	0.236	0.239	0.238	0.238	0.246	0.240	0.233	0.005	0.006	0.008	0.801	0.720	0.774
Energy losses, MJ/kg BW^{0.75}.d																	
Skin and viscera	0.163	0.157	0.156	0.164	0.160	0.150	0.175	0.163	0.162	0.153	0.157	0.005	0.006	0.008	0.409	0.595	0.108
Faeces	0.731	0.716	0.699 ^b	0.745 ^a	0.726 ^{ab}	0.693	0.763	0.738	0.706	0.727	0.715	0.009	0.011	0.016	0.242	<0.05	0.284
Urine + heat production	0.654	0.656	0.672	0.650	0.644	0.676	0.648	0.638	0.668	0.652	0.650	0.011	0.013	0.019	0.856	0.293	0.861

** Standard Error of Means (n = 11 animals/treatment).

** Low: 3.5% ADL; Medium: 4.5% ADL; High: 5.5% ADL.

^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$.

GEi: gross energy intake. DEi: digestible energy intake. ER: energy retained.

Skin and viscera (MJ/kg BW^{0.75} and d): (MJ gross energy retained in – MJ gross energy retained in carcass)/kg BW^{0.75} and d.

Faeces (MJ/kg BW^{0.75} and d): (Gross Energy intake -DEi)/kg BW^{0.75} and d.

Urine + heat production (MJ/ kg BW^{0.75} and d): (DEi – Gross energy retained in carcass – GE excreted in skin and viscera)/kg BW^{0.75} and d.

5.4. Experiment 4: Effect of feed restriction on growth performance, carcass yield and nitrogen and energy balance in growing rabbits.

5.4.1. Growth performance

Mortality in the whole fattening period was similar among treatments (12.8% on average) and any restriction strategy reduced it (Table 27). The observed symptoms were liquid stomach, and intestinal distension with gas and mucus, which were compatible with epizootic rabbit enteropathy.

During the first fattening phase (from 34 to 48 d), 80 and 70% of restriction from *ad libitum* feed intake, decreased the growth performance (DWG) (−24.5 and −34.7%, respectively; $P < 0.001$) and impaired the feed conversion ratio (FCR) (+5.2 and +6.7%, respectively; $P < 0.001$).

In the second fattening phase (from 48 to 61 d), there was a compensatory growth in the animals re-fed *ad libitum* (R80AL = +26.5% and R70AL = +34.8%, with respect AL; $P < 0.001$). On the opposite, animals that continued restricted during the second period (R80 and R70) did not show significant differences on DWG with the control group, although the rabbits with the highest feed restriction (R70) had the lowest DWG (43.8 g/d), with respect to the other treatments.

During this period, an interaction between feeding program and restriction level was found ($P < 0.001$). Feed intake in R80AL and R70AL groups were similar (158 g/d, on average), but higher (+7.5%, $P < 0.001$) than in the control group, whereas feed intake in R80 and R70 were lower than in control group (−8.2 and −19.0%, respectively), especially in R70 treatment. Nevertheless, because the increase of feed intake (DFI) was lower than the DWG,

R80AL and R70AL treatments improved the FCR with respect the animals fed *ad libitum* (+15.8 and +20.2% for R80AL and R70AL, respectively; $P < 0.001$). The same effect was observed for treatments R80 and R70, which also improved FCR with respect AL group (+12.0 and +13.9%, respectively; $P < 0.001$), despite the fact that there was no compensatory growth in the R70 group with respect to AL group.

Rabbits restricted at 80% and later *ad libitum* (R80AL) achieved a body weight and a DWG like non-restricted animals. However, the animals restricted up to 70% only during the first fattening period (R70AL), were not able to match, neither the weight (−5.2%) nor the DWG (−7.2%) than AL. The rabbits of the treatments R80 and R70 showed, as it was expected, a lower body weight (−9.1 and −14.9%, respectively) than the animals fed *ad libitum*. Moreover, FCR was lower in the restricted animals, but only R80AL and R70AL were significantly different from the control (+4 and +5%, respectively; $P < 0.001$).

5.4.2. Slaughter traits

Despite the carcass yield of restricted treatments was similar to that of the animals fed *ad libitum*, it tended to be different ($P = 0.075$; Table 28) between R70 and R80AL, being the lowest (54.2%) and the highest (55.6%) value, respectively.

Relative digestive tract weight of all restricted groups was similar to the control group. It only differed in those treatments refed *ad libitum* (19.3 and 20.6%BW for R80AL and R70AL, respectively; $P = 0.034$). Relative liver weight was higher in R80AL with respect AL (+11.3%). On the other hand, R70 showed the lowest liver weight of all treatments (3.51%BW), being 20% lower than the AL group. R80 and R70AL had similar relative liver weights than the AL treatment. Relative full stomach weight was heavier in rabbits refed *ad*

libitum than in the rest of treatments (+15.2%; $P < 0.001$), but the weight of the empty stomach was higher in all restricted animals compared with the control ones (+8.62%; $P < 0.001$). There were no differences in the caecal weight among treatments ($P = 0.25$).

5.4.3. Apparent total-tract digestibility (ATTD)

The different restriction strategies did not affect the nutrient digestibility (Table 29), except for CP digestibility ($P = 0.017$), which were 2.5 percentage units higher in animals restricted during the whole fattening period (R80 and R70) than those restricted 80% up to 48 d (R80AL), while AL and R70AL groups showed intermediate values.

Table 27. Effect of the feeding restriction strategies on zootechnical parameters in rabbits from 34 to 61 d of age. (*Experiment 4*)

Item	Treatments*					SEM**	<i>p-Treatment</i>
	AL	R80	R70	R80AL	R70AL		
1st phase: from 34 to 48 d***							
Weight at 34 d, g	670	673	654	678	673	13.3	0.76
Weight at 48 d, g	1514 ^a	1310 ^b	1219 ^c	1315 ^b	1209 ^c	8.50	<0.001
Daily weight gain, g	60.3 ^a	45.5 ^b	40.3 ^c	45.5 ^b	38.4 ^c	0.61	<0.001
Daily feed intake, g	110 ^a	86.9 ^b	77.8 ^c	87.5 ^b	75.5 ^c	1.00	<0.001
Feed conversion ratio, g/g	1.82 ^b	1.91 ^a	1.93 ^a	1.93 ^a	1.97 ^a	0.01	<0.001
Mortality, %	3.33	6.67	3.33	5.83	4.17	2.07	0.66
2nd phase: from 48 to 61 d***							
Weight at 61 d, g	2122 ^a	1936 ^c	1790 ^d	2085 ^{ab}	2019 ^{bc}	13.4	<0.001
Daily weight gain, g	46.8 ^{cd}	48.4 ^c	43.8 ^d	59.2 ^b	63.1 ^a	0.79	<0.001
Daily feed intake****, g	147 ^b	135 ^c	119 ^d	157 ^a	159 ^a	1.72	<0.001
Feed conversion ratio, g/g	3.16 ^a	2.78 ^b	2.72 ^b	2.66 ^{bc}	2.52 ^c	0.04	<0.001
Mortality, %	6.67	5.00	10.0	11.7	7.50	2.62	0.36
Whole phase: from 34 to 61 d							
Daily weight gain, g	53.9 ^a	46.8 ^c	42.1 ^d	52.1 ^a	50.0 ^b	0.49	<0.001
Daily feed intake, g	127 ^a	109 ^c	97.1 ^d	118 ^b	113 ^c	1.03	<0.001
Feed conversion ratio, g/g	2.36 ^a	2.33 ^{ab}	2.31 ^{abc}	2.27 ^{bc}	2.24 ^c	0.02	<0.001
Mortality, %	10.0	11.7	13.3	17.5	11.7	3.10	0.48

*AL = Ad-libitum; R80 = 80% of theoretical ad libitum feed intake during whole fattening period; R70 = 70% of theoretical ad libitum feed intake during whole fattening period; R80AL = 80% of theoretical ad libitum feed intake up to 48 d; R70AL = 70% of theoretical ad libitum feed intake up to 48 d.

** Standard Error of Means (n = 24 cages/treatment).

^{a-d} Values within a row with different superscripts differ significantly at P<0.05.

*** P-phase <0.001 and P-phase x treatment <0.001 for all variables of the phases 34–48 d and 48–61 d

**** P-feeding program (AL or R) x level restriction (70 or 80%) <0.001 for daily feed intake of phase 48–61 d

Table 28. Effect of the feeding restriction strategies on slaughter traits at 61 d. (*Experiment 4*)

Item	Treatments*					SEM**	p-Value
	AL	R80	R70	R80A L	R70AL		
Body weight at slaughter, g	2014 ^a	2092 ^a	1970 ^b	2117 ^a	2055 ^{ab}	26.3	<0.001
Cold carcass weight, g	1157 ^a	1147 ^a	1067 ^b	1178 ^a	1121 ^{ab}	17.2	<0.001
Carcass Yield, %	54.9	54.9	54.2	55.6	54.6	0.35	0.08
Digestive tract weight, %BW [#]	19.4 ^{ab}	19.6 ^{ab}	19.9 ^{ab}	19.3 ^b	20.6 ^a	0.31	<0.05
Liver weight, %BW	3.97 ^b	4.07 ^{ab}	3.51 ^c	4.42 ^a	4.37 ^{ab}	0.11	<0.001
Full stomach weight, %BW	4.99 ^{bc}	5.06 ^{bc}	4.79 ^c	5.51 ^{ab}	5.90 ^a	0.15	<0.001
Empty stomach weight, %BW	1.06 ^c	1.15 ^{ab}	1.22 ^a	1.11 ^{bc}	1.15 ^{ab}	0.02	<0.001
Full caecal weight, % BW	7.30	7.61	7.39	6.71	7.14	0.25	0.25

*AL = Ad-libitum; R80 = 80% of theoretical ad libitum feed intake during whole fattening period; R70 = 70% of theoretical ad libitum feed intake during whole fattening period; R80AL = 80% of theoretical ad libitum feed intake up to 48 d of life; R70AL = 70% of theoretical ad libitum feed intake up to 48 d

**Standard Error of Means (n = 24 cages/treatment).

[#] BW: Body Weight.

^{a-c} Values within a row with different superscripts differ significantly at P<0.05.

Table 29. Effect of the feeding restriction strategies on apparent total-tract digestibility (ATTD) from 53 to 57 d. (*Experiment 4*)

Item	Treatments*					SEM**	p-Value
	AL	R80	R70	R80AL	R70AL		
Dry matter intake, g/d	153 ^a	128 ^b	111 ^b	158 ^a	158 ^a	4.11	<0.001
Digestibility coefficients, %							
Dry matter	58.1	58.4	57.5	57.5	57.9	0.34	0.32
Organic matter	60.1	60.3	59.6	59.5	59.7	0.33	0.42
Crude protein	68.4 ^{ab}	70.3 ^a	70.3 ^a	67.8 ^b	70.0 ^{ab}	0.61	<0.05
Gross energy	59.0	60.0	59.3	59.2	59.6	0.32	0.32
Neutral detergent fibre	22.3	21.6	20.3	21.1	20.8	0.71	0.39
Ether extract	88.4	88.4	88.9	88.3	86.5	0.77	0.25

* AL = Ad-libitum; R80 = 80% of theoretical ad libitum feed intake during whole fattening period; R70 = 70% of theoretical ad libitum feed intake during whole fattening period; R80AL=80% of theoretical ad libitum feed intake up to 48 d; R70AL = 70% of theoretical ad libitum feed intake up to 48 d

** Standard Error of Means (n = 12 cages/treatment).

^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 30. Effect of the feeding restriction strategies on carcass chemical composition at 34 and 61 d. (*Experiment 4*)

Item	Treatments*					SEM**	P-Value
	AL	R80	R70	R80AL	R70AL		
Carcass composition at 34 d of age, % DM							
Body weight, g	664	675	651	682	666	13.5	0.56
Water, %	71.6	71.3	71.6	71.4	71.5	0.07	0.11
Crude Protein	60.7	60.7	60.4	60.6	60.6	0.14	0.60
Ash	17.1	16.9	17.2	16.9	17.0	0.09	0.10
Ether extract	20.2	20.3	20.5	20.2	20.1	0.17	0.41
Gross Energy kJ/100 g DM	2051	2066	2047	2064	2053	6.36	0.15
Carcass composition at 61 d of age, % DM							
Body weight, g	2132 ^a	2013 ^a	1860 ^b	2116 ^a	2004 ^a	33.1	<0.001
Water, %	66.9 ^b	67.5 ^{ab}	68.2 ^a	67.1 ^b	67.5 ^b	0.14	<0.001
Crude Protein	56.0 ^b	57.5 ^a	58.7 ^a	56.8 ^b	57.0 ^a	0.36	<0.001
Ash	13.3 ^b	14.1 ^b	14.9 ^a	13.6 ^b	14.0 ^b	0.16	<0.001
Ether extract	29.1 ^a	27.6 ^{ab}	26.1 ^b	28.5 ^a	27.8 ^a	0.31	<0.001
Gross Energy, kJ/100 g DM	2387 ^a	2326 ^b	2266 ^c	2365 ^{ab}	2338 ^{ab}	11.1	<0.001

* AL = Ad-libitum; R80 = 80% of theoretical ad libitum feed intake during whole fattening period; R70 = 70% of theoretical ad libitum feed intake during whole fattening period; R80AL=80% of theoretical ad libitum feed intake up to 48 d of life; R70AL = 70% of theoretical ad libitum feed intake up to 48 d of life.

** Standard Error of Means (n = 24 cages/treatment).

^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$.

5.4.4. Carcass chemical composition and nitrogen and energy balances

The feed restriction affected the carcass chemical composition (Table 30). Protein content increased in the two restricted groups at 70% (R70 and R70AL) and in the R80 group (+4.7%; +1.8% and +2.6%, respectively; $P < 0.001$), while the rabbits restricted 80% only up to 48d (R80AL) had a similar protein content than control group. The ash content increased in rabbits restricted at 70% during the whole period with respect to the other treatments (14.9% vs. 13.7% on average; $P < 0.001$). The fat, and therefore the energy of the carcass, decreased in the animals restricted during the whole growth period and more significantly in the group R70 (−10% fat than the control; $P < 0.001$). On the opposite, this group (R70) showed the highest water content compared with AL, R80AL and R70AL groups.

As it was expected, the lower feed intake (from 34 to 61 d) in some of the restriction strategies used affected the DN and DE intake significantly ($P < 0.001$; Table 31). Animals restricted at 70% during the whole growing period (R70) showed a lower feed intake, both DN_i and DE_i (−22% and −25%, respectively; $P < 0.001$), compared with animals fed *ad libitum*. The rest of the restricted animals (R80, R80AL and R70AL) had a similar DN_i and DE_i, but they were lower than in the animals fed *ad libitum* (−10% and −9% on average, respectively). Restricted animals tended to have a better nitrogen efficiency, both in body (+3.42%; $P = 0.099$) and carcass (+4.85%; $P = 0.074$) than AL group. As a consequence, even though the DN_i was lower, the nitrogen retained in the carcass and whole body was similar among the different treatments, with the exception of the animals restricted at 70% during the whole period (R70) that was on average 5.7% lower in the carcass and 7.14% in the body ($P < 0.05$) than in the rest of treatments. In this case, the efficacy of retention did not compensate the lower DN_i, despite being also the group with lower N excretion in skin, viscera, faeces and

urine ($P < 0.001$). Nitrogen losses in faeces decreased in all restricted animals with respect to the control ($P < 0.001$), especially in R80 (-15.1%), R70 (-22.4%) and R70AL (-14.8%). Moreover, R70 and R80AL showed the lowest nitrogen losses in urine ($P < 0.001$).

The efficiency of ER was not affected by the different feeding restriction strategies, but DEi decreased when voluntary intake was restricted, especially in the rabbits of group R70 that had the lowest value (-17%) compared to AL. The effects of restriction strategies on energy losses in skin and viscera and through the faeces, followed a similar evolution than the observed for the nitrogen excretion. Restricted rabbits also showed lower urine energy + heat increment losses compared with animals fed *ad libitum*.

Table 31. Effect of the feeding restriction strategies on nitrogen and energy balance from 34 to 61 d. (*Experiment 4*)

Item	Treatments*					SEM**	P-Value
	AL	R80	R70	R80AL	R70AL		
Nitrogen intake[#], g/kg BW^{0.75}.d							
Ni	2.529 ^a	2.271 ^c	2.116 ^d	2.351 ^b	2.305 ^{bc}	0.019	<0.001
DNi	1.730 ^a	1.593 ^b	1.496 ^c	1.595 ^b	1.625 ^b	0.013	<0.001
Nitrogen retained[#], g/kg BW^{0.75}.d¹							
NR body	1.101 ^a	1.027 ^{ab}	0.989 ^b	1.083 ^a	1.049 ^{ab}	0.010	<0.05
NR carcass	0.538 ^a	0.507 ^{ab}	0.490 ^b	0.531 ^a	0.518 ^{ab}	0.019	<0.05
Nitrogen efficiency							
NR body/DNi	0.636	0.644	0.662	0.679	0.646	0.012	0.099
NR carcass/DNi	0.309	0.317	0.327	0.333	0.319	0.006	0.074
Nitrogen losses, g/kg BW^{0.75}.d							
Skin and viscera	0.563 ^a	0.520 ^{ab}	0.499 ^b	0.552 ^a	0.532 ^{ab}	0.011	<0.001
Faeces	0.799 ^a	0.678 ^c	0.620 ^d	0.756 ^b	0.681 ^c	0.006	<0.001
Urine	0.630 ^a	0.566 ^{ab}	0.506 ^b	0.512 ^b	0.512 ^b	0.020	<0.001
Energy intake[#], MJ/kg BW^{0.75}.d							
GEi	1.760 ^a	1.586 ^c	1.477 ^d	1.645 ^b	1.609 ^{bc}	0.013	<0.001
DEi	1.054 ^a	0.953 ^b	0.875 ^c	0.975 ^b	0.960 ^b	0.008	<0.001
Energy retained[#], kJ/kg BW^{0.75}.d							
ER body	367 ^a	323 ^b	295 ^b	354 ^{ab}	327 ^b	8.410	<0.001
ER carcass	219 ^a	193 ^{bc}	174 ^c	212 ^{ab}	195 ^b	5.151	<0.001
Energy efficiency							
ER body/DEi	0.348	0.338	0.337	0.363	0.341	0.009	0.24
ER carcass/DEi	0.208	0.202	0.198	0.216	0.204	0.005	0.19
Energy losses, MJ/kg BW^{0.75}.d							
Skin and viscera	0.149 ^a	0.129 ^c	0.122 ^c	0.142 ^{ab}	0.132 ^{bc}	0.004	<0.001
Faeces	0.706 ^a	0.633 ^c	0.603 ^d	0.670 ^b	0.649 ^{bc}	0.005	<0.001
Urine + heat production	0.687 ^a	0.630 ^b	0.580 ^c	0.621 ^{bc}	0.632 ^b	0.010	<0.001

* AL = Ad-libitum; R80 = 80% of theoretical *ad libitum* feed intake during whole fattening period; R70 = 70% of theoretical *ad libitum* feed intake during whole fattening period; R80AL = 80% of theoretical *ad libitum* feed intake up to 48 d; R70AL = 70% of theoretical *ad libitum* feed intake up to 48 d. ** Standard Error of Means (n = 24 cages/treatment).

^{a-d} Values within a row with different superscripts differ significantly at P < 0.05.

[#] Ni: nitrogen intake. DNi: digestible nitrogen intake. NR: nitrogen retained. GEi: gross energy intake. DEi: digestible energy intake. ER: energy retained. Skin and viscera (g/kg BW^{0.75} and d): (g N retained in body – g retained in carcass)/kg BW^{0.75} and d. Faeces (g/kg BW^{0.75} and d): (Total N intake – DNi)/kg BW^{0.75} and d. Urine (g/kg BW^{0.75} and d): (DNi – N Retained in carcass – N excreted in skin and viscera)/kg BW^{0.75} and d. Skin and viscera (MJ/kg BW^{0.75} and d): (MJ gross energy retained in – MJ gross energy retained in carcass)/kg BW^{0.75} and d. Faeces (MJ/kg BW^{0.75} and d): (Gross Energy intake – DEi)/kg BW^{0.75} and d. Urine + heat production (MJ/kg BW^{0.75} and d): (DEi – Gross energy retained in carcass – GE excreted in skin and viscera)/kg BW^{0.75} and d.

GENERAL DISCUSSION

VI. GENERAL DISCUSSION

6.1. Effect of enzyme supplementation (Experiments 1 and 2)

6.1.1. Growth performance and slaughter traits (Experiments 1 and 2)

Many studies have shown that enzymes supplementation have a positive effect on the feed efficiency (Gutiérrez *et al.*, 2002a; Eiben *et al.*, 2004; Cachaldora *et al.*, 2004; Attia *et al.*, 2012; Khalil 2018), specially on the first phases of the fattening period. This effect could be explained by a more efficient utilization of the nutrients (Gutiérrez *et al.*, 2002a; García-Palomares *et al.*, 2006), particularly in young animals, because the native enzymatic capacity develops with age (Dojana *et al.*, 1998). In this study, the quadratic effect found in the second phase have been previously observed by other authors. In this sense, Attia *et al.* (2012) reported a lower live weight at 8 weeks of age in the rabbits fed with the highest dose (750 ppm) of a multi enzymatic complex. El-Sagheer and Hassanein (2014) found higher positive effect on feed conversion ratio, body weight and BWG with 1 g/kg of an enzyme complex and probiotic instead of 2 g/kg. Fraiha *et al.* (1997) also described an optimal enzyme dose supplementation according to the substrate. However, in this work during the whole fattening phase these beneficial effects were lost and this lack of effect might be explained by the low mortality observed during this trial (1.77%) and the optimal management conditions. The limited effect of enzyme supplementation was also described by Cachaldora *et al.* (2004) in two experiments with α -amylases. In one of them, mortality was high (27% in control group and with a great incidence of diarrhoea) and amylases supplementation showed a very positive effect compared with the control group (mortality decreased up to 12.2 %, $P=0.03$; FCR improved +16%, $P=0.04$), whereas in the other experiment, mortality was very low (2.6 % and almost any diarrhoea) and enzymes had no positive effects. In the same way, Gutiérrez *et al.* (2002a)

found that the enzymatic supplementation reduced mortality in the whole fattening period compared with control group when it was moderate (6.9 vs. 13.9%).

The results obtained in carcass traits match with Abaza and Omara (2011). They also found no effects on carcass weight and carcass yield, but observed that rabbits fed with enzymes had high relative viscera weight, as in our study, where treatments supplemented with enzymes showed higher weight of digestive tract, which could be related to the worst digestibility found in these treatments. Khalil (2018) also reported no significant differences in carcass parameters. El Sagheer and Hassanein (2014) found neither any effect on carcass yield, although they did not measure the total digestive tract, but the liver relative weight, was increased with enzyme supplementation. Neither Olorutola *et al.* (2018) found a significant effect with a multi-enzyme supplementation on carcass traits or in any internal organs. In contrast, El-Aziz *et al.* (2019) reported a significant differences in most of the carcass traits studied (carcass yield (%), forequarter (%) and hindquarter (%)) in rabbits supplemented with a multi-enzymes preparation fortified with sodium butyrate and housed in a semi closed rabbitry of 180 m² with natural ventilation. Likewise, a better carcass yield with enzyme supplementation were also found by Hernandez-Martínez *et al.* (2017) and Shanmuganathan *et al.* (2004). In the experiment 2, in contrast to results found in experiment 1, none of the performance traits studied showed a dose effect, probably because in experiment 2 the highest dose was only 200 ppm. The reason why a higher enzyme dose, based on the results of experiment 1, was not used in this experiment was because experiment 1 and 2 were done at the same time. The lack of effects with the enzyme supplementation on productive parameters have been observed previously by other authors (Falcao-e-Cunha *et al.*, 2007; Gutiérrez *et al.*, 2002a; Cachaldora *et al.*, 2004) and the low mortality (1.85%) and optimal conditions could explain this lack of effects observed.

The higher feed intake of diet B was due to the higher proportion of NDF (+5,05 %) and the lower DE (- 6,76%) compared to diet A. Due to the homeostatic mechanism, rabbits can regulate the feed intake to consume a constant energy, within reasonable limits (Xiccato and Trocino, 2010). According to Maertens *et al.* (1997) and De Blas *et al.* (1999), fibrous diets decrease the digesta retention time and increase feed intake but impair the FCR.

In the first phase, the worst growth of the rabbits fed with diet B could be due to two effects. On one hand, because rabbits do not have completely developed the digestive physiology at 34 d of age (Gutiérrez *et al.*, 2002b) and they could not compensate the decrease in energy with a higher feed intake (although a tendency was observed) impairing the growth. On the other hand, young animals have high requirement in protein and AA, not only for tissues growth, but also for the development of the digestive tract and the maintenance of gut mucosa function (Carabaño *et al.*, 2009). In this sense, Maertens *et al.* (1997) and Trocino *et al.* (2000) reported a detrimental effect on growth during the first days of fattening when CP content in feed was lower than 15.6%. In the second phase, rabbits were able to intake more feed and growth was similar in both groups.

The difference in DWG between both periods could show that the protein requirements in rabbits during the fattening period depends on the age. A protein level under 15% during the first days decrease growth performance (Maertens *et al.*, 1997; Trocino *et al.*, 2000 and 2013; Martínez-Vallespín *et al.*, 2011). However, in contrast to the results observed by Maertens *et al.* (1997), Trocino *et al.* (2000 and 2013) and Martínez-Vallespín *et al.* (2011) reported that growth performance only tended to decrease during the whole phase.

In our Experiment, diet B had higher NDF and lower energy content, increasing the development of the digestive tract and increasing the feed intake, what could have favoured

the compensatory growth, as reported by Martínez-Vallespín *et al.* (2011) in feeds with high fibre content. As a consequence of the higher feed intake (in the second and whole fattening phases) and the lower growth (in the first phase) FCR was impaired in diet B.

Although diet B had higher digestive tract, and full and empty stomach weights, the slaughter weight and carcass weight were higher in diet A. In general, feeds with higher fibre content used to have more stomach content in rabbits (De Blas *et al.*, 1986; Parigi Bini *et al.*, 1994), and also lower caecal content (Gidenne and Pérez 1994, Gidenne, 2000, Álvarez *et al.*, 2007) although it depends on the variations of fibre content in the diets. Values over or under 39.5 % NDF (%DM) in feed could increase caecal content (De Blas *et al.*, 1999), because it is the level that minimized the caecal content weight. Due to the lower slaughter live weight and carcass weight in animals fed diet B, carcass yield was similar between both types of diets. Many authors have reported no effect on carcass yield using both different protein levels or diets with different fibre and energy content (Parigi Bini *et al.*, 1994; Maertens *et al.*, 1997). Some studies have shown an increased in carcass yield with increasing levels of NDF (Fabre *et al.*, 2006); but always that these levels are close to the optimal one that minimized the caecal content. On the contrary, an excess of NDF can impair carcass yield (Margüenda *et al.*, 2006 and 2012; Villena *et al.*, 2008; Trocino *et al.*, 2011). El-Kelawy and El-Shafey (2017) showed a decrease in carcass yield with higher doses of date stone meal (with high NDF and low energy and protein levels), but enzyme supplementation was able to compensate partly or completely, depending on date stone meal dose, the drop in carcass yield.

6.1.2. Apparent total-tract digestibility (ATTD) (Experiments 1 and 2)

The negative results on digestibility observed in experiment 1, were also found by Bolis *et al.* (1996), who found a detrimental effect on DE and ME when an enzymatic compound,

also from fungus origin, was included in a basal feed with high fibre content (52.5% NDF) in adult rabbits. A negative effect on DM and OM digestibility with enzyme supplementation was also reported by Castrovilli *et al.* (1995). Most of the studies (Falcao-e-Cunha *et al.* 2007) have not found significant improvements on digestibility when enzymes are used, although some of them have observed an improvement on digestibility (Makled *et al.*, 2005; García-Ruiz *et al.*, 2006; Abaza and Omara, 2011).

The impairment on digestibility detected, could be explained by some kind of interaction between the enzymatic activity and the digestive physiology of the rabbits, or between endogenous enzymes of the animal (Bolis *et al.*, 1996), as well as among the exogenous enzymes themselves. According to Barekattain *et al.*, (2013), in a work done in chickens, the addition of xylanases and proteases, together or separated, showed that could have been a negative interaction between them, because the same positive results were not found whether enzymes were added together or separately. This hypothesis, of possible interaction among proteases with other exogenous enzymes, has been also proposed by other authors (Naveed *et al.*, 1998; Olukosi *et al.*, 2007). Otherwise, Attia *et al.* (2012) observed no interaction with the endogenous proteases in rabbits when adding an enzymatic compound that contained β -glucanases, celulases, α -amilases, proteases and lipases. In this study, a positive effect of the exogenous enzymes was reported on the final live weight and in the activity of amylase, lipase and protease in serum, pancreatic tissues, and intestinal content. However, and as it is evidenced by the literature, nowadays it is not deeply understand the mode of action of the exogenous proteases on the digestive physiology, and even less on the interaction between proteases and xylanases (Barekattain *et al.*, 2013).

In experiment 2, enzyme supplementation had no effect on digestibility of any of the nutrients studied, probably because of the lower doses used. Type of diet impaired digestibility, probably due to the higher content in NDF in diet B, as many studies have shown the negative correlation between fibre content and energy and fibre digestibility (de Blas *et al.*, 1999).

6.1.3. Carcass chemical composition and nitrogen and energy balances (Experiments 1 and 2)

Regarding the carcass composition there was no effect of enzyme supplementation, because to affect carcass composition significantly, energy and/or protein intake should be quite higher or lower than the optimal dairy recommendation (Xiccato, 1999).

The lower content in EE and GE on the carcasses of animals fed diet B could be due to the lower DE intake (-6.2%) and the lower GE retention (-0.20 kJ/kg PV^{0.75} and d). Also, the lower growth during the first phase could have delayed the deposit of perirenal, inguinal, and scapular fat (Lebas and Ouhayoun, 1987; Ouhayoun, 1989). Dalle Zotte *et al.* (1997) reported higher fat deposition in carcasses of rabbits fed with high ratios of DE/DP during post-weaning. Moreover, some authors (Ouhayoun and Cheriet, 1983; Fraga *et al.*, 1983) have observed an increase of fat content and a decreased of nitrogen content in animals that consumed diets with lower ratio DP/DE. On the other hand, García *et al.* (1993) reported a lower fat content in carcasses of rabbits fed with higher NDF content, substituting barley by sugar beet pulp and Xiccato *et al.* (1993) using different DP/DE ratios during the fattening phase did not observe any variation neither on carcass composition nor in meat. El-Kelawy and El-Shafey (2017) found lower protein and lipid percentage in carcass with 10 and 20% of date stone meal replacement, in a diet with more NDF and less energy and protein levels compared to the control.

In the experiment 1, the lack of effect of enzymes on nitrogen and energy balances is according to the lack of significant effects of enzymes on the whole fattening period, where any effect on FCR, as well as nor on CP digestibility was observed. However, due to the lower digestibility of GE, there was significant differences in GE excretion in faeces, being higher in the treatments supplemented with enzymes.

The amount of N excreted is lower than the calculated by Maertens *et al.* (2005), for rabbits from 0.8 to 2.5 kg of LW (0.094 vs. 0.071 kg of N/rabbit, on average, in our experiment). In Spain, rabbits are slaughtered at lower LW (2-2.2 kg LW), this could explain this difference in N excretion, since the higher the live weight the higher the N excretion. This could be the cause why nitrogen balance obtained in this study are more similar to the results from Calvet *et al.* (2008), because growing conditions are more similar to ours than in the study of Maertens *et al.* (2005).

In diet B, GE excretion in faeces increased due to the lower GE digestibility compared to diet A. As a consequence, DE intake, energy efficiency and retained energy, both in whole body and in carcass, were also lower in diet B. The interaction observed between diet and enzyme supplementation could be related with the higher efficiency of enzymes when feed digestibility decreases (Cowan *et al.*, 1996).

Regarding N balance, animals of diet B consumed less DN (-16.4 g N/kg BW^{0.75}.d), and had a higher retention efficacy in carcass (0.514 vs. 0.538), but the N retained was lower (-0.18 g N/kg^{0.75} and d), probably due to the lower growth showed in the first phase. The N excreted in faeces was lower in diet B due to its lower protein content because protein digestibility was similar. Nitrogen in urine was also reduced, due to the lower DN consumption and to the higher nitrogen retention efficacy. Maertens *et al.* (1997) also observed an

improvement in N efficacy in rabbits with lower protein content. However, Trocino *et al.* (2000) did not find any effect on N retention efficacy, although they found that a low protein level decreased N excretion, as well as Maertens *et al.* (1997). Maertens *et al.* (1998) also reported that a lower DP/DE ratio decreased the N excretion.

6.2. Effect of lignin source and level (Experiment 3)

6.2.1. Growth performance and slaughter traits (Experiment 3)

Chemical composition and physical structure of plant cell walls varies widely among the different sources of fibre. The botanical origin of the lignin can have important effects on digestibility (De Blas *et al.*, 1999), caecal fermentation and enzymatic activity in caecum (Gidenne, 2000; García *et al.*, 2002b), and performance (Pérez *et al.*, 1994; Nicodemus *et al.*, 1999). For example, according to Gidenne (2000) it seems that the botanical origin affects the FCR or could modulate the effect of the lignin level on FCR. However, Gidenne (2000) and Pérez *et al.* (1994) did not demonstrate an effect of lignin origin on OM digestibility, although demonstrated that botanic origin of the lignin could affect the average retention time of the digesta, probably it is related with the particle size (García *et al.*, 1999).

According to Colin *et al.* (2007) Arbocel® did not affect productive performance or final live weight when is compared with feeds or with lignin provided by “traditional” raw materials like alfalfa, whereas García *et al.* (2002a), reported an over intake of feed in animals fed with defatted grape seed meal and a FCR impairment. However, in this experiment, FCR was slightly increased with Arbocel® compared to the grape meal, both in individual and collective fattening, without any significant effect on growth or feed intake. Griess *et al.* (1990) showed a higher caecum motility in rabbits fed diets supplemented with lignin derived from wood. In addition, García *et al.* (2002a) observed a lower caecal content weight with defatted

grape seed meal. In our trial any differences were appreciated on total digestive tract nor caecum weights, probably because the effect of both lignin sources on passage rate was similar. Any effect was neither observed on carcass yield, although Colin *et al.* (2007) reported an increase of +1.1 points more with Arbocel®.

The influence on slaughter live weight observed among different levels of lignin contrasted with the no effect observed on the final live weight in the zootechnical parameters. The explanation could be due to the selection of the 24 rabbits per treatment for the carcass traits. Animals were selected based on the average weight, having the animals with medium lignin level a lower average weight. In fact, when DWG, FCR and DFI were calculated, only for the animals selected for the carcass traits there were significant differences on DWG in the animals fed with the medium lignin level. But as both, the slaughter and carcass weight, were lower, the carcass yield was not affected by any treatment.

Low lignin level tended to increase the relative weight of the total digestive tract (+0.45 percentage units, on average). This tendency is most probably related with the higher caecum weight ($P < 0.05$) also found in low lignin treatment. However, the greater weight of the digestive tract did not impair the carcass yield.

Digesta retention time in the digestive tract is an important feature of the diets because it is related with the voluntary intake and it can vary from 16 to 54 hours, depending on the fibre source. In standard diets average retention time is 18 hours on average (De Blas *et al.*, 1999). Caecal retention is around a 60% of the total retention time, for this reason there is a negative correlation between caecal content weight and passage rate (De Blas *et al.*, 1999) and dry matter intake (García *et al.*, 2002b).

Lignin level influences the average digesta retention time and, therefore, the passage rate, and modifies the caecum weight as it was noted by several authors (Gidenne and Pérez 1994; Nicodemus *et al.*, 1999; Gidenne, 2000; Nicodemus *et al.*, 2007). Nicodemus *et al.* (1999) reported that lower lignin level, increased caecal content weight and decreased feed intake in isoenergetic diets. Recently, Caïsin *et al.* (2020) also observed lower full caecum and full gastrointestinal tract in rabbits fed with high lignin diets (over 7.5% DM) when soluble fibre was low (8.2%). Usually, if feeds energy concentration is between 9.2 and 13.4 MJ DE/kg (Gidenne and Pérez, 1994), rabbits are able to adjust their feed intake in order to keep the daily intake of DE, maintaining the growth rate during the fattening period (Lebas, 1975; Partridge *et al.*, 1989, Gidenne and Pérez, 1994) and reaching similar final weights (Nicodemus *et al.*, 1999; Gidenne and Pérez, 1994; Colin *et al.*, 2007). But if the lignin level is very high the feed consumption increases (Gidenne and Pérez, 1994; Gidenne, 2000; Caïsin *et al.*, 2020), but an impairment in FCR is produced (Gidenne, 2000; Trocino *et al.* 2013; Caïsin *et al.*, 2020). Below a minimum of 4.1% ADL (%DM), Pérez *et al.*, (1994) and Nicodemus *et al.* (1999), have reported a lower growth rate and feed intake, for this reason is recommended to incorporate a minimum of 4.13% ADL (% DM) in diets of rabbits.

These results agree with our work, where the medium and high lignin levels also had lower relative caecum weight, compare with low level lignin, that could be linked with a faster passage rate, but without significant effect on feed intake. In the same way, final live weight nor DWG were significantly affected, because energy supply satisfies rabbits requirements, and therefore, DE intake was similar in all treatments, as noted by Gidenne (2000) and Pérez *et al.* (1994).

Although total digestive tract weight was lower with the medium and high lignin levels, carcass yield was not significantly affected, as it was also observed by Caîsin *et al.* (2020). According to Nicodemus *et al.* (1999) fibre level has an opposite effect in the stomach than in the caecum. However, in this experiment this effect was not observed in the stomach but was noted in caecum, for this reason differences in carcass yield could have been found. Wu *et al.* (2018) reported increased stomach relative weight, stomach content relative weight, caecal relative weight, and caecal content weight with increasing NDF levels. In fact, Colin *et al.* (2007) reported a tendency to increase 0.8 points carcass yield with increasing lignin level. However, according to Xiccato (1999), neither the fibre nor any of its fractions play a specific role on carcass quality, in addition to its role to modify energy concentration. only if the fibre concentration modifies it in excess and impair rabbit's growth, carcass yield is not modified.

Some papers (Pérez *et al.*, 1994; Gidenne *et al.*, 2001; Wu *et al.*, 2018) have reported a better mortality, sanitary risk index and morbidity in rabbits fed with higher lignin levels, however, due to the low mortality in this experiment, no significant differences were found. However, in order to avoid the use of antibiotics and prevent the epizootic rabbit enteropathy (ERE), the latest researches have recommended the inclusion of moderate levels of soluble fibre (11-12%; Martínez-Vallespín *et al.*, 2011; Trocino *et al.*, 2013; Delgado *et al.*, 2018), but as long as ADL and particle size requirements are met.

6.2.2. Apparent total-tract digestibility (ATTD) (Experiment 3)

Lignin source influenced on OM and DM digestibility in agreement with the results noted by Gidenne (2000). García *et al.* (2002a), reported a positive effect of defatted grape seed meal on the enzymatic activity of the small intestine, what could show a higher functional capacity of gut mucosa in rabbits fed with this fibre source.

GE digestibility was decreased in medium and high levels. As it was reported by De Blas *et al.* (1999) an increase in ADF content in the diet decreases the digestible energy. Other authors (Gidenne and Pérez, 1994; Nicodemus *et al.*, 1999; Gidenne, 2000; Trocino *et al.* 2013, Caîsin *et al.*, 2020) have also observed worse digestibility rates (DM, OM, CP, GE and/or NDF) with increasing levels of lignin, due to a reduction of the retention time (Gidenne, 2000) and the own negative effect of the lignin on the feed digestion/fermentation (Gidenne and Pérez, 1994; Trocino *et al.*, 2013). Like in other species, lignin could hinder the rabbit's digestion due to the covalent links with other components of the cell wall (Van Soest, 1994). Generally, it is observed a protein digestibility reduction when lignin content is increased (Falcao-e-Cunha, 1986, Gidenne, 1987 and Motta, 1990), although Nicodemus *et al.* (1999) and Gidenne (2000) reported an increased in CP with higher levels of lignin. On the other hand, García *et al.* (2002a) and Gidenne and Pérez (1994) did not find any difference on protein digestibility. However, in this experiment a decreased in protein digestibility was only observed with a medium level of lignin. Other authors, as it was observed in this experiment, did not find any difference on fibre digestibility, despite is usually observed an impairment on NDF digestibility with higher lignin levels (Gidenne and Pérez, 1994; Nicodemus *et al.*, 1999; García *et al.*, 2002). Regarding EE digestibility there is a lineal increased with the increase of lignin level. As previously it has been described, higher lignin level usually impairs nutrient digestibility, therefore, this EE digestibility increment could be due to the slightly higher fat content and more soya oil in diets of higher levels of lignin. According to Maertens, (1998) vegetable oils are highly digestible and Casado *et al.* (2010) also found that EE digestibility of vegetable oils was higher. Moreover, Fernández *et al.* (1994) and Fernández and Fraga (1996), reported that a higher EE in diets improved EE digestibility, although ADF content was higher, without any effect on the GE intake nor in the energy efficacy retention. Caîsin *et al.* (2020)

also observed a higher EE digestibility in high lignin diets, because in order to keep diets isoenergetic, higher levels of fat in form of soya oil as well were used.

6.2.3. Carcass chemical composition and nitrogen and energy balances.

(Experiment 3)

In this experiment GE retention efficacy, digestible energy intake or energy excreted by skin and viscera, urine and heat production were not affected by the lignin level, despite a higher fibre content could decrease energy retention (De Blas *et al.*, 1999). On contrast, Caîsin *et al.* (2020) observed higher fat deposition in high lignin levels. However, in those diets, EE content was double than in low lignin level diets and could have played a role on fat deposition.

Due to the lower protein digestibility in medium and high lignin levels, nitrogen digestible intake was lower ($P < 0.05$) in these diets, whereas the N utilization increased, therefore, N retained was similar for the three lignin levels studied. These results agree with Maertens *et al.* (1997) that reported an improvement of the N retention in the rabbits fed with a lower protein intake. However, N excretion in faeces, despite the worse CP digestibility, was not modify. Nitrogen excreted via urine decreased in medium and high lignin feeds, probably because retained nitrogen was similar but digestible nitrogen was lower. Nitrogen amount excreted via faeces was lower than in the previous experiments (0.790 vs. 0.825 g/kg $PV^{0.75}.d$, on average) because the N digestibility was higher (68.0 vs 71.2 %). However, the N in urine was higher than in previous experiment (0.930 vs. 0.597 g/kg $PV^{0.75}.d$) due to the higher DN intake (1.962 vs. 1.731 g/kg) and the lower N retained (1.032 vs. 1.090 g N/kg $LW^{0.75}.d$). Even so, these results are lower than the ones calculated by Maertens *et al.* (2005), but more similar to the ones of Calvet *et al.* (2008).

Because N and energy retention were not modified by the different treatments, carcass composition was neither modified. In this sense, DP/DE ratio and/or amount of fibre in feed must be extremely high or extremely low to have an influence on energy and protein content in the rabbits (Maertens, 1999 and Xiccato, 1999).

6.3. Effect of feed restriction (Experiment 4)

6.3.1. Growth performance and slaughter traits (Experiment 4)

One of the practical applications of this technique is the reduction of post-weaning mortality (Gidenne *et al.*, 2009a, 2012, and 2017a; Romero *et al.*, 2010; Alabiso *et al.*, 2017). In this trial was not observed any difference among groups, that may have been because the mortality was moderate (12.8% on average). In fact, in other studies the feed restriction did not reduce the mortality rate below 9–17% when that of control group was between 15 and 23% (Gidenne *et al.*, 2009c; Knudsen *et al.*, 2014; 2017), or even tended to increase it (Birolo *et al.*, 2020), although other authors found very positive effects (Birolo *et al.*, 2016; Romero *et al.*, 2010), even compared to a medicated feed (Alabiso *et al.*, 2017). There is no clear explanation for these differences among studies, and a combination of factors like the farm health status, diet, method of feed restriction employed, or type of housing might be behind them.

During the restriction period there is an impairment of growth (Romero *et al.*, 2010; Gidenne *et al.*, 2012 and 2017a). However, the effect on FCR is not so clear. Romero *et al.* (2010) also observed an impairment of FCR during the restriction period, but other authors have not found any effect (Gidenne *et al.*, 2012; Birolo *et al.*, 2016), or describe an improvement that could range between 5 and 10% (Alabiso *et al.*, 2017; Gidenne *et al.*, 2017a; Knudsen *et al.*, 2017).

The compensatory growth observed in the treatments re-fed ad libitum in the second phase (R80AL and R70AL) has also been shown by many authors (Romero *et al.*, 2010; Birolo *et al.*, 2016; Gidenne *et al.*, 2012, 2017a; Knudsen *et al.*, 2017) and its intensity depends on the restriction level. The animals restricted also in the second phase had a similar growth rate than the control group, that might be explained because the effect of feed restriction on growth is more severe at the beginning of the restriction period (just after the weaning), than later (Gidenne *et al.*, 2012). However, as the increase of feed intake was moderate compared to the compensatory growth in the second phase, the FCR improved with respect to the ad libitum animals, as it has been described by Gidenne *et al.* (2017a). Considering the whole fattening period, animals of R80AL group had a similar growth rate and lower feed conversion ratio than ad libitum, whereas R70 group had the lowest daily gain and was the only restriction group that reduced cold carcass weight compared with the ad libitum, although the FCR was similar than in the control. These results agree with those observed by Birolo *et al.* (2016) that did a restriction up to 58 d and Knudsen *et al.* (2017) until 63 – 64 d. The reasons for a better feed efficiency have been described by a better digestibility of nutrients (Gidenne *et al.*, 2012; Birolo *et al.*, 2016; Knudsen *et al.*, 2017). However, in this trial, digestibility (Table 4) was measured in the second phase, and there was only a significant improvement in the digestibility of CP in the animals that remained restricted, what could explain only partially the improvement in FCR during the second phase. Therefore, the compensatory growth and better FCR cannot be completely explained by the feed digestion and neither by a better nitrogen or energy retention efficiency (Table 6). Thus, the compensatory growth could also be of metabolic origin as it has been described in other species. In poultry, the compensatory growth observed in previously restricted animals has been rather associated to a decrease on maintenance costs or heat production. So, the increase of feed intake leads to an improvement

in growth efficiency (Sahraei, 2012). In other studies, this better efficiency could be associated to an increase in the concentration, absorption and utilization of VFA, as it was observed in restricted animals (Gidenne and Feugier, 2009; MacDonald *et al.*, 2011), although in this work they have not been measured. This hypothesis could be supported with the results of the nitrogen and energy excretion because it was observed lower excretion in both parameters (Table 6) in restricted animals. Unlike the observations by other authors (Romero *et al.*, 2010; Gidenne *et al.*, 2017a; Knudsen *et al.*, 2017), the rabbits restricted only during the first phase (R70AL and R80AL), reached an acceptable body weight at slaughter for the Spanish market (2 kg BW). Birolo *et al.* (2016) reached also a similar final weight, but feed restriction was moderate (93% of ad libitum intake).

The lack of differences on dressing out percentage after a restriction period is in agreement with other studies (Tumová *et al.*, 2006; Birolo *et al.*, 2016; Chodová *et al.*, 2019). However, other authors (Gidenne *et al.*, 2012; Knudsen *et al.*, 2017; Birolo *et al.*, 2020) reported an impairment of the carcass yield. Normally, the impairment is related to higher weight of the full tract associated with the development of internal organ such as small and large intestines, caecum and the liver during the restriction period and refeeding (Tumová *et al.*, 2016). In this work, R70AL had the higher digestive tract and full stomach weight, but without any impairment in carcass yield. In the same way, animals of treatment R70AL had the greater liver weight, neither without effect in carcass yield. Gidenne *et al.* (2012) and Knudsen *et al.* (2017) also reported a heavier stomach and livers at slaughter when animals were restricted, based on the faster growth of the digestive organs after a restriction period, during the compensatory growth. The higher weight of liver after restriction period could be related to the major functional demands of liver, such as glycogen storage (Knudsen *et al.*, 2017) or a larger diameter of the hepatocytes (Tumová *et al.*, 2016) detected in restricted

animals. Greater differences could be expected when restriction is more severe, <70%, or for long periods (from 4 to 12 weeks of age) (Birolo *et al.*, 2016).

6.3.2. Apparent total-tract digestibility (ATTD) (Experiment 4)

The improvement on protein digestibility with the restriction has been previously observed in various works (Xiccato *et al.*, 1992; Gidenne and Feugier, 2009; Birolo *et al.*, 2016; Knudsen *et al.*, 2017), and it is probably accounted for the larger mean retention time associated to restricted rabbits compared to the ad libitum group (Gidenne and Feugier, 2009). This effect was higher in the treatments that continued restricted all the whole fattening period. It seems that the animals refed ad libitum lost this effect and tended to have a similar digestibility than the control group, as it has been observed by Tumová *et al.* (2003, 2007) and Knudsen *et al.* (2014).

Regarding the digestibility of the other nutrients, according to Gidenne *et al.* (2012) is necessary a restriction period between 8 and 10 d to observe an improvement, however, we did not observe any positive effect on digestibility after 16 d of restriction. The lack of consistency among results of the different works on digestibility improvement may be caused by the variation on the intensity, duration, and the feed composition (Gidenne *et al.*, 2012). There are results that observed an improvement in digestibility of different nutrients (CP, CE, fibre, etc.), both during (Di Meo *et al.*, 2007; Tumová *et al.*, 2007; Gidenne *et al.*, 2009a y b) and after the restriction phase (Ledin, 1984; Birolo *et al.*, 2016), but there are others that did not observe any effect (Díaz Arca *et al.*, 1999; Gidenne and Feugier, 2009; Tumová *et al.* 2016).

6.3.3. Carcass chemical composition and nitrogen and energy balances (Experiment 4)

The increase of carcass protein content in restricted animals is in agreement with Schlolaut *et al.* (1978) who described with two restriction levels (80 and 60%) a sharp increase in carcass protein content (12 percentage units, on average). A higher protein content is usually accompanied by lower fat content (Schlola *et al.*, 1978), but in this experiment it was observed only in the R70 group. However, Xiccato (1999) and Birolo *et al.* (2016) did not find considerable changes in the empty body protein. This could be partially explained by the expression of the data, done in DM in our trial and in fresh matter in these studies. The higher ash content in the carcass of restricted animals could be explained because, according to Pálsson (1955), the restriction should impair more the tissues that have been developed earlier (digestive tract) than those of later development (bones, muscle and fat).

The lower fat and energy content in the carcass of restricted animals are usually observed during or even after the restriction, independently of the strategy followed (Xiccato, 1999; Xiccato and Trocino, 2010; Gidenne *et al.*, 2012; Knudsen *et al.*, 2017). Gondret *et al.* (2000) showed that changes in the nutritional status regulate intramuscular lipid deposition without changing muscular fibre type composition. It might be related to the reduction of the activity of the enzymes involved in the biosynthesis of fatty acids (enzyme malic and glucose-6-phosphate dehydrogenase), observed even after the feed restriction (Gondret *et al.*, 1997). However, we observed that the treatments re-fed from 48 d onwards had a similar fat content than rabbits fed *ad libitum*. Despite most of the studies described a lower fat content in carcass, Birolo *et al.* (2016) observed a higher content after the re-feeding phase; probably, the lower feed restriction and the longer fattening period might have affected those results compared to those in this paper.

In general, there is an improvement in the nitrogen balance with feed restriction (Gidenne *et al.*, 2017b). However, the discrepancies observed among studies could depend on the different slaughter weights (Calvet *et al.*, 2008) or dietary N content (Xiccato *et al.*, 2005; Gidenne *et al.*, 2013; Tazzoli *et al.*, 2015) or the restriction program (Birolo *et al.*, 2016 and 2017). Protein retention efficacy is usually improved when digestible protein intake or the ratio DP/DE decreases (Motta Ferreira *et al.*, 1996; Maertens *et al.*, 1997). The energy balance followed the same trend than the nitrogen one. Except R80AL, restricted groups had less energy retained in carcass, skin and viscera, and less energy losses via urine and heat production, what could explain partially their compensatory growth.

6.4. Effect of slaughter weight on carcass yield

The main objective of this Thesis was to improve the dressing out percentage with two nutritional strategies: 1) increasing carcass weight due to a greater deposition of protein and/or fat with the use of enzymes and 2) reducing the relative weight of the digestive tract by using an enzymatic supplementation, increasing lignin levels and applying different levels of feed restriction.

Contrary to the hypothesis, enzymes impaired digestibility and, consequently, increased the relative weight of the total tract by 2.7% (experiment 1). However, this negative effects on digestibility has been also found by other authors (Bolis *et al.*, 1996). In experiment 2, total digestive tract and stomach weight increased by 5% in the diet with the lowest DE and DP. This effect has also been observed in diets with high fibre content (De Blas *et al.*, 1986; Parigi Bini *et al.*, 1994). In experiment 4, feed restriction did not reduce relative weight of the digestive tract, except in the animals restricted 70% of AL intake that had an opposite effect

and increased its digestive tract weight by 6.2%. On the contrary, total digestive tract decreased 1.43%, on average, with the increase of ADL level in experiment 3.

Considering that digestive tract is between 20–21% of the live weigh of the animals (Margüenda *et al.*, 2012), some effects on carcass yield could not have been expected. In part, this lack of effects in carcass yield might be explained by de differences observed in the slaughter weights among experiments. The average slaughter weight in experiments 1, 2, 3 and 4 was 2349, 2319, 2450 and 2151 g, respectively. In order to analyse the effect of slaughter weight on carcass yield it was performed a regression analysis. Four regression equations were obtained, but the slope was common for all of them (Figure 4). These results indicated that for each increase of 100 g in slaughter weight, the carcass yield increases by 0.24 percentage units. Comparing the four experiments, the dressing out percentage was significantly lower ($P<0.001$) only in experiment 4, due to the lower final weight of the animals. This lower live weight could be explained, by two causes: the lower final weight of rabbits as a consequence of the restriction and because the fattening trial was carried out in collective cages, with 5 rabbits per cage, where animals usually finish growing period with lower weight than in individual cages.

Figure 4. Effect of slaughter weight on carcass yield.

GENERAL CONCLUSIONS AND IMPLICATIONS

VII. GENERAL CONCLUSIONS AND IMPLICATIONS

7.1. Conclusions

1. The nutritional strategies studied in this Thesis had no effect on carcass yield. However, feed restriction increases crude protein content and decreases ether extract in carcass composition, without impairing performance, what could have a positive influence nowadays that people is looking for healthier, low fat food.

2. Enzyme supplementation in rabbits with good sanitary conditions:

- Seems not to have a clear positive effect in the productive performance during the whole fattening period.
- In case of using an enzyme complex in diets that supply all the nutritional requirements would be recommended only for the first phase (34 to 48 d).
- In the second phase (48 – 60 d) would not be recommended to supplement more than 400 ppm of the enzyme complex.
- In diets with lower nutritional value enzyme complex improved nitrogen and energy balance, reducing the energy losses and nitrogen excretion to the environment.

3. Feeds with lower DP/DE ratio and more NDF impair nutrient digestibility and FCR but reduce fat carcass content and the N excretion to the environment up to 12%.

- A feeding program by phases would be more recommendable, more adapt to rabbits needs in each moment, to reduce N excretion via faeces and urine.

4. Lignin source and level have no important effect on growth performance and carcass traits, despite a slightly negative effect of lignin level on digestibility and Arbocel® on feed conversion rate.

5. Feed restriction up to 80% of *ad libitum* intake in the first fattening phase, followed by *ad libitum* intake in the second phase had a similar growth rate, lower feed conversion ratio, and tended to have higher digestible nitrogen and energy retention efficiency than the group fed *ad libitum*.
 - However, restriction of *ad libitum* intake up to 70% during the whole fattening period reduced the cold carcass weight, although it increased the protein content of the carcass and reduced the fat one.
6. Feed restriction during post-weaning period of fattening (34–48 d) reduce the farm environmental impact, without impair growth performance and dressing out percentage.
7. In non-enteropathy conditions, none of the strategies studied had effect on mortality.

7.2. Implications

Carcass yield is an important quality and profitable trait for the meat industry. Nutrition plays an important role on it, but the strategies studied in this Thesis have not shown an effect and should not be recommended for that purpose. However, if the objective was to obtain lower fat carcasses, maybe due to the customer demand for lean meat, restrict feed intake 80% of AL intake in the first fattening phase (35-49 d) and AL in the second phase (49-61 d) would be interesting. In addition, performance is globally improved.

From the environmental point of view, feed restriction and formulation with lower CP, GE and higher NDF strategies decreased the N excretion, and therefore, the environmental impact.

Based on the present work, future lines of research could be:

- Study the nutrient requirements of fattening rabbits in the different growing phases.
- Differences among enzyme supplements and types. Interaction among exogenous and/or endogenous enzymes of the rabbit.
- Study other nutritional strategies that could improve carcass yield and/or meat quality.

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VIII. REFERENCES

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