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EFFECT OF DIETARY SOLUBLE FIBRE LEVEL AND PROTEIN SOURCE ON GROWTH, DIGESTION, CAECAL ACTIVITY AND HEALTH OF FATTENING RABBITS

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Abstract: From weaning (34 d of age) until slaughter (76 d), 216 hybrid rabbits were divided into six experimental groups and fed *ad libitum* six iso-protein (CP: 15.9% as-fed), iso-starch (9.1%) and iso-ADF (21.4%) diets formulated in a bi-factorial arrangement, with three soluble fibre levels (LS: 5.8% *vs*. MS: 7.4% *vs*. HS: 8.5%) and two protein sources (Soy: soybean meal *vs*. Sun: sunflower meal). The increase in dietary soluble fibre level linearly increased (P_L<0.001) in vivo digestibility of dry matter (from 55.4 to 61.3%), gross energy and fibre fractions (from 14.8 to 25.7% for acid detergent fibre; from 40.3 to 49.2% for insoluble hemicelluloses; from 85.0 to 93.9% for soluble fibre). Replacing soybean with sunflower meal tended to decrease crude protein digestibility (73.1 *vs*. 72.6%, *P*=0.058) and reduced acid detergent fibre digestibility (22.0 *vs*. 18.4%, *P*<0.001), while improving ether extract digestibility (69.3 *vs*. 70.8, *P*<0.001). The nutritive values of the experimental diets increased with soluble fibre level (digestible energy from 9.0 to 9.9 MJ/kg) regardless of the protein source. Intestinal mucosa traits at 56 d of age were not affected, while caecal pH linearly decreased (from 6.19 to 5.97; P_L=0.017) with increasing dietary soluble fibre. During the trial, health problems were moderate (mortality 3.4% and morbidity 15.6%) and not affected by feeding treatments. With increasing soluble fibre level, rabbit daily weight gain and final live weight linearly increased (P_L=0.045) and feed conversion improved (from 3.55 to 3.30, P_L<0.001). Protein source did not affect intestinal traits or growth performance. Slaughter results and meat quality were unaffected by feeding treatments.

Key Words: soluble fibre, soybean meal, sunflower meal, health, growth performance, caecal fermentation.

INTRODUCTION

The spread of Epizootic Rabbit Enteropathy (ERE) in Europe has remarkably increased animal mortality and morbidity in rabbit breeding stocks (Licois *et al*., 2006). Lacking the definitive identification of an etiological agent responsible for ERE and due to the multi-factorial nature of this syndrome (Pérez de Rozas *et al*., 2005), research has been called to define nutritional requirements and feeding strategies in relation to rabbit intestinal health (Gidenne and García, 2006; Carabaño *et al*., 2008).

The practice of feeding low-energy high-fibre diets to growing rabbits as a way to reduce digestive disorders (Gidenne, 2003; Gidenne and Licois, 2005) has greatly impaired overall farm feed efficiency (Maertens, 2009; Xiccato and Trocino, 2010). On the contrary, increasing high-digestible fibre fractions, such as hemicelluloses and especially pectins, may stimulate caecal fermentation (García *et al*., 2000; Falcão-e-Cunha *et al*., 2004), modulate the microbiota composition (Gómez-Conde *et al*., 2009), positively affect

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intestinal mucosa integrity (Álvarez *et al*., 2007; Gómez-Conde *et al*., 2007) and reduce mortality caused by diarrhoea (Soler *et al*., 2004; Gómez-Conde *et al*., 2009).

In addition, as stated by Carabaño *et al*. (2009), the role of protein in digestive health level need to be further investigated because little is known about the interaction between dietary fibre composition and the protein level and source. As occurs with protein excess, the use of less digestible protein sources may increase the nitrogen flux at caecum and favour the development of pathogenic strains of *E. coli* and *Clostridium spp.*, thus impairing animal health and performance (Gutiérrez *et al*., 2003; García-Ruiz *et al*., 2006; Chamorro *et al*., 2007).

The present paper aimed to assess whether the increase in soluble fibre fractions with rather constant levels of acid detergent fibre (ADF) and starch and the complete replacement of soybean meal by sunflower meal might improve rabbit intestinal conditions, reduce the incidence and severity of digestive pathologies, increase feed efficiency and guarantee high growth performance and slaughter results.

MATERIALS AND METHODS

Animals and diets

Two hundred and sixteen rabbits of both genders from a hybrid line (Hyplus, Grimaud Frères, France) were reared in individual cages from weaning at 34 d of age until slaughter at 76 d. The rabbits were kept in a brick shed equipped with a forced heating system to maintain a minimum temperature of 18°C and submitted to a natural photoperiod.

Four diets (Soy-LS, Soy-HS, Sun-LS and Sun-HS) were formulated and pelleted using soybean meal (Soy) or sunflower meal (Sun) as main protein source and varying the inclusion levels of fibrous raw materials (mainly alfalfa meal and dried beet pulp) to obtain low soluble (LS, 5-6% as-fed diet) and high soluble (HS, 8-9%) fibre levels (Tables 1 and 2). Dilution technique was used to prepare 2 more diets with moderate soluble fibre level (MS): Soy-MS diet (0.5 Soy-LS + 0.5 Soy-HS) and Sun-MS diet (0.5 Sun-LS + 0.5 Sun-HS). Crude protein concentration (CP) was quite constant among diets, i.e. 15.9% as-fed on average (Table 2). Similarly, ether extract (EE; 2.3 to 2.8%) did not differ substantially among diets and the concentrations of the low-digestible fibre fractions were rather constant (ADF 20.6 to 21.8%; acid detergent lignin, ADL 3.7 to 4.2%). The main change in chemical composition concerned the soluble fibre, which varied from 5.5-6.0% in LS diets to 7.2-7.5% in MS diets to 8.5% in HS diets. In Soy diets, the increase of 3.0 points in soluble fibre from LS to HS level mainly corresponded to a reduction of 0.4 points in starch and 1.4 points in insoluble hemicelluloses. In Sun diets, the increase of 2.4 points in soluble fibre corresponded to a reduction of 1.4 points in starch and 1.1 points in ADF. The ratio between soluble fibre and starch increased from 0.60 to 0.82 to 0.98 from LS to MS to HS diets, respectively.

From 34 d of age until slaughter, rabbits were divided into 6 groups (36 rabbits each), homogeneous in average live weight and variability, and given *ad libitum* access to the six experimental diets. One hundred and eighty rabbits (30 per dietary treatment) were used for the growth trial and 36 rabbits (6 per dietary treatment) were used for sampling caecal content and intestinal mucosa.

Individual live weight and feed intake were recorded three times a week. The health status of rabbits was controlled daily. Rabbits were considered ill when showing clear signs of diarrhoea as well as strong reduction in feed intake (−30% compared to the previous recording). In the morbidity calculation, the ill rabbits were counted only once and dead animals were considered only in the mortality calculation. The sanitary risk was calculated as the sum of morbidity and mortality (Bennegadi *et al*., 2000). No antibiotics, drugs or additives were added to feed and water.

Table 1: Ingredient composition of experimental diets[1] (%, as fed).

	Soy-LS	Soy-HS	Sun-LS	Sun-HS
Alfalfa meal 16% CP	48.30	37.10	44.40	27.70
Wheat bran	28.00	13.00	30.00	20.00
Barley meal	4.00	10.00	3.00	7.00
Dried beet pulp	10.00	25.00	10.00	25.00
Soybean meal 46% CP	7.00	11.50	0.00	0.00
Sunflower meal 36% CP	0.00	0.00	10.00	17.50
Cane molasses	1.30	1.30	1.30	1.30
Dicalcium phosphate	0.35	1.05	0.15	0.35
Salt	0.40	0.40	0.40	0.40
Lysine (liquid form)	0.00	0.00	0.10	0.10
DL-methionine	0.08	0.08	0.08	0.08
Choline	0.07	0.07	0.07	0.07
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50

[1]Soy-MS diet = (0.5 Soy-LS + 0.5 Soy-HS); Sun-MS diet = (0.5 Sun-LS + 0.5 Sun-HS).
[2]Premix provided per kg compound diet: vit. A, 12,000 IU; vit. D_3, 1,000 IU; vit. E acetate, 50 mg; vit. K_3, 2 mg; Biotin, 0.1 mg; Thiamine, 2 mg; Riboflavin, 4 mg; vit. B_6, 2 mg; vit. B_{12}, 0.1 mg; Niacin, 40 mg; Pantothenic acid, 12 mg; Folic acid, 1 mg; Choline chloride, 300 mg; Fe, 100 mg; Cu, 20 mg; Mn, 50 mg; Co, 2 mg; I, 1 mg; Zn, 100 mg; Se, 0.1 mg.

Digestibility trial

The apparent digestibility coefficients of dry matter (DM) and nutrients and the digestible energy (DE) and digestible protein (DP) concentrations of the experimental diets were measured in an *in vivo* digestibility assay carried out according to the European harmonised method (Perez *et al*., 1995) on 60 rabbits (10

Table 2: Chemical composition of experimental diets (%, as fed).

	Soy-LS	Soy-MS	Soy-HS	Sun-LS	Sun-MS	Sun-HS
Dry matter	89.5	89.6	89.8	89.6	89.5	89.4
Crude protein	16.0	16.2	15.9	15.6	15.7	15.9
Ether extract	2.8	2.4	2.3	2.8	2.6	2.5
Ash	7.9	7.8	7.8	7.6	7.3	7.2
Starch	9.3	9.0	8.9	9.9	8.9	8.5
Total dietary fibre (TDF)	43.5	44.2	45.0	44.0	44.7	45.1
Neutral detergent fibre (NDF)	38.0	37.0	36.5	38.0	37.2	36.7
Acid detergent fibre (ADF)	21.8	21.8	21.7	21.7	21.0	20.6
Acid detergent lignin (ADL)	3.9	3.9	3.7	4.2	4.0	3.7
Soluble fibre[1]	5.5	7.2	8.5	6.0	7.5	8.4
Insoluble hemicelluloses[2]	16.2	15.2	14.8	16.3	16.2	16.1
Sugars[3]	4.6	4.9	5.2	4.6	4.7	4.8
Soluble fibre to starch ratio	0.59	0.80	0.96	0.61	0.84	1.00

[1]TDF-NDF. [2]NDF-ADF. [3]Values estimated on the basis of sugar content of raw materials (Maertens *et al*., 2002).

animals of both genders per diet) among those on trial and placed in the same room. The digestibility trial started at 53 d of age with a 4 d collection period. Faeces were collected and analysed individually.

Caecal content and intestinal mucosa sampling

Thirty six rabbits out of the initial 216 rabbits were slaughtered at 56 d of age (6 rabbits per dietary treatment) to sample caecal content and intestinal mucosa. The slaughtered animals were representative of the 6 experimental groups in terms of average live weight and variability. They were weighed immediately before slaughter and killed by cervical dislocation from 8:00 to 12:00 h. Full gut, stomach and caecum were removed and weighed. The caecal content pH was measured and the caecal content was diluted with 15% HPO_3 solution (25% wt/wt) and stored at −20°C until chemical analyses.

Mucosa samples were taken from the intermediate tract of jejunum, gently washed and fixed in para-formaldehyde at 4% in PBS, then dehydrated and included in paraffin. Sections of 4 µm were obtained after cutting by microtome and used for morphometric evaluations on the preparations coloured with haematoxylin/eosin. Villi length and crypt depth were measured by image analysis software (DP-soft, Olympus Optical, Co., Hamburg, Germany).

Commercial slaughter and carcass and meat quality recordings

The rabbits were slaughtered at 76 d of age in a commercial slaughterhouse and the carcasses chilled at 2°C for 24 h. The chilled carcasses were then dissected following harmonised methods (Blasco *et al*., 1993). The pH was measured in *longissimus lumborum* and *biceps femoris* muscles using a pH meter (HI 9025C, Hanna Instruments, Sarmeola di Rubano, Padova, Italy) fitted with a combined Ingold electrode (406 M3). The L*a*b* colour (CIE, 1976) was measured in the same muscles using a colorimeter (Minolta CR100 Chroma Meter, Minolta Corp., Ramsey, NJ).

Chemical analysis

Diets and faeces were analysed to determine the concentrations of DM (934.01), ash (967.05), CP (2001.11) and starch (amyloglucosidase-α-amylase method, 996.11) by AOAC (2000) methods following harmonised procedures (EGRAN, 2001). Ether extract (EE) was determined after acid-hydrolysis treatment (EC, 1998). Fibre fractions, NDF, ADF and ADL, were analysed according to Mertens (2002), AOAC (2000, procedure 973.187) and Van Soest *et al*. (1991), using the sequential procedure. Total dietary fibre (TDF) was determined by gravimetric-enzymatic procedure, with α-amylase, protease and amyloglucosidase (Megazyme Int. Ireland Ltd., Wicklow, Ireland) (Method AOAC 991.43). Soluble fibre was calculated by subtracting NDF to TDF (Van Soest *et al*., 1991). Gross energy (GE) was measured by adiabatic bomb calorimeter.

The thawed samples of caecal content were centrifuged for 10 min at 9000 *g*. Caecal N-ammonia was determined in the supernatant by pH-meter (PHM 84, Research pH-meter, Radiometer, Copenhagen, Denmark) equipped with an ammonia-specific electrode (mod. 9512, Orion Research Incorporated, Boston, USA). Volatile fatty acid (VFA) concentration in the supernatant was measured by gas-chromatography (HRGC 5300 Carlo Erba, Milano, Italy) on a cross bond capillary column (25 m × 0.32 mm I.D., 3.5 mm film thickness) (JRX, Mega, Milano, Italy) using the method of Osl (1988).

Statistical analysis

The data recorded were analysed by a three-way ANOVA with soluble fibre level, protein source and gender as main effects and their interactions. The gender effect was neither reported nor discussed because it was not relevant to the objectives of the present paper. The GLM procedure by SAS (SAS, 1991) was used. Orthogonal polynomial contrasts were used to compare means by group of soluble fibre level in order to estimate the probability of linear (P_L) component of variance. Mortality, morbidity and sanitary risk were analysed by the CATMOD procedure from SAS.

RESULTS AND DISCUSSION

Digestibility and nutritive value of diets

The DM and GE digestibility increased linearly (P_L<0.001) with increasing soluble fibre (Table 3). In consequence, the DE content of the diets increased from 9.0 to 9.9 MJ/kg with soluble fibre level both in Soy and Sun diets. The increased DM and GE digestibility (+6 points) cannot be ascribed only to the higher concentration (+3 points) of highly-digestible soluble fibre, or to changes in the digestibility of CP, EE or starch, which showed similar or even lower digestive utilisation rates as soluble fibre increased. The improved digestive efficiency was the consequence of the linear (P_L<0.001) and substantial increase (+9-10 points) of digestibility of all fibre fractions (ADF from 14.8 to 25.7%; insoluble hemicelluloses from 40.3 to 49.2%; soluble fibre from 85.0 to 93.8%). This dramatic change can be ascribed to the lower lignification and complexity of cell walls, but also to variations in the ingredient composition of diets which affected the nature of the high digestible fibre fractions (e.g. the ratio pectins to soluble arabinoxylans; the hemicellulosic constituents) and made fibre more susceptible to microbiota fermentation activity in the ileum and caecum (Gidenne, 1992; Carabaño *et al*., 2001; García *et al*., 2002). A similar increase in dietary nutritive value was found when replacing insoluble fibre from alfalfa with soluble fibre from beet pulp (Trocino *et al*., 1999; Falcão-e-Cunha *et al*., 2004) or beet-apple pulp (Gómez-Conde *et al*., 2009).

Protein source did not affect DM and GE digestibility and had a weak effect on nutrient digestibility (Table 3). CP digestibility tended to be lower (P=0.058) in Sun diets than in Soy diets. Other authors (Gutiérrez *et al*., 2003; García-Ruiz *et al*., 2006) did not find differences in faecal apparent digestibility in diets based on sunflower meal (36% CP) or soybean meal (46-48% CP), while measuring a higher ileal digestibility of CP and DM in the former than in the latter (Gutiérrez *et al*., 2003). EE and hemicelluloses were less digested (P<0.001) in Soy diets than in Sun diets, while ADF and soluble fibre resulted more digested in the former ones. These results could not be ascribed to differences in soybean or sunflower digestibility, but likely to the effects of other ingredients included in different proportions in Soy and Sun diets.

Moreover, the significant interactions (Table 3) for CP, soluble fibre and starch digestibility may be attributable to a not perfectly linear response of the digestibility values.

The DP to DE ratios lowered from 12.9 to 11.7 g/MJ as soluble fibre increased and were higher than values recommended for fattening rabbits (10-11 g DP/MJ DE; De Blas and Mateos, 1998; Carabaño *et al*., 2009).

Caecal content and intestinal tissue characteristics

At 56 d of age, the relative weight of full stomach and gut tended to decrease as soluble fibre level increased (P_L<0.10), while full caecum proportion did not vary (Table 4). In contrast, no effect of protein source on gut filling was observed.

The increased gut content found in previous studies (García *et al*., 1993; Carabaño *et al*., 1997; Falcão-e-Cunha *et al*., 2004) in rabbits fed diets containing high levels of soluble fibre from beet pulp likely depended on the contemporary relevant changes in NDF, ADF and starch concentrations. On the contrary, when iso-ADF diets were compared, as in the present study, no real impact of beet pulp inclusion on the filling of digestive organs was found (Jehl and Gidenne, 1996; Gidenne and Perez, 2000). Besides, as reviewed by García *et al*. (2002), the weight of caecal content mainly depends on dietary NDF and its

Table 3: Effect of soluble fibre and protein source on digestibility coefficients and nutritive value of experimental diets from 53 to 57 d of age.

	Diet						*P*-value			
	Soy-LS	Soy-MS	Soy-HS	Sun-LS	Sun-MS	Sun-HS	S_L[1]	P[2]	S×P[3]	RSD[4]
Rabbits, No.	10	10	10	10	10	10				
Digestibility coefficients, %										
Dry matter	55.5	58.7	61.5	55.2	58.0	61.0	<0.001	0.27	0.93	1.72
Crude protein	72.8	73.4	73.2	73.6	71.6	72.5	0.32	0.058	0.003	1.14
Ether extract	72.8	67.8	67.4	73.5	69.1	69.8	<0.001	<0.001	0.14	1.30
Starch	96.6	96.0	96.6	97.0	96.8	96.3	<0.001	<0.001	<0.001	0.15
Total dietary fibre	33.8	40.7	47.0	33.2	40.1	44.2	<0.001	0.30	0.99	2.44
Neutral detergent fibre	26.2	30.1	35.7	25.0	30.4	35.6	<0.001	0.68	0.70	2.87
Acid detergent fibre	16.5	21.9	27.7	13.0	18.5	23.6	<0.001	<0.001	0.94	3.27
Insoluble hemicelluloses	39.3	42.0	47.4	41.2	45.7	50.9	<0.001	<0.001	0.44	2.31
Soluble fibre	86.1	92.8	95.6	83.8	88.6	92.1	<0.001	<0.001	<0.001	0.39
Gross energy	54.7	57.7	61.0	54.8	57.2	60.2	<0.001	0.43	0.76	1.75
Diet nutritive value:										
DE[5], MJ/kg (as fed)	8.90	9.39	9.93	9.05	9.38	9.79				
DP[6], g/kg (as fed)	116	119	116	115	112	115				
DP to DE ratio, g/MJ	13.1	12.7	11.7	12.7	12.0	11.8				

[1]S_L = Probability of soluble fibre level (linear component of variance). [2]P = Probability of protein source. [3]S×P = Probability of the interaction soluble fibre level×protein source. [4]RSD = Residual standard deviation. [5]DE = Digestible energy. [6]DP = Digestible protein.

degree of lignification. These variables remain relatively constant among diets in our experiment, which might explain the lack of effect of soluble fibre on this trait.

Caecal fermentation activity, in terms of N-ammonia, total VFA concentration and VFA molar proportions, was not influenced by feeding treatments, while caecal content pH linearly decreased by increasing soluble fibre (P_L=0.017). This variation, even though not associated to an increase in total VFA concentration, as also found by Carabaño *et al*. (1997), may be considered favourably in view of the maintenance of balance in the caecal bacterial population and fermentation. Previous studies (García *et al*., 2000; Gidenne and Bellier, 2000; Falcão-e-Cunha *et al*., 2004) showed a favourable increase in caecal fermentation activity when differences in soluble fibre content among diets were greater than in the present trial. In fact, Gómez-Conde *et al*. (2009) did not find any effect on caecal environment with a limited increase in neutral detergent soluble fibre (from 9.1 to 12.0%).

Intestinal mucosa morphometry, in terms of jejunum villi height and crypt depth, was unaffected by treatments (Table 5) and the digestive functionality and the capacity of intestinal barrier to face the attack of pathogens were probably not altered. While in weaning pigs a positive effect of the dietary inclusion of soluble fibre on intestinal mucosa condition has been proven (Vente-Spreeuwenberg and Beynen, 1997), in rabbits Gómez-Conde *et al*. (2007) found an improved gut-barrier function in rabbits fed more soluble fibre fractions, but Xiccato *et al*. (2008) did not detect differences in villi length or crypt depth according to dietary high-digestible fibre/starch ratio. These controversial results likely depend on the differences in weaning age of rabbits, age at sampling and, finally, on possible interactions with the farm sanitary status. Contrary to the findings of studies on pigs (Vente-Spreeuwenberg *et al*., 2004a, b), in our assay the protein source did not affect intestinal mucosa traits. Similarly, Chamorro *et al*. (2007) found no effects of the vegetable protein source (alfalfa hay *vs*. soybean protein concentrate) on rabbit intestinal mucosa. On

Table 4: Effect of soluble fibre and protein source on gut and fermentation characteristics at 56 d of age.

	Soluble fibre level (S)			Protein source (P)		*P*-value			
	LS	MS	HS	Soy	Sun	S_L[1]	P[2]	S×P[3]	RSD[4]
Rabbits, No.	12	12	12	18	18				
Full stomach, %LW[5]	7.18	6.77	6.47	6.93	6.68	0.049	0.37	0.19	0.8
Full caecum, %LW	8.43	8.69	8.42	8.34	8.69	0.98	0.13	0.84	0.7
Full gut, %LW	23.9	23.3	22.8	23.6	23.1	0.086	0.30	0.38	1.5
Caecal content traits:									
pH	6.19	6.14	5.97	6.08	6.12	0.017	0.53	0.27	0.21
N-NH3, mmol/L	4.27	5.12	5.69	5.57	4.49	0.17	0.20	0.061	2.47
Total VFA[6], mmol/L	58.3	64.1	62.4	62.0	61.2	0.32	0.79	0.18	9.8
C2, %mol VFA	81.6	81.4	82.7	82.2	81.9	0.23	0.68	0.54	2.22
C3, %mol VFA	4.19	4.16	4.11	4.00	4.31	0.73	0.11	0.23	0.56
C4, %mol VFA	13.6	13.4	12.6	13.2	13.2	0.27	0.92	0.68	2.15
C5, %mol VFA	0.59	0.60	0.55	0.55	0.62	0.51	0.24	0.60	0.18
C3 to C4 ratio	0.32	0.32	0.34	0.31	0.34	0.49	0.34	0.62	0.08

[1]S_L = Probability of soluble fibre level (linear component of variance). [2]P = Probability of protein source. [3]S×P = Probability of the interaction soluble fibre level×protein source. [4]RSD = Residual standard deviation. [5]LW = Live weight. [6]VFA = Volatile fatty acids.

the contrary, mucosa integrity was improved in early-weaned rabbits fed diets containing animal plasma rather than soybean (Gutiérrez *et al*., 2000) and possible damage to mucosa depending on feeding legume anti-nutritional factors has been hypothesised (Gutiérrez *et al*., 2003; Cano *et al*., 2004).

Health status and growth performance

During the trial, health problems were limited and only six animals died, mostly during the 3rd and 4th wk of the experiment, and a 7th rabbit weighing less than the admitted market LW, 2.0 kg, was excluded from growth data. Statistical analysis did not reveal any effect of feeding regime (Table 6), even if the mortality associated with Soy diets was numerically higher (5.6 *vs*. 1.1%; P=0.13) in comparison with Sun diets. A certain number of animals (12-20% depending on the experimental group; P>0.10) presented digestive disorders (diarrhoea, constipation, weight loss, decreased feed intake), mostly around the fourth week of the trial, which were quickly recovered without the need of antibiotic supply.

Table 5: Effect of soluble fibre and protein source on morphometry of jejunal mucosa at 56 d of age.

	Soluble fibre level (S)			Protein source (P)		*P*-value			
	LS	MS	HS	Soy	Sun	S_L[1]	P[2]	S×P[3]	RSD[4]
Rabbits, No.	12	12	12	18	18				
Villi height, μm	537	542	543	553	528	0.82	0.23	0.63	59
Crypt depth, μm	70	70	72	72	69	0.56	0.44	0.46	9
Villi to crypt ratio	7.78	7.95	7.66	7.83	7.77	0.78	0.90	0.25	1.34

[1]S_L = Probability of soluble fibre level (linear component of variance). [2]P = Probability of protein source. [3]S×P = Probability of the interaction soluble fibre level×protein source. [4]RSD = Residual standard deviation.

Table 6: Effect of soluble fibre and protein source on mortality, morbidity and sanitary risk from weaning (34 d) to slaughter (76 d)[1].

	Soluble fibre level (S)			Protein source (P)		*P*-value		
	LS	MS	HS	Soy	Sun	S[2]	P[3]	S×P[4]
Mortality, %	3.3 (2)	5.0 (3)	1.7 (1)	5.6 (5)	1.1 (1)	0.61	0.13	-[5]
Morbidity, %	15.0 (9)	20.0 (12)	11.7 (7)	15.6 (14)	15.6 (14)	0.47	0.89	0.70
Sanitary risk[6], %	18.3 (11)	25.0 (15)	13.4 (8)	21.2 (19)	16.7 (15)	0.32	0.65	0.32

[1]In parenthesis the number of dead or sick animals. [2]S = Probability of soluble fibre level. [3]P = Probability of protein source. [4]S×P =Probability of the interaction soluble fibre level×protein source. [5]No statistical analysis was possible. [6]Sanitary risk = mortality + morbidity. The initial number of rabbits was 30 per experimental diet, corresponding to 60 per soluble fibre level and 90 per protein source.

The good sanitary status of the commercial farm from which the rabbits were taken and the optimal hygienic conditions of the experimental facilities could have helped maintain the rabbits in good health, whatever the diet. Previous studies carried out with animals in less favourable sanitary conditions showed a decrease of rabbit mortality and morbidity due to ERE and other digestive disorders by increasing the dietary concentration of high-digestible fibre fractions in replacement of ADF (Xiccato *et al*., 2006) or, in iso-ADF diets, in replacement of starch (Perez *et al*., 2000, Soler *et al*., 2004), hemicelluloses (Gómez-Conde *et al*., 2009) or CP (Gidenne *et al*., 2001). The older weaning age (33 d) of the rabbits used in the present trial compared to the above-mentioned studies (25-30 d) could also account for the low susceptibility to digestive disorders (Lebas, 1993).

In our conditions, all animals fully expressed their growth potential with an average daily weight gain above 50 g/d, reaching an average LW over 3 kg at 76 d of age (Table 7). Increasing soluble fibre level linearly increased rabbit daily weight gain and final LW (P_L=0.045). As a result of the higher nutritive value of high-soluble fibre diets and the chemostatic control of appetite (Xiccato and Trocino, 2010), feed intake tended to decrease linearly (P_L=0.073), thus permitting a significant improvement of feed conversion (from 3.55 to 3.42 and 3.30 for rabbits fed LS, MS and HS diets, respectively, P_L<0.001).

Other authors also found decreased feed intake when rabbits were fed diets with increasing high-digestible fibre fractions (from beet pulp) and decreasing insoluble fibre levels (from alfalfa hay) (Carabaño *et al*., 1997), but growth rate and LW decreased only at the highest beet pulp levels (>30%) (Falcão-e-Cunha *et al*., 2004). When high-digestible fibre replaced starch, with beet pulp inclusion rates of over 35%, an increase in feed intake and a reduction in growth were observed (García *et al*., 1993). Conversely, Gidenne *et al*. (2004) reported negative effects on feed intake or growth only in very young rabbits (25-38 d) and with 50% of beet pulp inclusion.

The complete substitution of soybean meal by sunflower meal did not affect growth performance (Table 7) as found in other studies (Gutiérrez *et al*., 2003). Nevertheless, García-Ruiz *et al*. (2006) observed higher daily weight gain and feed intake in rabbits fed diets based on soybean meal rather than sunflower meal, which were associated to a higher mortality ascribed to the higher ileal flux of soybean protein.

Slaughter results and meat quality

Live weight at the slaughterhouse averaged 2925 g, with losses during transportation around 3.2-3.3%. Cold dressing percentage resulted rather high (59.9% on average) mainly as a consequence of the high body weight at slaughter (Xiccato, 1999). Reference carcass presented on average 3.3% of dissectible fat, 32.4% of hind legs and 6.21 muscles/bone ratio of the hind leg.

Table 7: Effect of soluble fibre and protein source on growth performance from weaning (34 d) to slaughter (76 d).

	Soluble fibre level (S)			Protein source (P)		P-value			
	LS	MS	HS	Soy	Sun	S_L[1]	P[2]	S×P[3]	RSD[4]
Rabbits, No.	58	57	58	84	89				
Live weight at 34 d, g	898	897	900	897	900	0.83	0.68	0.72	57
Live weight at 55 d, g	2032	2039	2080	2041	2060	0.21	0.56	0.17	206
Live weight at 76 d, g	2990	2996	3079	3008	3034	0.045	0.46	0.18	236
First period (34-55 d)									
Daily weight gain, g/d	54.0	54.4	56.2	54.5	55.2	0.19	0.60	0.17	9.0
Daily feed intake, g/d	149	143	142	146	144	0.036	0.95	0.27	19
Feed conversion rate	2.78	2.67	2.57	2.68	2.67	0.003	0.78	0.61	0.37
Second period (55-76 d)									
Daily weight gain, g/d	45.6	45.6	47.6	46.0	46.4	0.15	0.73	0.90	7.2
Daily feed intake, g/d	204	198	200	201	201	0.34	0.99	0.55	21
Feed conversion rate	4.60	4.42	4.25	4.48	4.37	0.009	0.29	0.89	0.73
Whole trial (34-76 d)									
Daily weight gain, g/d	49.8	50.0	51.9	50.3	50.8	0.036	0.49	0.18	5.2
Daily feed intake, g/d	176	170	171	173	173	0.073	0.98	0.28	17
Feed conversion rate	3.55	3.42	3.30	3.44	3.41	<0.001	0.23	0.55	0.21

[1]S_L = Probability of soluble fibre level (linear component of variance). [2]P = Probability of protein source. [3]S×P = Probability of the interaction soluble fibre level×protein source. [4]RSD = Residual standard deviation.

In contrast to the final live weight recorded at the experimental rabbitry, the live weight at slaughter did not significantly differ between experimental groups (Table 8), probably due to the lower number of animals (20 *vs*. 30 per group) checked at slaughter. Similarly, no differences were ascribed to treatments for carcass weight and composition or for meat quality in terms of pH and colour of *longissimus lumborum* and *biceps femoris* muscles (Table 8).

These results are not surprising because feeding treatments hardly affect slaughter results and carcass characteristics or meat traits, if animals fed *ad libitum* balanced diets and with minor differences in final body weight are recorded (Xiccato, 1999; Hernández, 2008). On the other hand, an increased gut filling would have been expected as a consequence of the change in dietary fibre fractions (Parigi Bini *et al*., 1994) or the increase in beet pulp inclusion level (García *et al*., 1993; Cobos *et al*., 1995; Carabaño *et al*., 1997; Trocino *et al*., 1999; Falcão-e-Cunha *et al*., 2004). Similar rates of gastro-intestinal tract (18.7-19.0%) and dressing percentage were observed at final slaughter among groups, however, confirming what the slaughter observations at 56 d (see Table 4). This result suggests that the increase in beet pulp inclusion from 10 to 25% alone does not modify the gut filling and transit, when low-digestible fibre and starch levels are not modified substantially, as discussed above.

CONCLUSIONS

Increasing dietary soluble fibre from 5.8 to 8.5% by raising dried beet pulp inclusion improved diet digestive utilisation and nutritive value, resulting in better growth and feed conversion. In the tested

Table 8: Effect of soluble fibre and protein source on carcass and meat quality at slaughter (76 d).

	Soluble fibre level (S)			Protein source (P)		*P*-value			
	LS	MS	HS	Soy	Sun	S_L[1]	P[2]	S×P[3]	RSD[4]
Rabbits, No.	40	40	40	60	60				
Slaughter weight (SW), g	2904	2910	2961	2914	2935	0.22	0.56	0.14	203
Gastro-intestinal tract, % SW	19.0	18.7	18.7	18.7	19.0	0.43	0.27	0.51	1.5
Cold carcass weight, g	1735	1745	1780	1748	1758	0.14	0.70	0.16	132
Dressing percentage, % SW	59.7	60.0	60.1	60.0	59.9	0.26	0.59	0.60	1.4
Reference carcass (RC), g	1434	1448	1473	1445	1459	0.14	0.52	0.073	117
Dissectible fat, % RC	3.3	3.3	3.4	3.2	3.4	0.58	0.17	0.73	0.8
Hind legs, % RC	32.8	32.1	32.4	32.5	32.3	0.25	0.56	0.66	1.0
Hind-leg muscle to bone ratio	6.12	6.22	6.29	6.20	6.23	0.24	0.77	0.15	0.42
Longissimus lumborum									
pH	5.58	5.59	5.57	5.58	5.59	0.62	0.67	0.99	0.09
L*[5]	52.0	51.2	52.8	52.3	52.3	0.14	0.95	0.88	2.50
a*[6]	−2.40	−2.53	−2.41	−2.50	−2.38	0.97	0.42	0.044	0.75
b*[7]	0.06	−0.48	0.07	−0.13	−0.10	0.98	0.94	0.090	1.96
Biceps femoris									
pH	5.81	5.84	5.81	5.81	5.83	0.93	0.42	0.74	0.11
L*	50.7	50.4	51.5	50.7	51.0	0.18	0.57	0.028	2.60
a*	−2.34	−2.36	−2.31	−2.38	−2.27	0.73	0.27	0.18	0.40
b*	0.21	0.34	0.11	0.18	0.27	0.78	0.73	0.82	1.52

[1]S_L = Probability of soluble fibre level (linear component of variance). [2]P = Probability of protein source. [3]S×P = Probability of the interaction soluble fibre×protein source. [4]RSD = Residual standard deviation. [5]L*, lightness index; [6]a*, red-green component; [7]b*, yellow-blue component (CIE, 1976).

range, caecal fermentation and intestinal mucosa traits did not substantially change and, in the absence of ERE, health status was good in all rabbits. Moreover, sunflower meal fully replaced soybean meal with no consequences in growth performance or digestive health and without interactions with dietary soluble fibre level.

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