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Effect of increasing lignin in isoenergetic diets at two soluble fibre levels on digestion, performance and carcass quality of growing rabbits

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Running head: Lignin and soluble fibre in growing rabbits

Abstract

To assess the effect of increasing dietary lignin in isoenergetic diets at two soluble fibre (SF) levels on digestion, performance and carcass quality of growing rabbits, four diets were formulated according a 2×2 factorial design: low SF-low lignin (LSF/LL), low SF-high lignin (LSF/HL), high SF-low lignin (HSF/LL) and high SF-high lignin (HSF/HL). On average, in HSF diets SF was increased by 49 g/kg DM, mainly replacing starch (-53 g/kg DM), and in HL diets, lignin was increased by 40 g/kg, mainly reducing starch (-78 g/kg DM), with increasing EE (+31 g/kg DM). Two hundred and sixty crossbred weaned rabbits (35 days old) were assigned to the experimental diets, individually housed and fed *ad libitum* until 63 days of age. Digestibility (from 49 to 53 days old), growth performance (from 35 to 63 days old), carcass quality (at 63 days old) and caecal environment (at 63 days old) were studied in 12, 65, 45 and 16 rabbits per diet, respectively. High SF diets showed higher CTTAD of fibrous fractions (+0.206±0.011, +0.207±0.015, +0.214±0.011 and +0.167±0.015 for aNDFom, ADFom, hemicelluloses and cellulose, respectively, $P<0.001$), OM (+0.042±0. 004, $P<0.001$) and GE (+0.055±0.005, $P<0.001$), resulting in high DE content (10.6 *vs.* 9.30 MJ/kg DM). In contrast, CTTAD of CP was lower (-0.023±0.009, $P=0.013$), as well as the DP content (96.9 *vs.* 103 g/kg DM). This dietary variation reduced the DM content of caecal digesta (-28±3 g/kg, $P<0.001$), besides increasing its VFA concentration (+18.0±4.0 mmol/L, $P<0.001$) and reducing its pH (-0.28±0.05, $P<0.001$). Feed intake and LW gain decreased, with an improvement of feed to gain ratio (-13.8%, -4.7%, -9.4%, respectively; $P<0.001$). The proportion of gastrointestinal tract was increased, with a subsequent reduction in dressing out (+19±2 g/kg LW and -15±2 g chilled carcass weight/kg LW, respectively, $P<0.001$). High lignin diets showed lower CTTAD of OM (-0.055±0.004, $P<0.001$) and GE (-0.034±0.005, $P<0.001$) without affecting DE and DP contents. This dietary variation increased DM content of caecal digesta (+21±3 g/kg, $P<0.001$), but did not affect the other

caecal digesta traits. Feed intake was higher (+4.9%, $P<0.001$), although differences were dependent on the growth phase and the SF level (maximum difference at 35-49 days with low SF diets, +11.0%, $P<0.001$; minimum difference at 49-63 days with high SF diets, +1.0%, $P=0.689$), but did not affect LW gain and consequently impaired the feed to gain ratio (+5.1%, $P<0.001$). No effect was observed on dressing out, but the dissectible fat proportion increased (+6.7±1.1 g/kg reference carcass weight, $P<0.001$).

Keywords: lignin, soluble fibre, growing rabbits

Introduction

In recent years, the role of dietary soluble fibre (SF) in growing rabbits has been examined in numerous studies and the object of meta-analysis or review (Trocino *et al*., 2013a; Gidenne, 2015). The main practical results of increasing SF (ordinarily from sugar beet pulp) in diets for growing rabbits are usually the reduction in mortality due to digestive troubles and the increase in relative full gastrointestinal weight and a consequent decrease in dressing out. On the other hand, an increase in lignin content in diets for growing rabbits is associated with a lower frequency of digestive troubles and a faster digestive transit (Gidenne and Perez, 1994; Gidenne *et al*., 2001), and to a lower relative weight of caecal digesta (Nicodemus *et al*., 1999; García *et al*., 2002). Accordingly, the impairment of dressing out induced by high SF diets could be amended by increasing the dietary lignin content through reducing the relative caecal weight. The aim of the current study was to assess the effect of increasing dietary lignin in isoenergetic diets at two SF levels on digestion, performance and carcass quality of growing rabbits.

Material and Methods

Diets

Four experimental diets (LSF/LL, LSF/HL, HSF/LL, HSF/HL) were formulated according to a 2×2 factorial design with two levels of SF and lignin. The composition of the experimental diets is described in Table 1. From low SF diets (LSF), high SF diets (HSF) were essentially obtained by increasing the inclusion of beet pulp (+229 g/kg DM) at the expense of wheat (-102 g/kg DM), alfalfa hay (-83 g/kg DM) and cereal straw (-70 g/kg DM). From low lignin diets (LL), high lignin diets (HL) were essentially obtained by increasing the inclusion of defatted grape seed (+141 g/kg DM) at the expense of corn starch (-76 g/kg DM), grape marc (-40 g/kg DM) and cereal straw (-42 g/kg DM). On average, the SF level in

HSF diets was increased by 49 g/kg DM, mainly replacing starch (-53 g/kg DM), and lignin level in HL diets was increased by 40 g/kg, mainly reducing starch (-78 g/kg DM), with increasing EE (+31 g/kg DM). Diets were formulated to have the same DE, CP, amino acid and mineral contents, according to recommendations for fattening rabbits (De Blas and Mateos, 2010). However, differences in DE content were found between LSF and HSF (on average, 9.30 *vs* 10.6 MJ/kg DM, respectively). All the experimental diets included diclazuril (1 ppm) as coccidiostat. No antibiotics were used in feed or water.

Animals and experimental procedures

The experimental protocols followed the Spanish Royal Decree 53/2013 on the protection of animals used for scientific purposes (Boletín Oficial del Estado, 2013), as well as the recommendations for applied nutrition research in rabbits described by the European Group on Rabbit Nutrition (Fernández-Carmona *et al*., 2005), and were approved by the Committee of Ethics and Animal Welfare of the Universitat Politècnica de València.

Two hundred and sixty crossbred rabbits (H×LP does, inseminated with pooled semen from R bucks; lines H, LP and R from Universitat Politècnica de València, Spain) delivered in three batches were used. At weaning (35 days old), animals were blocked by litter, assigned at random to the experimental diets and individually housed in metabolic (44 × 52 × 32 cm; 48 animals) or conventional (26 × 50 × 31 cm; 212 animals) cages until 63 days old. Feed and water were provided *ad libitum*. Throughout the experimental period (February to April), animals were kept at 11ºC to 22ºC, with a photoperiod of 12 hours of light and 12 hours of darkness.

Animals in metabolic cages (12 rabbits per diet) were used to perform a digestibility trial according Perez *et al*. (1995), with a 4-day period for recording feed intake and collecting faeces (from 49 to 53 days old). Faeces were stored in identified sealed plastic bags and frozen at -20 ºC until analysis. The CTTAD of DM, OM, CP, aNFDom, ADFom,

hemicelluloses, cellulose and GE were determined for each animal. In addition, 20 g of the individual faecal samples from each diet were pooled to obtain an average sample used to estimate CTTAD of EE, TDF and SF. All animals (65 rabbits per diet) were used to carry out a growth trial recording feed intake and LW every two weeks. Sanitary status was monitored daily. A total of 37 rabbits (14.2%) died and another four rabbits (1.5%) were discarded as declared morbid due to diarrhoea symptoms, very low feed intake or LW gain. At 63 days of age, 45 rabbits per diet, non-fasted and randomly selected, were slaughtered in the morning (8:00-10:00 h) by electrical stunning (90V, 50Hz, 3s) and bleeding. After skinning, the full gastrointestinal tract was removed and weighed. Next, the caecum was separated and weighed. Carcasses were suspended for 30 min and then cooled in a chamber at 3 ºC for 24 h. Chilled carcass weight (CCW) and reference carcass weight (RCW, after removing liver, kidneys, thoracic viscera and head) were recorded according to Blasco and Ouhayoun (1996). Scapular and perirenal fat were separated and weighed. Caecal digesta from 16 rabbits per diet was collected and the pH was measured (GLP21 pHmeter, Crison, Barcelona, Spain). Samples were taken for later determination of VFA and ammonia concentrations, by adding 2 mL 0.35 M H_3PO_4 or 3 mL 0.35 M H_2SO_4 to 1 g of caecal digesta, respectively. Caecal digesta samples and the remaining caecal digesta were frozen at -20 ºC until analysis.

Chemical analyses

Methods of the AOAC (2002) were used for DM (934.01), ash (942.05), CP (990.03, Dumas method, CN628 Elemental Analyzer, LECO, St. Joseph, MI, USA) and EE (920.39, with acid-hydrolysis of samples prior to the extraction). Starch content was determined according to Batey (1982), by a two-step enzymatic procedure with solubilisation and hydrolysis to maltodextrins with thermostable α-amylase followed by complete hydrolysis with amyloglucosidase (both enzymes from Sigma-Aldrich, Steinheim, Germany), and the resulting glucose being measured by the hexokinase/glucose-6 phosphate

dehydrogenase/NADP system (R-Biopharm, Darmstadt, Germany). The TDF content was determined by a gravimetric-enzymatic method, AOAC procedure 985.29 (2002), with α-amylase, protease and amyloglucosidase treatments (Megazyme Int. Ireland Ltd., Wicklow, Ireland), correcting for ash and CP. The aNDFom, ADFom and lignin (sa) fractions were analysed sequentially according to Mertens *et al*. (2002), AOAC procedure 973.18 (2002) and Robertson and Van Soest (1981), respectively, with a thermostable α-amylase pre-treatment and expressed exclusive of residual ash, by using a nylon filter bag system (Ankom, Macedon, NY, USA). Hemicelluloses and cellulose were calculated by difference [aNDFom-ADFom and ADFom-lignin (sa), respectively]. The SF content was determined as proposed by Van Soest *et al*. (1991), by subtracting the aNDFom corrected for CP from the TDF content. The GE content was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, Loughborough, UK).

Determination of VFA was based on the method described by Jouany (1982). Samples were filtered through 0.45 µm cellulose syringe filters. Next, 100 µL of an internal standard solution (0.4 g of 4-methylvaleric acid diluted in 100 mL of deionised water) and 0.1 mL of a preservative (5% H_3PO_4 and 1% HgCl in deionised water) were added to 0.9 mL of filtrate. One µL from each sample was injected into a gas chromatograph (Fisons 8000 series, Milan, Italy) equipped with a split/splitless injector and FID detector. The separation of VFA was made in a DB-FFAP capillary column (30 m x 0.25 mm x 0.25 µm of film thickness, J&W Scientific, Folson, CA, USA). The carrier gas was N_2 at a constant pressure of 120 kPa. Injector and detector temperatures were set at 200 ºC and 245 ºC respectively. The initial oven temperature was set at 110 ºC, held for five min and increased to 230 ºC at 8.5 ºC/min and finally maintained at that temperature for 10 min. Finally, VFA were identified by comparing their retention times with a standard (46975-U from Supelco®, Bellefonte, PA, USA). Ammonia concentration was determined according to procedure 984.13 of the AOAC

(2002). The VFA and ammonia concentrations were expressed as mmol/L of the liquid phase of caecal digesta.

Statistical analyses

The data were analysed using the GLM procedure from SAS (2009) according to a model including the SF level, the lignin level and their interaction as main effects. In the case of growth performance and carcass traits, litter as block effect and weaning weight as linear covariate were also included in the model. When interaction was significant (P<0.05), the least square means of diets were compared by t-test.

Results

Digestibility

The CTTAD of DM, OM and GE were higher in HSF than in LSF diets (+0.034±0.004, +0.042±0.004 and +0.055±0.005, respectively, P<0.001, Table 2). Similarly, the CTTAD of the fibrous fractions were higher in HSF than in LSF diets (+0.206±0.011, +0.207±0.015, +0.214±0.011 and +0.167±0.015 for aNDFom, ADFom, hemicelluloses and cellulose, respectively, P<0.001). On the contrary, the CTTAD of CP was lower in HSF than in LSF diets (-0.023±0.009, P=0.013). Average CTTAD of TDF and SF, determined by analysing the faeces pools, were higher in HSF than in LSF diets (0.368 *vs.* 0.184 and 0.648 *vs.* 0.444, respectively). Thus, the DE content resulted higher and the DP content lower in HSF than in LSF diets (10.6 *vs.* 9.30 MJ/kg DM and 96.9 *vs.* 103 g/kg DM, respectively; Table 1). On the other hand, the CTTAD of DM, OM and GE were lower in HL than in LL diets (-0.051±0.004, -0.055±0.004 and -0.034±0.005, respectively, P<0.001). However, the CTTAD of hemicelluloses was higher in HL than in LL diets (+0.062±0.011, P<0.001). Average CTTAD of EE, determined by analysing the faeces pools, was higher in HL than in

LL diets (0.833 vs. 0.699). The DE and DP contents were very similar in HL and LL diets, having the same SF level (Table 1).

Growth performance and carcass traits

Compared to LSF diets, HSF diets reduced intake (-13.8%, $P<0.001$, Table 3) as well as, to a lesser extent, LW gain (-4.7%, $P<0.001$) and therefore improved the feed to gain ratio (-9.4%, $P<0.001$). Compared to LL diets, HL diets resulted in higher feed intake (+4.9%, $P<0.001$), but did not affect LW gain and consequently impaired the feed to gain ratio (+5.1%, $P<0.001$). Differences in feed intake between LL and HL diets were dependent on the growth phase and the SF level (Figure 1), being more relevant in the post-weaning phase (35-49 days old) than later (49-63 days old) and with LSF than HSF diets (maximum difference at 35-49 days with LSF diets, +11.0%, $P<0.001$; minimum difference at 49-63 days with HSF diets, +1.0%, $P=0.689$).

Compared to LSF diets, HSF diets led to a higher proportion of the gastrointestinal tract (+19±2 g/kg LW, $P<0.001$, Table 4), as observed mainly in the caecum (+9.4±1.2 g/kg LW, $P<0.001$), lower dressing out (-15±2 g CCW/kg LW, $P<0.001$) and higher reference carcass yield (+5.5±2.1 g RCW/kg CCW, $P=0.010$), but no differences were observed in the proportion of dissectible fat compared to the reference carcass. Additionally, with regard to LSF diets, HSF diets improved the feed to chilled carcass and feed to reference carcass ratios (-9.5% and -10.1%, respectively, $P<0.001$). Compared to LL diets, HL diets reduced the proportion of the caecum (-6.0±1.6 g/kg LW, $P<0.001$) and gastrointestinal tract (-8.3±3.0 g/kg LW, $P=0.006$) only in LSF diets, did not affect the dressing out and increased the reference carcass yield (+16±2 g RCW/kg CCW, $P<0.001$) and the proportion of dissectible fat in the reference carcass (+6.7±1.1 g/kg RCW, $P<0.001$), by increasing the proportion of the two considered fatty depots. Additionally, with regard to LL diets, HL diets impaired the

feed to chilled carcass (+4.2%, P<0.001) and feed to reference carcass (+2.1%, P=0.033) ratios.

Caecal environment

Compared to LSF diets, HSF diets induced lower DM content (-28±3 g/kg, P<0.001, Table 5) and pH (-0.28±0.05, P<0.001), besides higher VFA concentration (+18.0±4.0 mmol/L, P<0.001), without affecting the molar proportions of acetate, propionate and butyrate, as well as the ammonia concentration. Compared to LL diets, HL diets increased the DM content (+21±3 g/kg, P<0.001), more clearly in the HSF diets (+30±4 g/kg, P<0.001) than in LSF diets (+12±4 g/kg, P=0.011), but did not affect the other caecal digesta traits.

Discussion

Soluble fibre replacing starch

In the last two decades, great efforts have been made in researching the effects of increasing SF in rabbit diets. However, the dietary changes involved varied widely among the different experiments. For this reason, it is mainly experiments where SF equivalently replaced starch without important changes in the dietary levels of insoluble fibres, CP and EE that are taken into account in the following discussion.

In the current study, increasing SF at the expense of starch also involved important simultaneous changes in the origin of both soluble and insoluble fibre, resulting in higher CTTAD of SF (determined from pools of faeces), aNDFom, ADFom, hemicelluloses and cellulose. Trocino *et al*. (2013a) emphasised that faecal digestibility of both soluble and insoluble fibre increases linearly with the dietary SF level (as well as with the level of inclusion of beet pulp in the diet) due to the high faecal digestibility of all the fibre constituents from sources of SF (mainly beet pulp). Although starch is almost completely digested in rabbits (Blas and Gidenne, 2010) and the CTTAD of SF in the current study was

lower than those usually reported (0.70-1.00; Trocino *et al*., 2013a; Gidenne, 2015), increases in CTTAD of the different fibrous fractions explain the higher CTTAD of DM, OM and GE as well as dietary DE content (+14%) when SF replaced starch, as also observed in other experiments (Grueso *et al*., 2013; Trocino *et al*., 2013b). In contrast, Xiccato *et al*. (2011) and Delgado *et al.* (2018, 2019) found no effects on the CTTAD of DM and GE, as well as on dietary DE content, as no differences or lesser increase in CTTAD of SF and lesser increase in CTTAD of aNDFom were observed when SF replaced starch, probably because the dietary changes in these studies involved different variations in the proportion of fibre constituents coming from wheat bran or from alfalfa and straw, respectively. The effect of SF replacing starch on the CTTAD of CP would be also dependent on the influence of the concomitant changes in the nature of the dietary CP, associated with variations in the contribution of the diverse ingredients. In the current study, increasing SF at the expense of starch slightly reduced the CTTAD of CP, in parallel to increased neutral detergent insoluble CP (37 and 8 g/kg DM in HSF and LSF diets, respectively. These results agree with those by Grueso *et al.* (2013), where no data on neutral detergent insoluble CP are indicated, and Delgado *et al.* (2019). Conversely, no differences were found in CTTAD of CP in studies where changes in neutral detergent insoluble CP were negligible (Xiccato *et al*., 2011; Trocino *et al*., 2013b).

The amount of TDF apparently digested in the gastrointestinal tract during the growing period averaged 23.1 and 12.0 g/d in HSF and LSF diets, respectively. Consistently, fermentative activity estimated as the VFA concentration in caecal digesta was 26% higher in HSF than LSF diets. This effect of SF replacing starch has been also previously reported (Xiccato *et al*., 2011; Martínez-Vallespín *et al*., 2013; Trocino *et al*., 2013b; Soler, 2014; Ocasio-Vega *et al*., 2018), in accordance with caecal VFA concentration increasing linearly with dietary SF content (Trocino *et al*., 2013a). Higher VFA concentration in ileal digesta when SF replaced starch has been also reported (Ocasio-Vega *et al*., 2018). The effect on the

caecal fermentation profile is controversial, as depending on the studies no changes (Trocino *et al*., 2013b; current study), an increase of butyrate at the expense of acetate (Martínez-Vallespín *et al*., 2013; Soler, 2014), and the opposite (Xiccato *et al*., 2011; Ocasio-Vega *et al*., 2018) have been observed. The discrepancies are probably due to methodological differences between experiments or/and to concurrent dietary changes induced by increasing SF at the expense of starch, which varied greatly between experiments, particularly those affecting the origin of the dietary soluble and, particularly, insoluble fibre. Decreases in the ammonia caecal concentration reported when SF replaced starch (Xicatto *et al*., 2011; Martínez-Vallespín *et al*., 2013; Trocino *et al*., 2013b; Soler, 2014) could be explained by an increase in ammonia uptake for microbial protein synthesis supporting the enhanced microbial activity, but this effect was not detected in the current study. In addition to the methodological differences mentioned above, interactions with changes in caecal proteolytic activity due to variations in the origin of protein cited above can be hypothesised. Caecal pH was lower in HSF than LSF diets, as found in some experiments (Xiccato *et al*., 2011; Martínez-Vallespín *et al*., 2013; Trocino *et al*., 2013b, Delgado, 2017) but not in others (Martínez-Vallespín *et al*., 2013; Soler, 2014; Delgado, 2017). Caecal pH decreases linearly as SF increases (Trocino *et al*., 2013a) but VFA and ammonia concentrations in caecal digesta explain only 12% of caecal pH variability, also depending on physicochemical characteristics of caecal DM (García *et al*., 2002).

The DM in caecal digesta decreased when SF replaced starch, probably because of the high water holding capacity of pectins (Gidenne *et al*., 2010). However, this effect would be dependent on the dietary lignin content, as this reduction was greater in LL than HL diets (averaging 42 g and 82 g lignin/kg DM, respectively) and was also observed in 4-6 week old rabbits when diets had 57 g lignin/kg DM, but disappeared in diets containing 97 g lignin/kg DM (Martínez-Vallespín *et al*., 2013; Soler, 2014). This interaction could be explained by

faster digestive transit of fine particles (and probably liquid phase) and not of large ones, as a result of increased dietary lignin content (Gidenne and Perez, 1994), as well as by the hydrophobic nature of lignin, which could reduce the water holding capacity of caecal digesta.

Higher DE content in HSF diets compared to LSF diets explains lower feed intake and improved feed to gain ratio. These effects have previously been reported (Martínez-Vallespín *et al*., 2011; Trocino *et al*., 2013b; Soler, 2014; Delgado *et al.*, 2018). However, the effect of SF replacing starch on LW gain is controversial and would be dependent on protein supply. Thus, while DE intake was very similar with HSF and LSF diets (1.44±0.02 *vs*. 1.46±0.02 MJ/d, P=0.224), DP intake was clearly lower in HSF than in LSF diets (13.1±0.2 *vs*. 16.1±0.2 g/d, -23%, P<0.001). In fact, the usual recommendation for the DP to DE ratio for growing rabbits is 10.5-11.0 g/MJ (Xiccato and Trocino, 2010) and HSF but not LSF diets would be unbalanced (9.12 *vs*. 11.0 g/MJ, respectively). In this line, lower LW gain has been observed when SF replaced starch in low protein diets (144-147 g CP/kg DM) but not in high protein diets (172-179 g CP/kg DM) (Martínez-Vallespín *et al*., 2011; Soler, 2014) and when DP intake was hardly reduced while SF replaced starch (Trocino *et al*., 2013b; Delgado *et al.*, 2018). Logically, no effects on feed intake, LW gain or feed to gain ratio were observed when SF replaced starch without altering the dietary DE content and the DP to DE ratio (Xiccato *et al*., 2011).

A negative impact of increasing SF at the expense of starch on dressing out was observed, associated with higher relative weight of the gastrointestinal tract, mainly as a result of the effect on caecum, in line with other studies (Martínez-Vallespín *et al*., 2013; Pascual *et al*., 2014; Soler, 2014). However, no effects of SF replacing starch on dressing out have been reported (Trocino *et al*., 2013b), even in spite of increasing the relative weight of the gastrointestinal tract (Xiccato *et al*., 2011). Trocino *et al*. (2013a) underlined that

elucidating the effects of fibrous fractions on gastrointestinal weight and dressing out can be difficult due to differences in age and LW at slaughter, as well as in pre-slaughter conditions (fasting or not, transport and wait length, etc.) among experiments. On the other hand, SF replacing starch increased the reference carcass yield, as also reported by Pascual *et al.* (2014), who detected a concomitant reduction in the relative weight of liver. No effects of SF replacing starch on dissectible fat in the reference carcass have been reported in studies where neither LW nor chilled and reference carcass weight were affected (Xiccato *et al*., 2011; Trocino *et al*., 2013b). Similarly, Delgado *et al.* (2018) found no differences in fat content of the carcass estimated *in vivo* by bioelectrical impedance analysis when SF replacing starch had no effect on LW. However, Pascual *et al*. (2014) observed less dissectible fat when SF replaced starch, impairing LW as well as chilled and reference carcass weight. In the current study, SF replacing starch also impaired LW (-71±21 g, P=0.001), CCW (-74±13 g, P<0.001) and RCW (-52±10 g, P<0.001), but no effect was observed on dissectible fat in the reference carcass, probably because of the above commented low PD to DE ratio in HSF diets, resulting in poor protein supply and more energy for the synthesis of body fat. In this situation, the lower body fat associated with lower carcass weight would be compensated by the higher body fat associated with low PD to DE ratio (De Blas and Mateos, 2010). Interestingly, the feed to chilled or reference carcass ratios remained noticeably improved in HSF diets compared to LSF diets.

Lignin and fat replacing starch

In spite of much effort in research on rabbit nutrition involving changes in dietary levels of lignin, fat and starch, to the authors' knowledge no experiments have approached the effects of increasing the dietary lignin and fat contents, replacing starch in growing rabbit diets with negligible variations in the level of other insoluble or soluble fibrous fractions.

In the current study, this dietary change reduced the CTTAD of DM, OM and GE, but the dietary DE content was unaffected, mainly due to the higher GE content in HL than in LL diets. On the other hand, in spite of the higher CTTAD of hemicelluloses in HL than in LL diets, probably associated with changes in the origin of this fibrous fraction, the amount of TDF apparently digested in the gastrointestinal tract during the growing period was similar in HL and LL diets (averaging 18.4 and 17.1 g/d, respectively), explaining the lack of differences in VFA concentration and pH in caecal digesta. However, Martínez-Vallespín *et al*. (2013) found higher a VFA concentration in caecal digesta when ADFom, essentially lignin, replaced starch without changing fat content, although in younger animals (5-week old) sampled in the evening.

The role of lignin in stimulating the rate of passage of fine particles (and probably liquid phase) and not of large ones, as well as its previously cited hydrophobic nature, would explain the DM content of caecal digesta being higher in HL than in LL diets, particularly in HSF diets. These results closely agree with those reported by Martínez-Vallespín *et al*. (2013). However, the consequences on caecal weight are controversial, as increasing lignin in the current study reduced caecal weight in LSF diets, but not in HSF diets, whereas Martínez-Vallespín *et al*. (2013) found the opposite. Age and diet dependent differences in the role of lignin affecting feed intake and stimulating the digestive transit can be hypothesised to explain discrepancies between both studies. During the post-weaning period (4 to 7 weeks of age; Martínez-Vallespín *et al*., 2011), lignin similarly increased feed intake with high and low SF diets, whereas the reduction in relative caecal weight of 5-week old rabbits was detected in high but not in low SF diets (Martínez-Vallespín *et al*., 2013), suggesting a greater effect of lignin stimulating caecal rate of passage in high SF diets. In contrast, during the late growing period (7 to 9 weeks of age; current study), lignin increased feed intake (194±3 *vs*. 185±3 g DM/day, P=0.027) and reduced relative caecal weight with LSF diets, but did not

affect feed intake (167±3 *vs*. 166±3 g DM/day, *P*=0.689) and relative caecal weight with HSF diets, paradoxically suggesting the persistence of the specific role of lignin stimulating feed intake and caecal rate of passage in low SF-low DE diets but not in high SF-high DE diets, where feed intake seemed essentially regulated by a chemostatic mechanism to maintain DE intake constant (Xiccato and Trocino, 2010).

Feed intake during the overall growing period was higher in HL than in LL diets, although HL and LL diets were iso-energetic in terms of DE. Consequently, DE intake was higher with HL than with LL diets (1.48±0.02 *vs*. 1.41±0.02 MJ/d, +5.0%, *P*<0.001), although LW gain was unaffected and, therefore, feed to gain ratio was impaired in HL diets compared to LL diets. On the other hand, the above mentioned effect of lignin decreasing relative caecal weight with LSF diets was paralleled by decreasing relative weight of the gastrointestinal tract, although dressing out did not improve significantly. Moreover, contrary to what was hypothesised, increasing lignin did not improve dressing out in HSF diets due to the lack of effect on relative caecal and gastrointestinal weights. As fat content and digestibility was higher in HL than in LL diets, the amount of digested fat and its contribution to DE intake were greater with HL than with LL diets (averaging 8.2 and 3.5 g/d, 21.6% and 9.6%, respectively). Higher DE intake and digested fat with HL than with LL diets without affecting LW (-4±21 g, *P*=0.856) and CCW (+1±13 g, *P*=0.919) would lead to higher carcass adiposity, as suggested by the higher dissectible fat proportion in the reference carcass with HL than with LL diets. Fernández and Fraga (1996) reported that dietary fat replacing starch increases the body fat content and reduces the relative weight of liver, probably as a consequence of higher availability of dietary fat and lower extent of hepatic lipogenesis. Higher fat accretion would explain higher reference carcass weight (+24±10 g, *P*=0.013) and, together with the hypothetical reduction in relative liver weight, higher reference carcass

yield (+16±2 g/kg CCW, P<0.001) with HL than with LL diets. Nevertheless, the feed to chilled or reference carcass ratios were still impaired in HL diets compared to LL diets.

Conclusion

With respect to low SF diets, high SF diets with SF replacing starch with minor changes in insoluble fibre level, but involving important changes in the origin of both soluble and insoluble fibre, showed higher CTTAD of all fibrous fractions, OM and GE, resulting in high DE content. This dietary variation also affected caecal environment, reducing DM content of caecal digesta besides increasing its VFA concentration and reducing its pH, reduced feed intake and impaired LW gain but improved feed to gain ratio, and had negative impact on dressing out.

Increasing lignin and fat replacing starch reduced CTTAD of OM and GE without affecting DE content. This dietary variation increased the DM content of caecal digesta, increasing feed intake except for high SF diets in the late growing period, without affecting LW gain and, consequently, impaired feed to gain ratio. Increasing lignin and fat replacing starch failed to improve dressing out in high SF diets, but increased carcass adiposity.

The research over the last two decades has shown that the use of diets enriched in soluble or insoluble fibre is of interest to reduce the incidence of digestive problems in growing rabbits, in the context of a rabbit production that aims to eliminate or at least minimise the use of antibiotics. However, such diets can reduce the animals' performance. In the current study, the negative impact that the increase of soluble fibre replacing starch had on dressing out could not be corrected by increasing lignin and fat replacing starch. More research is needed to provide diets that simultaneously optimise digestive health and performance of growing rabbits.

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Figure caption

Figure 1. Effect of diet on feed intake in rabbits during (1) the post-weaning phase (35 to 49 days old) and (2) the late growing phase (49 to 63 days old). LSF: low soluble fibre; HSF: high soluble fibre; LL: low lignin; HL: high lignin. $^{a, b, c, b}$ Means not sharing any common superscript are significantly different ($P<0.05$).