Combined feeding of rabbit female and young: partial replacement of starch with acid detergent fibre or/and neutral detergent soluble fibre at two protein levels

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Abstract

To evaluate the effects of the composition of weaning diets used for combined feeding both on the performance and body condition of rabbit does and on the growth and health of young rabbits, eight experimental diets were formulated according to a factorial design 2x2x2, the three factors being: i) partial replacement of starch with acid detergent fibre (ADF), ii) partial replacement of starch with neutral detergent soluble fibre (NDSF) and iii) reduction of the crude protein (CP) content from 175 to 145 g kg^{-1} dry matter (DM). The study involved 138 rabbit does and 318 litters fed experimental diets from 17^{th} to 28^{th} day of lactation (weaning). Thereafter, 2371 weaned rabbits (784 in individual cages and 1587 in collective ones) continued receiving the same experimental diets until 49 days of age and a commercial finishing diet until 60 days of age. Replacing starch with ADF increased feed intake of does (+26±5 g DM day^{-1}, P<0.001) but, only with high CP diets, impaired their milk yield (-15±6 g day^{-1}, P<0.01) and perirenal fat thickness (PFT) change (-0.41±0.13 mm, P<0.01), as well as litter weight at weaning (-154±52 g, P<0.01); from weaning to 49 days of age, this replacement increased feed intake (+14±2 g DM day^{-1}, P<0.001) and impaired live weight gain (-1.6±0.7 g day^{-1}, P<0.05), but reduced mortality rate (-10.2%, P<0.001). Replacing starch with NDSF reduced feed intake of does, in greater extent with high CP diets (-34±6 g DM day^{-1}, P<0.001) than with low CP diets (-19±7 g DM day^{-1}, P<0.01), as well as their milk yield (-16±4 g day^{-1}, P<0.001), with impairing PTF change only with high CP diets (-0.30±0.13 mm, P<0.05); litter feed intake and weight at weaning were also negatively affected (-14.4±2.5 g DM day^{-1}, -202±38 g, P<0.001); from weaning to 49 days of age, this replacement reduced feed intake (-8±2 g DM day^{-1}, P<0.001), impaired live weight gain only with low CP diets (-2.3±1.0 g day^{-1}, P<0.05) and reduced mortality rate (-15.1%, P<0.001). Reducing CP content affected negatively feed intake of does (-22±5 g DM day^{-1}, P<0.001), as well as their milk yield, this impairment being more important with more starch-less ADF diets (-24±5 g day^{-1}, P<0.001) than with less starch-more ADF diets (-13±6 g day^{-1}, P<0.05), and, only with more starch-less ADF diets, PFT change (-0.36±0.13 mm, $P<0.01$); litter feed intake was affected negatively (-20.1±2.4 g DM day^{-1}, $P<0.001$), as well as litter weight at weaning, in great extent when fed more starch-less ADF diets (-581±52 g, $P<0.001$) than when fed less starch-more ADF diets (-403±54 g, $P<0.001$); from weaning to 49 days of age, this dietary change reduced feed intake (-6±2 g DM day^{-1}, $P<0.001$) and impaired live weight gain (-5.0±0.7 g, $P<0.001$), but reduced mortality rate (-19.9%, $P<0.001$). Overall, the three dietary changes under study impaired milk yield, body condition of does and litter weight at weaning, although the negative effect of replacing starch with ADF was only observed in the high CP diets; moreover, growth during post-weaning period also decreased. However, in an Epizootic Rabbit Enteropathy context, mortality rate during this period was reduced by these three dietary changes, which effects seemed additive.

Keywords: rabbit, combined feeding, weaning diet, fibre, starch, protein.

Introduction

One of the main handicaps of current intensive rabbit production is the frequent appearance of digestive troubles during the growing period, compromising farm viability. In the last decade, the digestive physiology and nutrition of young rabbits around weaning has been widely reviewed in order to define better their requirements and the adequate diet for minimizing these troubles. In fact, the level and type of some nutrients (such as fibre, starch or protein) have been frequently related with digestive health of young rabbit, although their respective effects are sometimes difficult to elucidate because dietary changes are usually complex and involve several nutrients. Moreover, diet should not be commonly considered as a primary cause of digestive troubles but as an epidemiological risk factor depending on other factors such as hygienic or sanitary status of farm, feeding management (age at weaning, diet switching, etc…) and use of antimicrobials agents; thus, it is not infrequent that the same dietary variations behave differently with respect to digestive health depending on the mentioned factors.

The beneficial effect of increasing dietary fibre replacing starch on digestive health of young rabbits is well established (Blas and Gidenne, 2010; De Blas *et al*., 1999; Gidenne and García, 2006; Gidenne *et al.*, 2010). Depending on if low-digestible fibre (lignin, cellulose) or high-digestible fibre (pectins, hemicelluloses) are involved, promoted rate of passage of digesta in the caeco-colic segment or favourable changes in carbohydrate substrate for microbiota, fermentative activity and gut ambient are mainly concerned. Reduction of dietary crude protein content has also positive effect on digestive health of young rabbits (Carabaño *et al.*, 2009). This reduction has been related with lower ileal flow of nitrogen, caecal *Clostridium perfringens* population and mortality (Chamorro *et al.*, 2007).

In spite of these nutritional advances, the standard management in rabbit farms makes difficult the use of specific diets for young rabbits before weaning, as usual in other mammals. Modifications in the cages and feeders has been proposed (Fortun-Lamothe *et al.*, 2000), but a practical alternative would be a combined feeding of rabbit does and litters during late lactation (from 16-18th day postpartum onward) with a weaning diet formulated to meet the requirements of young rabbits around weaning. However, the dietary changes above described as improving digestive health of young rabbits could be inadequate to meet the nutritive concentration required in diets for lactating rabbit does (De Blas and Mateos, 2010). Nevertheless, there is scarce knowledge about how these dietary changes could affect performance and body condition of rabbit females when applied exclusively during late lactation.

In view of the foregoing, the aim of the present work was to evaluate, in a factorial design, the effects of partial replacement of starch with acid detergent fibre (ADF), partial replacement of starch with neutral detergent soluble fibre (NDSF) and reduction in the crude protein (CP) content in the weaning diet used for combined feeding both on the performance and body condition of rabbit does and on the growth and health of young rabbits.

Material and Methods

Diets

Eight experimental weaning diets were formulated according to a factorial design 2×2×2. The factors were the replacement of 50 g kg^{-1} dry matter (DM) of starch with ADF, the replacement of 50 g kg^{-1} DM of starch with NDSF and the reduction of the CP content from 175 to 145 g kg^{-1} DM; this design allowed to compare all the combinations of these factors (LLH, HLH, LHH, HHH, LLL, HLL, LHL and HHL), where the first, second and third letters were respectively the dietary ADF, NDSF and CP contents (low or high; L or H). The ingredient and chemical composition of the experimental diets are described in Tables 1 and 2, respectively. Starch was replaced by ADF mainly by reducing wheat grain (-90 g kg^{-1} DM), wheat bran (-40 g kg^{-1} DM) and grape pomace (-70 g kg^{-1} DM) and increasing oat hulls (+60 g kg^{-1} DM) and defatted grape seed (+130 g kg^{-1} DM); starch was replaced by NDSF mainly by reducing wheat grain (-90 g kg^{-1} DM), soybean hulls (-40 g kg^{-1} DM) and treated wheat straw (-80 g kg^{-1} DM) and increasing beet pulp (+170 g kg^{-1} DM) and oat hulls (+50 g kg^{-1} DM); CP content was reduced mainly by replacing sunflower meal (-360 or -355 g kg^{-1} DM) with soybean protein concentrate (+75 g kg^{-1} DM), beet pulp (+60 g kg^{-1} DM), defatted grape seed (+60 g kg^{-1} DM) and treated wheat straw (+130 or +127.5 g kg^{-1} DM). All the experimental diets included zinc bacitracin (100 ppm) and robenidine (66 ppm).

Chemical analyses of diets were performed according to the methods of the Association of Official Analytical Chemists (2000): 934.01 for DM, 942.05 for ash, 976.06 for CP and 920.39 for ether extract, 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 thermo-stable 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). Neutral detergent fibre (NDF), ADF and acid detergent lignin (ADL) fractions were analysed sequentially (Van Soest *et al.*, 1991) with a thermo-stable α-amylase pre-treatment and expressed exclusive of residual ash, using a nylon filter bag system (Ankom, Macedon, NY, USA). NDSF content was determined according to Hall *et al.* (1997), adapting the method to the nylon filter bag system and with the following modifications: a Soxhlet extraction of the samples with petroleum ether (6 hours) was performed prior to the ethanol-water extraction and the NDF fraction was determined from the ether-ethanol insoluble residue instead of from the raw sample. Pellet hardness was determined by manual spring durometer (Amandus-Kahl, Reinbek, Germany) measuring 25 pellets from each experimental diet.

Animals and experimental procedure

The experimental protocols followed both the Spanish Royal Decree 1201/2005 on the protection of animals used for scientific purposes (Boletín Oficial del Estado, 2005) and the recommendations for applied nutrition research in rabbits described by the European Group on Rabbit Nutrition (Fernández-Carmona *et al.*, 2005), being approved by the Committee of Ethics and Animal Welfare of the Universidad Politécnica de Valencia.

Animals were housed in an experimental farm having a history of Epizootic Rabbit Enteropathy (ERE). The building was equipped with a forced ventilation and cooling system to maintain the temperature within the range of 12ºC to 25ºC throughout the experimental period (November to July). A photoperiod of 16 hours of light and 8 hours of darkness was established using artificial lights just in the months in which was needed. A lactation trial was carried out in the breeding unit, equipped with cages which dimensions were 50 × 70 × 32 cm and provided with nesting boxes, and a growing trial was conducted in the fattening unit using individual (26 × 50 × 31 cm) and collective cages (50 × 80 × 32 cm, 8 rabbits).

Lactation trial

One hundred and thirty-six rabbit does (maternal line A, Universidad Politécnica de Valencia, Spain) providing a total of 318 litters were used. Litters were standardized to 9-10 kits at birth. Until 17th day of lactation, females received *ad libitum* a commercial diet for reproductive rabbit does (177 g CP, 155 g starch and 191 g ADF kg^{-1} DM). The does were inseminated at 11th day postpartum, and each 21 days afterwards if needed, with pooled semen (growth line R, Universidad Politécnica de Valencia, Spain).

At 17th day of lactation, females were separated from their litters to avoid free suckling, litters were re-standardised to 8-9 kits, transferring the remainder kits to nursing does, and both females and litters had free access to one of the experimental diets, randomly assigned. Until weaning (28th day of lactation), females were taken to the litter cage once daily in the morning for a short time and milk production was measured from 21st to 25th day of lactation by weighing the doe immediately before and after suckling. After weaning, females were again fed *ad libitum* with the commercial feed for reproductive rabbit does.

Live weight and perirenal fat thickness (PFT) of females was measured at 17th and 28th day of lactation by echography (Pascual *et al.*, 2000), using an ultrasound unit (JustVision 200, Toshiba Medical Systems, Tokyo, Japan) equipped with image analyser software to determine distances. Estimated body energy (EBE) content of does was determined from live weight and PFT of does, using the equation proposed by Pascual *et al.* (2004). Feed intake of females was controlled from 17th day of lactation to weaning. Litter size and weight was registered at 17 days of age and weaning. Solid feed intake of litters was monitored from 17 days of age to weaning.

Growing trial

After weaning, 2371 young rabbits were distributed in two different allocations: 784 animals were housed in individual cages and 1587 in collective ones. All of them continued with the respective experimental diets until 49 days of age, when they were switched to a non-medicated commercial finishing diet (168 g CP, 163 g starch and 180 g ADF kg^{-1} DM) until 60 days of age. Live weight and feed intake were controlled for the animals allocated in individual cages at 28, 49 and 60 days of age. Mortality was monitored in both individual and collective cages.

Statistical analysis

Data were analysed by SAS software (Statistical Analysis Systems Institute, 2002). Traits from rabbit does and litters were analysed using the mixed procedure, the model including the diet (LLH, HLH, LHH, HHH, LLL, HLL, LHL or HHL), the parity order (from 1 to 8) as repeated measure factor and the pregnancy status at 17th day of lactation (pregnant or not pregnant), with a permanent effect of each rabbit doe and the error as random factors; litter size and weight at 17th day of lactation were included as covariates when analysing data at weaning. Growing traits were analysed using the mixed procedure, the model including the diet, the age (28, 49 or 60 days) as repeated measure factor, the interaction between diet and age and the growing batch (from 1 to 5), with a permanent effect of each animal (nested to diet and growing batch) and the error as random factors. Mortality rate during the growing period was analysed by the genmod procedure. Pellet hardness was analysed by the glm procedure.

Orthogonal contrasts were computed to test the differences due to replacement of starch with ADF [(HLH+HHH+HLL+HHL)/4 – (LLH+LHH+LLL+LHL)/4], to replacement of starch with NDSF [(LHH+HHH+LHL+HHL)/4 – (LLH+HLH+LLL+HLL)/4] and to CP reduction [(LLL+HLL+LHL+HHL)/4 – (LLH+HLH+LHH+HHH)/4]. Additional contrasts were performed to look for interactions between the CP content and the replacement of starch with ADF (high CP diets: [(HLH+HHH)/2 – (LLH+LHH/2]; low CP diets: [(HLL+HHL)/2 – (LLL+LHL)/2]) or the replacement of starch with NDSF (high CP diets: [(LHH+HHH)/2 – (LLH+HLH/2]; low CP diets: [(LHL+HHL)/2 – (LLL+HLL)/2]).

Results

Lactation trial

The effect of the experimental diets on the performance of rabbit does and litters from 17^{th} to 28^{th} day of lactation is shown in Table 3.

Feed intake of rabbit does increased when ADF replaced starch (+7.0%, P<0.001), in spite of which digestible energy (DE) intake decreased (-4.8%, P<0.001). The effects of this dietary variation on milk yield and PFT change depended on the CP content, as illustrated in Figure 1. Thus, this replacement impaired milk yield in high CP diets (-15±6 g day^{-1}, P<0.01) and not in low CP diets (-3±6 g day^{-1}, P>0.1). Although lower live weight increase of rabbit does from 17^{th} day of lactation to weaning was also observed when ADF replaced starch, the negative effect on PFT change was only observed in animals fed high CP diets (-0.41±0.13 mm, P<0.01), not in those fed low CP diets (+0.17±0.13 mm, P>0.1); similarly, EBE change was only affected in animals fed high CP diets (-1.43±0.46 MJ, *P*<0.01), not in those fed low CP diets (-0.77±0.47 MJ, *P*>0.1). Litters showed similar feed intake but lower DE intake as feed (-12%, *P*<0.001) when ADF replaced starch. The overall effect of this replacement on litter weight at weaning was near to significance level (*P*=0.08) because differences were recorded with high CP diets (-154±52 g, *P*<0.01) and not with low CP diets (+24±54 g, *P*>0.1), as illustrated in Figure 1.

On the other hand, NDSF replacing starch reduced feed intake (-6.6%, *P*<0.001), in an extent depending on the CP content, greater for high CP diets (-34±6 g DM day^{-1}, *P*<0.001) than for low CP diets (-19±7 g DM day^{-1}, *P*<0.01), as shown in Figure 2; as a consequence, this replacement decreased DE and CP intake of rabbit does (-5.5% and -7.4%, respectively, *P*<0.001), more extensively with high CP diets (-0.33±0.07 MJ day^{-1}, *P*<0.001; -6.2±1.0 g day^{-1}, *P*<0.001) than with low CP diets (-0.15±0.07 MJ day^{-1}, *P*<0.05; -3.4±1.1 g day^{-1}, *P*<0.01). This replacement also reduced milk yield (-6.1%, *P*<0.001). Live weight increase, PFT change and EBE change of rabbit does throughout lactation were negatively affected when NDSF replaced starch in high CP diets (-59±25 g, -0.30±0.13 mm and -0.9±0.4 MJ, respectively, *P*<0.05) but not in low CP diets (-10±26 g, -0.04±0.13 mm and -0.2±0.5 MJ, respectively, *P*>0.1), as illustrated in Figure 2 for PFT change. Litter feed intake (and, similarly, DE and CP intake as feed) and weight at weaning were reduced by NDSF replacing starch (-19% and -4.1%, respectively, *P*<0.001).

The reduction of CP content affected negatively feed, DE and, particularly, CP intake of rabbit does (-5.6%, -13% and -22%, respectively, *P*<0.001), as well as their milk yield (-7.2%, *P*<0.001). Although live weight increase and EBE change of rabbit does from 17th day of lactation to weaning were also lower when reducing CP content, this effect was not significant for PFT change, as a consequence of interactions above commented (Figures 1 and 2). Litter feed intake (and, similarly, DE and CP intake as fed) and weight at weaning were negatively affected by the reduction of CP content (-25% and -9.5%, respectively, *P*<0.001).

Throughout the trial, the mortality rate was 12% for rabbit does and negligible for suckling rabbits from 17th to 28th day of age (20 from a total of 2836 (0.7%)).

Growing trial

Table 4 shows the effect of the experimental diets on the performance of growing rabbits reared in individual cages from weaning to 60 days of age.

From weaning to 49 days of age, the replacement of starch with ADF increased feed intake (+13%, P<0.001) and reduced live weight gain (-3.2%, P<0.05), the feed conversion rate (FCR) being so worsened (+17%, P<0.001). After switching to commercial finishing diet, feed intake continued higher in animals previously fed more ADF-less starch diets (+3.5%, P<0.01), which had higher live weight gain (+4.1%, P<0.01) than those previously fed less ADF-more starch diets.

The inclusion of NDSF instead of starch reduced feed intake (and, similarly, DE and CP intake) from weaning to 49 days of age (-6.7%, P<0.001), without affecting live weight gain in animals fed high CP diets (0.1±1.1 g day^{-1}, P>0.1) but reducing it in those fed low CP diets (-2.3±1.0 g day^{-1}, P<0.05), thus the FCR resulting improved more extensively with high CP diets (–0.11±0.3, P<0.001) than with low CP diets (–0.07±0.2, P<0.01). After switching to commercial finishing diet, feed intake continued lower in animals previously fed more NDSF-less starch diets (-2.7%, P<0.05), which had lower live weight gain (-3.6%, P<0.01) than those previously fed less NDSF-more starch diets.

The reduction of CP content affected negatively feed, DE and, particularly, CP intake from weaning to 49 days of age (-5.7%, -12% and -21%, respectively, P<0.001), as well as live weight gain (-9.2%, P<0.001) and FCR (+4.0%, P<0.001). After switching to commercial finishing diet, animals previously fed low CP diets had similar feed intake but improved live weight gain (+8.0%, P<0.001) and FCR (-6.6%, P<0.001) compared with those previously fed high CP diets.

Finally, Table 5 shows the mortality rate registered in growing rabbits depending on the weaning diet. During the first 3 weeks after weaning mortality was high because of an ERE outbreak. Nevertheless, the three evaluated dietary variations (ADF replacing starch, NDSF replacing starch, CP reduction) led to lower mortality during this period (−10.2, −15.1, −19.9%, respectively, $P<0.001$). No effects of the above mentioned strategies were detected on mortality rate after switching to commercial finishing diet.

Discussion

Replacement of starch with fibre

In the current study, dietary starch was partially replaced with ADF or/and NDSF. Whatever the fibre nature is, the increase of fibre content at the expense of starch impairs dietary energy value and the general physiological response of rabbits to this effect includes an elevation of feed intake to meet energy requirements unless energy dilution is excessive (Xiccato and Trocino, 2010). However, other aspects concerning feed intake regulation must be considered. Between them, low-digestible fibre as cellulose and mainly lignin stimulates the intestinal rate of passage (Gidenne, 2003) which could promote feed intake, whereas soluble fibre as mainly pectins reduces feed intake, probably as consequence of its high water holding capacity to form gels in solution, higher gastric and caecal content weight and slower digestive transit (Fraga *et al*., 1991; Carabaño *et al*., 1997; Gidenne *et al*., 1998).

Below 11.7-12.2 MJ DE kg^{-1} DM, lactating rabbit does fed *ad libitum* reduce its DE intake as dietary content decreases (Xiccato and Trocino, 2010). The experimental diets used in the current study presented estimated DE contents below the indicated minimum and, in spite of increased feed intake, the calculated DE intake decreased when ADF replaced starch which impaired milk yield, as well as live weight, body reserves estimated by PFT and EBE balance with diets having 175 g CP kg^{-1} DM. However, with diets having 145 g CP kg^{-1} DM the effect on both milk yield and EBE balance was not significant (PFT even increased although not significantly), because protein instead of energy supply became limiting and increased feed intake as a result of ADF replacing starch turned out useful in sustaining milk yield and EBE balance by increasing protein intake.

On the other hand, feed intake of young rabbits before weaning does not fit the regulation pattern based on dietary DE content observed in rabbit does and weaned rabbits. Thus, it has been previously reported higher feed intake as a result of increasing its energy concentration both when milk intake remain unchanged (Fortun-Lamothe *et al*., 2001) or even increased (Debray *et al*., 2002). In the present study, litter feed intake was unaffected when ADF replaced starch (DE intake as feed being then reduced) and a negative effect on this trait might be hypothesized as a result of harder pellets (+1.7±0.2 kg, +18%, $P<0.001$). Lower feed intake of young rabbits before weaning or early weaned has been reported when pellet hardness increased as a result of reducing pellet diameter (Gidenne *et al.*, 2003; Maertens, 1994). Differences in litter weight at weaning as result of ADF replacing starch were essentially an expression of those observed in milk yield of rabbit does since nutrient supply in suckling rabbits from 17 to 28 days of age is basically provided by milk (Gallois *et al*., 2005) and, consequently, were detected only when using high CP diets.

Similarly to that occurred in lactating rabbit does, increased feed intake observed in weaned rabbits when ADF replaced starch was associated with impaired performance, even though the calculated DE intake was similar either expressed as absolute or relative to metabolic weight at 28 days of age (+0.04±0.03 MJ $kg^{-0.75}$ day^{-1}, $P>0.1$), although it must be taken into account that calculated instead of measured dietary DE contents were applied. Nevertheless, Gidenne (2000) noted that growth rate decreases when dietary ADF is above 275 g kg^{-1} DM and all less starch-more ADF diets of the current study exceeded this limit (averaging 294 g kg^{-1} DM). Increased feed intake maintained after switching to finishing commercial diet (probably linked to greater gastrointestinal development) and completed compensatory growth led to lack of differences in live weight at 60 days of age as consequence of ADF replacing starch in weaning diets.

On the other hand, the replacement of starch with NDSF affected negatively feed intake of both lactating rabbit does and litters, as well as of weaned rabbits. The effect of high-digestible fibre replacing starch on feed intake is controversial. In growing rabbits, Trocino *et al.* (2011) have also reported lower feed intake as a consequence of replacing starch with soluble fibre (parallel increases of cellulose and insoluble hemicelluloses content accounted only for 14% of replaced starch). However, other studies reported no variation or even an increase of feed intake in growing rabbits having similar or faster hindgut transit when high-digestible fibre replaced starch (Gidenne and Bellier, 2000; Gidenne and Jehl, 1996; Gidenne and Perez, 2000; Perez *et al.*, 2000). In the current study, the less starch-more NDSF diets had also slightly higher levels of cellulose and insoluble hemicelluloses (overall accounting for 28% of replaced starch), whereas in the above cited studies diets with increased high-digestible fibre had greater increments in content of cellulose and insoluble hemicelluloses (overall accounting for 40-60% of replaced starch). Greater increases of the content of these insoluble fibres in the diets richer in high-digestible fibre of these works could have induced higher feed intake, by diluting more extensively the dietary energy content or/and stimulating the rate of passage in the hindgut, especially if considering the low lignin levels of these diets (27 to 49 g kg^{-1} DM), compared with those of less starch-more NDSF diets in the current study (56 to 112 g kg^{-1} DM). On the other hand, with changes in levels of cellulose and insoluble hemicelluloses similar to those in the current study (overall accounting for 25% of replaced starch), Xiccato *et al.* (2011) have reported replacement of starch with soluble fibre having no effect on feed intake in growing rabbits; great differences in the ingredients involved to induce this replacement and in the analytical methodology to estimate soluble fibre could be hypothesized to explain the discrepancy with the current work, especially if taking into account the diversity of compounds included in the soluble fibre fraction.

The reduction of nutrient supply in lactating rabbit does when NDSF replaced starch would explain the impairment in milk yield. Live weight, body reserves estimated by PFT and EBE balance weakened with diets having 175 g CP kg^{-1} DM but differences were not significant with those having 145 g CP kg^{-1} DM, because of the difference in the extent of nutrient supply fall and the above mentioned limiting effect of protein intake. Less milk availability together lower nutrient supply as feed before weaning in litters receiving less starch-more NDSF diets led to an important decline in its weight at weaning compared with those receiving more starch-less NDSF diets.

The reduction in DE intake observed in weaned rabbits when NDSF replaced starch disappeared if expressed relative to metabolic weight at 28 days of age (-0.01±0.03 MJ $kg^{-0.75}$ day^{-1}, P>0.1) and live weight gain was negatively affected only in animals fed low CP diets, because also in such a case protein intake became limiting and, moreover, CP intake relative to metabolic weight at 28 days of age decreased when NDSF replaced starch in low CP diets (-1.2±0.5 g $kg^{-0.75}$ day^{-1}, P<0.05) and not in high CP diets (-0.1±0.6 g $kg^{-0.75}$ day^{-1}, P>0.1). With diets having standard CP contents (166-182 g kg^{-1} DM), Trocino *et al.* (2011) and Xiccato *et al.* (2011) have found no effect on live weight gain when soluble fibre replaced starch. After switching to commercial finishing diet, lower live weight gain was observed in animals previously fed less starch-more NDSF diets, even though feed intake expressed relative to metabolic weight at 49 days of age was similar than in those previously fed more starch-less NDSF (-0.5±1.4 g DM $kg^{-0.75}$ day^{-1}, P>0.1); this fact could be explained by live weight overestimating body weight at 49 days of age in animals fed these diets, as a consequence of increased digestive contents. Differences in live weight at 60 days of age as consequence of NDSF replacing starch in weaning diets reflected both lower weight at weaning and lower live weight gain during the full growing period.

Mortality rate during the growth trial was high, as usual in ERE outbreaks; values till 70-80% had been reported (Le Bouquin *et al.*, 2010; Rosell *et al.*, 2000). Results of current study closely agree with those obtained in several works recently reviewed by Blas and Gidenne (2010) indicating that, even if dietary fibre meets essentially the requirements proposed by Gidenne and García (2006) to prevent digestible troubles in young rabbits during post-weaning period, increasing levels of low- or high-digestible fibre replacing starch reduce mortality rate in a context of ERE. The beneficial effect of both replacements seems to be additive, since reduction in mortality rate was stronger when made simultaneously.

Reduction of CP content

The reduction of CP content from 175 to 145 g kg^{-1} DM produced a decrease in feed intake of both lactating rabbit does and litters, as well as of weaned rabbits. Prasad and Karim (1998) also found this effect in lactating rabbit does when comparing diets with 186 or 152 g CP kg^{-1} DM. Similarly, Martínez-Vallespín *et al.* (2008) observed a reduction in feed intake of lactating rabbit does, litters and weaned rabbits in a previous work where standard and reduced protein levels were compared (123 and 93 g digestible protein kg^{-1} DM, respectively), also reported in lactating rabbit does by Partridge and Allan (1982) when comparing diets with 175 or 135 g CP kg^{-1} DM; however, in both studies, level of ADF differed among diets being lower in those with less protein. On the other hand, no effect on feed intake was observed as a result of reducing CP content from 201 to 175 g kg^{-1} DM in lactating rabbit does and litters (García-Palomares *et al.*, 2006a) or from 175 to 154 g kg^{-1} DM in weaned rabbits (García-Palomares *et al.*, 2006b). The role of dietary protein content in controlling voluntary feed intake is complex and incompletely understood (Tome, 2004). Forbes (1995) noted that voluntary feed intake is not affected by protein content within the normal range, but it is depressed by diets of too low protein, as well as by imbalance, deficiency or excess of essential amino acids. In the current study, diets with 145 g CP kg^{-1} DM might pass the critical limit in protein content causing a reduction of feed intake secondary to lower performance and, moreover, they had similar content in total lysine, sulphur amino acids, threonine and tryptophan (respectively, 11.3-11.7, 8.1-8.4, 8.0-8.3 and 3.7-3.8 g kg^{-1} DM, calculated according Fundación Española para el Desarrollo de la Nutrición Animal, 2010) but higher in added DL-methionine, L-threonine and L-tryptophan than diets with 175 g CP kg^{-1} DM (averaging 4.13 *vs.* 1.13, 3.50 *vs.* 1.50 and 2.25 *vs.* 1.50 g kg^{-1} DM, respectively). An excess of added DL-methionine has been reported to depress feed intake in suckling and weaned rabbits (Colin *et al*., 1973; Gidenne *et al*., 2002), although using higher addition levels (>8 g kg^{-1} DM). Added amino acids are more rapidly available than those constituting dietary protein and, since the ability of animals to store amino acids in excess of immediate requirements is very limited and they must be available at the same time, this imbalance might produce both higher concentration in blood having central satiating effect and lower performance, causing respectively primary and secondary reduction of feed intake (Forbes, 1995); besides, essential amino acids other than those mentioned above could become limiting in diets having only 145 g CP kg^{-1} DM.

The reduction of nutrient supply, particularly protein, in lactating rabbit does when fed low CP diets impaired milk yield. Less milk availability and reduction in DE and, especially, protein intake as feed before weaning in litters receiving diets having 145 g CP kg^{-1} DM led to large reduction in their weight at weaning compared with those having 175 g CP kg^{-1} DM. Current recommendations in diets for lactating rabbits does are >185 g CP kg^{-1} DM (De Blas and Mateos, 2010; Xiccato and Trocino, 2010), although García-Palomares *et al.* (2006a) found no negative effects on performance of does and suckling rabbits when reducing dietary protein content to 175 g CP kg^{-1} DM in late lactation.

Reduced feed intake in weaned rabbits fed diets with 145 g CP kg^{-1} DM disappeared if expressed relative to metabolic weight at 28 days of age (+0.0±2.4 g DM $kg^{-0.75}$ day^{-1}, *P*>0.1) but reduction in DE and, especially, CP intake remained (-0.13±0.03 MJ $kg^{-0.75}$ day^{-1}, -6.7%, *P*<0.001; -4.9±0.4 g $kg^{-0.75}$ day^{-1}, -16%; *P*<0.001), leading to great reduction in live weight gain compared with those fed diets with 175 g CP kg^{-1} DM. Current recommendations in diets for growing rabbits are >165 g CP kg^{-1} DM (De Blas and Mateos, 2010; Xiccato and Trocino, 2010), although García-Palomares *et al.* (2006b) found no negative effects on growth performance when reducing protein content to 154 g CP kg^{-1} DM. After switching to commercial finishing diet, higher feed intake as expressed relative to metabolic weight at 49 days of age (+7.5±1.4 g DM $kg^{-0.75}$ day^{-1}, +7.0%, *P*<0.001) allowed compensatory growth still incomplete at 60 days of age, in such a way that differences in live weight at this age as consequence of reducing CP content in weaning diets reflected both lower weight at weaning and lower live weight gain during the full growing period.

As in the current study, reduction on mortality rate of young rabbits after weaning by reducing CP content as been also reported by Chamorro *et al*. (2007) comparing diets with 207 and 176 g CP kg^{-1} DM, as well as by Carabaño *et al*. (2009) comparing diets with 177 and 155 g CP kg^{-1} DM. The positive effect of this dietary change seems to be additive to those induced by ADF or/and NDSF fibre replacing starch and mortality rate was lowest in the diet which accumulated the three dietary changes (HHL).

Conclusion

The partial replacement of starch with ADF or with NDSF or the reduction of CP content from 175 to 145 g CP kg^{-1} DM in weaning diets for lactating rabbit does in late lactation and young rabbits around weaning, impaired milk yield and body condition of does, as well as litter weight at weaning, although the negative effect of replacing starch with ADF was only observed in the high CP diets; moreover, growth during post-weaning period also decreased. However, in an ERE context, mortality rate during this period was reduced by these three dietary changes, which effects seemed additive.

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Figure captions

Figure 1. Interaction between the replacement of starch with acid detergent fibre (ADF) and the crude protein (CP) content on milk yield (a), perirenal fat thickness change (b) and litter weight at weaning (c). 1: more starch-less ADF and high CP diets (LLH and LHH); 2: less starch-more ADF and high CP diets (HLH and HHH); 3: more starch-less ADF and low CP diets (LLL and LHL); 4: less starch-more ADF and low CP diets (HLL and HHL)

Figure 2. Interaction between the replacement of starch with neutral detergent soluble fibre (NDSF) and the crude protein (CP) content on feed intake (a) and perirenal fat thickness change (b). 1: more starch-less NDSF and high CP diets (LLH and HLH); 2: less starch-more NDSF and high CP diets (LHH and HHH); 3: more starch-less NDSF and low CP diets (LLL and HLL); 4: less starch-more NDSF and low CP diets (LHL and HHL)

Table 1. Ingredient composition of the experimental diets (g kg^{-1} dry matter)

Diets	LLH	HLH	LHH	HHH	LLL	HLL	LHL	HHL
Nutrient level[1]								
Acid detergent fibre	L	H	L	H	L	H	L	H
Neutral detergent soluble fibre	L	L	H	H	L	L	H	H
Crude protein	H	H	H	H	L	L	L	L
Ingredients								
Wheat grain	180	90	90	-	180	90	90	-
Wheat bran	40	-	40	-	40	-	40	-
Sunflower meal 28	360	355	360	355	-	-	-	-
Soybean protein concentrate 61	-	-	-	-	75	75	75	75
Fish meal 70	20	25	20	25	20	22.5	20	22.5
Beet pulp	40	40	210	210	100	100	270	270
Oat hulls	40	100	90	150	40	100	90	150
Defatted grape seed	20	150	20	150	80	210	80	210
Grape pomace	80	10	70	-	100	30	90	20
Soybean hulls	40	40	-	-	40	40	-	-
Treated wheat straw	80	80	-	-	210	207.5	130	127.5
Soybean oil	50	50	50	50	50	50	50	50
Sugarcane molasses	10	10	10	10	10	10	10	10
Corn starch	-	10	-	10	-	10	-	10
L-Lysine HCl	5	5	4.5	4.5	5.75	5.75	5	5

DL-Methionine	1	1.25	1	1.25	4	4.25	4	4.25
L-Threonine	1.5	1.75	1.25	1.5	3.5	3.75	3.25	3.5
L-Tryptophan	1.5	1.5	1.5	1.5	2.25	2.25	2.25	2.25
Calcium carbonate	12	8.5	11.25	7.75	3	-	3	-
Dicalcium phosphate	10	13	11.5	14.5	27.5	30	28.5	31
Sodium chloride	5	5	5	5	5	5	5	5
Vitamin/trace element premix[2]	4	4	4	4	4	4	4	4

[1] L, low; H, high.

[2] Supplied per kg of feed: Vitamin A: 8.375 IU; Vitamin D3: 750 IU; Vitamin E: 20 mg; Vitamin K_3: 1 mg; Vitamin B_1: 1 mg; Vitamin B_2: 2 mg; Vitamin B_6: 1 mg; Nicotinic acid: 20 mg; Choline chloride: 250 mg; Magnesium: 290 mg; Manganese: 20 mg; Zinc: 60 mg; Iodine: 1.25 mg; Iron: 26 mg; Copper: 10 mg; Cobalt: 0.7 mg; Butyl hydroxylanysole and ethoxiquin mixture: 4 mg.

Table 2. **Chemical composition of the experimental diets (g kg^{-1} dry matter)**

Diets	LLH	HLH	LHH	HHH	LLL	HLL	LHL	HHL
Nutrient level[1]								
Acid detergent fibre	L	H	L	H	L	H	L	H
Neutral detergent soluble fibre	L	L	H	H	L	L	H	H
Crude protein	H	H	H	H	L	L	L	L
Chemical composition								
Organic matter	921	923	925	923	911	914	918	919
Crude protein	172	177	172	176	147	145	145	144
Ether extract	89	74	89	77	86	77	85	69
Starch	133	84	81	36	129	87	87	30
Neutral detergent fibre, NDF	435	495	439	513	418	475	433	502
Acid detergent fibre, ADF	236	292	236	310	228	282	229	290
Acid detergent lignin, ADL	64	102	65	112	57	96	56	96
Insoluble hemicelluloses, NDF-ADF	199	203	203	203	190	193	204	211
Cellulose, ADF-ADL	172	190	171	198	170	186	173	195
Neutral detergent soluble fibre	145	153	185	185	143	140	188	180
Digestible energy[2] (MJ kg^{-1} dry matter)	11.5	10.3	11.6	10.4	10.7	9.5	10.8	9.6
Hardness (kg)	9.3	12.3	9.5	10.9	9.1	11.2	10.5	10.9

[1] L, low; H, high.

[2] Calculated according to Villamide *et al.* (2010) and Fundación Española para el Desarrollo de la Nutrición Animal (2010).

Table 3. Effect of the weaning diet on performance of rabbit does and litters at weaning (28th day of lactation)

	Diet[1]								Contrast[2] (estimate ± standard error)		
	LLH	HLH	LHH	HHH	LLL	HLL	LHL	HHL	ADF for starch	NDSF for starch	CP reduction
Number of observations	46	42	43	36	38	38	39	36			
Rabbit does											
17th day of lactation											
Live weight (g)	4384	4347	4398	4365	4384	4389	4435	4351	-37±23	11±24	16±22
PFT[3] (mm)	5.85	6.05	5.91	6.05	6.02	5.90	6.12	5.96	0.01±0.07	0.06±0.07	0.04±0.07
EBE[4] (MJ)	37.2	36.4	37.4	36.5	36.8	37.5	38.0	37.0	-0.5±0.4	0.3±0.4	0.5±0.4
17th day of lactation to weaning											
Feed intake (g dry matter day^{-1})	402^{b}	424^{a}	366de	391^{bc}	367de	399^{b}	352^{e}	377^{cd}	26±5***	-26±5***	-22±5***
Digestible energy intake[5] (MJ day^{-1})	4.64^{a}	4.37^{b}	4.27^{b}	4.08^{bc}	3.93^{cd}	3.78^{de}	3.81^{de}	3.61^{e}	-0.21±0.05***	-0.24±0.05***	-0.56±0.05***
Crude protein intake (g day^{-1})	69.3^{b}	75.1^{a}	63.0^{c}	69.0^{b}	53.7^{e}	57.6^{d}	50.6^{f}	53.8^{e}	4.7±0.8***	-4.8±0.8***	-15.2±0.7***
Milk yield (g day^{-1})	273^{a}	260^{ab}	258^{b}	242^{cd}	248^{bc}	246^{bc}	234^{cd}	230^{d}	-9±4*	-16±4***	-19±4***

Live weight change (g)	185^{a}	120^{ab}	118^{ab}	69^{bcd}	90^{b}	18^{cd}	88^{bc}	0^{d}	$-68\pm18^{***}$	-35±18	$-74\pm18^{***}$
PFT[3] change (mm)	0.43^{a}	-0.03^{b}	0.09^{ab}	-0.28^{b}	-0.08^{b}	0.09^{ab}	-0.12^{b}	0.05^{b}	-0.12±0.09	-0.17±0.09	-0.07±0.09
EBE[4] change (MJ)	3.35^{a}	1.61^{bc}	2.14^{ab}	1.01^{bcd}	0.94^{bcd}	0.61^{cd}	1.13^{bcd}	-0.07^{d}	$-1.10\pm0.33^{***}$	-0.57±0.33	$-1.37\pm0.33^{***}$
Litters											
17th day of lactation											
Litter size	8.95	8.97	8.99	8.95	8.98	8.95	8.93	8.93	-0.01±0.04	-0.01.04	-0.02±0.04
Litter weight (g)	2699	2709	2728	2729	2718	2729	2753	2689	-10±27	11±27	6±26
17th day of lactation to weaning											
Feed intake (g dry matter day^{-1})	88.3^{a}	87.5^{a}	76.8^{b}	71.6^{b}	68.6^{b}	68.3^{b}	52.5^{c}	54.4^{c}	-1.1±2.4	$-14.4\pm2.5^{***}$	$-20.1\pm2.4^{***}$
Digestible energy intake[5] (MJ day^{-1})	1.01^{a}	0.91^{b}	0.89^{b}	0.74^{c}	0.74^{c}	0.64^{cd}	0.56^{de}	0.52^{e}	$-0.10\pm0.03^{***}$	$-0.14\pm0.03^{***}$	$-0.27\pm0.03^{***}$
Crude protein intake[6] (g day^{-1})	15.0^{a}	15.6^{a}	13.2^{b}	12.4^{b}	10.1^{c}	9.7^{c}	7.5^{d}	7.8^{d}	-0.1±0.4	$-2.4\pm0.4^{***}$	$-5.3\pm0.4^{***}$
Weaning											
Litter size	8.91	8.90	8.92	8.88	8.89	8.84	8.86	8.85	-0.03±0.03	-0.00±0.03	-0.04±0.03
Litter weight (g)	5327^{a}	5212^{a}	5200^{a}	5006^{b}	4812^{c}	4814^{c}	4553^{d}	4598^{d}	-65±37	$-202\pm38^{***}$	$-492\pm37^{***}$

[1]First, second and third letters show acid detergent fibre (ADF), neutral detergent soluble fibre (NDSF) and crude protein (CP) levels (low, L; high, H), respectively.

[2]ADF for starch: [(HLH+HHH+HLL+HHL)/4–(LLH+LHH+LLL+LHL)/4]; NDSF for starch: [(LHH+HHH+LHL+HHL)/4–(LLH+HLH+LLL+HLL)/4]; CP reduction: [(LLL+HLL+LHL+HHL)/4–(LLH+HLH+LHH+HHH)/4].

[3]Perirenal fat thickness.

[4]Estimated body energy using the equation provided by Pascual *et al.* (2004).

[5]As feed; digestible energy content calculated according Villamide *et al*. (2010) and Fundación Española para el Desarrollo de la Nutrición Animal (2010).

[6]As feed.

*P<0.05; **P<0.01; ***P<0.001

[a, b, c, d, e] Within a row, means not sharing any common superscript are significantly different (P<0.05)

Table 4. Effect of the weaning diet on performance of growing rabbits (individual cages)

	Diet[1]								Contrast[2] (estimate ± standard error)		
	LLH	HLH	LHH	HHH	LLL	HLL	LHL	HHL	ADF for starch	NDSF for starch	CP reduction
Number of rabbits[3]	41	38	42	42	46	43	47	49			
28 to 49 days of age											
Feed intake (g dry matter day^{-1})	112^{cd}	126^{a}	108^{d}	116^{bc}	105^{e}	122^{ab}	98^{f}	112^{cd}	14±2***	-8±2***	-6±2***
Digestible energy intake[4] (MJ day^{-1})	1.27^{a}	1.30^{a}	1.25^{a}	1.22^{ab}	1.13^{cd}	1.17^{bc}	1.06^{d}	1.10^{cd}	0.02±0.02	-0.06±0.02**	-0.15±0.02***

Crude protein intake (g day^{-1})	19.0^{c}	22.3^{a}	18.4^{c}	20.7^{b}	15.4^{e}	17.8^{d}	14.1^{f}	16.4^{e}	$2.5\pm0.3^{***}$	$-1.2\pm0.3^{***}$	$-4.1\pm0.3^{***}$
Live weight gain (g day^{-1})	55.1^{a}	53.5^{ab}	55.4^{a}	53.5^{ab}	51.4^{bc}	49.6^{cd}	48.9^{cd}	47.5^{d}	$-1.6\pm0.7^{*}$	-1.1 ± 0.7	$-5.0\pm0.7^{***}$
Feed conversion rate	2.02^{d}	2.35^{b}	1.95^{e}	2.19^{c}	2.04^{d}	2.45^{a}	1.98^{e}	2.39^{ab}	$0.34\pm0.02^{***}$	$-0.09\pm0.02^{***}$	$0.09\pm0.02^{***}$
49 to 60 days of age[5]											
Feed intake (g dry matter day^{-1})	159^{bc}	166^{ab}	155^{c}	162^{abc}	161^{abc}	166^{a}	157^{c}	160^{abc}	$6\pm2^{**}$	$-4\pm2^{*}$	1 ± 2
Live weight gain (g day^{-1})	50.8^{d}	54.8^{ab}	50.5^{d}	51.2^{cd}	56.2^{ab}	57.7^{a}	53.8^{bc}	56.1^{ab}	$2.1\pm0.7^{**}$	$-2.0\pm0.7^{**}$	$4.1\pm0.7^{***}$
Feed conversion rate	3.15^{ab}	3.01^{bc}	3.19^{a}	3.20^{a}	2.99^{c}	2.89^{c}	2.93^{c}	2.90^{c}	-0.07 ± 0.04	0.05 ± 0.04	$-0.21\pm0.04^{***}$
Live weight at 60 days of age (g)	2294^{ab}	2342^{a}	2264^{bc}	2242^{bc}	2219^{c}	2210^{cd}	2149^{de}	2135^{e}	1 ± 18	$-68\pm18^{***}$	$-107\pm18^{***}$

[1]First, second and third letters show acid detergent fibre (ADF), neutral detergent soluble fibre (NDSF) and crude protein (CP) levels (low, L; high, H), respectively.

[2]ADF for starch: [(HLH+HHH+HLL+HHL)/4–(LLH+LHH+LLL+LHL)/4]; NDSF for starch: [(LHH+HHH+LHL+HHL)/4–(LLH+HLH+LLL+HLL)/4]; CP reduction: [(LLL+HLL+LHL+HHL)/4–(LLH+HLH+LHH+HHH)/4].

[3]Alive at 60 days.

[4]Digestible energy content calculated according Villamide *et al*. (2010) and Fundación Española para el Desarrollo de la Nutrición Animal (2010).

[5]Commercial finishing diet.

**P*<0.05; ***P*<0.01; ****P*<0.001

[a, b, c, d, e, f] Within a row, means not sharing any common superscript are significantly different (*P*<0.05)

0

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Table 5. Effect of the weaning diet on mortality rate of growing rabbits (collective and individual cages)

	Diet[1]								Contrast[2] (estimate ± standard error)		
	LLH	HLH	LHH	HHH	LLL	HLL	LHL	HHL	ADF for starch	NDSF for starch	CP reduction
Number of rabbits[3]	345	319	333	263	286	268	290	267			
Mortality rate (%)											
28 to 49 days of age	71.6^{a}	68.3^{a}	58.6^{b}	43.4^{cd}	51.4^{bc}	41.8^{d}	42.8^{d}	29.2^{e}	-10.2^{***}	-15.1^{***}	-19.9^{***}
49 to 60 days of age[4]	1.0	6.9	6.5	2.0	5.8	1.3	3.0	3.2	-1.3	-0.0	-0.9

[1]First, second and third letters show acid detergent fibre (ADF), neutral detergent soluble fibre (NDSF) and crude protein (CP) levels (low, L; high, H), respectively.

[2]ADF for starch: [(HLH+HHH+HLL+HHL)/4–(LLH+LHH+LLL+LHL)/4]; NDSF for starch: [(LHH+HHH+LHL+HHL)/4–(LLH+HLH+LLL+HLL)/4]; CP reduction: [(LLL+HLL+LHL+HHL)/4–(LLH+HLH+LHH+HHH)/4].

[3]Alive at 28 days.

[4]Commercial finishing diet.

***P<0.001

[a, b, c, d, e] Within a row, means not sharing any common superscript are significantly different (P<0.05)