Partial replacement of starch with acid detergent fibre or/and neutral detergent soluble fibre at two protein levels: effects on ileal apparent digestibility and caecal environment of growing rabbits

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Abstract

The effects of the composition of peri-weaning diets on apparent ileal digestibility of nutrients and caecal environment were studied in growing rabbits. Eight diets were formulated according to a 2x2x2 factorial design to increase acid detergent fibre (ADF) from 230 to 290 g kg^{-1} dry matter (DM) at the expense of mainly starch, to increase neutral detergent soluble fibre (NDSF) from 145 to 185 g kg^{-1} DM at the expense of mainly starch and to reduce crude protein (CP) content from 175 to 145 g kg^{-1} DM. A total of 32 litters of eight kits were randomly assigned to the diets at 17 days of age, weaned at 28 days of age and slaughtered at 35 days of age to collect samples of ileal and caecal contents. The substitution of ADF for starch reduced the coefficient of apparent ileal digestibility (CAID) of DM (0.292 *vs.* 0.229; P<0.01). The substitution of NDSF for starch increased the CAID of NDSF (-0.040 *vs.* 0.099; P<0.01). An interaction between dietary ADF and NDSF levels was found for the CAID of CP, higher in the low ADF-low NDSF diets than in the rest (+0.074; P<0.05). The reduction of dietary CP content decreased the CAID of CP (0.578 *vs.* 0.525; P<0.05) and NDSF (0.086 *vs.* -0.038; P<0.01). Interactions between dietary ADF and NDSF levels were found affecting full caecum weight as well as DM content and pH of caecal digesta. The increase of ADF in the high NDSF diets reduced full caecum weight (-9.1 g kg^{-1} live weight; P<0.01), increased DM content of caecal digesta (+31 g kg^{-1}; P<0.001) and reduced caecal pH (-0.10; P<0.05). In the low NDSF diets, higher ADF increased DM content of caecal digesta to a lesser extent (+16 g kg^{-1}; P<0.001) and increased caecal pH (+0.08; P<0.05). The increase of NDSF in the high ADF diets increased full caecum weight (+8.5 g kg^{-1} live weight; P<0.01) and reduced caecal pH (-0.14; P<0.001). In the low ADF diets, higher NDSF increased full caecum weight more extensively (+15.5 g kg^{-1} live weight; P<0.001) and reduced DM content of caecal digesta (-16 g kg^{-1}; P<0.001). Both ADF and NDSF replacing starch increased total volatile fatty acid (VFA) concentration of caecal digesta (+11.5% and +13.9%, respectively; $P<0.01$). The replacement with NDSF increased molar proportion of butyrate (0.095 *vs.* 0.112; $P<0.001$) at the expense of mainly acetate (0.841 *vs.* 0.820; $P<0.001$) and reduced ammonia concentration of caecal digesta (-8.1%; $P<0.05$). The reduction of dietary CP content decreased total VFA (-14.4%; $P<0.001$) and ammonia (-23.5%; $P<0.001$) concentrations of caecal digesta and increased caecal pH (5.87 *vs.* 5.93; $P<0.05$). Overall, the three dietary changes under study led to changes in the caecal environment which might be effective in reducing mortality rate in a context of rabbit epizootic enteropathy.

Keywords: growing rabbit, apparent ileal digestibility, caecal fermentation, fibre, starch, protein.

1. Introduction

The incidence of digestive disorders in rabbit commercial farms has encouraged the research on peri-weaning diets. Studies have been carried out to determine how some dietary components influence the development of the gastrointestinal tract and the caecal microbial ecosystem in young rabbits. Level and type of fibre, starch and protein are topics of concern, although their effects on digestive health are difficult to elucidate because dietary changes are usually complex and involve several nutrients.

The beneficial effect of increasing dietary fibre at the expense of starch on digestive health of growing rabbits is well established (De Blas et al., 1999; Gidenne et al., 2010b). Even if dietary fibre meets the requirements proposed by Gidenne and García (2006) to prevent digestive troubles in the post-weaning period, increased levels of poorly- or highly- digestible fibre replacing starch might reduce the mortality rate in a context of rabbit epizootic enteropathy (REE), as reviewed by Blas and Gidenne (2010). Depending on the nature of fibre, increased digesta passage rate in the caeco-colic segment (by increasing lignin and cellulose) or favourable changes in caecal fermentative activity and environment (by increasing pectins and hemicelluloses) are mainly involved in the beneficial effect of fibre. A reduction in dietary crude protein (CP) has also been shown to have positive effects on digestive health of growing rabbits (Carabaño et al., 2009), probably because of lower ileal flow of nitrogen and a reduced caecal *Clostridium perfringens* population (Chamorro et al., 2007).

Martínez-Vallespín et al. (2011a) studied the effects of increasing acid detergent fibre (ADF) content at the expense of mainly starch, increasing neutral detergent soluble fibre (NDSF) content at the expense of mainly starch and reducing the CP content in the peri-weaning diet on the performance of both rabbit does and growing rabbits. They showed that, in a context of REE, mortality rate during the post-weaning period was reduced by these three dietary changes. The effects seemed additive. The present work evaluated the effects of the above mentioned dietary changes of the peri-weaning diet on apparent ileal digestibility of nutrients and caecal environment in young rabbits that might have a relationship with REE incidence.

2. Material and methods

2.1. Diets

Eight peri-weaning diets were formulated according to a 2×2×2 factorial design i.e. increase of the ADF content from 230 to 290 g kg^{-1} dry matter (DM) at the expense of mainly starch; increase of the NDSF content from 145 to 185 g kg^{-1} DM at the expense of mainly starch; and reduction of the CP content from 175 to 145 g kg^{-1} DM. Ingredients and chemical composition of the diets are described in Tables 1 and 2, respectively. Starch was replaced by ADF mainly by reducing the amounts of wheat grain, wheat bran and grape pomace and increasing oat hulls and defatted grape seed inclusion rates. Starch was replaced by NDSF mainly by reducing the amounts of wheat grain, soybean hulls and treated wheat straw and increasing sugarbeet pulp and oat hull inclusion rates. The CP content was reduced mainly by replacing sunflower meal with soybean protein concentrate, sugarbeet pulp, defatted grape seed and treated wheat straw. All diets included 5 g kg^{-1} DM of alfalfa hay marked with ytterbium (García et al., 1999). Zinc bacitracin (100 mg kg^{-1}) and robenidine (66 mg kg^{-1}) were added to all diets.

2.2. Animals and housing

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

A total of 32 litters (maternal line A × growth line R; Universitat Politècnica de València, Spain) were standardized to eight kits at 17 days of age and received their respective diets, randomly assigned (4 litters/diet). Litters were weaned at 28 days of age and samples of ileal and caecal contents were collected at 35 days of age. From 28 to 35 days of age, 17 animals (6.6%) died. Samples from 52 animals (20.3%) showing signs of digestive troubles (dirty perineum, abnormal caecal or colic contents) were discarded.

Rabbits were housed in an experimental farm with a temperature of 16ºC to 24ºC throughout the experimental period. From birth to weaning, the litters were maintained in cages (50×70×32 cm) provided with nesting boxes, and then litters were transferred to growing cages (50×80×32 cm), each litter being split into two cages of four rabbits. Throughout the experiment, animals had free access to feed and water. Feed intake was daily controlled from weaning.

2.3. Sampling of ileal and caecal contents

At 35 days of age, animals were weighed and slaughtered by intracardiac injection of sodium thiopental (75 mg kg^{-1} live weight) between 20:00 and 23:00 h., to minimize the influence of caecotrophy on the composition of digestive contents (Gidenne and Poncet, 1985; Merino and Carabaño, 2003). Samples of ileal content were obtained by pooling digesta from the distal part of the small intestine (around 30 cm before the ileo-caeco-colic valve) of two to four animals per replicate, frozen at -20 ºC, freeze-dried and ground. Full caecum weight and pH (GLP21 pH-meter, Crison, Barcelona, Spain) of caecal digesta were recorded. Two mL of 0.35 M H_3PO_4 or three mL of 0.35 M H_2SO_4 were added to one g of caecal digesta for later determination of volatile fatty acid (VFA) and ammonia concentrations, respectively. The remaining caecal content was used to determine the DM content. Caecal digesta samples were frozen at -20 ºC until analysis.

2.4. Chemical analyses

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). The content of NDSF was determined according to Hall et al. (1997), after adapting the method to the modifications proposed by Martínez-Vallespín et al. (2011b).

Ytterbium was analysed according to García et al. (1999), by atomic absorption spectrometry (Smith-Hieftje 22, Thermo Jarrell Ash, Franklin, MA, USA) using predosed samples of faeces to prepare common-matrix standards. Samples were previously ashed (550 °C) and digested by boiling with a solution of 1.5 M HNO_3 and 0.05 M KCl. The coefficients of apparent ileal digestibility (CAID) of nutrients were calculated according to Chamorro et al. (2007), with the following equation (where concentrations are expressed as g kg^{-1} DM):

$$\text{CAID of nutrient} = 1 - \frac{\text{dietary ytterbium concentration} \times \text{ileal nutrient concentration}}{\text{ileal ytterbium concentration} \times \text{dietary nutrient concentration}}$$

For VFA analysis, samples were previously filtered through a cellulose filter (0.45) and 250 μL were transferred to the injection vials; two μL from each sample were injected into the gas chromatograph (Fisons 8000 series, Milan, Italy) equipped with an AS800 automatic injector. The column used was a BD-FFAP 30×0.25×0.25 mm. Injector and detector temperatures were maintained at 220ºC and 225ºC, respectively. Ammonia concentration was determined according to procedure 984.13 of the AOAC (2000). The VFA and ammonia concentrations were expressed as mmol L^{-1} of the liquid phase of caecal content.

2.5. Statistical analysis

Data were analysed using the GLM procedure of SAS (Statistical Analysis Systems Institute, 2002). The model included the main effect of the diet and the litter (nested to diet) as a random effect. The effects of the three factors (ADF for starch, NDSF for starch, CP reduction) and all their interactions were assessed by orthogonal contrasts. All data are presented as least-squares means and statistical significance was declared at $P<0.05$.

3. Results

3.1. Apparent ileal digestibility of nutrients

The coefficients of apparent ileal digestibility (CAID) of DM, CP, starch and NDSF of the diets are shown in Table 3. The substitution of ADF for starch reduced the CAID of DM (0.292 *vs*. 0.229; $P<0.01$). The substitution of NDSF for starch increased the CAID of NDSF (-0.040 *vs*. 0.088; $P<0.01$). The reduction of CP content decreased the CAID of CP (0.578 *vs*. 0.525; $P<0.05$) and NDSF (0.086 *vs*. -0.038; $P<0.01$).

An interaction between the ADF and NDSF levels was found for the CAID of CP (Figure 1), which was lower with the substitution of ADF for starch only in the low NDSF diets (-0.066±0.033; $P<0.05$) and with the substitution of NDSF for starch only in the low ADF diets (-0.088±0.035; $P<0.05$), resulting in higher CAID of CP in the low ADF-low

NDSF diets than in the rest (+0.074±0.028; P<0.05). An interaction between the NDSF and CP levels was also detected for the CAID of starch, which was higher with the substitution of NDSF for starch only in the high CP diets (+0.029±0.012; P<0.05) and with the reduction of CP content only in the low NDSF diets (+0.026±0.011; P<0.05).

Because of the high incidence of mortality (6.6%) and digestive troubles (20.3%), data on feed intake, obtained from collective cages, were not reliable and consequently ileal flow of nutrients could not be presented.

3.2. Caecal environment

The dietary effects on full caecum weight (relative to live weight) and on major parameters describing caecal fermentation and environment are shown in Table 4.

The substitution of ADF for starch reduced the full caecum weight (89.4 *vs.* 83.8 g kg^{-1} live weight; P<0.01) and increased the DM content (214 *vs.* 238 g kg^{-1}; P<0.001) and total VFA concentration (+11.5%; P<0.01) of caecal digesta. On the contrary, the substitution of NDSF for starch increased the full caecum weight (80.6 *vs.* 92.6 g kg^{-1} live weight; P<0.001) and reduced the DM content of caecal digesta (231 *vs.* 222 g kg^{-1}; P<0.01). This dietary change also increased the total VFA concentration (+13.9%; P<0.01), raising the molar proportion of butyrate (0.095 *vs.* 0.112; P<0.001) at the expense of mainly acetate (0.841 *vs.* 0.820; P<0.001) and decreased the ammonia concentration (-8.1%; P<0.05) of caecal digesta. The reduction of dietary CP decreased both total VFA and ammonia concentrations (-14.4% and -23.5%, respectively; P<0.001) of caecal digesta, with increased pH (5.87 *vs.* 5.93; P<0.05).

Interactions between the ADF and NDSF levels were found to affect full caecum weight as well as DM content and pH of caecal digesta (Figure 2). Thus, the full caecum weight decreased when ADF replaced starch only in the high NDSF diets (-9.1±3.0 g kg^{-1} live weight; P<0.01) and increased more extensively when NDSF replaced starch in the low ADF diets (+15.5±3.2 g kg^{-1} live weight; P<0.001) than in the high ADF diets (+8.5±2.7 g kg^{-1} live weight; P<0.01). The DM content of caecal digesta increased to a greater extent when ADF replaced starch in the high NDSF diets (+31±4 g kg^{-1}; P<0.001) than in the low NDSF diets (+16±4 g kg^{-1}; P<0.001). The DM content of caecal digesta decreased when NDSF replaced starch only in the low ADF diets (-16±5 g kg^{-1}; P<0.001). The caecal pH increased when ADF replaced starch in the low NDSF diets (+0.08±0.04; P<0.05) but decreased when this replacement was done in the high NDSF diets (-0.10±0.04; P<0.05) and also when NDSF replaced starch only in the high ADF diets (-0.14±0.04; P<0.001).

Interactions between the NDSF and CP levels were detected affecting full caecum weight and DM content of caecal digesta. Thus, the full caecum weight increased more extensively when NDSF replaced starch in the high CP diets (+16.9±3.2 g kg^{-1} live weight; P<0.001) than in the low CP diets (+7.1±2.6 g kg^{-1} live weight; P<0.01). The DM content of caecal digesta decreased when NDSF replaced starch only in the high CP diets (-16±5 g kg^{-1}; P<0.001) and with the reduction of CP content only in the low NDSF diets (-12±4 g kg^{-1}; P<0.01). An interaction between the ADF and CP levels was also detected for the molar proportion of propionate, which was higher when ADF replaced starch only in the low CP diets (+0.007±0.003; P<0.05) and lower with the reduction of CP content only in the low ADF diets (-0.006±0.003; P<0.05).

4. Discussion

4.1. Replacement of starch with fibre

The replacement of starch with ADF impaired the CAID of DM, which is consistent with the high ileal digestibility of starch (Blas and Gidenne, 2010), whereas a low precaecal digestion of ADF can be assumed. In adult rabbits, CAID of ADF from 0.08

to 0.18 have been reported by Gidenne et al. (2000). Other studies in adult rabbits have reported CAID of glucose from non-starch polysaccharides (mainly from cellulose) being close to zero (Gidenne, 1992; Carabaño et al., 2001).

The replacement of starch with NDSF impaired the CAID of DM, likely because of the low ileal digestibility of this fibrous fraction, whereas it increased the CAID of NDSF probably because of the higher contribution of sugarbeet pulp to this fraction (averaging 0.22 and 0.60 in the low and high NDSF diets, respectively). Sugarbeet pulp is rich in pectic substances which have a high CAID (0.2 to 0.5) in adult rabbits (Gidenne, 1992; Carabaño et al., 2001). This fact might be explained as a consequence of microbial enzyme activity in the stomach and small intestine (Marounek et al., 1995). Moreover, the inclusion of sugarbeet pulp in the diet increases intestinal viscosity in rabbits (Volek et al., 2005) and reduces the CAID of CP because of greater endogenous losses (Chamorro et al., 2007). On the other hand, the contribution of fibrous raw materials to the CP supply was larger in the high than in the low ADF diets (0.32 *vs*. 0.24). Lower CAID of CP has been reported for fibrous raw materials than for concentrates (Carabaño et al., 2009). These facts could explain why the CAID of CP was higher in the low ADF-low NDSF diets.

Dietary level and type of fibre were the main dietary factors affecting caecal traits as reported by García et al. (2002) in a meta-study with data from different experiments. According to these authors, caecal weight depends quadratically on the dietary NDF content, being minimal with 393 g NDF kg^{-1} DM, and decreases linearly with the increase of degree of lignification of NDF and of feed intake. Diets rich in pectins, usually from sugarbeet pulp, increased caecal weight regardless of variations in the level of insoluble fibre (NDF, ADF, ADL) and starch (García et al., 1993; Carabaño et al., 1997; Gidenne and Bellier, 2000; Falcão-e-Cunha et al., 2004), as observed in the current study. Gómez-Conde et al. (2009) also found higher caecal weight when NDSF increased in iso-ADF and iso-starch diets. On the contrary, Trocino et al. (2010) found no variation in caecal weight with diets differing in soluble fibre content. The lack of effect on full caecum weight when increasing ADF (and ADL to NDF ratio) in the low NDSF diets observed in the current study could be explained by their ADF content being already high enough to maximize this rate of passage, as caecal weight is related to ileo-rectal rate of passage (Gidenne et al., 2010a). On the other hand, the effect of insoluble fibre in reducing caecal weight would persist above the limit proposed by García et al. (2002) in the high NDSF diets.

The increase of DM content of caecal digesta with dietary ADF could be a consequence of higher ileal flow of DM. The ileal flow of DM calculated using feed intake from a previous study using the same diets (Martínez-Vallespín et al., 2011a) would be higher in the high than in the low ADF diets (92 *vs.* 75 g day^{-1}). In the high NDSF diets, this effect of dietary ADF was larger, which could be explained by a differentiated transit, faster for liquid than for solid phase, associated to stimulated caecal rate of passage. Faster oro-rectal rate of passage of fine particles (and not of large ones) has been reported as a result of increasing the dietary lignin content (Gidenne and Perez, 1994). Similarly, Gidenne and Perez (2000) and Gidenne and Jehl (1996) found faster oro-rectal rate of passage of liquid phase and fine particles (and not of large ones) or faster ileo-rectal rate of passage of liquid phase (and not of solids), respectively, when increasing the dietary fibre level (cellulose+insoluble hemicelluloses+pectins). The increase of dietary NDSF in the low ADF diets induced lower DM content of caecal digesta probably because of higher water retention capacity of pectins (Gidenne et al., 2010a). However, no differences were found in the high ADF diets because this effect could have been compensated by the differentiated transit of liquid and solid phases.

García et al. (2002) reported that VFA concentration of caecal digesta increases with the dietary content in uronic acids (monomers constituting the backbone of pectins) and NDF, whereas it is reduced by increasing lignification of NDF. Likewise, higher VFA concentration has been observed when several fibrous constituents (pectins, insoluble hemicelluloses, cellulose) were increased simultaneously at the expense of starch (Jehl and Gidenne, 1996; Gidenne and Bellier, 2000). Recent studies (Trocino et al., 2011; Xiccato et al., 2011) also reported higher VFA concentration when soluble fibre replaced starch. Similarly, in the current study, VFA concentration of caecal digesta increased with the high NDSF or ADF diets (the latter being also high in NDF, although with higher degree of lignification) and the maximum VFA concentration was observed when NDSF and ADF increased concurrently at the expense of starch. Nevertheless, the difference in fermentative activity depending on the NDSF level cannot be supported by the difference in the calculated ileal flow of this fibrous fraction (17.5 and 18.2 g day^{-1} for the low and high NDSF diets, respectively), which might be related to the high residual variability of its CAID (mean: 0.010, residual standard deviation: 0.102).

Regarding the VFA profile of caecal digesta, García et al. (2002) reported that acetate increased and butyrate decreased when increasing dietary NDF, whereas propionate was positively correlated with the dietary content in uronic acids. The same effect on acetate and butyrate molar proportions was found by Xiccato et al. (2011) when soluble fibre replaced starch. On the contrary, in the current work, no effect was found on VFA profile when ADF replaced starch whereas when NDSF replaced starch it consistently reduced the contribution of acetate and increased that of butyrate.

The ammonia concentration of caecal digesta was reduced when NDSF replaced starch in the diet, which could be explained by enhanced microbial activity and subsequent increase of ammonia uptake for microbial protein synthesis, since the calculated ileal flow of CP was very similar in low and in high NDSF diets (8.0 *vs.* 8.1 g day^{-1}). Xiccato et al. (2011) also reported lower ammonia concentration as a consequence of a large increase of soluble fibre at the expense of starch; Trocino et al. (2011) found no effect when this replacement was slighter. On the other hand, enhanced microbial activity when ADF replaced starch was not reflected in a reduction of ammonia concentration because higher ammonia uptake by microbiota could be compensated by an increase in ammonia production as a result of enhanced proteolytic activity; in fact, the calculated ileal flow of CP increased with the high ADF diets (7.3 *vs.* 8.7 g day^{-1}).

Uronic acids are the only dietary constituent explaining a relevant part (67%) of the variation observed in caecal pH, which depends not only on VFA and ammonia concentration in caecal digesta (both explaining only 12% of caecal pH variability) but also on physicochemical characteristics of caecal DM (García et al., 2002). In the current study, a stepwise multiple linear regression analysis to examine the relationship between caecal pH and other caecal traits revealed VFA concentration as the only variable retained in the model [pH = 6.31 (±0.03; P<0.001) − 0.0058 (±0.0004; P<0.001) × VFA (mmol L^{-1}); RSD=0.13, R^2=0.51, n=187]. In this line, maximum VFA concentration and minimum pH value were recorded with diets which NDSF and ADF contents were increased concurrently at the expense of starch.

4.2. Reduction of CP content

A reduction of dietary CP content from 175 to 145 g kg^{-1} reduced the CAID of this nutrient. The contribution of fibrous raw materials to the CP supply was larger in the low than in the high CP diets (0.37 *vs.* 0.19) and, as mentioned above, lower CAID of CP has been reported for fibrous raw materials than for concentrates (Carabaño et al., 2009). The higher proportion of ileal endogenous nitrogen in low protein diets should also be taken into account. The reduction of CP content also impaired the CAID of NDSF, in spite of the higher contribution of sugarbeet pulp to this fraction (0.55 *vs.* 0.36). Lower precaecal microbial activity because of insufficient N availability could be hypothesized. In fact, as commented below, caecal microbial activity was depressed with the low CP diets. The combined effect of the reduction of CAID of CP and NDSF content in the low CP diets would explain a major part of the impairment of CAID of DM in these diets.

In the current study, a reduction of dietary CP content reduced VFA concentration, as found also by Xiccato et al. (2011), and increased pH in caecal digesta, which may indicate lower microbial activity. In fact, the lower ileal flow of CP (7.5 *vs.* 8.5 g day^{-1}; calculated values) and N availability (illustrated in the lower ammonia concentration of caecal digesta) could have limited the microbial activity.

5. Conclusions

The replacement of around 50 g kg^{-1} DM of starch with ADF or with NDSF or the reduction of CP content from 175 to 145 g kg^{-1} DM in diets for young rabbits around weaning led to changes in the caecal environment that might be effective in reducing mortality rate from REE.

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Figure captions

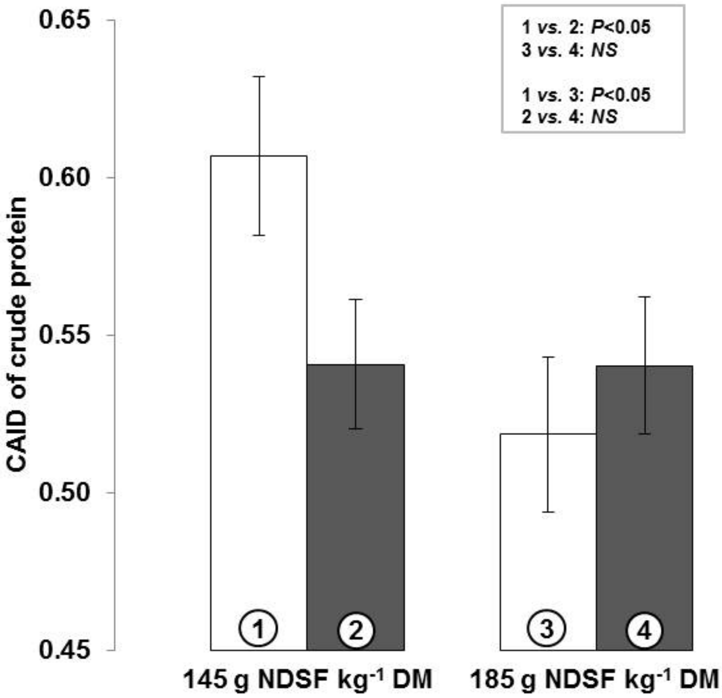

Figure 1. Interaction between acid detergent fibre (ADF) and neutral detergent soluble fibre (NDSF) dietary levels on coefficient of apparent ileal digestibility (CAID) of crude protein. 1: low ADF-low NDSF [(LLH+LLL)/2]; 2: high ADF-low NDSF [(HLH+HLL)/2]; 3: low ADF-high NDSF [(LHH+LHL)/2]; 4: high ADF-high NDSF [(HHH+HHL)/2]. *NS*: non-significant (*P*>0.05).

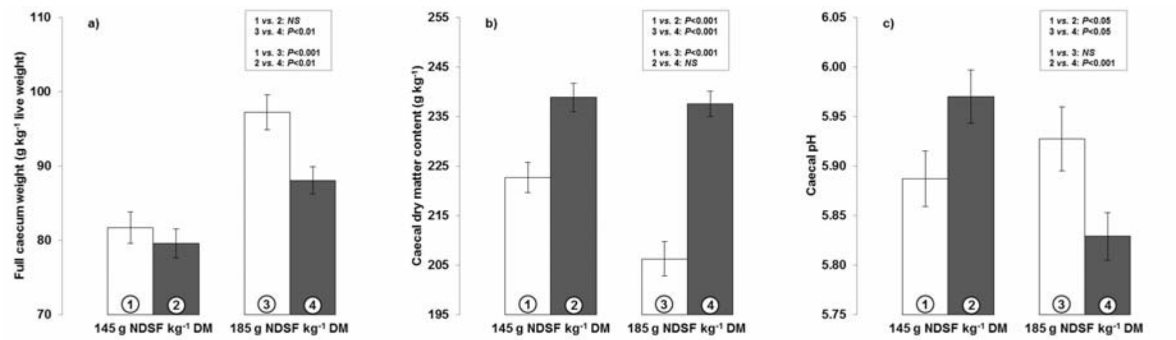

Figure 2. Interaction between acid detergent fibre (ADF) and neutral detergent soluble fibre (NDSF) dietary levels on full caecum weight (a), caecal dry matter content (b) and caecal pH (c). 1: low ADF-low NDSF [(LLH+LLL)/2]; 2: high ADF-low NDSF [(HLH+HLL)/2]; 3: low ADF-high NDSF [(LHH+LHL)/2]; 4: high ADF-high NDSF [(HHH+HHL)/2]. *NS*: non-significant (*P*>0.05).

Table 1. Ingredients 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
Sugarbeet 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	922	923	925	924	914	916	919	920
Crude protein	174	176	175	174	147	147	145	144
Ether extract	83	76	84	74	81	75	79	72
Starch	130	83	79	31	129	83	85	28
Neutral detergent fibre, NDF	412	480	424	497	407	464	423	486
Acid detergent fibre, ADF	231	289	235	302	226	281	228	287
Acid detergent lignin, ADL	59	97	61	104	54	93	55	93
ADL to NDF ratio	0.14	0.20	0.14	0.21	0.13	0.20	0.13	0.19
Neutral detergent soluble fibre	145	153	185	185	143	140	188	180

[1] L: low, H: high.

Table 3. Effect of the experimental diets on the coefficients of ileal apparent digestibility of dry matter, crude protein, starch and neutral detergent soluble fibre in 35 day-old rabbits

	Diet								Contrast[2]			
	LLH	HLH	LHH	HHH	LLL	HLL	LHL	HHL	ADF for starch	NDSF for starch	CP reduction	SED[3]
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				
Ileal apparent digestibility												
Number of observations	8	8	8	8	8	8	8	8				
Dry matter	0.314[a]	0.258[abc]	0.293[ab]	0.244[abc]	0.316[a]	0.221[bc]	0.245[abc]	0.193[c]	-0.063 **	-0.033	-0.034	0.019
Crude protein	0.613[a]	0.569[abc]	0.574[abc]	0.557[abc]	0.601[ab]	0.512[cd]	0.463[d]	0.524[bcd]	-0.022	-0.044	-0.053 *	0.023
Starch	0.936[ab]	0.921[b]	0.945[ab]	0.970[a]	0.957[a]	0.953[ab]	0.934[ab]	0.952[ab]	0.006	0.009	0.006	0.008
Neutral detergent soluble fibre	0.043[abc]	0.048[ab]	0.133[a]	0.121[ab]	-0.092[c]	-0.157[c]	0.096[ab]	0.002[bc]	-0.042	0.127 **	-0.124 **	0.036

[1] L: low, H: high.
[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] Standard error of difference.
*P<0.05, **P<0.01, ***P<0.001
[a, b, c, d] Means within a row with unlike superscripts differ (P<0.05)

Table 4. Effect of the experimental diets on caecal traits in 35 day-old rabbits

	Diet								Contrast[2]			
	LLH	HLH	LHH	HHH	LLL	HLL	LHL	HHL	ADF for starch	NDSF for starch	CP reduction	SED[3]
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				
Caecal traits												
Number of observations	18	18	21	28	29	27	21	25				
Full caecum weight (g kg^{-1} live weight)	81.9[ab]	73.9[b]	98.9[a]	90.7[ab]	81.5[ab]	85.2[ab]	95.4[a]	85.5[ab]	-5.6 **	12.0 ***	0.6	2.1
Caecal pH	5.88[abc]	5.91[abc]	5.90[abc]	5.80[c]	5.89[abc]	6.03[a]	5.95[ab]	5.86[bc]	-0.01	-0.05	0.06 *	0.03
Caecal digesta composition												
Dry matter (g kg^{-1})	228[abc]	246[a]	205[c]	238[a]	218[bc]	231[ab]	208[c]	237[a]	24 ***	-9 **	-6	3
Total VFA[4] (mmol L^{-1})	71.8[abc]	74.8[abc]	79.8[abc]	84.5[a]	58.7[c]	64.4[bc]	62.5[bc]	80.5[ab]	7.9 **	9.4 **	-11.2 ***	3.0
Acetate (molar proportion)	0.836	0.847	0.816	0.821	0.844	0.836	0.827	0.818	-0.001	-0.021 ***	0.001	0.005
Propionate (molar proportion)	0.052	0.049	0.057	0.056	0.046	0.057	0.050	0.053	0.003	0.003	-0.002	0.002
Butyrate (molar proportion)	0.099	0.093	0.108	0.113	0.094	0.095	0.108	0.117	0.002	0.016 ***	0.000	0.004
Ammonia (mmol L^{-1})	16.0[ab]	17.2[a]	15.1[abc]	14.8[abc]	12.5[bc]	12.4[bc]	11.1[c]	12.3[bc]	0.5	-1.2 *	-3.7 ***	0.6

[1] L: low, H: high.
[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] Standard error of difference.
[4] Volatile fatty acids.
*$P<0.05$, **$P<0.01$, ***$P<0.001$
[a, b, c] Means within a row with unlike superscripts differ ($P<0.05$)