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A META-ANALYSIS ON THE ROLE OF SOLUBLE FIBRE IN DIETS FOR GROWING RABBITS

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Abstract: In this review, the methods used to measure fibre and soluble fibre fractions are briefly presented and the effects of the soluble fibre content in diets for growing rabbits reviewed by a meta-analysis of studies available in literature, with the aim of elucidating the relationships with other dietary nutrients. Soluble fibre was assumed as the difference between total dietary fibre (TDF) and neutral detergent fibre (NDF), as it is simple to obtain and has been measured in numerous studies. Dietary soluble fibre content affects the digestive utilisation of soluble and insoluble fibre fractions and its increase is associated with reduced mortality in growing rabbits affected by epizootic rabbit enteropathy. This effect could be attributed to the high fermentability of soluble fibre, the consequent changes in the intestinal microbiota and an enhanced gut barrier function just after weaning. A supply of 12-14% of soluble fibre (as-fed) is recommended in diets for post-weaning and growing rabbits containing around 30% NDF and 18% acid detergent fibre. The positive effects of increasing dietary soluble fibre are linked to the use of sugar beet pulp as primary source of soluble fibre and should be confirmed with other sources of soluble fibre.

Key Words: growing rabbits, soluble fibre, digestibility, caecal fermentation, health.

INTRODUCTION

Total dietary fibre (TDF) is the major fraction of commercial diets for rabbits (35-50% as-fed). Its importance is related to the influence on the rate of passage of *digesta* and the function as substrate for microbiota, which in turn affect and regulate rabbit growth performance and digestive health (Gidenne *et al.*, 2010a, 2010b). Insoluble fibre [corresponding to neutral detergent fibre (NDF)] has been widely recognised as the most important fibre fraction and used to express fibre requirements: it accounts for about 65-90% of TDF in commercial diets for rabbits and is quantified by rather standardised methodologies. The chemical composition (degree of lignification) and the physical properties (particle size) of insoluble fibre also affect the rate of passage of *digesta* and the susceptibility of fibre to fermentation (Gidenne *et al.*, 2010a). Current recommendations state that diets for rabbits should contain at least 30% NDF and 16% acid detergent fibre (ADF) (De Blas and Mateos, 2010).

In contrast, soluble fibre (SF) has been neglected in rabbit nutrition until recently: it comprises non-starch and non-NDF polysaccharides (Hall, 2003) and is a minor and heterogeneous fraction of the total dietary fibre (around 10-35% of TDF). However, a positive role of SF in rabbit digestive health has been claimed (Perez *et al.*, 2000; Soler *et al.*, 2004; Gómez-Conde *et al.*, 2007) in a context of epizootic rabbit enteropathy (ERE), which has been associated to improved intestinal mucosa integrity and modulation of intestinal microbiota (Gómez-Conde *et al.*, 2007, 2009). However, results are not always consistent among studies. A previous review (Gidenne, 2003) associated increased levels of dietary pectins and hemicelluloses with an increase of the sanitary risk and recommended a ratio (pectins+hemicelluloses)/ADF below 1.3 in diets with at least 19% ADF from 28 to 42 d of age. Moreover, some questions have not yet been clarified, such as the amount of SF utilised at ileum and caecum or the interactions

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between SF and other dietary nutrients. In fact, the most common feedstuff rich in SF (i.e. sugar beet pulp) used in commercial and experimental diets also contains high levels of fermentable insoluble fibre, which makes it difficult to distinguish between the effects of the 2 fractions.

The present review is intended as a starting point for discussion on SF in rabbit nutrition: methods to measure soluble and insoluble fibre fractions will be briefly presented; the role of SF in digestive physiology will be discussed; the effects of SF on performance and health will be reviewed by a meta-analysis of studies available in literature, also with the aim of elucidating the relationships with other main nutrients. Finally, recommendations for dietary SF for growing rabbits will be proposed.

DEFINITION AND QUANTIFICATION OF FIBRE FRACTIONS

Total dietary fibre

In human nutrition and in other mammals, total dietary fibre is defined as the polysaccharides and associated substances resistant to mammalian enzymatic digestion and absorption that can be partially or totally fermented in the gut (Hipsley, 1953; Burkitt *et al.*, 1972; Trowell, 1974; DeVries, 2010). From a chemical point of view, TDF is mainly constituted by the plant cell walls composed by a backbone of cellulose microfibrils embedded in a matrix of lignin, hemicelluloses, pectins and proteins, as well as other substances linked to the cell wall (polyphenols, cutin, gums, etc.) or in the cytoplasm (resistant starches, oligosaccharides, fructans, etc.) (Gidenne *et al.*, 2010a).

TDF is primarily analysed by enzymatic-gravimetric methods based on the Association of Official Analytical Chemists (AOAC, 2000) procedures 985.29 and 991.43, which solubilise the different non-fibrous substances with enzymes and solvents and measure the weight of fibrous residues after these treatments (as reviewed by Bach Knudsen, 1997, 2001; DeVries, 2010; Elleuch *et al.*, 2011). These procedures may differentiate the insoluble and soluble fibre fractions. Besides, a recent update of the analytical methods also allows inclusion of non-digestible oligosaccharides and resistant starch by the use of liquid chromatography (McCleary, 2007; McCleary *et al.*, 2010).

In contrast, enzymatic-chemical methods first separate the fibre from the other nutrients enzymatically and then quantify the fibre residue chemically by hydrolysing the polysaccharides in the residue and determining the content of neutral sugars and uronic acids by means of gas-liquid chromatography or high-performance liquid chromatography and colorimetry and the lignin residue (Klason lignin) gravimetrically (Englyst *et al.*, 1994; Theander *et al.*, 1995). They may also differentiate the insoluble and soluble fibre fractions of the sample. However, these enzymatic-chemical methodologies, designed to measure non-starch polysaccharides, may underestimate dietary fibre content and are somewhat complex, expensive and difficult to implement as routine analysis, and characterised by a rather low reproducibility (Mertens, 2003; Elleuch *et al.*, 2011).

Insoluble dietary fibre

With relevance to animal digestive physiology, insoluble dietary fibre for herbivores is defined by Mertens (2003) as "indigestible or slowly digesting organic matter of feeds that occupies space in the gastrointestinal tract", that is, from a chemical point of view, lignin (indigestible) and mostly hemicelluloses and celluloses (slowly digesting and fermenting organic matter of feeds). Accordingly, insoluble dietary fibre does not comprise those polysaccharides of plant cell walls which may be rapidly fermented (e.g. pectins), and those soluble ones which do not occupy space in a liquid environment (e.g. fructans and gums) and are highly digested.

Insoluble dietary fibre may be quantified by the above mentioned AOAC methods for TDF by preventing the recovery of water-soluble structural polysaccharides. However, the enzymatic-gravimetric determination of NDF is the most simple, low-cost, rapid and reproducible method used to quantify insoluble fibre (Mertens, 2003). Due to the different procedures available (Van Soest and Wine, 1967; Robertson and Van Soest, 1980; Mertens, 2002) and adaptations in laboratories, the procedure of Mertens (2002) is recommended (Uden *et al.*, 2005), where NDF is obtained with α-amylase and sodium sulphite treatments and is expressed free from ash.

Weende crude fibre and acid detergent fibre (ADF) determinations may also be used to quantify insoluble fibre, but neither of them fit with the aforementioned definitions of total or insoluble dietary fibre. In fact, the chemical composition of crude fibre residue is highly variable depending on the feed; ADF does not quantify all the insoluble fibre, as hemicelluloses, and may contain some pectins when it is not obtained sequentially from NDF residue. As a consequence, crude fibre or ADF cannot fully explain the effects exerted by insoluble fibre on herbivorous digestive physiology. However, both of them are very useful to predict the nutritive value of diets for rabbits (Wiseman *et al.*, 1992; Villamide *et al.*, 2009) and their analytical determinations have similar or even higher reproducibility compared with NDF analysis (Xiccato *et al.*, 1996, 2012).

Finally, the characterisation of insoluble fibre may be completed by determination of the lignin concentration by treating the ADF residue with sulphuric acid (ADL), according to Robertson and Van Soest (1980). The method is widely adopted, but it is more laborious and less reproducible compared with NDF determination (Xiccato *et al.*, 1996; Gidenne *et al.*, 2001b).

The EGRAN (European Group on Rabbit Nutrition) proposed some recommendations to improve the reproducibility among laboratories when determining insoluble fibre in compound feeds and raw materials for rabbits (Xiccato *et al.*, 1996; Gidenne *et al.*, 2001b).

In addition to the dietary insoluble fibre level, the type of insoluble fibre (i.e. the degree of lignification and particle size) is also relevant in rabbit nutrition and digestive physiology (Nicodemus *et al.*, 1999, 2006). To this end, besides the lignin content, the proportion of lignin on insoluble fibre (ADL to NDF ratio) may give additional information (García *et al.*, 2002).

Soluble fibre

SF is the part of TDF that comprises the non-starch and non-NDF polysaccharides, including pectic substances, β-glucans, fructans and gums (Hall, 2003).

SF may be quantified as soluble dietary fibre (SDF) according to the Prosky enzymatic-gravimetric procedure (Prosky *et al.*, 1992; AOAC, 2000; Megazyme Ltd, 2005): the carbohydrates are solubilised in phosphate buffer or MES (4-morpholine-ethanoesulfonic acid)/TRIS buffer; α-glucans are hydrolysed by amyloglucosidase; insoluble fibre is separated by filtration; solubilised fibre is precipitated with an ethanol solution from the solvent extract and measured gravimetrically after correction for protein and ash contents. Inaccuracies may arise from the partial degradation of carbohydrates, incomplete extraction and/or precipitation with the addition of ethanol, the interference with other substances and differences in the nature of the feeds analysed (Theander *et al.*, 1995; Hall *et al.*, 1997).

In addition, SF may be measured according to Hall *et al.* (1999) as the neutral detergent soluble fibre (NDSF) obtained gravimetrically as the difference between the weight of the 80% ethanol-insoluble residue and those of starch and NDF after correction for protein and ash. The NDSF determination may be affected by the accumulation of errors in measurement of the different components, as well as the error linked to the coefficient (N×6.25) used for protein correction (Hall, 2003; Martínez-Vallespín *et al.*, 2011).

Using enzymatic-gravimetric methods, SF may also be obtained as the difference between TDF and any measurement of insoluble fibre. According to Van Soest *et al.* (1991), SF may be obtained by subtracting the content of NDF after correction for ash and protein from the TDF value, thus including non-starch polysaccharides, i.e. fructans, galactans, β-glucans and pectins (Xiccato *et al.*, 2012). Finally, SF content may be calculated by difference as: organic matter–(crude protein+crude fat+soluble sugars+starch+NDF).

In the present review, SF measured as the difference between TDF (which includes non-starch polysaccharides, lignin and some resistant starches) (Elleuch *et al.*, 2011) and NDF (which includes hemicelluloses, celluloses and lignin) (Mertens, 2003) will be extensively used, since it was available in most studies on the role of SF in growing rabbits.

SOLUBLE FIBRE IN DIETS FOR GROWING RABBITS

In rabbit nutrition, Gidenne (2003) first proposed identifying SF and less lignified insoluble fibre by the term “digestible fibre”, which is the sum of hemicelluloses (NDF–ADF) and pectins (estimated from tables of raw material composition) and may also be directly calculated as TDF–ADF. Later, the majority of published papers rather referred to SF measured as TDF–NDF or as NDSF.

The studies carried out until now on the effect of SF or "digestible fibre" in growing rabbits have elucidated some points, but also posed some questions: Is it possible to isolate the effect of SF from that of the other nutrients, in view of the strict relationships among SF, insoluble fibre, starch and/or protein? As changes in SF levels are primarily obtained by varying sugar beet pulp (SBP) inclusion rate, should the effects of SF level be ascribed to the SBP inclusion rate itself?

In order to draw some general conclusions and isolate the effect of SF from that of the other nutrients, we collected the data from 18 experiments performed in 4 laboratories in 3 countries (France, Italy and Spain) in which 90 experimental diets were fed to rabbits from weaning until slaughter (Table 1). Diets were designed to evaluate the effect of SF or "digestible fibre" in replacement of insoluble fibre (ADF) or starch at different protein levels.

On average, around 70% of the experimental diets were based on dehydrated alfalfa meal, cereals (barley or wheat meal), SPB and wheat bran (Table 2), as usual in rabbit feeding (Maertens *et al.*, 2002). SF levels were primarily increased by increasing the rates of SBP and, in a few cases (Gidenne *et al.*, 2004b; Gómez-Conde *et al.*, 2007), of citrus or apple pulp. The correlation between SF content and SBP rate was high and significant (r=0.70; P<0.001). Insoluble fibre primarily came from dehydrated alfalfa meal and sometimes from wheat straw.

Table 1: Studies on the effects of digestible fibre or soluble fibre in diets for growing rabbits performed at the University of Padova (Italy), INRA of Toulouse (France), Polytechnic University of Madrid and Polytechnic University of Valencia (Spain).

Definition used in the paper	Analytical/calculation method	Experiment arrangement	Reference (Code)
Digestible fibre	Hemicelluloses (NDF–ADF)+ pectins[a]	DF/starch ratio	Gidenne and Jehl, 1996; Jehl and Gidenne, 1996
Digestible fibre	Hemicelluloses (NDF–ADF)+ pectins[a]	DF/starch ratio	Gidenne and Perez, 2000; Perez *et al.*, 2000
Digestible fibre	Hemicelluloses (NDF–ADF)+ pectins[a]	DF/starch ratio	Gidenne and Bellier, 2000
Digestible fibre	Hemicelluloses (NDF–ADF)+ pectins[a]	DF/starch ratio	Gidenne *et al.*, 2004a
Digestible fibre	Hemicelluloses (NDF–ADF)+ pectins[a]	DF/starch ratio×ADF level	Gidenne *et al.*, 2004b
Digestible fibre	Hemicelluloses (NDF–ADF)+ pectins[a]	DF/starch ratio	Soler *et al.*, 2004
Digestible fibre	Hemicelluloses (NDF–ADF)+ pectins[a]	DF/ADF ratio×CP level	Xiccato *et al.*, 2006
Digestible fibre	TDF–ADF	DF/ADF ratio×starch level	Carraro *et al.*, 2007
Digestible fibre	TDF–ADF	DF/starch ratio	Xiccato *et al.*, 2008
Digestible fibre	TDF–ADF	DF/starch ratio	Tazzoli *et al.*, 2009
NDSF	Hall *et al.* 1997	NDSF/ADF ratio	Gómez-Conde *et al.*, 2007 and 2009
Soluble fibre	TDF–NDF without corrections[b]	SF/starch ratio×CP source	Trocino *et al.*, 2010
Soluble fibre	TDF–NDF with correction[b]	Source of pectins	Abad, 2011; El Abed *et al.*, 2011a
Soluble fibre	TDF–NDF with corrections[b]	SF/starch ratio×CP level	Xiccato *et al.*, 2011
Soluble fibre	TDF–NDF with corrections[b]	Starch/ADF ratio×SF level	Trocino *et al.*, 2011
Soluble fibre	TDF–NDF with corrections[b]	(SF+starch)/ADF ratio×CP level	Tazzoli, 2012 (exp. 1 and 2)
Soluble fibre	TDF–NDF with corrections[b]	SF/starch ratio×CP level	Trocino *et al.*, 2013

ADF: acid detergent fibre; CP: crude protein; DF: digestible fibre; NDF: neutral detergent fibre; NDSF: neutral detergent soluble fibre; SF: soluble fibre; TDF: total dietary fibre.

[a]Pectin contents of diets were calculated on the base of ingredient composition and tables on chemical composition of raw materials.

[b]Corrections for ash and protein contents of NDF residue.

Table 2: Variation of the ingredient inclusion levels (%) of the 90 diets tested in the 18 experiments listed in Table 1.

	Average	Standard deviation	Minimum	Maximum
Dehydrated alfalfa meal (%)	20.1	17.0	0	72.0
Barley+wheat meal (%)	19.5	14.9	0	86.0
Sugar beet pulp[a] (%)	16.0	11.8	0	49.0
Wheat bran (%)	14.3	9.81	0	35.2

[a]Citrus and apple pulp were used in 2 experiments (Gidenne *et al.*, 2004b; Gómez-Conde *et al.*, 2007).

Chemical composition differed among experimental diets (Table 3): changes in crude protein contents of the diets were in a narrow range (from 14 to 18% as-fed) and close to recommendations for growing rabbits (De Blas and Mateos, 2010); the contents of NDF, ADF, lignin and starch were sometimes far from recommendations; the degree of lignification of the insoluble fibre (ADL to NDF ratio) varied between 8 and 15%. SF ranged from 1.8 to 14.7% and was not homogeneously measured in all experiments: in most cases, it was calculated as TDF–NDF (the latter not always corrected for protein and ash contents); sometimes, it corresponded to the estimated pectin content; only once (Gómez-Conde *et al.*, 2007), SF was measured as NDSF (Hall *et al.*, 1997).

A number of traits measured in the different studies were taken into account in order to find relationships between dietary nutrients and *in vivo* response of the animal (Table 3). In details, the feed conversion ratio was selected to evaluate the performance of growing rabbits and the farm efficiency; the faecal digestibility of dry matter (DM) and fibre fractions (NDF, ADF, SF) was used to evaluate the diet utilisation; data on caecal pH and total volatile fatty acids (VFA) content were considered to describe the fermentation activity and gut health. In addition, when the

Table 3: Variation of the chemical composition (% as-fed) of the diets and the traits measured in the 18 experiments listed in Table 1.

	No.	Average	Standard deviation	Minimum	Maximum
Chemical composition					
NDF (%)	90	32.4	3.79	19.4	42.3
ADF (%)	90	18.0	2.81	9.20	25.3
ADL (%)	90	3.63	1.01	0.80	5.10
SF[a] (%)	90	7.69	3.08	1.76	14.7
Hemicelluloses (NDF–ADF) (%)	90	14.4	1.91	9.32	19.3
Starch (%)	90	15.0	5.92	2.50	34.0
Crude protein (%)	90	15.8	0.97	13.7	18.3
Traits					
Digestible energy (MJ/kg)	76	10.2	0.99	8.11	12.4
Feed conversion ratio	73	3.05	0.33	2.54	4.04
DM faecal digestibility (%)	62	62.7	5.4	49.7	75.8
NDF faecal digestibility (%)	80	31.3	9.4	14.3	51.7
ADF faecal digestibility (%)	76	21.8	7.9	2.6	41.7
SF faecal digestibility (%)	62	89.1	8.3	69.7	100
Caecal pH	67	5.92	0.24	5.23	6.57
Caecal volatile fatty acids (mmol/L)	63	65.6	9.68	49.5	89.1
Mortality[b] (%)	59	19.8	13.2	2.4	47.2
Gut proportion[c] (%)	55	18.2	0.80	16.7	20.2
Dressing percentage[d] (%)	55	60.4	0.78	58.2	61.9

ADF: acid detergent fibre; ADL: acid detergent lignin; DM: dry matter; NDF: neutral detergent fibre; SF: soluble fibre.
[a]When the soluble fibre was not available as TDF–NDF (Van Soest *et al.*, 1991) or as neutral detergent soluble fibre (NDSF, Hall *et al.*, 1997), the pectin content of the diets given in the paper was used. [b]Only experiments which reported the occurrence of digestive diseases were considered. [c]Full gut weight/slaughter weight×100. [d]Cold carcass weight/slaughter weight×100.

experiments reported the occurrence of digestive diseases the mortality rate was also considered. Finally, to provide some information on the effects of dietary treatments on carcass performance at commercial slaughter, the proportion of gut and the dressing percentage values were included in the data analysis.

"Digestible fibre" *vs.* soluble fibre

As a first step of our analyses of published studies, both SF (TDF–NDF) and "digestible fibre" (TDF–ADF) were used to calculate regressions to predict the above traits on the most homogeneous dataset, which was the 10 experiments performed at the University of Padova (Table 4). The analysis of variance was performed by PROC GLM (SAS, 1991) which included experiment as a class, and SF or "digestible fibre" and their interaction with the experiment as covariates.

The use of SF or "digestible fibre" contents to predict feed conversion, digestive utilisation of diets, caecal fermentation activity and health of rabbits was rather equivalent (Table 4), even if R^2 increased and the error decreased when using SF. "Digestible fibre" played a role only in the prediction of the gut proportion (P=0.09) and dressing percentage at slaughter (P=0.05).

The lower performance of regressions based on "digestible fibre" is likely explained by the low prediction weight of hemicelluloses. In fact, hemicellulose content in our dataset showed a lower variability (coefficient of variation, CV=13%) compared with the variability of pectin content (CV=40%). Moreover, the digestive utilisation of hemicelluloses may vary greatly depending on the different nature of insoluble fibre (in terms of lower lignification and complexity of the cell walls or different hemicellulose constituents) (Gidenne, 1992; Carabaño *et al.*, 2001; García *et al.*, 2002). In fact, the content of digestible hemicelluloses may be lower than that of digestible cellulose, as usually occurs in diets containing high levels of alfalfa and straw compared to those with high levels of SBP and wheat bran, and the contents of the 2 fractions are not correlated (Figure 1). Moreover, the digestible hemicellulose content decreases with the lignification degree of the insoluble fibre (Figure 2).

Based on the discussion and regressions above, in the meta-analysis of the 18 experiments we preferred the SF content because *i*) the "digestible fibre" is not likely to measure the fibre really digested by the rabbit with all types of diets; *ii*) the SF corresponds to chemically defined substances; *iii*) regressions based on SF explain more variability and are more accurate than regressions based on "digestible fibre".

Table 4: Explained variance (R^2) and residual standard deviation (RSD) of regressions[a] based on soluble fibre and digestible fibre content (n=55[b]).

	Soluble fibre (TDF–NDF)			Digestible fibre (TDF–ADF)		
	R^2	RSD	P-value	R^2	RSD	P-value
Feed conversion ratio	0.93	0.12	<0.001	0.82	0.18	<0.001
Digestible energy (MJ/kg)	0.89	0.37	<0.001	0.73	0.57	<0.001
DM faecal digestibility (%)	0.86	2.52	<0.001	0.66	3.91	<0.001
NDF faecal digestibility (%)	0.89	3.53	<0.001	0.73	5.51	<0.001
ADF faecal digestibility (%)	0.88	3.34	<0.001	0.71	5.25	<0.001
SF faecal digestibility (%)	0.90	3.28	<0.01	0.87	3.81	<0.01
Caecal pH	0.74	0.11	0.04	0.71	0.11	0.02
Caecal VFA (mmol/L)	0.78	5.76	<0.001	0.73	6.36	<0.001
Mortality (%)	0.82	5.66	<0.001	0.76	6.70	0.02
Gut proportion (% SW)	0.87	0.37	0.11	0.86	0.37	0.09
Dressing percentage (%)	0.82	0.40	0.14	0.84	0.38	0.05

ADF: acid detergent fibre; DM: dry matter; NDF: neutral detergent fibre; SF: soluble fibre; SW: slaughter weight; VFA: volatile fatty acids. [a]Analysis of variance by PROC GLM (SAS, 1991) included the experiment as a class and soluble fibre or digestible fibre and their interaction with the experiment as covariates. [b]Dataset of 55 dietary treatments from 10 experiments performed at the University of Padova (see Table 1).

Results of meta-analysis of studies on SF

As a first calculation step, the ADF, SF, hemicellulose, starch and crude protein contents of diets were selected out of all chemicals as independent variables due to their low variance inflation (<3). Then, the meta-analysis of the data from the 18 experiments was performed using the mixed model proposed by Sauvant *et al.* (2008), which included the experiment and the interaction experiment×nutrient as random effects and the nutrients as fixed factors.

Only the ADF and SF content entered the regression equations with a significant effect on the tested variables. The other nutrients were not included in equations due to lack of significant effect, low F-value and small decrease in the error term when they were added to the first nutrient in the regressions.

Feed conversion ratio and faecal digestibility: Feed conversion ratio, digestible energy content of diets and faecal digestibility of dry matter measured in growing rabbits were significantly affected by the dietary content of ADF and not by the SF content (Table 5). Feed conversion ratio was impaired when the dietary ADF level increased. The slope coefficient for ADF (–0.295 on as-fed basis) in the equation for the prediction of digestible energy content was higher than those previously obtained by other authors (De Blas *et al.*, 1992; Fernández-Carmona *et al.*, 1996; Villamide *et al.*, 2009) (from –0.12 to –0.21 ADF on DM basis). In all cases, however, ADF content was negatively correlated with the digestible energy content of diets. Xiccato and Trocino (2010) calculated that feed conversion ratio was impaired by 0.029 points when the DE content decreased by 1 MJ/kg diet, as a consequence of the increased feed intake (+12 g/d).

The digestibility of NDF and ADF depended on both ADF and SF content, with a negative coefficient for the former and a positive coefficient for the latter, whereas the faecal digestibility of SF was explained by the SF content itself (Table 5).

In fact, when the SF to TDF ratio increased, the faecal digestibility of TDF improved (Figure 3) because of the high digestibility of SF itself (on av. 89.1%; Table 3) and the low lignification of the insoluble fibre contained in raw materials used to increase dietary SF level (i.e. SBP) (Martínez and Fernández, 1980; Gidenne, 1987a, 1987b; De Blas and Villamide, 1990; Carabaño *et al.*, 1997). Moreover, when the SF to TDF ratio increased, the digestive utilisation of TDF either in the ileum or the caecum also increased (Figure 4) (Abad, 2011;

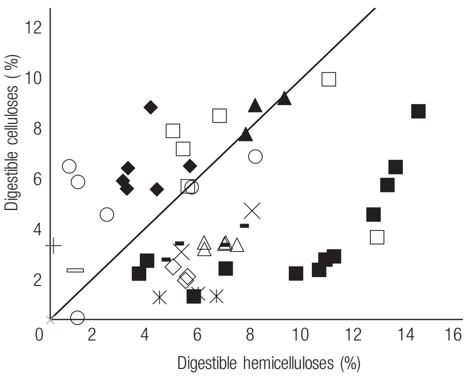

Figure 1: Relationships between digestible hemicellulose content and digestible cellulose content in diets for growing rabbits. Digestible celluloses=2.50(±0.84)+0.27(±0.088) Digestible hemicelluloses, n=52; P=0.005; R^2=0.027; residual standard deviation=1.77. ◆ Falcão-e-Cunha and Lebas, 1986. □ Gidenne, 1987a, 1987b. △ Gidenne and Perez, 1994. ✕ Gidenne and Jehl, 1996. ✳ Gidenne *et al.*, 1998. ○ García *et al.*, 1999. + García *et al.*, 2000. ▬ Gidenne and Perez, 2000. ◇ Gidenne *et al.*, 2001a. ▭ García *et al.*, 2002. ■ Falcão-e-Cunha *et al.*, 2004. ▲ Volek *et al.*, 2007.

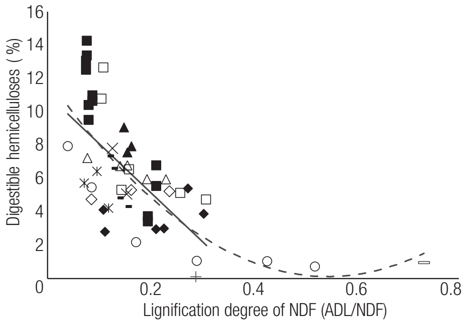

Figure 2: Relationships between NDF lignification degree (ADL/NDF ratio) and digestible hemicellulose content in diets for growing rabbits. —Digestible hemicelluloses=9.723(±0.90)–0.18(±0.032)ADL/NDF. n=52; P<0.001; R^2=0.40; residual standard deviation=2.37. ---Digestible hemicelluloses=12.1 (±1.13)–0.43(±0.083) ADL/NDF+0.0041(±0.0013)$(ADL/NDF)^2$. n=52; P<0.001; R^2=0.49; residual standard deviation=2.16. NDF: neutral detergent fibre; ADL: acid detergent lignin. ◆ Falcão-e-Cunha and Lebas, 1986. □ Gidenne, 1987a, 1987b. △ Gidenne and Perez, 1994. ✕ Gidenne and Jehl, 1996. ✳ Gidenne *et al.*, 1998. ○ García *et al.*, 1999. + García *et al.*, 2000. ▬ Gidenne and Perez, 2000. ◇ Gidenne *et al.*, 2001a. ▭ García *et al.*, 2002. ■ Falcão-e-Cunha *et al.*, 2004. ▲ Volek *et al.*, 2007.

Table 5: Regression equations[a] calculated on the chemical composition of the diets used in the 18 experiments listed in Table 1.

Variable	Equation	RSD	*P*-value
Feed conversion ratio	1.43+0.089 ADF	0.003	<0.001
Digestible energy (MJ/kg)	15.40–0.29 ADF	0.13	<0.001
DM faecal digestibility (%)	101–2.07 ADF	0.87	<0.001
NDF faecal digestibility (%)	56.7–2.32 ADF+1.93 SF	1.36	<0.001
ADF faecal digestibility (%)	39.8–1.89 ADF+1.88 SF	1.20	<0.001
SF faecal digestibility (%)	81.7+0.09 SF	1.95	<0.01

RSD: residual standard deviation; DM: dry matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; SF: soluble fibre.
[a]The mixed model included the experiments and the experiment×nutrient interactions as random effects and the nutrients as fixed factors (Sauvant *et al.*, 2008). Regressions with no significant effect (gut proportion and dressing percentage) are not presented in the table. Dietary ADF and SF are expressed in % (as-fed).

Abad *et al.*, 2012). Accordingly, the average ileal digestibility of SF was 40% and ranged from 10 to 65% in diets with different SF level and ingredient composition (Abad, 2011; Abad *et al.*, 2012), consistently with previous data on ileal digestibility of arabinose and uronic acids (main components of pectins) (average value: 35%; range: 20-50%) (Gidenne, 1992; Carabaño *et al.*, 2001).

In fact, increasing dietary SF content may stimulate the growth of fibrolytic microbiota in the gut, thus increasing the utilisation at both ileum and caecum of insoluble fibre fractions (hemicelluloses and ADF) (Gómez-Conde *et al.*, 2007 and 2009).

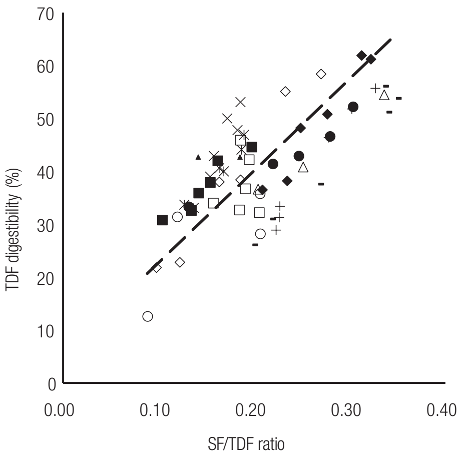

Figure 3: Relationships between soluble fibre (SF)/total dietary fibre (TDF) ratio of diets and TDF faecal digestibility (TDFd). RSD: residual standard deviation. TDFd=5.49(±1.79)+174(±3.74) SF/TDF. RSD=1.94; *P*<0.001. ■ Xiccato *et al.*, 2006. □ Carraro *et al.,* 2007. △ Gómez Conde *et al.*, 2007. ▲ Xiccato *et al.*, 2008. ╳ Tazzoli *et al.,* 2009. ⋇ Trocino *et al.*, 2010. ○ Abad., 2011. + Trocino *et al.*, 2011. ● Xiccato *et al.*, 2011. ◇ Tazzoli, 2012 (exp.1). ◆ Tazzoli, 2012 (exp.2). ▬ Trocino *et al.*, 2013.

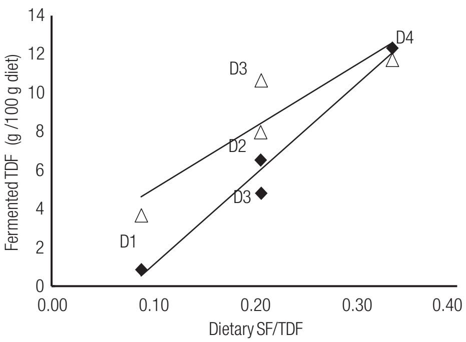

Figure 4: Ileal and caecal fermentation of total dietary fibre (TDF) as affected by soluble fibre (SF)/TDF ratio of the diets. Fermented TDF=–0.87(±1.79)+0.39(±0.079)SF/TDF. RSD: residual standard deviation. n=8; $P_{SF/TDF}$=0.004; R^2=0.90; RSD=1.53; $P_{segment}$=0.092. $P_{SF/TDF\times segment}$=0.30. Diet D1, 100% of dietary neutral detergent fibre (NDF) and TDF from sunflower hulls and straw; Diet D2, 65% of NDF from sunflower hulls and straw and 35% from sugar beet pulp (Abad, 2011). Diet D3, 50% of NDF from oat and sunflower hulls and 50% from alfalfa, wheat and soybean meal; Diet D4, 20% of NDF from oat and sunflower hulls, 30% sugar beet pulp and 50% from alfalfa, wheat and soybean meal (Abad *et al.*, 2012). ◆ Ileal TDF. △ Caecal TDF.

Caecal fermentation traits: The SF level affected caecal pH and caecal total volatile fatty acids concentration (Figure 5 and 6). As commented above, by modifying the amount and type of substrate reaching the caecum, the SF level affects ileal and caecal microbiota composition (Gómez-Conde *et al.*, 2007 and 2009) and, thus, caecal fermentation activity.

The increase in dietary SF at the expense of insoluble fibre or starch is known to promote caecal fermentation in fattening rabbits (Jehl and Gidenne, 1996; Falcão-e-Cunha *et al.*, 2004; Xiccato *et al.*, 2006, 2008, 2011; Trocino *et al.*, 2011). In some studies, moreover, higher proportions of acetate and lower rates of butyrate were found in the caecum of rabbits fed diets with high levels of SBP (Fraga *et al.*, 1991; Falcão-e-Cunha *et al.*, 2004; Gidenne *et al.*, 2004a; Xiccato *et al.*, 2011), which have been associated with a greater availability of substrate fermentable by fibrolytic bacteria (Falcão-e-Cunha *et al.*, 2004) and a lower activity of amylolytic microflora in the caecum (Parigi Bini *et al.*, 1990; Gidenne *et al.*, 2000; Blas and Gidenne, 2010). However, in other studies, caecal fermentation pattern did not vary according to the SF level (Carabaño *et al.*, 1997; Gómez-Conde *et al.*, 2009; Trocino *et al.*, 1999, 2010, 2011; Belenguer *et al.*, 2011). The contemporary variations in the insoluble fibre level and its degree of lignification are responsible for the different results among studies (García *et al.*, 2002).

Finally, whether the increase of caecal VFA and the decrease of pH may be considered positive for rabbit health is not definitively stated. In fact, reviewing several studies, neither García *et al.* (2002) nor Gidenne *et al.* (2010b) found a clear relationship between the 2 traits or between these 2 traits and the development of some enteric pathogens (such as *E. coli*).

Health status: The variability in the data of mortality linearly depended on SF content (Figure 7), while other dietary characteristics (e.g. ADF, starch or protein contents) did not enter into the equation. From the dataset we used, mortality fell below 5% when dietary SF content was at about 12% and reached 0% when dietary SF content reached about 14% (Figure 7). In fact, some studies have shown a decrease in rabbit mortality and morbidity caused by epizootic rabbit enteropathy and other digestive disorders when SF replaced insoluble fibre (Gómez-Conde *et al.*, 2007; Xiccato *et al.*, 2006) or starch (Jehl and Gidenne, 1996; Perez *et al.*, 2000; Soler *et al.*, 2004; Xiccato *et al.*, 2008, 2011).

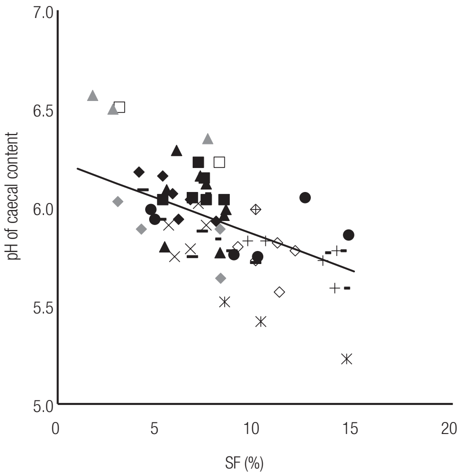

Figure 5: Relationships between dietary soluble fibre (SF) level (% as-fed) and caecal pH. RSD: residual standard deviation. pH=6.23(±0.26)–0.037(±0.08)SF. RSD=0.047. *P*<0.001. □ Gidenne and Jehl, 1996. ▲ Gidenne and Bellier, 2000. ◆ Xiccato *et al.*, 2006. ■ Carraro *et al.*, 2007. ✳ Gómez-Conde *et al.*, 2007. ▲ Xiccato *et al.*, 2008. ✕ Tazzoli *et al.*, 2009. ▲ Trocino *et al.*, 2010. ◆ El Abed *et al.*, 2011. + Trocino *et al.*, 2011. ● Xiccato *et al.*, 2011. ▬ Tazzoli, 2012 (exp.1). ◇ Tazzoli, 2012 (exp.2). ▬ Trocino *et al.*, 2013.

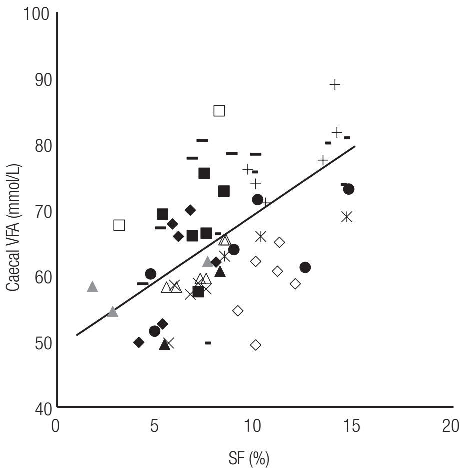

Figure 6: Relationships between dietary soluble fibre (SF) level (% as-fed) and caecal total volatile fatty acids (VFA). RSD: residual standard deviation. VFA=48.9(±1.74)+2.05(±0.60) SF. RSD=2.41. *P*<0.001. □ Gidenne and Jehl, 1996. ▲ Gidenne and Bellier, 2000. ◆ Xiccato *et al.*, 2006. ■ Carraro *et al.*, 2007. ✳ Gómez-Conde *et al.*, 2007. ▲ Xiccato *et al.*, 2008. ✕ Tazzoli *et al.*, 2009. △ Trocino *et al.*, 2010. + Trocino *et al.*, 2011. ● Xiccato *et al.*, 2011. ▬ Tazzoli, 2012 (exp.1). ◇ Tazzoli, 2012 (exp.2). ▬ Trocino *et al.*, 2013.

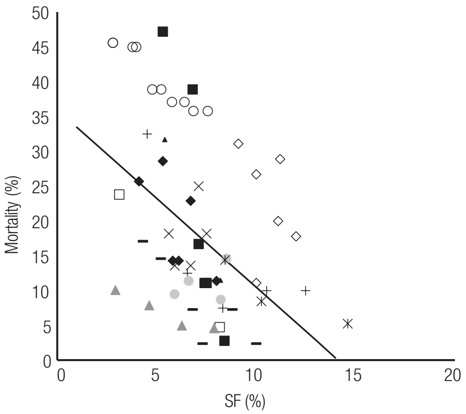

Figure 7: Relationships between dietary soluble fibre (SF) level (% as-fed) and mortality rate of growing rabbits. RSD: residual standard deviation. Mortality=36.0(±2.1)–2.59(±0.71)SF. RSD=3.14 P<0.001. □ Gidenne and Jehl, 1996. ▲ Gidenne and Perez, 2000. + Gidenne *et al.*, 2004a. ● Gidenne *et al.*, 2004b. ○ Soler *et al.*, 2004. ◆ Xiccato *et al.*, 2006. ■ Carraro *et al.*, 2007. ⋇ Gómez-Conde *et al.*, 2007. ▴ Xiccato *et al.*, 2008. × Tazzoli *et al.,* 2009. ▬ Tazzoli, 2012 (exp.1). ◇ Tazzoli, 2012 (exp.2).

The reduction in the mortality rate has been associated with higher jejunal villi height to crypt depth ratio and disaccharidase activity in young rabbits (35 d of age) fed increasing levels of SF and fermentable insoluble fibre (Gómez-Conde *et al.*, 2007). Indeed, higher villi height to crypt depth ratio was also recorded in 35-d-old rabbits when a diet containing non fermentable insoluble fibre (from wheat straw, sunflower hulls) was supplemented with SF (pectins from SBP) or with fermentable insoluble fibre (insoluble fibre from SBP), or, especially, with SBP (as source of both soluble and insoluble fermentable fibre) (El Abed *et al.*, 2011a). SF and fermentable insoluble fibre from SBP may also exert their positive effects on rabbit health after weaning by increasing the number of Goblet cells per villi and the ileal viscosity as a consequence of a high mucin production (El Abed *et al.*, 2011b). However, these changes in gut mucosa morphology might be age-dependent as they were lower in animals at 45 d of age (Àlvarez *et al.*, 2007) or not observed in older rabbits (51-56 d of age) (Trocino *et al.*, 2010, 2011; Xiccato *et al.*, 2011). The sampling site (jejunum *vs.* ileum), time after weaning and health status of the animals may also contribute to these differences.

Gut proportion and slaughter results: Several studies reported an increase of full gut weight at slaughter and a consequent decrease in dressing percentage when rabbits received diets containing high levels of SF (and SBP) (García *et al.*, 1993; Carabaño *et al.*, 1997; Falcão-e-Cunha *et al.*, 2004; Trocino *et al.*, 2011). The contemporary wide variations in insoluble fibre (NDF or ADF) in diets used in those experiments might explain most of these variations (García *et al.*, 2002). However, also when iso-ADF diets were used, the results were not consistent among studies: some authors did not find a real impact of SBP inclusion on the filling of digestive organs (Jehl and Gidenne, 1996; Gidenne and Perez, 2000; Trocino *et al.*, 2010; Margüenda *et al.*, 2012) while others authors did (Fraga *et al.*, 1991; Carabaño *et al.*, 1997). A special role in increasing the weight of caecal content has been attributed to the insoluble fibre of SBP, which may reach the caecum and increase the amount of fibre here fermented (Abad, 2011).

In our meta-analysis, no nutrient was selected at a significant level (P<0.05) in equations to predict gut proportion and dressing percentage at commercial slaughter. Large differences in final slaughter age and live weight at slaughter among animals of the different trials as well as different pre-slaughter treatments (starvation or not; the duration of transport and pre-slaughter wait, etc.) likely contributed to hide the effects of the fibre fractions on gut proportion and dressing percentage of rabbits at slaughter (Trocino *et al.*, 2003; Cavani *et al.*, 2009).

Effect of SBP inclusion rate: When regressions were calculated on the SBP inclusion rate of diets, digestible energy content of diets and feed conversion ratio were not affected (Table 6), which indicates that the insoluble fibre coming from other ingredients also helps explain the variability of these data.

Gut proportion on slaughter weight and dressing percentage were not affected by the SBP level, as they were not affected by the SF level. In contrast, faecal digestibility of DM and all fibre fractions increased with the SBP inclusion rate, which agrees with the above discussion on the effect of SF level on the digestive utilisation of soluble and insoluble fibre fractions. Similarly, the effect of SF on caecal fermentation traits (pH and VFA concentration) as well as on mortality was associated with changes in SBP inclusion rate, even if the estimate errors were generally higher when SPB rate was used in the equation instead of SF level.

Table 6: Regression equations[a] calculated on the sugar beet pulp inclusion rate of the diets used in the 18 experiments listed in Table 1.

Variable	Equations	RSD	*P*-values
DM faecal digestibility (%)	58.0+0.303 SBP	1.09	<0.01
NDF faecal digestibility (%)	22.8+0.545 SBP	1.54	<0.001
ADF faecal digestibility (%)	13.7+0.506 SBP	1.52	<0.001
SF faecal digestibility (%)	85.9+0.207 SBP	1.73	0.001
Caecal pH	6.04–0.007 SBP	0.045	<0.001
Caecal volatile fatty acids (mmol/L)	58.8+0.432 SBP	2.39	<0.001
Mortality (%)	23.6–0.374 SBP	3.41	<0.01

ADF: acid detergent fibre; DM: dry matter; NDF: neutral detergent fibre; RSD: residual standard deviation; SBP: sugar beet pulp inclusion rate (% of the diet); SF=soluble fibre.

[a]The mixed model included the experiment and the experiment×SBP interaction as random effects and the SBP as fixed factors (Sauvant *et al.*, 2008). Regressions with no significant effect (digestible energy, feed conversion ratio, gut proportion, dressing percentage) are not presented in table.

SOLUBLE FIBRE REQUIREMENTS

With a view to preventing digestive troubles, fibre requirements should not be based only on insoluble fibre, but also on SF. According to the French researchers at the Institut National de Recherche Agronomique (INRA), the dietary supply of "digestible fibre" must be considered in strict relation to dietary ADF and the "digestible fibre" to ADF ratio should not exceed 1.3 (Gidenne *et al.*, 2010b). This limit implies a maximum level of SF at 12-13% as-fed for a maximum SF to ADF ratio of 0.63, since INRA also recommends a minimum dietary supply of 19-17% ADF and 12-10% hemicelluloses (the higher values during post-weaning period and the lower ones during fattening period).

The Spanish researchers at the Polytechnic University of Madrid prefer to express recommendations in terms of dietary NDSF content around 12% in diets fed during the post-weaning period, without limits during the fattening (Gidenne *et al.*, 2010b) once insoluble fibre (NDF, ADF and ADL) requirements are satisfied (De Blas and Mateos, 2010). These latter recommendations are in agreement with the optimal SF level (12% as-fed) to control mortality below 5%, as mentioned above, whereas higher SF levels (14%) would allow us to abate mortality based on the meta-analysis of available results.

However, few experimental data are available with diets containing SF levels higher than 12% and further research would therefore be useful to elucidate the response of rabbits to a higher dietary SF supply. Moreover, these results need to be confirmed with diets containing SF sources other than SBP (e.g. apple pulp, citrus pulp, chicory), even if the formulation of diets containing such high levels of SF would not be easy without SBP, which is currently the most widely used and cheap source of SF (24.3% DM) (Xiccato *et al.*, 2012).

CONCLUSIONS

SF may be measured and calculated by different methods and procedures. In this review, we referred to SF as the difference TDF-NDF due to its simplicity and the numerous studies that measured it. The increase in dietary SF has a positive effect on the reduction of mortality in growing rabbits affected by epizootic rabbit enteropathy and this result could be ascribed to the high SF fermentability and the changes exerted on the intestinal microbiota and the enhanced gut barrier function. Fermentable insoluble fibre likely shares with SF the responsibility for these effects. A minimum SF supply of 12% as-fed should be guaranteed in diets for post-weaning and growing rabbits containing around 30% NDF and 18% ADF to maintain mortality below 5%. These conclusions are linked to the use of SBP as primary source of SF and should be confirmed with other SF sources.

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