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ROLE OF SOLUBLE FIBRE IN DIETS FOR GROWING RABBITS: A REVIEW

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ABSTRACT

In this review, the methods to measure fibre and soluble fibre fractions have been briefly presented; we have referred to soluble fibre as the difference between Total Dietary Fibre (TDF) and Neutral Detergent Fibre (NDF) due to its simplicity to be obtained and the numerous studies that measured it; the effects of soluble fibre on performance, digestive efficiency and physiology, caecal activity and health of growing rabbits have been reviewed by a meta-analysis of studies available in literature, also with the aim of elucidating the relationships with other dietary nutrients. The level of soluble fibre affects the digestive utilizations of soluble and insoluble fibre fractions at ileum and caecum, ileal and caecal microbiota and caecal fermentations by modifying the amount and the type of substrate reaching the caecum. The increase of soluble fibre has a positive effect on the reduction of mortality in growing rabbits affected by epizootic rabbit enteropathy, which could be related to the high fermentability of soluble fibre, the changes exerted in the intestinal microbiota, and an enhanced gut barrier function just after weaning. A minimal supply around 12% of soluble fibre (as fed) is recommended in diets for post-weaning and growing rabbits containing about 30% NDF and 18% ADF. These conclusions are linked to the use of sugar beet pulp as primary source of soluble fibre and should be confirmed with other soluble fibre sources.

Key words: Growing rabbits, soluble fibre, digestibility, caecal fermentation, health.

INTRODUCTION

The fibre is the major fraction of diets for rabbits (35-50%); 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 the growth performance and the digestive health of rabbits (Gidenne *et al.,* 2010a and 2010b). In rabbits, insoluble fibre has been widely recognized as the most important fibre fraction and used to express fibre requirements: it accounts for about 65-90% of the total dietary fibre (TDF) and is quantified by rather standardized methodologies; current recommendations state that diets for rabbits should contain at least 30% NDF and 16% ADF (De Blas and Mateos, 2010). The chemical (degree of lignification) and physical characteristics (particle size) of insoluble fibre also affect the rate of passage of digesta and the susceptibility of fibre to fermentation (Gidenne *et al.,* 2010b).

Differently, soluble fibre (SF) has been neglected in rabbits until recent times: it is a minor and heterogeneous fraction of the total dietary fibre (about 10-35% of TDF) and there is no agreement on how to define, quantify and characterize it. The most common source of soluble fibre in rabbit diets is sugar beet pulp (SBP) that has been traditionally used as a substitute of cereal grains to limit dietary starch content, thanks to its high digestible energy concentration (De Blas and Carabaño, 1996; Jehl and Gidenne, 1996). There is a recent interest in the role of soluble fibre in rabbit feeding due to claimed positive effects on rabbit digestive health (Perez *et al.,* 2000; Soler *et al.,* 2004; Gómez-Conde *et al.,* 2007) in a context of epizootic rabbit enteropathy (ERE), which have been associated to an improved intestinal mucosa integrity and a modulation of intestinal microbiota (Gómez-Conde *et al.,* 2007, 2009). However, results on the effects of soluble fibre in rabbits are not always consistent among studies and some questions have not yet been clarified, e.g. the site of soluble fibre fermentation in the gut; the effect of soluble fibre on viscosity in the digestive tract; the interactions between soluble fibre and other dietary nutrients. In fact, feedstuffs rich in soluble fibre (*e.g.* sugar beet pulp, citrus pulp) also contain high levels of fermentable insoluble fibre, which make difficult to distinguish the peculiar effects of the two fractions.

The present review aims to be a starting point of discussion on soluble fibre in rabbit nutrition: methods to measure fibre and soluble fibre fractions will be briefly presented; the role of soluble fibre on digestive physiology will be discussed; the effects of soluble fibre 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, minimum requirements of soluble fibre for growing rabbits will be proposed.

DEFINITION AND QUANTIFICATION OF FIBRE FRACTIONS

Total dietary fibre

The concept of dietary fibre used in human nutrition and extended to other mammals has been periodically reviewed (Hispley, 1953; Burkitt *et al.,* 1972; Trowell, 1974; DeVries, 2010), and it is generally defined as the polysaccharides and associated substances resistant to mammal enzyme digestion and absorption that can be partially or totally fermented in the gut. As described in Figure 1, from a chemical point of view, dietary fibre 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, etc.) or in the cytoplasm (resistant starches, oligosaccharides, fructans) (Gidenne *et al.,* 2010a). Currently, total dietary fibre (TDF) is primarily analysed by enzymatic-gravimetric methods based on AOAC procedures 985.29 and 991.43 that solubilise the different fibre fractions with enzymes and solvents and measure the weight of residues after these treatments (as reviewed by Bach Knudsen, 1997 and 2001; DeVries, 2010; Elleuch *et al.,* 2011).

<table>
<tr><td rowspan="10">Dietary fibre</td><td rowspan="4">Cell content</td><td>Resistant starch</td><td colspan="4" rowspan="2"></td><td rowspan="10">Total dietary fibre[3] (resistant starch has been included only in the latest adaptation of the method)</td></tr>
<tr><td>Oligosaccharides</td></tr>
<tr><td>Fructans</td><td colspan="2" rowspan="6"></td><td rowspan="5">NDSF[2]</td><td rowspan="5">Soluble dietary fibre[3]</td></tr>
<tr><td>Mannans-Galactomannans</td></tr>
<tr><td rowspan="6">Cell wall</td><td>Pectic substances</td></tr>
<tr><td>Glucomannans, Galactoglucomannans</td></tr>
<tr><td>β-glucans</td></tr>
<tr><td>Hemicelluloses
Arabinoxylans
Arabinogalactans
Xyloglucans</td><td rowspan="3">aNDF[1]</td><td rowspan="3">Insoluble dietary fibre[3]</td></tr>
<tr><td>Cellulose</td><td></td><td rowspan="2">ADF[1]</td></tr>
<tr><td>Lignin</td><td>Lignin(sa)[1]</td></tr>
</table>

Figure 1: Enzymatic gravimetric-methods for measuring major constituents of dietary fibre: [1]Mertens, 2002; [2]Hall *et al.,* 1997; [3]McCleary *et al.,* 2010 (adapted from Hall, 2003 and Gidenne *et al.,* 2010a).

These procedures have been updated to include also non-digestible oligosaccharides and resistant starch (McCleary *et al.,* 2010). Differently, enzymatic-chemical methods quantify the fibre residue chemically, by hydrolyzing the polysaccharides in the residue and determining their contents in 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 differentiate the insoluble and soluble fractions of the sample. The combination of fibre monosaccharide composition with additional chemical information allows a better description of fibre structure and its physic-chemical properties. However, these enzymatic-chemical methodologies, designed to measure non starch polysaccharides, may underestimate dietary fibre content, and are rather complex, expensive, difficult to implement as routine analysis and characterized by a rather low reproducibility (Mertens, 2003; Elleuch *et al.,* 2011).

Insoluble dietary fibre

With relevance to the special features of animal digestive physiology, insoluble dietary fibre (IDF) for herbivores is defined 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) (Mertens, 2003). Accordingly, IDF 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 at similar levels of starch or cytoplasmatic proteins. Insoluble dietary fibre may be easily quantified by the above mentioned AOAC method for TDF, by avoiding the recovery of water-soluble structural polysaccharides. However, the enzymatic-gravimetric determination of neutral detergent fibre (NDF) is the most simple, low-cost, rapid and reproducible method, among those derived from the dietary fibre definition, which is used to quantify insoluble fibre (Mertens, 2003). The different procedures (Van Soest and Wine, 1967; Robertson and Van Soest, 1980; Mertens, 2002) and adaptations in laboratories may bring about a somewhat weakness in the reproducibility of NDF analyses. For this reason, Uden *et al.* (2005) recommend to adopt the procedure of Mertens (2002), where NDF is obtained with amylase and sodium sulphite treatments and is expressed ash free (aNDFom), as well as to describe accurately the methodology used. The European Group on Rabbit Nutrition also proposed some recommendations for determining insoluble fibre in compound feeds and raw materials for rabbits to improve the reproducibility among laboratories (Xiccato *et al.,* 1996; EGRAN, 2001).

Since these methods might not measure the fibre that is really insoluble in the rabbit digestive tract, the ileal *in vitro* dry matter indigestibility (i-*iv*DMi) has been proposed to quantify insoluble fibre under conditions of temperature, pH and time which simulate the digestion in the small intestine (Carabaño *et al.,* 2008; Abad *et al.,* 2011). When the insoluble fibre contents of seven diets and six fibrous feedstuffs for rabbits measured as IDF (AOAC), aNDFom and i-*iv*DMi were compared, the values were the lowest for aNDFom, while similar for IDF and i-*iv*DMi (Table 1). The differences among values were higher in the feedstuffs rich in soluble fibre (e.g. sugar beet pulp) and lower in those rich in insoluble fibre (e.g. sunflower hulls). However, the correlation among the three values of insoluble fibre was high ($r \geq 0.97$; $P<0.001$; n=13) indicating an acceptable agreement among these methodologies.

There are other analytical procedures to quantify insoluble fibre, like Weende crude fibre and acid detergent fibre (ADF) determinations, but neither of them fit with the above mentioned definitions of total or insoluble dietary fibre. In fact, the chemical composition of crude fibre residue is highly variable depending on the feed, and 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 the animal digestive physiology. On the other hand, both of them are very useful to predict dietary nutritive value (Wiseman *et al.,* 1992; Villamide *et al.,* 2009) and their determinations have similar or even higher reproducibility compared with NDF analysis (Xiccato *et al.,* 1996). Dietary insoluble fibre can also be estimated by using Near Infrared Reflectance Spectroscopy (NIRS), especially when ADF is considered (Xiccato *et al.,* 2003).

Table 1: Comparison of the different methodologies used to determine insoluble and soluble fibre in seven compound diets for rabbits and six feedstuffs[1] (Abad *et al.,* 2011).

Item[2]	**Mean value, % DM**[3]	**rsd**	**P-value**
Insoluble dietary fibre			
Insoluble dietary fibre (IDF) (AOAC)	47.0^{B}	3.52	0.029
Ileal *in vitro* DM indigestibility (i-*iv*DMi)	48.1^{B}		
aNDFom	44.3^{A}		
Soluble dietary fibre			
Soluble dietary fibre (SDF) (AOAC)	10.5^{b}	3.02	0.002
TDF – IDF	12.4^{b}		
TDF – i-*iv*DMi	11.3^{b}		
TDF - aNDFom	15.3^{a}		

[1]Sugar beet pulp, insoluble sugar beet pulp, sugar beet pectin, lignocellulose, sunflower hulls and wheat straw. [2]TDF, IDF, Ileal *in vitro* DM indigestibility, aNDFom, values corrected for ash and protein. [3] Mean values in the same column with a different superscript significantly differ ($P<0.05$).

Besides the dietary insoluble fibre level, the type of insoluble fibre (characterized by the degree of lignification and particle size) is also relevant on rabbit nutrition and digestive physiology. Lignin content is usually determined according to Robertson and Van Soest (1981) by treating the ADF residue by sulphuric acid (ADL): the method is largely applied but is more laborious and less reproducible compared with NDF determination (Xiccato *et al.,* 1996).

Soluble dietary fibre

Soluble fibre is the part of TDF that comprises the non-starch, non-NDF polysaccharides, including pectic substances, β-glucans, fructans and gums (Hall, 2003). This fraction is more complex to assess than insoluble fibre and may be measured by different methods.

When soluble dietary fibre (SDF) is determined according to the AOAC 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 hydrolyzed by amyloglucosidase, insoluble fibre is separated by filtration, solubilised dietary fibre is precipitated with an ethanol solution from the solvent extract and measured gravimetrically after correction for protein and ash contents. Inaccuracies in the SDF determination may arise by the partial degradation of carbohydrates, the incomplete extraction and/or precipitation with the addition of ethanol, the interference by other substances, and differences in the nature of the analysed feed (Theander *et al.,* 1994; Hall *et al.,* 1997).

When soluble fibre is measured according to Hall *et al.* (1999), the neutral detergent soluble fibre (NDSF) is obtained gravimetrically as the difference between ethanol/water insoluble residue and starch and NDF after correction for protein and ash (Hall *et al.,* 1999). The NDSF measurement may be affected by the accumulation of errors in the measurement of the different components as well as the error linked to the value used for protein correction (N x 6.25) (Hall, 2003; Martínez-Vallespín *et al.,* 2011).

When soluble fibre content is measured using enzymatic-gravimetric methods, it could be obtained as the difference between TDF and any measurement of insoluble fibre. According to Van Soest *et al.* (1991), soluble fibre 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. Finally, soluble fibre content may be calculated by difference as: organic matter – (protein + fat + soluble sugars + starch + NDF).

As mentioned above, to measure the fibre that is really soluble in the rabbit digestive tract, soluble fibre can be calculated as the difference between TDF and the ileal *in vitro* dry matter indigestibility (i-*iv*DMi) (Table 1). When the soluble fibre contents of seven diets and six fibrous feedstuffs for rabbits were measured directly (by AOAC), or calculated as TDF-IDF or TDF- i-*iv*DMi, the values were similar among them and lower than soluble fibre measured as TDF-aNDFom (Abad *et al.,* 2011). The correlation among the four values was high ($r \geq 0.96$; $P \leq 0.001$; n=13), but decreased (r=0.84-0.93) when data of sugar beet pulp and sugar beet pulp pectins were excluded (n=10, r=0.84-0.93).

Regardless of the advantages and disadvantages of the different methods and calculation procedures, the choice of the method to quantify SF will depend also on the correlation with *in vivo* data collected in rabbits. In the present review, soluble fibre measured as TDF-aNDF will be extensively used, since it was available in most studies concerning the role of soluble fibre in growing rabbits.

EFFECT OF SOLUBLE FIBRE LEVEL IN GROWING RABBITS

During the last years, several studies have been conducted on the effect of dietary soluble fibre or digestible fibre in growing rabbits which have elucidated some points, but have also posed some questions:

1) Is there any fibre fraction that explains better the *in vivo* response of the rabbit?

2) May we isolate the effect of soluble fibre from that of the other nutrients in the diets, in view of the strict relationships among soluble fibre, insoluble fibre, starch and/or protein?
3) Since changes in soluble fibre levels are mainly obtained (in the practice and in experiments) by varying sugar beet pulp inclusion rate, have the effects of soluble fibre level to be ascribed simply to the sugar beet pulp rate?

In order to draw some general conclusions and to isolate the effect of soluble fibre from that of the other nutrients in the diet, we collected the data coming from 18 experiments performed in four laboratories of three countries (Italy, France and Spain) in which 90 experimental diets were fed to rabbits from weaning until slaughter (Table 2). Diets were designed to evaluate the effect of soluble fibre or digestible fibre in replacement of insoluble fibre (ADF) and starch and in relationships with the dietary protein content.

Table 2: Studies on the effects of digestible fibre (DF) or soluble fibre (SF) in diets for growing rabbits performed at the University of Padova (Italy), INRA of Toulouse (France), Polytechnical University of Madrid and Polytechnical University of Valencia (Spain)

Soluble fibre definition used in the paper	Analytical/calculation method	Experimental arrangement	Reference (Code)
Digestible fibre	Hemicelluloses (aNDF-ADF) + pectins[1]	Ratio DF/ADF x protein level	Xiccato *et al.,* 2006 (I01)
Digestible fibre	TDF-ADF	Ratio DF/ADF x starch level	Carraro *et al.,* 2007 (I02)
Digestible fibre	TDF-ADF	Ratio DF/starch	Xiccato *et al.,* 2008 (I03)
Digestible fibre	TDF-ADF	Ratio DF/starch	Tazzoli *et al.,* 2009 (I04)
Soluble fibre	TDF-aNDF without corrections[2]	Ratio SF/starch x protein source	Trocino *et al.,* 2010 (I05)
Soluble fibre	TDF-aNDF with corrections[2]	Ratio SF/starch x protein level	Xiccato *et al.,* 2011 (I06)
Soluble fibre	TDF-aNDF with corrections[2]	Ratio starch/ADF x SF level	Trocino *et al.,* 2011 (I07)
Soluble fibre	TDF-aNDF with corrections[2]	Ratio DF/starch x protein level	Tazzoli *et al.,* 2011 (I08)
Soluble fibre	TDF-aNDF without corrections[2]	Ratio (SF+starch)/ADF x protein level	Tazzoli, 2012a (I09)
Soluble fibre	TDF-aNDF with corrections[2]	Ratio (SF+starch)/ADF x protein level	Tazzoli, 2012b (I10)
Digestible fibre	Hemicelluloses (aNDF-ADF) + pectins[1]	Ratio DF/starch	Gidenne and Jehl 1996; Jehl and Gidenne, 1996 (F11)
Digestible fibre	Hemicelluloses (aNDF-ADF) + pectins[1]	Ratio DF/starch	Gidenne and Perez, 2000; Perez *et al.,* 2000 (F12)
Digestible fibre	Hemicelluloses (aNDF-ADF) + pectins[1]	Ratio DF/starch	Gidenne and Bellier 2000 (F13)
Digestible fibre	Hemicelluloses (aNDF-ADF) + pectins[1]	Ratio DF/starch x ADF level	Gidenne *et al.,* 2004b (F14)
Digestible fibre	Hemicelluloses (aNDF-ADF) + pectins[1]	Ratio DF/starch	Gidenne *et al.,* 2004a (F15)
Digestible fibre	Hemicelluloses (aNDF-ADF) + pectins[1]	Ratio DF/starch	Soler *et al.,* 2004 (S16)
Soluble fibre	TDF-aNDF with correction[2]	Source of pectins	Abad *et al.,* 2011 (S17)
Neutral detergent SF (NDSF)	Hall *et al.* 1997	Ratio NDSF/ADF	Gómez Conde *et al.,* 2007 and 2009 (S18)

[1]Pectin content of diets calculated on the base of ingredient composition and tables on chemical composition of raw materials. [2]Corrections for ash and protein contents of NDF residue.

On average, about 70% of the experimental diets was represented by dehydrated alfalfa meal, cereals (barley or wheat meal), sugar beet pulp and wheat bran (Table 3), as common in diets for rabbits (Maertens *et al.,* 2002). Soluble fibre levels were increased by increasing the inclusion levels of sugar beet pulp and, in few cases (Gidenne *et al.,* 2004b; Gomez-Conde *et al.,* 2007), of citrus and apple pulp (correlation between soluble fibre content and sugar beet pulp rate, r=0.70; P<0.001). Insoluble fibre came mainly from dehydrated alfalfa meal, and sometimes also from wheat straw.

Table 3: Mean ingredients composition and variability in 90 diets from the experiments listed in Table 2.

	Average	SD	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[1]	16.0	11.8	0	49.0
Wheat bran	14.3	9.81	0	35.2

[1]In two experiments, citrus and apple pulp were used (Gidenne *et al.,* 2004b; Gomez-Conde *et al.,* 2007).

Chemical composition was different among experimental diets: changes in crude protein contents of diets were in a narrow range (from 14 to 18%) and close to recommendations for growing rabbits (De Blas and Mateos, 2010); the contents of aNDF, ADF, lignin and starch were sometimes different from recommendations; the degree of lignification (ADL/aNDF x 100) varied between 8 and 15%. Soluble fibre ranged from 1.8 to 14.7%; it was not homogeneously measured in all experiments (Table 2): in most cases, soluble fibre was calculated as TDF-aNDF (this latter not always corrected for protein and ash contents) (Van Soest *et al.,* 1991); in some cases, soluble fibre corresponded to the pectin content of the ingredients estimated from the literature; only once (Gómez-Conde *et al.,* 2007), soluble fibre was measured as neutral detergent soluble fibre (Hall *et al.,* 1997).

Some 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 4). In particular, to evaluate performance of growing rabbits and the efficiency of the farm, the feed conversion ratio was selected; to evaluate consequences on the diet

Table 4: Mean values and variability of the chemical composition of diets (as-fed) and traits obtained with 90 diets from the 18 experiments listed in Table 2.

	N	Average	SD	Minimum	Maximum
aNDF, %	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
Soluble fibre[1], %	90	7.69	3.08	1.76	14.7
Hemicelluloses (aNDF-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
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
Faecal dry matter digestibility, %	62	62.7	5.35	49.7	75.8
Faecal aNDF digestibility, %	80	31.3	9.35	5.18	51.7
Faecal ADF digestibility, %	76	21.8	7.89	2.60	41.7
Faecal SF digestibility, %	62	92.2	10.9	73.0	131
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, %	77	16.3	13.4	0	47.2
Gut incidence[2], % SW	55	18.2	0.798	16.7	20.2
Dressing out percentage[3], % SW	55	60.4	0.767	58.2	61.9

[1]When the soluble fibre was not available as TDF-aNDF (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. [2]Full gut weight/slaughter weight x 100. [3]Cold carcass weight/slaughter weight x 100.

utilization, the faecal digestibility of dry matter and fibre fractions (NDF, ADF, soluble fibre) was used; to take into account the fermentation activity and digestive health of rabbits, data of caecal pH and total volatile fatty acids content were included as well as the mortality rate associated to each experimental dietary treatment. Finally, to get some information on the effects of dietary treatments at commercial slaughter, the incidence of gut and the dressing out percentage data were considered.

Digestible fibre *vs* soluble fibre

As a first step of our analyses of published studies, soluble fibre (TDF-aNDF) and digestible fibre (TDF-ADF) were used to calculate regressions to predict the traits above on the most homogeneous set of data, that is the 10 experiments performed at the University of Padova (Table 5).

Table 5: Explained variance (R^2) and root mean square errors (RMSE) of regressions[1] based on soluble fibre and digestible fibre in 55 diets from 10 experiments performed at the University of Padova

	Soluble fibre (TDF-aNDF)			Digestible fibre (TDF-ADF)		
Variable	R^2	RMSE	*Prob.* covariate	R^2	RMSE	*Prob.* covariate
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
Faecal dry matter digestibility, %	0.86	2.52	<0.001	0.66	3.91	<0.001
Faecal aNDF digestibility, %	0.89	3.53	<0.001	0.73	5.51	<0.001
Faecal ADF digestibility, %	0.88	3.34	<0.001	0.71	5.25	<0.001
Faecal SF digestibility, %	0.86	5.25	0.10	0.87	5.09	0.12
Caecal pH	0.74	0.11	0.04	0.71	0.11	0.02
Caecal volatile fatty acids, 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 incidence, %LW	0.87	0.37	0.11	0.86	0.37	0.09
Dressing out percentage, % LW	0.82	0.40	0.14	0.84	0.38	0.05

[1]Analysis of variance included experiment as a class, and soluble fibre or digestible fibre and their interaction with the experiment as covariates.

The use of soluble or “digestible fibre” contents to predict feed conversion, digestive utilization of diets, caecal fermentation activity and health of rabbits in the dataset based on experiments performed at the University of Padova is rather equivalent (Table 5), even if R^2 increases and the error decreases when using soluble fibre. In fact, the lower performance of regressions based on digestible fibre is explained by the low prediction weight of hemicelluloses. In our data set, hemicelluloses content included in “digestible fibre” also show a lower variability (coefficient of variation, CVR=13%) than pectin content (CVR=40%).

In rabbit nutrition, Gidenne (2003) firstly proposed to identify the soluble and less lignified insoluble fibre by the term “digestible fibre”, that is the sum of hemicelluloses (aNDF-ADF) and pectins (estimated from tables of raw material composition) and which may also be directly calculated as TDF-ADF. According to this definition, in diets for rabbits, hemicelluloses mostly contribute to digestible fibre, while cellulose is considered as low digestible/low fermentable fibre. However, the digestible cellulose may be higher than digestible hemicelluloses and the two contents may be not correlated (Figure 2.a) like in diets containing high levels of alfalfa and straw. Besides, the digestible hemicelluloses widely change with the lignification degree of insoluble fibre (Figure 2.b).

On the other hand, the correlation between measured digestible TDF and "digestible fibre" content is surely high when sugar beet pulp rate is increased in the diets (Figure 3). In this case, the contents and digestibility of hemicelluloses (coming mainly from wheat bran) are rather constant and the increasing level of pectin and the lower lignification of sugar beet pulp insoluble fibre account for the high relationship between digestible TDF and "digestible fibre" contents.

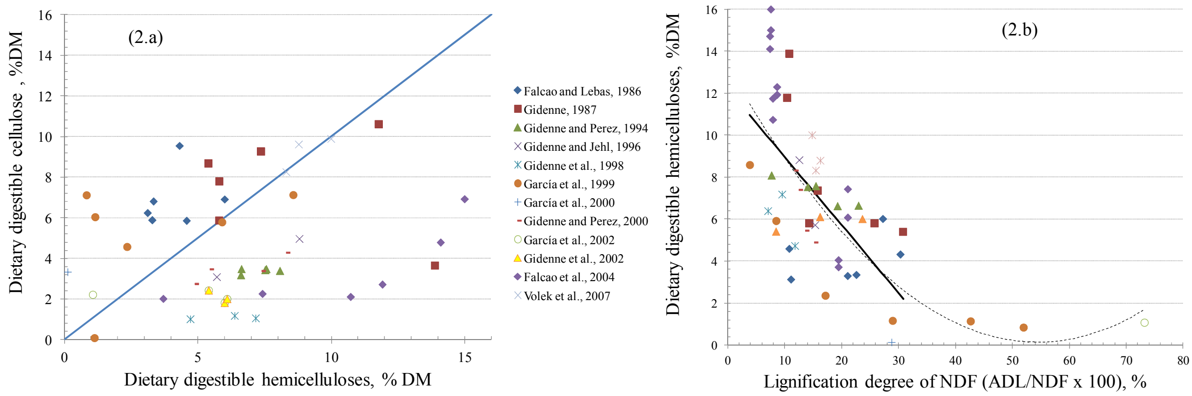

Figure 2: Relationships between the contents of dietary digestible hemicelluloses and celluloses (2.A) and between dietary NDF lignification degree and dietary digestible hemicelluloses content (2.B) in diets for rabbits.

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

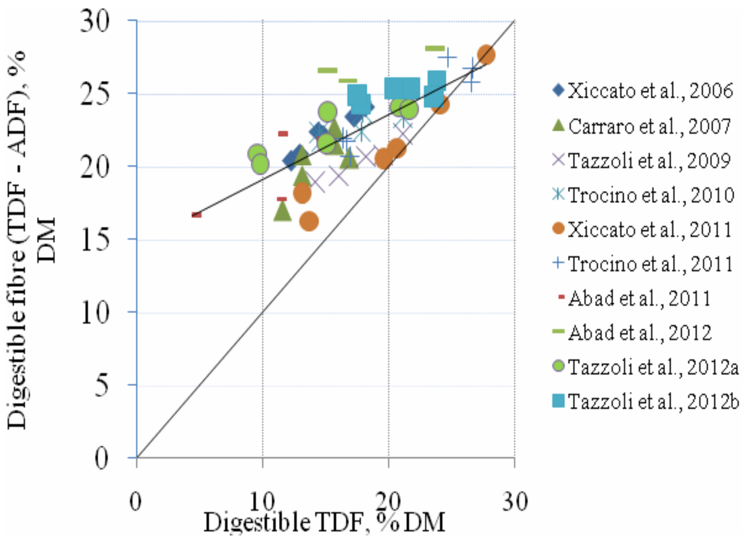

Figure 3: Relationship between digestible TDF and digestible fibre content

In the meta-analysis of the data of the 18 experiments, the ADF, soluble fibre, hemicelluloses, starch and crude protein contents of diets were selected out of all chemicals as independent variables due to their low variance inflation (<3) and, then, the best regressions on these terms were calculated according to Sauvant *et al.* (2008) (Table 6). Only the ADF and SF content entered into regression equations with a significant effect on the tested variables. The other nutrients (hemicelluloses, starch and crude protein) were not included in equations because of a lack of significant effect, low F value and small decrease in the error term when they were added to the first nutrient included in the regressions.

Table 6: Regression equations[1] calculated on the data set of the 18 experiments listed in Table 2

Variable	Equation	RMSE	*Prob. fixed effects*
Feed conversion ratio	1.42 + 0.089 ADF	0.003	<0.001
Digestible energy, MJ/kg	15.4 – 0.29 ADF	0.13	<0.001
Faecal dry matter digestibility, %	100 – 2.07 ADF	0.87	<0.001
Faecal aNDF digestibility, %	52.2 – 2.13 ADF + 2.17 SF	1.38	<0.001
Faecal ADF digestibility, %	36.0 – 1.70 ADF + 2.01 SF	1.29	<0.001
Caecal pH	6.23 - 0.042 SF	0.05	<0.01
Caecal volatile fatty acids, mmol/L	48.9 + 2.05 SF	2.41	<0.001
Mortality, %	43.7 – 5.86 SF + 0.245 SF^2	2.77	<0.001

[1]The mixed model included experiment and interaction experiment per nutrient as random effects and the nutrients as fixed factors (Sauvant *et al.,* 2008). Regressions with no significant effect were not presented in table (soluble fibre digestibility, gut incidence and dressing out percentage at commercial slaughter). ADF and SF are expressed on % as-fed.

The results of the meta-analysis and the weight of the different fibre fractions that entered in the equations are commented in the next paragraphs.

Feed conversion ratio and digestibility of dry matter and fibre fractions

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 (Table 6). Feed conversion ratio linearly impaired with increasing dietary ADF level. The slope coefficient for ADF (-0.29 on as-fed basis) in the equation for the prediction of digestible energy content was higher than that proposed by Villamide *et al.* (2009) (-0.19 ADF on DM basis). In fact, ADF is negatively correlated with the digestible energy content of diets and Xiccato and Trocino (2010) have calculated that, when the dietary content of DE decreases by 1 MJ/kg diet, feed conversion ratio impairs by 0.29 points as a consequence of the increased feed intake (+12 g/d).

The digestibility of aNDF and ADF significantly depended on both the content of ADF and soluble fibre, with a negative coefficient for the former and a positive coefficient for the latter (Table 6).

Basing on experiments in which TDF digestibility was available, the faecal digestibility of TDF linearly raised when the SF proportion on TDF increases in diets for rabbits (Figure 4.a), as a consequence of the high faecal digestibility of soluble fibre (73% to 100%; 90% on average; Figure 4.b) and of the low lignified insoluble fibre contained in sugar beet pulp (Martínez and Fernández, 1980; Gidenne, 1987; De Blas and Villamide, 1990; Carabaño *et al.,* 1997).

Moreover, faecal NDF and soluble fibre digestibility values were positively correlated when faecal soluble fibre digestibility was rather “low” (84% on average), while they did not vary in a consistent manner when soluble fibre digestibility was high (94% on average) (Figure 4.a), despite soluble fibre level increased. In fact, when data of all experiments were taken into account, the variability in the faecal digestibility of soluble fibre was not significantly explained by any of the nutrients tested in the regressions equations (Table 6).

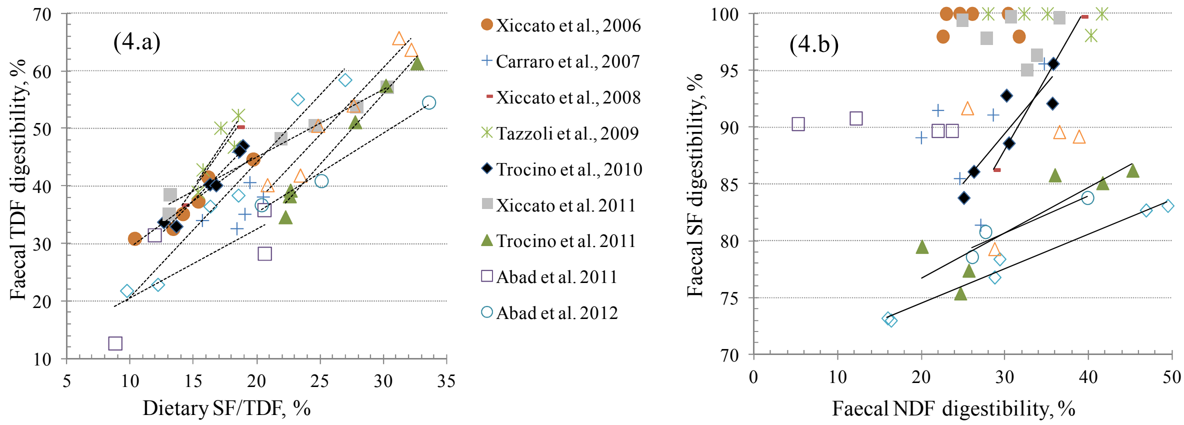

Figure 4: Effect of dietary soluble fibre (SF) proportion on total dietary fibre (TDF) digestibility (4.A) and relationship between SF and insoluble fibre (NDF) digestibility (4.B). Data refer to 11 experiments and a total of 62 diets.

We can get more information on the effect of soluble fibre level on digestive physiology of rabbits when distinguishing the ileal and caecal digestibility of fibre fractions, besides faecal digestibility. In fact, the increase of the soluble fibre proportion on TDF content improves the digestive utilization of TDF (expressed as grams of fermented TDF per 100 g diet) (Figure 5) and this result depends on the higher digestive utilization of both soluble fibre and insoluble fibre both at ileum and caecum (Abad *et al.*, 2011, 2012). Basing on the same data, moreover, TDF utilization is higher in the caecum compared with the ileum until SF/TDF ratio is 20% while it is similar in the two tracts at higher values (30%) (Figure 5).

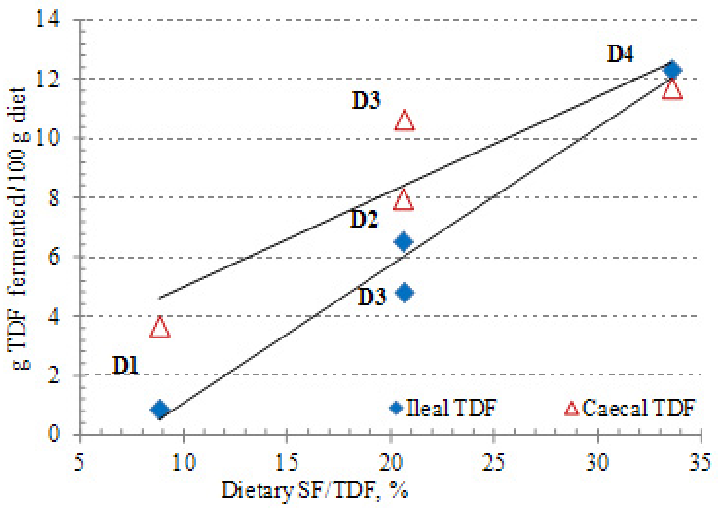

Figure 5: Ileal and caecal fermentation of total dietary fibre

Diets D1 and D2 from Abad *et al.* (2011), rabbits at 2.4 kg LW; D1, 100% of dietary NDF and TDF from sunflower hulls and straw; D2, 65% of NDF from sunflower hulls and straw and 35% from sugar beet pulp; Diets D3 and D4 from Abad *et al.* (2012), and with cannulated does; D3**,** 50% of NDF from oat and sunflower hulls and 50% from alfalfa, wheat and soybean meal. D4, 20% of NDF from oat and sunflower hulls, 30% sugar beet pulp and 50% from alfalfa, wheat and soybean meal

In diets varying in soluble fibre level and ingredient composition, ileal digestibility of soluble fibre may range from 10 to 65% (Abad *et al.*, 2011 and 2012). The mean value of ileal digestibility for soluble fibre (40%) measured by the same Authors is in accordance with previous data on ileal digestibility of arabinose and uronic acids (main components of pectins) (from 20 to 50%; 35% on average) (Gidenne, 1992; Carabaño *et al.*, 2001; Gidenne *et al.*, 2010a). The ileal digestibility of insoluble fibre is lower: in fact, the major monomers of fibre coming from cellulose and hemicelluloses (glucose and xylose) may be digested at ileum at rates ranging from 0 to 20% (Gidenne *et al.,* 2010b).

Therefore, the soluble fibre level may contribute to modify the faecal digestibility of insoluble fibre fractions (NDF and ADF) and affect the utilization of both soluble and insoluble fibre at ileum and caecum. As a consequence, the soluble fibre level is likely to affect ileal and, especially, caecal microbiota (Gómez-Conde *et al.,* 2007 and 2009) by modifying the amount and type of substrate reaching the caecum.

Caecal fermentation traits

The soluble fibre level affected the caecal fermentation traits which were included in the meta-analysis, that is caecal pH and caecal total volatile fatty acids concentration (Table 6 and Figure 6). The concentration of total caecal volatile fatty acids increased also with the increase of the dietary crude protein level (P<0.01), even if the inclusion of crude protein in the regression equation with SF fairly reduced the error term (RMSE=2.22) (data not presented in tables). As commented above, by modifying the amount and the type of substrate reaching the caecum, the soluble fibre level affects ileal and caecal microbiota composition (Gómez-Conde *et al.,* 2007 and 2009) and, thus, caecal fermentation activity. These changes in microbiota may be also related to the modified immune response observed in young rabbits fed soluble/insoluble fermentable fibre

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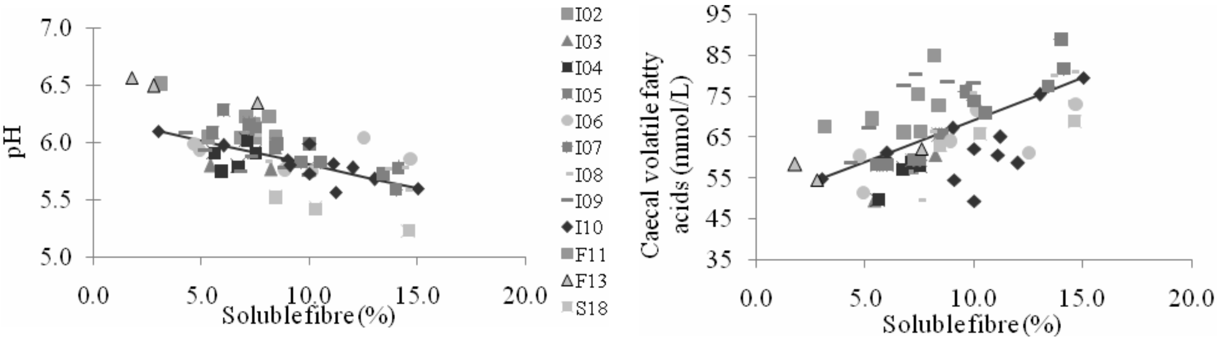

Figure 6: Effect of dietary soluble fibre level (% as fed) on caecal pH and caecal total volatile fatty acids

Whether this type of effect may be considered positively for rabbit health is not definitively stated. In fact, reviewing several studies, neither García *et al.* (2002) nor Gidenne *et al.* (2010b) found a constant and clear relationships between caecal pH and total volatile fatty acids and they did not identify a clear effect of caecal pH and total volatile fatty acids level on some pathogens (like *E. coli*) in rabbits.

The increase in dietary soluble fibre at the expenses of insoluble fibre or starch is known to promote caecal fermentations in fattening rabbits (Jehl and Gidenne, 1996; Falcão-e-Cunha *et al.,* 2004; Xiccato *et al.,* 2007, 2008 and 2011; Trocino *et al.,* 2011), whereas less agreement exists on the effects of soluble fibre on the proportion of caecal volatile fatty acids. In some studies, higher proportions of acetate and lower rates of butyrate were found in the caecum of rabbits fed diets with high levels of sugar beet pulp (Fraga *et al.,* 1991; Falcão-e-Cunha *et al.,* 2004; Gidenne *et al.,* 2004a; Xiccato *et al.,* 2011) which have been associated to a greater availability of substrate fermentable by fibrolitic 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 VFA profile did not vary (Carabaño *et al.,* 1997; Trocino *et al.,* 1999, 2010 and 2011). The contemporary variations in the insoluble fibre level and its degree of lignification might be the responsible for the different results among studies (García *et al.,* 2002) and need further studies.

Health status

The variability in the data of mortality associated to the experimental treatments significantly depended on soluble fibre both with a linear and quadratic component (Table 6), while other dietary characteristics (like ADF, starch or protein contents) did not enter into the equation. From the data set we used, a minimal mortality rate would be found at 12% soluble fibre (13% on DM basis). In fact, some studies have shown a decrease of rabbit mortality and morbidity caused by epizootic rabbit enteropathy and other digestive disorders by increasing the dietary concentration of soluble fibre in replacement of insoluble fibre (Gómez-Conde *et al.,* 2007; Xiccato *et al.,* 2007) or in replacement of starch (Jehl and Gidenne, 1996; Perez *et al.,* 2000; Soler *et al.,* 2004; Xiccato *et al.,* 2008 and 2011), but such a positive effect had been never so directly correlated to the level of soluble fibre *per se*.

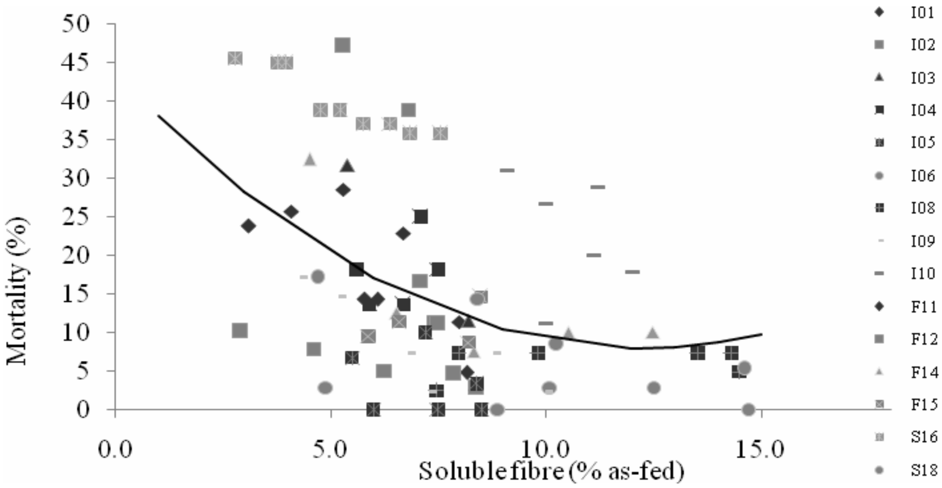

Figure 7: Effect of dietary soluble fibre level on mortality of growing rabbits

The reduction of the mortality rate may be associated with higher jejunal villi height/crypt depth ratio and disaccharidase activity in young rabbits (35 d) fed increasing levels of soluble fibre and insoluble fermentable fibre (Gómez-Conde *et al.,* 2007). In fact, higher villous height/crypt depth was recorded also in 35 d-old rabbits when a diet containing non fermentable insoluble fibre (wheat straw, sunflower hulls) was supplemented with soluble fibre (sugar beet pulp pectin) or partially substituted with insoluble fermentable fibre (insoluble sugar beet pulp fibre), or, especially, when substituted with sugar beet pulp as source of both soluble and insoluble fermentable fibre (El Abed *et al.,* 2011a). Soluble fibre and insoluble fermentable fibre from sugar beet pulp may also exert their positive effect on rabbit health after weaning by increasing the number of Goblet cells per villi and ileal viscosity (as a consequence of a higher mucin production) (El Abed *et al.,* 2011b). However, these changes in mucosa morphology might be age-dependent as they were not observed in older rabbits (51-56 d of age) (Trocino *et al.,* 2010 and 2011; Xiccato *et al.,* 2011).

Gut incidence and slaughter results

Several studies observed an increase in gut incidence when rabbits received diets containing high levels of soluble fibre (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, ADF) in diets used in those experiments might explain most of this variation in gut incidence (García *et al.,* 2002). Abad *et al.*

(2011), using iso-ADF diets, have attributed a special role in increasing weight of caecal content to the insoluble fibre of sugar beet pulp, which may reach the caecum and increase the amount of fibre here fermented. In contrast, also using iso-ADF diets, no real impact of sugar beet pulp inclusion on the filling of digestive organs was found by other authors (Jehl and Gidenne, 1996; Gidenne and Perez, 2000; Trocino *et al.,* 2010).

However, when using the whole data set, no nutrient was selected at a significant level (P<0.05) in equations to predict gut incidence and dressing out percentage at commercial slaughter (Table 6). The results of our analysis do not evidence a clear role of one fibre fraction compared to another because of large differences in final slaughter age and live weight at slaughter among animals of the different trial as well as different pre-slaughter treatments (starvation or not; the duration of transport and pre-slaughter wait, etc.) (Parigi Bini *et al.,* 1992; Dalle Zotte, 2000; Trocino *et al.,* 2003). These factors likely contributed to hide the effect of fibre on gut incidence and dressing out percentage at slaughter.

Effect of sugar beet pulp inclusion rate

When regressions were calculated using the sugar beet pulp inclusion rate of diets as fixed effect, digestible energy content of diets and feed conversion ratio were not significantly affected by the sugar beet pulp level, which indicates that the insoluble fibre coming from other ingredients also contributes to explain the variability of these data (Table 7). Faecal digestibility of soluble fibre, gut incidence and dressing percentage of animals at slaughter are not significantly affected by the sugar beet pulp level, as they have not been affected by the soluble fibre level. Differently, faecal digestibility of dry matter and insoluble fibre fractions increases with the inclusion level of sugar beet pulp, which is consistent with the above discussion on the effect of soluble fibre level on the digestive utilization of soluble and insoluble fibre fractions. Similarly, the effect of soluble fibre on caecal fermentation traits (pH and volatile fatty acids concentration) as well as on mortality is associated with changes in sugar beet pulp inclusion rate.

Table 7: Regression equations[1] calculated on data of a set of 90 diets from 18 experiments (SBP= sugar beet pulp inclusion rate)

Terms	Equations	RMSE	*Prob. fixed effect*
Faecal dry matter digestibility, %	57.99 + 0.303 SBP	1.09	<0.001
Faecal aNDF digestibility, %	22.82 + 0.303 SBP	1.54	<0.001
Faecal ADF digestibility, %	13.71 + 0.55 SBP	1.52	<0.001
Caecal pH	6.038 – 0.007 SBP	0.04	<0.001
Caecal volatile fatty acids, mmol/L	58.76 + 0.43 SBP	2.38	<0.001
Mortality, %	19.78 – 0.30 SBP	2.98	<0.01

[1]The mixed model included experiment and interaction experiment per nutrient as random effects and the nutrients as fixed factors (Sauvant *et al.,* 2008). Regressions with no significant effect were not presented in table (digestible energy, faecal soluble fibre digestibility, feed conversion ratio, gut incidence, dressing out percentage).

SOLUBLE FIBRE REQUIREMENTS

In view of preventing digestive troubles, fibre requirements cannot be only based on insoluble fibre but also on soluble fibre. According to INRA recommendations, the dietary supply of ”digestible fibre” must be considered in strict relation to dietary ADF and the ratio digestible fibre/ADF must not overcome 1.3 (Gidenne *et al.,* 2010b). This limit implies a maximum level of soluble fibre at 12-13%

soluble fibre (about 14% on DM basis) for a maximum soluble fibre/ADF ratio of 0.63, since INRA recommends also a minimum dietary supply of 19-17% ADF and 12-10% hemicelluloses (the higher values during post weaning and the lower ones during growth).

The Politechnical University of Madrid rather expresses recommendations in terms of dietary neutral detergent soluble fibre content around 12% (13% on DM basis) in diets fed during the post-weaning period, without limits during the later growth, once insoluble fibre (aNDF, ADF and ADL) requirements are satisfied and with no additional information for lower or upper limits (De Blas and Mateos, 2010). These latter recommended values are in agreement with the optimal soluble fibre level to minimise mortality (12%) we commented above, even if these results need to be confirmed with diets containing soluble fibre sources other than sugar beet pulp.

Finally, few experimental data are available with diets containing more than 12% SF and, therefore, more research would be useful to elucidate the response of rabbits to a higher dietary soluble fibre supply. It is worth to note, however, that the formulation of diets containing such high levels of soluble fibre would not be so easy taking into account that soluble fibre mainly comes from sugar beet pulp and that its soluble fibre content has been measured at 24.3% DM (Xiccato *et al.*, 2012).

CONCLUSIONS

Soluble fibre fractions may be measured and calculated by different methods and procedures. In this review, we referred to soluble fibre as the difference TDF-aNDF due to its simplicity to be obtained and the numerous studies that measured it. The increase of dietary soluble fibre has a positive effect on the reduction of mortality due to digestive diseases in growing rabbits affected by ERE and this result could be ascribed to the high fermentability of soluble fibre and the changes exerted on the intestinal microbiota and the enhanced gut barrier function. Insoluble fermentable fibre may partially share with soluble fibre the responsibility of these effects. A supply around 12% soluble fibre should be guaranteed in diets for post-weaning and growing rabbits containing about 30% aNDF and 18% ADF. These conclusions are linked to the use of sugar beet pulp as primary source of soluble fibre and should be confirmed with other soluble fibre sources.

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