The dietary fibres: definition and analysis in animal feeding, and their role in rabbit nutrition and health
Thierry Gidenne

To cite this version:
Thierry Gidenne. The dietary fibres: definition and analysis in animal feeding, and their role in rabbit nutrition and health. 19. ESCVN conference, Feb 2015, Toulouse, France. hal-02046864

HAL Id: hal-02046864
https://hal.science/hal-02046864
Submitted on 5 Jun 2020

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European Society of Veterinary and Comparative Nutrition

The dietary fibres: definition and analysis in animal feeding, and their role in rabbit nutrition and health.

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19th ESVCN congress
Toulouse : september 17-19

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- Context: fibres and digestive health in the growing rabbit
- Recalls on fibres : definition, structure, methods
- Recalls: effects of fibres on rabbit ingestion and digestion
- Fibres and digestive health of the growing rabbit
- Fibres requirements and European tables
- Conclusions & perspectives
1. Context

Digestive health in the growing rabbit

* Digestive troubles after weaning = 10 - 15% mortality
  => preventive antibiotherapy

* Digestive health / troubles = ??
  diarrhoea, caecal impaction, mucus +
  + intake troubles, weak growth, behaviour troubles (caecotrophy ...)

Origins:
- infectious: coccidiosis, colibacillosis, REE
- non-infectious risk factors:
  * stress, environnement (ventilation, heating),
  * unbalanced feeds

impact of nutrition on rabbit digestive health => YES
The rabbit is herbivorous: unsufficient fibres intake =>
higher RISK of digestive trouble after weaning

Main nutrients in a feed for a growing rabbit

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibres</td>
<td>39%</td>
</tr>
<tr>
<td>Sugars+oligo</td>
<td>5%</td>
</tr>
<tr>
<td>Starch</td>
<td>15%</td>
</tr>
<tr>
<td>Fats</td>
<td>3%</td>
</tr>
<tr>
<td>Proteins</td>
<td>17%</td>
</tr>
<tr>
<td>Ashes</td>
<td>10%</td>
</tr>
<tr>
<td>Water</td>
<td>11%</td>
</tr>
</tbody>
</table>

Fibres intake = 51 g / day
  + 11 g/d from soft faeces
Previous studies:
relationship among crude fibre and "mortality by diarrhoea"

Results: requirements in CRUDE FIBRE ??
very variable / unprecise ..... 6 to 14% !

Niehaus 1968 : 6 - 14 %
Morand Fehr 1970 : 12 - 17 %
Heckmann & Menner 1970 : 8 - 9 %
Martina 1974 : 6 - 8,4 %
Lebas 1975, 1984 : 13 - 14 %
Colin 1976 : 12 %
NRC 1977 : 10 - 12 %
Lang 1981 : 12-14 %
Maertens 1992 : > 14,5 %

How to improve the precision of these requirements?
Other criteria to be studied?

2. Recalls on fibres: origin, structure, methods

Standard plant cell wall
inside the plant cell = starch, lipids, proteins, oligonucleotides, sugars, ...

Schematic "3D" representation of the primary cell wall

A complex structure

Plant Cell Wall Structure

- Pectin
- Cross-Linking Glycan
- Cellulose Microfibrils
Simple CLASSIFICATION OF DIETARY FIBRES

- Polyphenolic compounds
- Lignins
- Cellulose
- Hemicelluloses
- Pectic Substances

Total Fibres
- Non Starch Polysaccharides
- = NSP

Water Insoluble Cell Wall
- Water-soluble Fibres
  - Pectines, arabinans, arabinogalactans
  - Hemicelluloses: arabinoxylans,
  - Beta-glucans (DP>60)

MAIN GRAVIMETRIC METHODS FOR DETERMINATION OF DIETARY FIBRES IN ANIMAL FEEDS

- Carré & Brillouet (1989)
- Van Soest (sequential) (1963-1991)
- Henneberg et Stohmann (1864)*

Classes of Polymers
- Lignins
- Cellulose
- Hemicelluloses
- Pectines (water insoluble)
- Water-soluble Fibres

NDSF = sum of pectines (water-solubles & insolubles) + β-glucanes + fructanes + oligosaccharides [DP>15]
Importance of the different fibres fractions in diet for growing rabbit

Interest of “Van-Soest” criteria:
allow to assess the quality of fibre
"routine" method

<table>
<thead>
<tr>
<th>Fibres</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignins</td>
<td>2-8%</td>
</tr>
<tr>
<td>Cellulose</td>
<td>10-20%</td>
</tr>
<tr>
<td>Hemicelluloses</td>
<td>7-15%</td>
</tr>
<tr>
<td>Pectins water-insoluble</td>
<td>4-10%</td>
</tr>
<tr>
<td>Water soluble fibres</td>
<td>≈ 2-6%</td>
</tr>
</tbody>
</table>

Not accounted with Van-Soest meth.

Fibres composition of some raw materials

- Alfalfa meal
- Wheat bran
- Beet pulp

- Pectines w-ins.
- Hemicel. 'VS'
- Cellulose 'VS'
- Lignins ADL
3. Fibres: effects on rabbit digestion

3.1 Microbial activity in the caecum

VFA concentrations: interaction Age and dietary fibre level

Fibrolytic activity of caecal bacteria according to dietary fibre level

(Fidenne et al., 2000)
Microbial activity and biomass production according to dietary fibre quality

**Diets**
- ADF = 20%
- Diet S: + 15% starch
- Diet DF: + 15% pectines + hemicell.

<table>
<thead>
<tr>
<th>S</th>
<th>Diets</th>
<th>D.F</th>
</tr>
</thead>
<tbody>
<tr>
<td>68 mM VFA</td>
<td>7.3 g DM/d. Biomass</td>
<td>13.3 g DM/d</td>
</tr>
</tbody>
</table>

Stimulation of the microbial activity with "digestible fibres"

### 3.2 Microbial community

**cellulolytic bacteria**

- According to the dietary fibre level
- Higher abundance of the cellulolytic bacteria with high fibre diet

Cultivation method
- Adult rabbit

Boualahrouf et al., 1991
Caecal microbiota according to the dietary fibre level

Diets:
- High fibre 19% ADF
- Fibre Deficient 9% ADF

**Bacteria “total”**
- **Archaea**
  - a
  - b
- **Bacteroides**
  - a
  - b
- **Fibrolytic bacteria**
  - NS

Molecular microbiology: 16S RNA probes

Age = 70d / adult rabbits

Bennegadi et al., 2003

Progress in caecal microbial ecosystem analysis

Diets:
- Control 19% ADF
- Fibre Deficient 9% ADF

Molecular microbiology: 16S RNA sequencing
- fingerprint “SSCP”
- + PCA analysis

Michelland et al., 2011

One point = one sample = 1 rabbit
Further progress in caecal microbiota characterisation

Molecular microbiology:
16S RNA high throughput sequencing
+ improved statistical analysis

3.2 Effect of fibre on digestion

<table>
<thead>
<tr>
<th>Class of dietary fibres</th>
<th>Mean, %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignins (ADL)</td>
<td>-10 + 15</td>
<td>-13 to +50</td>
</tr>
<tr>
<td>Cellulose (ADF – ADL)</td>
<td>15 - 18</td>
<td>5 to 40</td>
</tr>
<tr>
<td>Hemicelluloses (NDF – ADF)</td>
<td>25 - 35</td>
<td>10 to 60</td>
</tr>
<tr>
<td>Pectins (total uronic acids)</td>
<td>70 - 76</td>
<td>30 to 85</td>
</tr>
<tr>
<td>Soluble fibre (TDF-NDF)</td>
<td>70-90</td>
<td>??</td>
</tr>
</tbody>
</table>

Digestion of other nutrients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>90 - 100%</td>
</tr>
<tr>
<td>Proteins</td>
<td>70 - 75</td>
</tr>
<tr>
<td>Lipids</td>
<td>70 - 80</td>
</tr>
</tbody>
</table>

Conclusion:
fibres => reduction of diet digestibility
4. Fibres and digestive health of the growing rabbit

For a pure nutritionist, any reduction of fibre level will induce a reduction of nutritional costs

Thus: the BEST FIBRE LEVEL Should be THE LOWEST….?

BUT “breeders” and “pathologists” have observed:

low dietary fibres levels are associated with a higher risk of digestive troubles and mortality, after weaning

Thus, 2 questions:
1 - How to estimate the sanitary risk for digestive troubles?
2 - Which type of fibre to reduce the risk, and at which level?

4.1 Question 1: estimating the sanitary risk for digestive troubles, after weaning

Criteria to assess the sanitary status

Classical criterion = Mortality ➔ Yes/No criterion

Example:
fibre deficiency and digestive health (Bennegadi et al., 2001)

(N= 80 rabbits)
MORBIDITY =
- low growth (DWG +/- 2 S.D.), punctual loss of weight
- transitory diarrhoea
- intake troubles

Importance of the frequency of the measure
e.g. daily measure for Bennegadi et al. (2001).

Measure of the health status

17% mortality
36% morbidity

Example:
- fibre deficiency and digestive health
- (Bennegadi et al., 2000)

Health risk index, HRi
= 53%

IMPORTANT
necessary to measure those criteria on large groups of animal

10% mortality = ? 1 dead/10, very different from 100 deads/1000

NEED: large experimental design,
e.g. network of experimental units (french network GEC: 6 units)
4.2 Fibres and digestive health of the growing rabbit

**Question:** only one fibre criterion for mortality prediction?

Poorly digested fibres “ADF” and mortality from digestive troubles = ?

**Conclusions**

ADF alone is NOT sufficient to predict digestive health

Requirement: 14% to 22% ADF in diets ?

**Question:** effects of fibres quality

**Y = 1.49X² - 19.8X + 85.4**  
\[ R^2 = 0.77 \]

**Effects of lignins on rabbit digestive health??**

**Requirement:** minimum 5% ADL

**Beware:** ADL is not true lignin…

Overestimation in some cases (tannins -> grape marc, …)
Role of other fibre fractions?

- **Lignins**
- **Cellulose**

= NDF

= ADF

- **Hemicelluloses**
- **Water-insoluble Pectins WIP**
- **Water-soluble fibres**: pectins, β-glucanes, α-galactosides.

Not analysed with Van-Soest method

**Digestion**

- **Cellulose**
- **Lignins**

≈ ADF

- **NDF**

15-20 %

- **ADF**

25-40 %

- **Poorly digestible fibres**

40-70 %

- **Digestible fibres**

70-90 %

**Highly digestible, or rapidly fermentable fibres**

---

**Importance of digestible fibres “DgF”**

**Meta analysis**

6 studies, 31 diets

without antibiotics

Mortality rate is globally reduced with addition of digestible fibres (DgF, in iso-ADF diets)

Gidenne et al., 2013

Trocino et al., 2010

Soler et al., 2004

Xiccato et al., 2011

Xiccato et al., 2012

Tazzoli et al., 2009

Perez et al., 2000

---

Mortality, after weaning, %

0 5 10 15 20 25 30 35 40 45 50

10 15 20 25 30

dietary DgF concentration, % as fed

(for isoADF diets within a study)
Health risk and respective supply in: digestible fibres and lignocellulose (ratio "DgF/ADF").

\[ Y = 3.67e^{1.61X} \]

\[ R^2 = 0.69 \]

Conclusions
HRi depends from a balance between poorly digested (ADF) and highly digested (DgF) fibres

Requirement: DgF/ADF > 1.3

5 INRA studies 16 diets, without antibiotics

5. Fibre requirements & European tables of ingredients

Lignins + Cellulose

Poorly digestible fibres favourable for digestive health

Hemicelluloses + Pectines w-insol.

Digestible Fibres contribute favourably to health and growth

Balance the two "types" of fibres
Fibres requirements to prevent digestive troubles in the young and growing rabbit

3 key points

<table>
<thead>
<tr>
<th>Unit</th>
<th>Post weaning (28-42d old)</th>
<th>End of fattening (42-70d old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignocellulose &quot;ADFom&quot; *</td>
<td>≥190</td>
<td>≥170</td>
</tr>
<tr>
<td>Lignins &quot;ADL&quot; *</td>
<td>≥55</td>
<td>≥50</td>
</tr>
<tr>
<td>DgF/ADFom</td>
<td>≤1.3</td>
<td>≤1.3</td>
</tr>
<tr>
<td>Cellulose &quot;ADF-ADL&quot; *</td>
<td>&gt;130</td>
<td>≥110</td>
</tr>
<tr>
<td>Ratio Lignins/Cellulose</td>
<td>&gt;0.40</td>
<td>&gt;0.40</td>
</tr>
<tr>
<td>Hemicelluloses &quot;NDF-ADF&quot; *</td>
<td>&gt;120</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

### Table 4: Proximate fibre composition of some raw materials (90% DM) used in rabbit feeds, and with criteria used in fibre recommendations for the growing rabbit.

<table>
<thead>
<tr>
<th>g/kg on as fed basis</th>
<th>NDF</th>
<th>ADF</th>
<th>ADL</th>
<th>WIP</th>
<th>UAi</th>
<th>DgF</th>
<th>CF</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa meal 17</td>
<td>418</td>
<td>326</td>
<td>73</td>
<td>68</td>
<td>55</td>
<td>160</td>
<td>261</td>
<td>153</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>428</td>
<td>128</td>
<td>35</td>
<td>30</td>
<td>14</td>
<td>330</td>
<td>102</td>
<td>158</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>750</td>
<td>474</td>
<td>80</td>
<td>22</td>
<td>20</td>
<td>298</td>
<td>395</td>
<td>36</td>
</tr>
<tr>
<td>Sugarbeet pulp</td>
<td>428</td>
<td>212</td>
<td>78</td>
<td>250</td>
<td>190</td>
<td>466</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>Citrus pulp</td>
<td>220</td>
<td>155</td>
<td>16</td>
<td>120</td>
<td>80</td>
<td>185</td>
<td>83</td>
<td>59</td>
</tr>
<tr>
<td>Soya bean husks</td>
<td>588</td>
<td>426</td>
<td>21</td>
<td>92</td>
<td>60</td>
<td>254</td>
<td>85</td>
<td>122</td>
</tr>
<tr>
<td>Sunflower husks</td>
<td>693</td>
<td>562</td>
<td>202</td>
<td>100</td>
<td>75</td>
<td>231</td>
<td>68</td>
<td>54</td>
</tr>
<tr>
<td>Cocoa husk</td>
<td>390</td>
<td>300</td>
<td>140</td>
<td>30</td>
<td>20</td>
<td>120</td>
<td>83</td>
<td>164</td>
</tr>
<tr>
<td>Palm cake</td>
<td>520</td>
<td>317</td>
<td>90</td>
<td>30</td>
<td>10</td>
<td>233</td>
<td>50</td>
<td>185</td>
</tr>
<tr>
<td>Soyabean meal 48</td>
<td>104</td>
<td>65</td>
<td>5</td>
<td>55</td>
<td>24</td>
<td>94</td>
<td>50</td>
<td>468</td>
</tr>
</tbody>
</table>

*Whole seeds*

<table>
<thead>
<tr>
<th></th>
<th>NDF</th>
<th>ADF</th>
<th>ADL</th>
<th>WIP</th>
<th>UAi</th>
<th>DgF</th>
<th>CF</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya</td>
<td>117</td>
<td>73</td>
<td>8</td>
<td>60</td>
<td>25</td>
<td>104</td>
<td>56</td>
<td>369</td>
</tr>
<tr>
<td>Barley</td>
<td>175</td>
<td>55</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>126</td>
<td>46</td>
<td>108</td>
</tr>
<tr>
<td>Maize</td>
<td>100</td>
<td>25</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>82</td>
<td>19</td>
<td>92</td>
</tr>
</tbody>
</table>
6. Perspectives
further understanding of fibre effects on health?

Fibre – microbiota – immunity in the young rabbit

**Barrier role**
- Competition for adhesion to mucosa
- Competition for access to nutrients
- Production of antimicrobial substances

In the young rabbit:
* “feed” the microbiota (early during implantation)
* fibres? before weaning?

---

In the young rabbit:
* “feed” the microbiota (early during implantation)
* fibres? before weaning?

---

Rabbit research team

- 3 PhD students
  - Aïssa Benhouda
- Technical staff
  - Béatrice Gabinaud

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Thanks for your attention
Question 2: how to differentiate the effect of fibres from that of starch?

Answer 1:
factorial design $2 \times 2$ with: 2 starch (12% vs 19%) X 2 ADF (19% vs 16%) levels (4 diets)

Large scale study
n=507 rab./diet,
6 sites
weaning - slaughter

Effect of ADF: 7 vs 15%
P<0.05
Effect of starch = NS

Conclusions:
No effect of starch on mortality
ADF reduces the mortality

Answer 2:
isoADF diets but variations in starch quality: maize, wheat, barley, extruded maize

Diet with some "risks": ADF < 18% and starch ≈ 20%
n=446 rab./diet
5 sites
period: weaning - slaughter

Starch level at ileum (50d) % DM: 6b 5b 11a 2c

P=0.33 => NS

Conclusion: NO correlation among ileal starch and mortality,
NO starch overload

(Gidenne et al., 2005)