Stable C and N isotope analyses in muscle to discriminate dietary background in lambs

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Background

• Increasing interest in traceability
  – Need for markers in end-products that allow distinguishing and tracing back production and feeding systems
  – Plant biomarkers, indirect markers, physical markers…

• Various analytical and spectroscopic methods reported for this purpose
  – E.g. stable isotope ratios, NMR-techniques, chromatographic methods

Prache et al. (2005) Small Ruminant Research 59: 157
Franke et al. (2005) Eur Food Res Technol 221: 493
Stable isotope analyses

- Dietary isotope compositions influence isotope compositions in animal tissues → useful markers
  - Inference of C₃ and C₄ plant material in cattle diets based on large difference in δ¹³C value between C₃ (e.g. temperate grasses; Calvin cycle; mean δ¹³C value -27‰) and C₄ plant species (e.g. maize; Hatch-Slack cycle; mean δ¹³C value -13‰), e.g. beef : De Smet et al. (2004), Bahar et al. (2005)
  - Animal tissue δ¹⁵N less specific for dietary inputs and more dependent on metabolism, but may reflect presence of leguminous plants and application of N fertilisation, e.g. milk : Kornexl et al. (1997); beef : Schmidt et al. (2005)
  - Authentication of the geographic origin of lamb (Piasentier et al., 2003) and beef (Renou et al., 2004; Boner and Förstel, 2004; Schmidt et al., 2005), and production origin of Iberian pigs (Gonzalez-Martin et al., 1999, 2001)

Aim of this study

- Examine the potential of stable C and N isotope analyses in muscle for tracing back diets in lambs
  - Discriminate pasturing versus indoor feeding?
    ~ δ¹³C analyses
  - Discriminate organic versus conventional feeding?
    ~ δ¹⁵N analyses
Material and methods

• Trial 1
  – Pastured group (n=4, males): slaughtered at weaning, exclusively organic pasturing with ewe
  – Indoor fed groups (females) on hay/concentrate (50/50 DM) diet for 3 months

<table>
<thead>
<tr>
<th># lambs</th>
<th>Conventional feed</th>
<th>Organic feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% maize</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>18% maize</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

– *Longissimus*, plasma and feed samples

Material and methods (2)

• Trial 2
  – Three groups of lambs (each n=7) exclusively pastured for three months after weaning

  Botanically diverse: creeping bentgrass, soft brome, timothee ...

  Leguminosa rich: 40% white clover, 20% lucerne ...

  Intensive Ryegrass: 70% perennial ryegrass ...

– *Longissimus* samples at slaughter
Material and methods (3)

- Stable C and N isotope analyses by CF-IRMS

\[ \delta^{13}C \text{ or } \delta^{15}N (\text{‰}) = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \times 1000 \]

\[ R_{\text{sample}} = \frac{^{13}C / ^{12}C \text{ or } ^{15}N / ^{14}N}{R_{\text{standard}} = \text{international standard}} \]

δ\textsuperscript{13}C values (‰) (Trial 1)

Indoor fed hay / concentrate (50/50 DM)

- Plasma start
- Plasma month 1
- Plasma month 2
- Plasma month 3
- LT muscle
- Feed

18% maize 0% maize
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**δ¹³C values (‰)**

LT muscle (Trial 1)

- Pasture
- Hay/concentrate (18% maize)
- Hay/concentrate (0% maize)

**δ¹⁵N values (‰)**

(Trial 1)

- Plasma start
- Plasma month 1
- Plasma month 2
- Plasma month 3
- LT muscle
- Feed

Indoor fed hay / concentrate (50/50 DM)
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δ¹⁵N values (‰) LT muscle (Trial 1)

- pasturing
- organic feed
- conventional feed

Group discrimination (Trial 1)

Boner and Förstel (2004): "δ¹³C values below -20‰ threshold for organic farming" – questionable!
Stable isotope composition feeds

<table>
<thead>
<tr>
<th></th>
<th>δ^{13}C (‰)</th>
<th>δ^{15}N (‰)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Organic</td>
<td>Conventional</td>
</tr>
<tr>
<td>Maize</td>
<td>-12.0</td>
<td>-11.4</td>
</tr>
<tr>
<td>Wheat</td>
<td>-26.3</td>
<td>-26.6</td>
</tr>
<tr>
<td>Barley</td>
<td>-25.2</td>
<td>-27.6</td>
</tr>
<tr>
<td>Lucerne</td>
<td>-25.5</td>
<td>-28.6</td>
</tr>
<tr>
<td>Soybean</td>
<td></td>
<td>-25.4</td>
</tr>
<tr>
<td>Sunflower seeds</td>
<td></td>
<td>-26.0</td>
</tr>
<tr>
<td>Hay</td>
<td>-28.7</td>
<td>-29.3</td>
</tr>
</tbody>
</table>

Group discrimination (Trial 2)

95% correct classification after cross-validation
Conclusions

- Combined stable C and N isotope analyses in muscle or plasma did not allow discrimination between organic and conventional feeding.
- The use of concentrates versus pasturing and the type of pasture could be distinguished.
Thanks for your attention

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