Influence of diets of different abrasiveness on tooth wear, growth and shape in rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*)

Marcus Clauss¹, Jacqueline Müller¹, Daryl Codron¹, Ellen Schulz², Jürgen Hummel³, Mikael Fortelius⁴, Patrick Kircher⁵, Jean-Michel Hatt¹

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²Biocenter Grindel and Zoological Museum, University of Hamburg,  
³Department of Animal Sciences, University of Göttingen,  
⁴Department of Geosciences and Geography, University of Helsinki,  
⁵Division of Diagnostic Imaging, University of Zurich
Question

How does diet abrasiveness affect tooth wear (in terms of tissue loss, mesowear, 3D texture)?
Experimental work with goats:
- 4 groups of 7 animals
- kept for 9 months each on a specific diet
- CT scans at start, middle, end (measure mesowear and tissue loss)
- finally, teeth available for regular scoring incl. 3D texture analysis
- measuring abrasives in diet, digestive tract segments, faeces
Experimental work with goats:
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- measuring abrasives in diet, digestive tract segments, faeces

History

1. Research Grant of University Zurich
   - experiment initiated in 2011
   - candidate left after 5 months of experiment for permanent position
   - experiment had to be terminated after 6 months
   - lots of leftover food

2. Marie-Curie Fellowship
   - candidate could not start because offered a permanent position during evaluation stage
   - application handed in as first project of candidate’s permanent assignment, decision mid-June 2014

3. No grant but dedicated student
   - decision to add experiment with rabbits/guinea pigs
Doctoral student Jacqueline Müller
Growth and Wear of Incisor and Cheek Teeth in Domestic Rabbits (*Oryctolagus cuniculus*) Fed Diets of Different Abrasiveness

JACQUELINE MÜLLER¹, MARCUS CLAUSS¹, DARYL CODRON¹,², ELLEN SCHULZ³, JÜRGEN HUMMEL⁴, MIKAEL FORTELIUS⁵, PATRICK KIRCHER⁶, AND JEAN-MICHEL HATT¹

¹Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetuisse Faculty, University of Zurich, Zurich, Switzerland
²National Zoological Research, National Museum, Bloemfontein, South Africa
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⁴Department of Animal Sciences, Ruminant Nutrition, Georg-August University, Göttingen, Germany
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⁶Division of Animal Sciences, Vetuisse Faculty, University of Zurich, Zurich, Switzerland

Although patterns of tooth wear are crucial in palaeo-reconstructions, and dental wear abnormalities are important in veterinary medicine, experimental investigations on the relationship between diet abrasiveness and tooth wear are rare. Here, we investigated the effect of four different pelleted diets of increasing abrasiveness (due to both internal [phytoliths] and external abrasives [sand]) or whole grains hay fed for 2 weeks each in random order to 16 rabbits (*Oryctolagus cuniculus*) on incisor and premolar growth and wear, and incisor and cheek tooth length. Wear and tooth length differed between diets, with significant effects of both internal and external abrasives. While diet abrasiveness was linked to tooth length for all tooth positions, whole forage had an additional effect on upper incisor length only. Tooth growth was strongly related to tooth wear and differed correspondingly between diets and tooth positions. At 1.4–3.2 mm/week, the growth of cheek teeth measured in this study was higher than previously reported for rabbits. Dental abnormalities were most distinct on the diet with sand. This study demonstrates that concepts of constant tooth growth in rabbits requiring consistent wear are inappropriate, and that diet form (whole vs. pelleted) does not necessarily affect cheek teeth. Irrespective of the strong effect of external abrasives, internal abrasives have the potential to induce wear and hence exert selective pressure in evolution. Detailed differences in wear effects between tooth positions allow inferences about the mastication process. Elucidating feedback mechanisms that link growth to tooth-specific wear represents a promising area of future research. J. Exp. Zool. 321A:283–298, 2014. © 2014 Wiley Periodicals, Inc.

## Diet design

### Table 1. Composition of different complete pelleted diets (lucerne L, grass G, grass and rice hulls GR, grass and rice hulls and sands GRS) and grass hay (H).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>L</th>
<th>G</th>
<th>GR</th>
<th>GRS</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucerne meal (%)</td>
<td>60.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grass meal (%)</td>
<td></td>
<td>60.0</td>
<td>64.8</td>
<td>64.8</td>
<td></td>
</tr>
<tr>
<td>Rice hulls (%)</td>
<td></td>
<td></td>
<td>20.0</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Sand* (%)</td>
<td></td>
<td></td>
<td></td>
<td>5.0</td>
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<tr>
<td>Pure lignocellulose (%)</td>
<td>33.8</td>
<td>27.4</td>
<td>5.0</td>
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<tr>
<td>Soybean meal (%)</td>
<td></td>
<td>7.0</td>
<td>5.0</td>
<td>5.0</td>
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<tr>
<td>Molasses (%)</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
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<td></td>
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<tr>
<td>Lignobond (%)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
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<tr>
<td>Soy oil (%)</td>
<td>1.0</td>
<td>0.4</td>
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<tr>
<td>Mineral/vitamin premix (%)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
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<tr>
<td>Dry matter (% as fed)</td>
<td>91.4</td>
<td>91.9</td>
<td>91.8</td>
<td>92.2</td>
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### Nutrient composition (g/kg DM)

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<th>GR</th>
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<tr>
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<td>75</td>
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<tr>
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<tr>
<td>aNDFom b</td>
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<td>600</td>
<td>487</td>
<td>459</td>
<td>579</td>
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<tr>
<td>ADFom c</td>
<td>434</td>
<td>403</td>
<td>322</td>
<td>299</td>
<td>354</td>
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### Dry matter digestibility (%)

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<th>Diet</th>
<th>Dry matter digestibility (%)</th>
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<tbody>
<tr>
<td>L</td>
<td>39.7 ± 9.3</td>
</tr>
<tr>
<td>G</td>
<td>34.3 ± 8.1</td>
</tr>
<tr>
<td>GR</td>
<td>41.2 ± 5.7</td>
</tr>
<tr>
<td>GRS</td>
<td>40.7 ± 11.1</td>
</tr>
<tr>
<td>H</td>
<td>45.1 ± 4.1</td>
</tr>
</tbody>
</table>

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*aSand for playgrounds, grain size 0–1 mm, REDSUN garden products B.V., Heijen, Denmark; mean particle size measured by sieve analysis as dMEAN (Fritz et al., 2012) of 0.233 mm.*

*aNDFom neutral detergent fiber, determined using amylase and ash corrected.*

*ADFom acid detergent fiber, ash corrected.*

*ADL acid detergent lignin ash corrected.*

*ADIA acid detergent insoluble ash (a measure for abrasives).*
Diet design

### Table 1. Composition of different complete pelleted diets (lucerne L, grass G, grass and rice hulls GR, grass and rice hulls and sands GRS) and grass hay (H).

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<th>G</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Lucerne meal (%)</td>
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<tr>
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<td>—</td>
<td>64.8</td>
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<tr>
<td>Rice hulls (%)</td>
<td>—</td>
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<td>20.0</td>
<td>20.0</td>
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<tr>
<td>Sand (%)</td>
<td>—</td>
<td>—</td>
<td>5.0</td>
<td>5.0</td>
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<tr>
<td>Pure xylan cellulose (%)</td>
<td>33.8</td>
<td>27.4</td>
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<tr>
<td>Soybean meal (%)</td>
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<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
<td>Lignobond (%)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>—</td>
</tr>
<tr>
<td>Soy oil (%)</td>
<td>1.0</td>
<td>0.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mineral/vitamin premix (%)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
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<td>—</td>
</tr>
<tr>
<td>Dry matter (%) as fed</td>
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**Nutrient composition (g/kg DM)**

<table>
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<tr>
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<td>52</td>
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<tr>
<td>ADIA&lt;sup&gt;e&lt;/sup&gt;</td>
<td>05</td>
<td>16</td>
<td>24</td>
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<td>38</td>
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**Dry matter digestibility (%)**

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<tr>
<td>Molasses (%)</td>
<td>3.0</td>
<td>3.0</td>
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<tr>
<td>Lignobond (%)</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
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<tr>
<td>Soy oil (%)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Mineral/vitamin premix (%)</td>
<td>0.2</td>
<td>0.2</td>
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<tr>
<td>Dry matter (%) as fed</td>
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<td>91.4</td>
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<td>Nutrient composition (g/kg DM)</td>
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<td>05</td>
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<tr>
<td>Dry matter digestibility (%)</td>
<td>39.7±9.3</td>
<td>39.7±9.3</td>
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<td>39.7±9.3</td>
<td>39.7±9.3</td>
</tr>
</tbody>
</table>

Clear difference in abrasives - but no defined quantification of abrasive size - abrasives size not a factor in the experiments.
Method

16 animals, 5 diets, each diet fed for 2 weeks

burr marks on incisors and p3 (rabbits) – manual reading

measuring of food intake, faecal excretion, time required to eat 10 g (rabbits)
Experiment rabbits
Experiment guinea pigs
Method

CT scans after each diet period
Method

CT scans after each diet period

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general notes

May 1976

mean yearly total precipitation of 800 mm and experiences a mean monthly temperature of 13°C (81°F) from 1976.

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LITERATURE CITED


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Jenius E. Bennett, Jr., J. E. Smith, and J. H. C. Watson. Department of Biological Sciences, Purdue University, Lafayette, IN 47907 (present address: Bennett, Department of Physiology and Biophysics, UCSD, University of Washington, Seattle, WA 98195). Submitted 10 March 1975. Accepted 17 August 1975.

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A METHOD FOR DETERMINING GROWTH RATES IN CONTINUOUSLY GROWING MOLARS

Numerous herbivorous mammals have molars, continuously growing teeth, of which the best known example are the incisors of rodents. In a rodent's incisors, incremental growth have evolved in several mammalian orders (Maslow, 1974). The development of continuous growth in molar is
Method

CT scans after each diet period

(during final period, application of two fluorescence markers)

Preservation of teeth at the end of experiment for 3D texture

(additional experiment in rabbits: switchover from diet L to G and vice versa)
CT scans
CT scans
Histology (ongoing)
Food intake / Intake rate
Food intake / Body mass change
Food intake / Abrasives intake
Tooth growth compensates for wear; therefore we expect tooth length to be relatively constant across diets and growth tightly correlated with wear.

Nevertheless, differences in tooth length between diets, due to an incomplete compensation between growth and wear, can be detected.
Hypotheses II

Functional differences between incisors and cheek teeth lead to different wear and growth on different diets, i.e.

a) incisors are worn more heavily when feeding whole hay that needs more gnawing as compared to pellets;

b) cheek teeth, with a chewing action more independent from whether the diet is offered whole or pelleted, are worn more heavily with increasing dietary abrasiveness;

c) external abrasives (sand) lead to a gradient in wear along the maxillary cheek tooth row whereas increased internal abrasives (phytoliths in rice hulls) do not lead to such a gradient.
No hypothesis regarding differences between maxillary and mandibular teeth!
Incisor length

**Abrasives intake (g d⁻¹)**

- For L, G, GR, GRS, and H, the abrassives intake is shown, with L having the lowest and GRS having the highest intake.

**Total length (mm)**

- The total length for both upper and lower incisors is shown, with GRS having the longest total length compared to other groups.

- The data is represented graphically, with error bars indicating the standard deviation of each measurement.
Incisor length
Upper molar length

Abrasives intake (g d⁻¹)

L  G  GR  GRS  H

Abrasives intake (g d⁻¹)

L  G  GR  GRS  H

Total length (% on L)

L  G  GR  GRS  H

Total length (% on L)

P2  P3  P4  M1  M2

P4  M1  M2  M3
Upper/Lower molar length
Upper/lower incisor growth
Lower premolar growth

\[ y = 0.56 (0.44; 0.69) x + 0.82 (0.47; 1.17) \quad r^2 = 0.43 \]

\[ y = 0.60 (0.38; 0.83) x + 1.05 (0.51; 1.60) \quad r^2 = 0.37 \]

\[ y = 0.51 (0.40; 0.63) x + 1.02 (0.51; 1.52) \quad r^2 = 0.63 \]
## Absolute growth

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Diet</th>
<th>Upper incisor Growth</th>
<th>Lower incisor Growth</th>
<th>Lower cheek teeth Growth</th>
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<td>(Wolf and Kamphues, '95)</td>
<td>Tooth mark</td>
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<td></td>
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<td>G</td>
<td>1.53</td>
<td>1.31</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GR</td>
<td>1.57</td>
<td>1.34</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GRS</td>
<td>1.98</td>
<td>1.68</td>
<td>2.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H</td>
<td>2.39</td>
<td>2.52</td>
<td>2.42</td>
</tr>
</tbody>
</table>

$^1$: Tooth mark used for L, G, GR, GRS, and H.
Tooth growth compensates for wear on the basis of individual teeth; therefore we expect tooth length to be relatively constant across diets and growth tightly correlated with wear.

Nevertheless, differences in tooth length between diets, due to an incomplete compensation between growth and wear, can be detected.
Hypotheses I - confirmed

Tooth growth compensates for wear on the basis of individual teeth; therefore we expect tooth length to be relatively constant across diets and growth tightly correlated with wear.

Nevertheless, differences in tooth length between diets, due to an incomplete compensation between growth and wear, can be detected.

There must be a tooth-specific feedback mechanism probably using occlusion pressure as a feedback signal. This sensor remains to be identified.
Hypotheses II – mostly confirmed

Functional differences between incisors and cheek teeth lead to different wear and growth on different diets, i.e.

a) upper incisors are worn more heavily when feeding whole hay that needs more gnawing as compared to pellets;

b) cheek teeth, with a chewing action more independent from whether the diet is offered whole or pelleted, are worn more heavily with increasing dietary abrasiveness;

c) external abrasives (sand) lead to a gradient in wear along the maxillary cheek tooth row whereas increased internal abrasives (phytoliths in rice hulls) do not lead to such a gradient
Phytoliths abrade!
The cheek teeth gradient

Wear gradients on both intrinsic and extrinsic abrasives.
Different wear gradients (depending on diet) IN THE SAME INDIVIDUALS OF THE SAME SPECIES.

Wear gradients and functional grades in the diversification of the postcanine tooth row in mammalian dentitions

Wighart v. Koenigswald
Tooth wear in captive rhinoceroses (Diceros, Rhinoceros, Ceratotherium: Perissodactyla) differs from that of free-ranging conspecifics.

Lucy A. Taylor¹,², Dennis W.H. Müller³,⁴, Christoph Schütz⁵, Thomas M. Kaiser⁶, Daryl Codron⁷, Ellen Schulz⁸, Marcus Claus⁹,¹⁰

Contributions to Zoology, 83 (2) 107-117 (2014)
The cheek teeth gradient
Hypotheses III – ad hoc explanation

No hypothesis regarding differences between maxillary and mandibulary teeth!
Inverted pestle-and-mortar system:
Maxilla-Mandible-Difference

Inverted pestle-and-mortar system:
Maxilla-Mandible-Difference

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Maxilla-Mandible-Difference

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**Inverted pestle-and-mortar system:**
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Inverted pestle-and-mortar system:
Hypotheses IV

Abnormal tooth wear will occur more frequently with excessive external abrasives (sand), and affect the cheek teeth according to their position in the tooth row (anterior ones more affected).

If ‘bridge formation’ of the cheek teeth is caused by diets of low abrasiveness, the tooth angle of the cheek teeth should be flatter on low-abrasion diets and steeper on high-abrasion diets.
Abnormalities rabbit

Waviness

0 1 2 3

![Waviness images](image)

![Waviness score graph](graph)
Abnormalities rabbit

Tooth spurs

0 1 2 3

Graphs showing TSP score for different stages and locations.
Abnormalities rabbit

Tooth surface

-1 0 1
Abnormalities rabbit

Tooth angle

-1  0  1
Abnormalities guinea pig

http://www.ostseeschnuten.de/info/zahnprobleme.html
Abnormalities guinea pig

Bridge formation means a lesser angle of the tooth surface
Abnormalities guinea pig

bridge formation means a lesser angle of the tooth surface
Abnormalities guinea pig
Abnormalities guinea pig
more abrasive diet does not lead to a steeper angle
less abrasive diet does not lead to shallower angle

no indication of bridge formation
Malocclusion in inbred strain-2 weanling guineapigs

JOAN R. REST, TREVOR RICHARDS & SARAH E. BALL

Laboratory Animals (1982) 16, 84-87

The incidence of malocclusion was recorded for 4 years. The incidence was significantly reduced ($P > 0.001$) by breeding from animals without affected siblings: it is suggested that malocclusion in this colony has a genetic basis.
Aus dem Institut für Zuchthygiene der Universität Zürich
(Direktor: Prof. Dr. K. Zerobin)
Abteilung Zoo-Heim-Versuchstiere (Leiter: Dr. E. Isenbügel)

MALOÄKLUSION UND ZAHNÜBERWACHSTUM
Schädelmessungen bei
Cavia aperea f. porcellus Linnaeus, 1758

INAUGURAL—DISSERTATION
zur Erlangung der Doktorwürde
der Vet. Med. Fakultät der Universität Zürich

vorgelegt von
SILVIA STUDER
von Niederried/BE

Genehmigt auf Antrag von
Prof. Dr. H. U. Winzenried, Referent
Prof. Dr. J. Frewein, Korreferent

aku-Fotodruck
Zürich
1975
Breeding hygiene probably more important than the correct diet – because ‘good’ teeth will adapt to basically any situation.
3D texture analysis of different-diet teeth, incl. tooth row gradients and maxillary-mandible-gradient, and diet switch experiment.
Determine growth rates for all teeth via fluorescence microscopy.
Outlook III

Analyse diets in vitro (chewing machine)
Outlook IV

Evaluate goat CTs and teeth (mesowear, 3D texture, actual tissue loss)

Lucerne hay only (dicot)  Grass hay only (monocot)
Outlook IV

Evaluate goat CTs and teeth (mesowear, 3D texture, actual tissue loss)

dicot

monocot
Setup experiment with rabbits, dwarf goats & chewing machine.

More elaborate diets:

a) grass mowed daily – half fed fresh, half prepared as hay for other feeding period

b) standard diet with two kind of abrasives (phytolith-size; smaller)

c) how to manipulate ‘toughness’?
Something else

discuss this first