The extent and function of ‘food grinding’ in the laboratory mouse (Mus musculus)

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The extent and function of ‘food grinding’ in the laboratory mouse (Mus musculus)

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Short title: food grinding in laboratory mice
ABSTRACT

Many laboratory rodents grind their food into crumbs which are discarded at the bottom of the cage (sometimes called orts). This can have massive impacts on measures of food intake and assimilation efficiency. We quantified food grinding in two laboratory mouse strains on eight different diets and distinguished between two hypotheses of why food grinding occurs: a stereotypic behaviour due to a lack of environmental enrichment, or part of an optimal food intake strategy. Orts were quantified when mice were exposed to environmental enrichment and when offered diets of differing energetic quality. Grinding was significantly different between diets, but not between strains, although there was a significant diet by strain interaction. Ort production was lowest on the hardest diets. Not accounting for orts could affect food intake estimates by up to 31.8% and assimilation efficiency by up to 16.7%. Environmental enrichment increased physical activity, but did not reduce grinding. Mice selected the higher energy density components of the food. We suggest a refinement of the current methodology for measuring food intake is essential primarily because failure to take ort production into account created serious errors in estimates of food intake and assimilation efficiency in mice. Adding environmental enrichment is unlikely to reduce food grinding but careful choice of diet will reduce the errors.
Laboratory mice are usually offered food in a hopper so they must gnaw at food pellets through metal bars. Food is offered this way to reduce contamination by faeces and urine and for easy measurement of food intake; calculated as the mass of food missing from the hopper each day. As mice feed, large chunks of food occasionally fall through the bars, which are usually put back into the hopper by researchers prior to weighing the food to estimate intake. However, some mice also ‘grind’ food, resulting in a fine spillage discarded on the floor of the cage, sometimes called ‘orts’.

This term has been defined in the literature in different ways and in many cases the exact definition is not stated. Both captive and wild rodents have been reported to grind food. Koteja et al suggested that ‘food wastage should not be ignored without justification in calculations of food consumption’. An example of the importance of including orts in measurements of food intake comes from lines of mice selected for high and low food intake. Including or excluding orts can also impact on calculations of assimilation efficiency.

In a literature search using the search terms ‘food intake’ and ‘mouse’ or ‘rat’ in the publications database Web of Science, reveals more than 100,000 papers in the last 5 years alone. Out of 50 of these studies selected at random, only 5 mentioned correcting the intake for food grinding. This may be because ort production is negligible or it may be because some researchers are unaware of the extent of the potential problem with ignoring orts.

Two separate hypotheses for the behaviour have been suggested. First, food grinding in captivity may be a stereotypic or compulsive behaviour resulting from a lack of environmental enrichment. Normal behaviour patterns shown in wild mice, such as exploring and hiding may be lost in laboratory mice with no environmental enrichment. This
hypothesis predicts that grinding should be significantly reduced by providing mice with
enrichment. Also, grinding should be independent of the quality and availability of the diet.
Alternatively, optimal foraging theory predicts that ‘foragers should select a subset from the
set of potential food items, to maximise net energy intake per unit time spent foraging’\textsuperscript{15}. For
example, wild animals often discard the non-digestible parts of the food (such as seed
coatings) to select food of the highest energy density\textsuperscript{10,13,15}. For mice, it may be beneficial to
grind food to extract a more rewarding food component to maximise energy intake\textsuperscript{24}.
Therefore, as the heterogeneity of the diet increases and its quality decreases, it would be
predicted that mice grind more to select the more energetically profitable parts of the food.
Therefore, orts should have a lower energy content than the total diet as they should reflect
poorer components. The impact of greater selectivity should generate a positive correlation
between ort production and assimilation efficiency.

Koteja et al\textsuperscript{9} found that variation in grinding between individuals was high (from 2-40%),
but grinding was consistent within individuals. The trait was highly correlated in siblings
suggesting it has a genetic component. This suggestion is not independent of the two
hypotheses presented above as animals may genetically differ in their responses to lack of
enrichment and propensity to forage optimally. The hypothesis that individual variability in
grinding behaviour is genetic predicts consistency in grinding within individuals, independent
of the diet offered and the availability of the diet.

Food grinding may impact on measurements of food intake if it is not adequately accounted
for. We aim to evaluate the extent of the problem of ignoring ort production in food intake
measures and to point to conditions under which orts may be particularly significant. In the
context of “3Rs”, we aim to provide a refinement of the methodology for measuring food
intake. Food grinding was quantified in two laboratory mouse strains: one outbred: MF1 used extensively in studies of food intake during reproduction \(^1,2,5,26\) and one inbred: C57BL/6J, used extensively particularly in studies of obesity \(^27\), offered eight different commercially available pelleted rodent diets.

**ANIMALS, MATERIAL AND METHODS**

**Experiment 1**

Sixteen, 6 month old MF1 mice and fifteen, 3 month old C57BL/6J mice were used. All were female and individually housed in cages, dimensions 48cmx15cmx13cm (M3 base: North Kent Plastics, Kent, UK). Water was provided *ad libitum* and sawdust and shredded paper given as nesting material. Mice were housed at 20±2°C on a 12 hour light: 12 hour dark photoperiod at a relative humidity of 48±4%. Body mass and food intake were measured Monday to Friday at 09:00h (±0.01g; Sartorius top-pan balance). Food was provided *ad libitum* in a standard metal grid hopper; the bars of the hopper were on average 0.5cm apart.

Each diet was offered to 2 MF1 and 2 C57BL/6J mice for seven days and the diet order was randomised. Diets were changed every Friday after measurements so that mice had 2 full days acclimation to the new diet. The diets \((N = 8)\) were all pelleted (average length 2-3cm and diameter 1cm). First were the ‘Research Diets’ (Research Diets, Inc., New Brunswick, NJ, USA) diet-induced obesity ‘DIO series’ consisting of a low-fat control (D12450B: 10% kcal from fat), a medium fat (D12451: 45% kcal from fat) and a high-fat diet (D12492: 60% kcal from fat). The primary source of fat was lard. Also included was a custom-made high protein diet for use in macronutrient choice experiments (DX04080301). TestDiet\(^\text{®}\) (Richmond, IN, USA) created the TestDiet\(^\text{®}\) 21st Century Western Diet\(^\text{TM}\) Series. This consists of a low-fat control (5TJS: 12% kcal from fat), a medium fat (5TJN: 40% kcal from fat) and a high-fat diet (5TJP: 56% kcal from fat). These diets contained a variety of fats from
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different sources. Finally, a standard rodent chow was included (12% kcal from fat) (CRM(P) Special Diet Services; BP Nutrition Ltd, Essex, UK).

Any large pieces of uneaten food found in the cage (that had fallen through the hopper bars) were returned to the hopper, and the mass of food missing from the hopper was recorded. Each day the paper bedding was shaken out and the contents of the cage separated by hand into faeces and orts. Orts and a sample of each diet were dried at 60°C (Gallenkamp, Loughborough, UK) for a minimum of 14 days. Food intake was calculated as the amount of food removed from the hopper, corrected for hydration and dried orts. Ort production was expressed as the mean amount fragmented (g/mouse/day). The percentage of the food fragmented (PF) was calculated as: (ort production/food intake)*100%.

The hardness of each diet (Kg/mm$^2$) was measured using a digital micro-hardness tester fitted with a Vickers diamond indenter (Buehler, IL, USA). Five replicates of each diet were taken and a mean hardness value calculated for each. Two diets (5TJP and 5TJN) were unsuitable for testing because they were too soft and easily crumbled by hand. The hardness of the softest measureable diet (D12492) was 1.7Kg/mm$^2$. In the two cases where hardness could not be measured, it was set at the mid-point between 1.7 and 0Kg/mm$^2$.

**Experiment 2**

Fifteen, 4.5 month old female MF1 laboratory mice (*Mus musculus*) were used to explore the effects of diet quality, environmental enrichment and caloric restriction on food grinding. Housing and daily measurements were as detailed in experiment 1. Mice were sedated and implanted intraperitoneally with a wireless E-mitter (Model PDT-4000 E-Mitter, Mini-Mitter, Bend, OR, USA). Mice were sedated (isofluorane and oxygen mix) and maintained under...
anaesthesia throughout surgery via a nose tube. The chip (size: 23x8mm) was inserted intraperitoneally, and the wound sutured (Ethicon Vicryl; Dunlop’s Ltd, Dumfries, UK). The surgical procedure took 20-30 mins/mouse and they were monitored throughout the procedure. Mice were administered with an analgesic and left to recover on a heat pad for up to one hour. Mice were put back in their cage and monitored for wound healing but otherwise left alone for 4 weeks post-surgery. The implant recorded a series of activity counts each minute recorded by the Windows PC-based data acquisition system VitalView™ (Mini-Mitter, Bend, OR, USA).

Three independent experiments were carried out to determine if food grinding was affected by diet quality, environmental enrichment and caloric restriction. To test if ort production was affected by diet quality, mice were offered diets of three different energy densities consisting of standard pelleted rodent chow (Purina Mills chow #5001), with 0, 20, or 40% cellulose added by weight (Research Diets, Inc.). Diets were offered ad libitum in a hopper. Mice were offered the diets for 10 days in turn. To test if ort production was affected by the presence of environmental enrichment, on three separate days during exposure to 0 and 40% cellulose diets, enrichment (such as paper tubes and plastic houses) was added to the cages (Lillico Biotechnology, Surrey, UK). There were always at least 2 days without enrichment between the days enrichment was added. To test if ort production was affected by caloric restriction, mice were calorically restricted on the 20% cellulose diet for 10 days, during which they were offered 80% of their food intake during the ad libitum period. Each individual received each treatment (diet, enrichment and restriction), and the order of which was randomised across individuals.

Gross energy determinations and apparent energy assimilation efficiency (AEAE)
Faeces were collected from 8 individuals during experiment 2 after at least five days on each diet *ad libitum* feeding (0, 20, 40% cellulose). Orts were pooled from each individual, from each day on each diet. The gross energy (GE) content of the faeces, orts, diets and cellulose were measured using adiabatic bomb calorimetry (Parr, Moline, IL, USA). The apparent energy absorption efficiency (AEAE) was calculated as detailed previously, whereby both the actual GE, and the GE of only the ingested food were used in the calculation. These calculations were repeated using food intake with and without correction for orts.

**Statistics**

**Experiment 1**

A one-way analysis of variance (ANOVA) was used to compare ort production, PF, food intake and body mass between each diet for each strain. Food intake, strain, individual and diet were all analysed in a general linear model (GLM) to determine which significantly influenced ort production and PF, including body mass as a covariate. Interactions between all these factors were considered.

**Experiment 2**

The effects of diet quality (0%, 20% or 40% cellulose) on ort production and PF were analysed using one-way repeated measures ANOVA. GLM was used to first examine if ort production was influenced by food intake or diet, including body mass as a covariate and second to determine if ort production differed within individuals on the different quality diets. To examine the effects of environmental enrichment; mean food intake, ort production, percentage of the food fragmented (PF), body mass and activity were calculated for each mouse on three days: the day prior to, on the day of and the day following enrichment. One-way repeated measures analysis of variance (ANOVA) was used to find significant
differences between days for each parameter. Data for the 0% and 40% cellulose diets were  
analysed separately. A paired Student’s $t$ test was used to compare ort production and PF for  
the periods when the same mice were, and were not under caloric restriction on the 20%  
cellulose diet. One-way ANOVA was used to find differences in gross energy content for the  
diets, faeces and orts. Correlations between AEAE and the GE of the diets, also ort  
production and diet hardness were made using linear least-squares regression. To determine  
the primary composition of the orts, single-sample $t$ tests were used to compare the GE of  
orts produced on each diet (0, 20 and 40% cellulose) to a single mean GE value of the diets,  
cellulose and faeces measured by calorimetry. All statistical analysis was performed with  
MINITAB version 13.1 and significance values were taken as $P \leq 0.05$. Data are presented as  
the mean ± S.D.

RESULTS

Experiment 1

Mean ort production, food intake, percentage of the food fragmented (PF) and body mass for  
mice from each strain on each diet are shown in Table 1. MF1 mice were on average 41%  
heavier than C57BL/6J mice ($F_{(1,246)} = 1577.43$, $P < 0.001$). Food intake averaged 4.13g/day  
in MF1 and 2.92g/day in C57BL/6J which was a 29% difference ($F_{(1,246)} = 230.92$, $P <  
0.001$). Ort production across all diets averaged 1.10g per day in MF1 and 1.70g per day in  
C57BL/6J. Ort production was not significantly related to body mass ($F_{(1,229)} = 0.02$, $P =  
0.881$), or food intake ($F_{(1,229)} = 2.24$, $P = 0.136$), and did not differ between individuals  
($F_{(1,229)} = 0.00$, $P = 0.947$), or between strains ($F_{(1,229)} = 0.55$, $P = 0.461$). However, there was  
a significant difference in ort production between the diets ($F_{(7,229)} = 4.95$, $P < 0.001$) and a  
significant diet-by-strain interaction ($F_{(7,229)} = 2.77$, $P = 0.009$). Consequently, the way the  
two strains of mice reacted to the different diets was not the same. The diet that resulted in
the highest ort production averaged 4.30g/day for MF1 and 8.41g/day for C57BL/6 mice which was 13.3% and 43.3% of food intake respectively. Diets 5TJS and D12450B promoted no grinding in the MF1 (Figure 1a) and C57BL/6 strains (Figure 1b). The same significant effects were found when this analysis was repeated using the percentage of the food fragmented (PF). Ort production was averaged over the two strains on each diet. There was a significant negative correlation between ort production and diet hardness \( F_{(1,6)} = 7.63, P = 0.033 \) (Figure 2). The effect was non-linear and ort production in both strains increased enormously once the diet hardness fell below 3 kg/mm\(^2\).

**Experiment 2**

During the diet quality experiments, ort production was not significantly related to body mass \( F_{(1,40)} = 0.19, P = 0.663 \), or food intake \( F_{(1,40)} = 0.08, P = 0.785 \), but there was a significant dietary effect \( F_{(2,40)} = 4.81, P = 0.013 \). Ort production significantly increased as the proportion of cellulose in the diet increased \( F_{(2,42)} = 13.10, P < 0.001 \) and as hardness decreased \( F_{(2,12)} = 83.92, P < 0.001 \). The percentage of the food fragmented (PF) was 11.1, 12.9 and 31.8% on the 0, 20 and 40% cellulose diets respectively \( F_{(2,42)} = 22.99, P < 0.001 \).

When offered the three cellulose diets, ort production was significantly different between individuals \( F_{(1,384)} = 5.13, P = 0.024 \) and there was a significant individual-by-diet interaction \( F_{(2,384)} = 4.31, P = 0.014 \). This suggested that while individuals differed in the extent of grinding, these differences were not consistent across the different diets (Figure 3).

On the days that enrichment was added, activity levels increased by 5.8% and 5.5% for the MF1 and C57BL/6 strains respectively as compared to their average activity. Independent of the diet, activity levels significantly increased on the day enrichment was provided (0% cellulose diet: \( F_{(2,42)} = 8.67, P = 0.001 \), 40% cellulose diet: \( F_{(2,42)} = 9.04, P = 0.001 \) (Table 2).
However, there was no significant effect of environmental enrichment on ort production when compared with the day preceding or the day following enrichment for the 0% ($P = 0.197$) or 40% ($P = 0.315$) cellulose diets.

Average ort production on the 20% cellulose diet presented *ad libitum* was 1.10±0.71g/day and when the same animals were calorically restricted fell to zero ($t = 6.02$, $P < 0.001$). PF also significantly decreased from 13.0±5.8% to zero ($t = 8.69$, $P < 0.001$).

*Gross energy contents and apparent energy assimilation efficiency (AEAE)*

On all three diets of varying cellulose content, the GE of orts was significantly lower than the GE of the food (Table 3). Orts had significantly greater GE content than faeces and cellulose. This shows that the rejected portion of the diet was not pure cellulose and suggested that individuals were selecting higher energy density components of the food for ingestion.

Mean AEAE was 77.5±9.4, 68.9±6.1 and 45.4±8.8% for the 0, 20 and 40% cellulose diets respectively ($F_{(2,21)} = 31.70$, $P < 0.001$). Because the mice rejected the poorer constituents of the diets in the orts the mean GE of only the ingested food were estimated as 18.5 for 0% cellulose, 18.3 for 20% cellulose and 18.0kJ/g for 40% cellulose. This was an average increase of 1.23, 0.61 and 0.99% compared to the GE of the diets measured directly using calorimetry. Substituting the GE of the ingested food resulted in an average increase in the estimated energy intake of 0.25%, and a mean increase in AEAE of 0.28%. There was no significant correlation between AEAE (calculated using the GE of the diet or the ingested GE) and ort production on any diet. AEAE was calculated as 81.2, 70.9 and 62.6% for the 0, 20 and 40% cellulose diets using food intake not corrected for orts. This was an overestimation of 2.6, 1.9 and 16.7% respectively.
DISCUSSION

The magnitude of error induced by failing to take orts into account when calculating food intake and assimilation efficiency (AEAE) can be large. The present study suggests that the effect varies with diet and with mouse strain, but errors in food intake can be as large as 31.8%, and assimilation efficiency estimates can be in error by up to 16.7%. Peterson and Wunder found only 5 out of 30 studies accounted for orts in digestibility calculations. We also found that only 5 of 50 studies made reference to accounting for food grinding. It is unclear whether this failure to mention grinding is because researchers do not recognise its significance and do not account for it. However, our data suggest that on some diets, mice produce significant levels of orts. We therefore suggest that a refinement in experimental designs involving the measurement of food intake and assimilation efficiency in mice is required (by collecting orts) to gain accurate estimates of each.

Grinding was directly proportional to the hardness of the diet, as also reported by Ford. Rodent food manufacturers could strive to minimise the error in estimating food intake by making their pellets harder. Our data indicate that a target hardness of at least 2.5kg/mm² is desirable to minimise ort production. Currently, commercially available pellets containing high percentages of fat, or fat from a number of different sources tended to fall below this critical hardness and were ground more. Failure to account for grinding may seriously compromise studies examining the impact of high-fat diet feeding on food intake. The Research Diets 45% kcal from fat diet (D12451) was the only ‘high fat’ diet in our sample that combined a high level of fat with a hardness above this critical limit.
The difference between strains detected here supports the hypothesis that food grinding is heritable \(^9\). Recently, neuro-physiological signalling pathways involved in grinding behaviour have been examined, further suggestive of a genetic component to the behaviour \(^{28,29}\). The fact that grinding may have a significant genetic component is particularly important when considering studies which concern genetically manipulated mice, as observed by Hastings et al \(^17\).

The function of grinding: lack of enrichment or optimal foraging

It has been suggested that grinding may be a stereotypic or compulsive behaviour because of a lack of environmental enrichment \(^9\). In our experiments, physical activity was stimulated when enrichment was added, indicating mice were responding to the presence of enrichment. However, this did not reduce the level of ort production, as was also reported in laboratory rats \(^6\). In fact, the presence of some plastic enrichment items (igloos) resulted in additional grinding of the plastic. The present data suggest that grinding is not due to a lack of environmental enrichment and that adding enrichment to cages as a strategy to reducing grinding behaviour in mice is unlikely to be successful. In our experiments we only added enrichment for a single day and it might be argued that there would have been a more significant effect on grinding if it was in place for longer. However, this seems unlikely as the novelty aspect of enrichment would be lost over time and its capacity to distract the animals from grinding food probably also reduced.

Alternatively, grinding might be the consequence of an optimal food intake strategy. In support of this hypothesis, the energy content of the orts was lower than that of the food, suggesting mice were selecting the higher energy density parts of the pellets. Second, ort production fell to zero during caloric restriction when there was no advantage to be gained
from being selective. Third, the extent of grinding increased as the cellulose content of the food increased indicating mice were being more selective of the best parts of the diet when there was more incentive to do so. However, the energy density of the diet was correlated with hardness and since in experiment 1 we showed that hardness was a key feature influencing the level of grinding, this effect may have been a coincidence. Moreover, if grinding the food served to eliminate the poorest parts of the food, the impact would be an increase in the energy content of the ingested portion of the diet. The impact of selecting out the ort fraction on the energy content of the ingested food was however very minor. Koteja et al. also observed no impact of the extent of grinding on the AEAE. Our data suggest that grinding may be part of an optimisation strategy, but that its impact on gross energy intake and assimilation efficiency is only small because modern pelleted diets are extremely homogenous in their composition. Maintaining this homogeneity in chow manufacture may help to minimise the impact of grinding behaviour.

In conclusion, the present data suggest that food grinding by mice is not due to lack of environmental enrichment but may reflect an optimisation strategy. Grinding was dependent on diet hardness and was particularly high in high-fat diets. Moreover, the significant strain effect on levels of grinding supported the idea that grinding may have a genetic basis. Together, these effects mean that quantifying ort production is paramount in studies of different genotypes of mice and high-fat feeding. Failure to take ort production into account may cause serious errors in estimates of food intake and assimilation efficiency.

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Newcastle University for assistance with diet hardness measurements.

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Figure 1. Mean ort production for each mouse, on each diet in experiment 1. The MF1 strain is shown in panel A ($N = 16$) and the C57BL/6J strain in panel B ($N = 15$).

Figure 2. Ort production on each diet for mice from both strains combined in experiment 1 ($N = 31$ mice) against the hardness of the diet. Ort production decreased as the hardness of the diet increased.

Figure 3. Mean ort production for each mouse ($N = 15$) in experiment 2 on diets of three differing energetic qualities, with 0, 20 or 40% cellulose. Each mouse is shown to illustrate the significant individual variation.
Table 1 Average values of each parameter measured in experiment 1

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<th>Ort production/day (g)</th>
<th>Food intake/day (g)</th>
<th>PF (%)&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.088</td>
</tr>
</tbody>
</table>

<sup>a</sup> PF: percentage of the food fragmented
Table 2 Changes in each parameter over 3 days in experiment 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Difference from mean</th>
<th>0% added cellulose</th>
<th>40% added cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (calculated over 3 days)</td>
<td>Day prior</td>
<td>Day of enrichment</td>
</tr>
<tr>
<td>Ort production (g)</td>
<td>0.60±0.37</td>
<td>0.05±0.24</td>
<td>0.02±0.24</td>
</tr>
<tr>
<td>Food intake (g) (corrected for orts)</td>
<td>5.01±0.65</td>
<td>0.02±0.27</td>
<td>0.08±0.27</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>37.53±3.23</td>
<td>0.02±0.25</td>
<td>0.03±0.16</td>
</tr>
<tr>
<td>PF (%)a</td>
<td>9.96±5.16</td>
<td>0.94±1.67</td>
<td>−0.18±2.40</td>
</tr>
<tr>
<td>Activity (arbitrary units)</td>
<td>13.90±4.46</td>
<td>−0.38±1.09</td>
<td>0.80±0.86</td>
</tr>
</tbody>
</table>

|                                  | Mean (calculated over 3 days) | Day prior | Day of enrichment | Day after | F_{G×D} - value | P -value |
| Ort production (g)               | 4.23±3.99                | −0.64±2.89     | 0.50±1.95          | −0.07±0.40 | 1.19            | 0.315    |
| Food intake (g) (corrected for orts) | 7.09±1.16        | −0.05±1.30      | −0.17±0.70         | 0.20±0.68  | 0.62            | 0.543    |
| Body mass (g)                    | 36.13±2.78             | 0.03±0.20       | −0.05±0.16         | 0.03±0.14  | 0.94            | 0.398    |
| PF (%)a                          | 33.98±16.02            | 1.24±7.68       | 0.01±3.04          | 1.81±5.20  | 1.10            | 0.342    |
| Activity (arbitrary units)       | 12.05±3.30             | 0.36±0.84       | 0.65±0.84          | 0.42±0.63  | 9.04            | 0.001    |

* PF: percentage of the food fragmented
Table 3 Mean values of each parameter measured in the diet quality experiment

<table>
<thead>
<tr>
<th>Gross energy content (kJ/g)</th>
<th>0% cellulose</th>
<th>t - value</th>
<th>P - value</th>
<th>20% cellulose</th>
<th>t - value</th>
<th>P - value</th>
<th>40% cellulose</th>
<th>t - value</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orts</td>
<td>16.67±0.33</td>
<td></td>
<td></td>
<td>17.28±0.53</td>
<td></td>
<td></td>
<td>17.25±0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>18.29±0.09</td>
<td>-14.01</td>
<td>&lt;0.001</td>
<td>18.19±0.10</td>
<td>-4.89</td>
<td>0.002</td>
<td>17.81±0.12</td>
<td>-3.26</td>
<td>0.014</td>
</tr>
<tr>
<td>Faeces</td>
<td>16.05±0.43</td>
<td>5.39</td>
<td>0.001</td>
<td>16.55±0.20</td>
<td>3.94</td>
<td>0.006</td>
<td>16.70±0.16</td>
<td>3.25</td>
<td>0.014</td>
</tr>
<tr>
<td>Cellulose</td>
<td>16.67±0.05</td>
<td>0.02</td>
<td>0.983</td>
<td>16.67±0.05</td>
<td>3.27</td>
<td>0.014</td>
<td>16.67±0.05</td>
<td>3.39</td>
<td>0.012</td>
</tr>
</tbody>
</table>