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Impact of diet on faecal output and caecotroph consumption in rabbits

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1 Structured Summary

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5 Methods: Thirty-two Dutch rabbits were studied over 17 months. Faecal pellet size and 6 7 weight were measured in weeks 3, 9, 21 and 43 and faecal output in weeks 10, 22 and 45. 8 Number of uneaten caecotrophs was recorded weekly. 9 10 **Results:** Faecal pellets were consistently smaller and lighter in rabbits fed muesli only, and 11 the size of pellets produced by those fed muesli with hay decreased over the course of the 12 study. Faecal output was greatest in rabbits with the highest hay intake. Uneaten 13 caecotrophs were found in greatest frequency in rabbits fed muesli. 14 Clinical Significance: Muesli diets have a negative effect on faecal output and caecotroph 15 ingestion and may therefore predispose to digestive conditions. Higher hay intake is 16 associated with greater faecal output and less uneaten caecotrophs and may assist in preventing gastrointestinal stasis. 17 18 19 Keywords: Rabbit, Digestive, Gastrointestinal Stasis, Caecotrophs, Fly strike 20 21

Objectives: To assess the impact of four rabbit diets (hay only; extruded diet with hay;

muesli with hay; muesli only) on faecal pellet size, faecal output and caecotrophy.

22 Introduction

Whilst rabbits are popular pets (PFMA 2015; PDSA 2015), studies to determine their specific 23 24 nutritional requirements have not been conducted. Published nutritional guidelines (NRC 25 1977; FEDIAF 2013) are based on research conducted on laboratory and commercially farmed rabbits. Pet rabbits were traditionally fed muesli-type diets however the number fed 26 in this way swas reduced with more being fed hay, pelleted foods and vegetables (PDSA 27 2015). Muesli-type diets, especially if fed without hay, have been suggested to play a role in 28 29 a range of disease processes including gastrointestinal stasis (Mullan and Main 2006: Meredith 2012; RSPCA 2013), and previous studies have found that they are also 30 associated with obesity (Prebble et al. 2015a), dental disease (Meredith et al. 2015), and 31 32 behavioural changes (Prebble et al. 2015b).

33 Dietary fibre particles are separated in the proximal colon of the rabbit according to their size (Jilge 1982), with small particle (<0.3mm) retained in the caecum for microbial fermentation 34 (Gidenne 1993) and larger particles passed rapidly through the colon to be expelled in faecal 35 36 pellets. Larger fibre particles are necessary to maintain normal caecal-colic motility (Cheeke 1994). Increasing dietary fibre levels increases the rate of passage of ingesta (Hoover and 37 Heitmann 1972; Fraga et al. 1991; Gidenne 1992; Bellier and Gidenne 1996) conversely fine 38 39 grinding of fibre results in prolonged retention times and reduced food intake (Laplace et al. 1977; Fraga et al. 1991; Gidenne 1992). The caecal contents are expelled once or twice in a 40 41 24-hour period as mucus coated caecotrophs that are consumed intact directly from the 42 anus (Madsen 1939; Taylor 1939). Caecotrophy provides a source of B complex vitamins, 43 vitamin K and microbial protein (Kulwich et al. 1953; Hirakawa 2001). The amount of 44 caecotrophs consumed is influenced by diet; all are eaten when food is scarce, but when 45 food is provided ad libitum protein and fibre content affect consumption. Increased levels of dietary fibre increase consumption and high protein levels reduce consumption. Increased 46 dietary fibre content also reduces the protein and volatile fatty acid content of caecotrophs 47 (Carabaño et al. 1988). 48

49 Gastrointestinal (GI) disorders are common in pet rabbits (Varga 2014); Harcourt-Brown 50 2014)) and are often considered to be related to diet rather than enteric pathogens. GI stasis 51 is characterised by progressive hypomotility and eventual cessation of intestinal movement 52 with clinical signs including anorexia, reduced/absent faecal production and accumulation of 53 gas within the digestive tract (Harcourt-Brown 2002; Oglesbee and Jenkins 2012). Faecal 54 pellets gradually reduce in size and frequency of production and may also be misshapen 55 (Saunders and Rees Davies 2005). Pet rabbits also commonly present with uneaten 56 caecotroph accumulation around the anus (Saunders and Rees Davies 2005; Eatwell 2006; 57 Varga 2014; Harcourt-Brown 2014;) which predisposes to flystrike (Harcourt-Brown 2002; 58 Meredith 2014). Low fibre diets are frequently implicated in both GI stasis (Rees Davies and 59 Rees Davies 2003; Saunders & Rees Davies 2005; Varga 2014) and reduced caecotroph 60 consumption (Fekete and Bokori 1985).

A low fibre level alone may not cause GI stasis but is believed to act as a predisposing 61 factor; other factors such as stress, pain, infectious disease (e.g. coccidiosis) or medication 62 63 may be required to cause clinical disease (Harcourt-Brown 2002). Provision of high fibre diets to commercially farmed rabbits reduces the incidence of diarrhoea and associated 64 65 mortality rates (de Blas et al. 1986; Bennegadi et al. 2001; Blas et al. 2010) and may 66 therefore be providing a protective function and promoting health. Reduced faecal output 67 and faecal pellet size are often seen in the early stages of gastrointestinal stasis before 68 faecal output ceases completely (Oglesbee and Jenkins 2012). Wild rabbits (Oryctolagus cuniculus) generally produce between 300 and 500 pellets/24 hours (Lockley 1962; 69 Simonetti 1989; Gonzalez-Redondo 2009) although up to 820 have been reported (Taylor 70 and Williams 1956), and mean faecal pellet diameter of wild rabbits was 7.90±0.14 mm in 71 one study (Simonetti 1989). Diet may be responsible for these differences in numbers as, 72 although not studied in European rabbits (Oryctolagus cuniculus), higher fibre diets 73 significantly increase the number of faecal pellets produced by wild cottontails (Sylvilagus 74 75 floridanus) (Cochran and Stains 1961). Feeding muesli and/or a lack of hay may contribute

to gastrointestinal stasis and lack of caecotrophy in pet rabbits. This study aims to assess
the effect of feeding two concentrate types, extruded nugget (EH) and muesli (MH)
alongside ad lib hay with ad lib hay (HO) fed alone and ad lib muesli (MO) on faecal output,
faecal pellet size and the quantity of caecotrophs left uneaten.

80 Materials and methods

81 This study was conducted as a part of a long term study to assess the effect of diet on the 82 health and welfare of pet rabbits as previously described by Prebble & Meredith (2014), 83 Prebble et al. (2015a.b) and Meredith et al (2015). Study design and methodology was approved by the Ethical Review Committees of the Royal (Dick) School of Veterinary Studies 84 85 and the Food and Environment Research Agency (FERA). The rabbits were housed in a facility licensed by the Home Office; however, a project licence under the Animals (Scientific 86 87 Procedures) Act 1986 (ASPA) was not required for this study. The study was continually monitored by the FERA Ethics Committee and Home Office inspector throughout its 88 duration. 89

90 <u>Diets</u>

On arrival, 32 eight week old rabbits were housed in pairs and acclimatised over 40 days
(days –54 to –14) by maintaining their weaning diet of 50 g per rabbit of an extruded diet
(Burgess® Excel-Junior and Dwarf Rabbit; Burgess Pet Care, Thornton Le Dale, North
Yorkshire, UK) once a day plus ad lib Timothy Hay provided in wall-mounted hayracks.
Water was provided ad lib in 700 ml bottles. At Day –14, the rabbits were randomly allocated
to one of four diet groups each consisting of eight rabbits:

97 1. Hay only (HO) – ad lib supply of Timothy hay (n=8);

2. Extruded diet and hay (EH) – 50 g per rabbit Burgess Excel Adult Rabbit (Burgess Pet
Care, Thornton Le Dale, North Yorkshire, UK) with ad lib supply of hay (n=8);

100 3. Muesli and hay (MH) – 60 g per rabbit Russell Rabbit Complete Muesli (Supreme

101 Petfoods Limited, Ipswich, Suffolk, UK) with ad lib hay (n=8);

4. Muesli only (MO) – ad lib supply (125 g per rabbit) of Russell Rabbit Complete Muesli

103 (Supreme Petfoods Limited, Ipswich, Suffolk, UK) (n=8).

The rabbits were gradually transitioned on to the new diet over a 2-week period (Day –14 to 0) and remained on that diet for 17 months. All concentrates were weighed out daily to ensure accurate and consistent weights of food were offered. Food remaining after 24 hours was weighed to determine food consumed. The nutritional composition of the four diets

offered is detailed in Table 1. Hay intake and selective feeding of muesli were recorded in
months 3, 6 and 12 as reported by Prebble & Meredith (2014).

A rabbit in the EH group died suddenly on day 209, but the cause of death was not evident on post-mortem examination. A rabbit in the MO group was removed at month 7 following the development of clinical dental disease. Data from these two rabbits were not included in

113 any subsequent analyses.

114

115 Data collection

Faecal samples were collected from individual rabbits in weeks 3, 9, 21 and 43. The rabbits were separated for up to three hours in the light period using a Perspex divider within their home pen. Fifteen (or the total number produced if less than 15) of these faecal pellets were then randomly selected and weighed using digital scales (Fisherbrand DP 300) and a mean faecal pellet weight calculated. The size of the faecal pellet was measured using digital callipers; as the faecal pellets are not spherical or symmetrical the longest diameter was measured.

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Following the observation that longer periods of separation (>3 hrs) were required in the MO group in order to get sufficient quantities of faecal pellets, total faecal output in all groups was measured in weeks 10, 22 and 45. Bedding was removed from the pens for a 24 hour period and all faeces passed during this period collected and weighed. A sample of 20
pellets was weighed and the mean faecal pellet weight calculated. The total weight of faecal
pellets produced was then divided by the mean faecal pellet weight to give an indication
of the number of pellets produced.

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A count of caecotrophs found in the pens during weekly cleaning out was done by the sameresearcher on 25 of the weeks during the period between week 4 and week 31.

Caecotrophs were not measured in weeks 7 and 12 as the researcher was unable to bepresent during cleaning out.

136 Statistical Analysis

Data were analysed using Minitab (v16.1.1 © 2010 Minitab Inc.) and R software (v2.15.1 © 137 2012 The R Foundation for Statistical Computing). For most of the measurements, data from 138 139 each time point were analysed using standard analysis of variance (ANOVA) also taking the sex of the rabbit into account, using standard Tukey's post-hoc tests to assess pair-wise 140 141 differences between the groups where overall differences were obtained. Residuals were examined for adequate normality before analysis. A Pearson product moment correlation 142 143 was carried out to assess the relationship between rabbit weight, faecal pellet weight and faecal pellet diameter and also between faecal weight and number of faecal pellets. P<0.05 144 was taken to indicate the statistical significance throughout. The number of caecotrophs 145 produced from particular time points were analysed with General linear models with Poisson 146 errors were used to incorporate the integer nature of the caecotroph data with pairwise post-147 hoc Tukey analysis carried out if there were overall differences. 148

150 <u>Results</u>

151 Faecal pellet weight and diameter

152 Faecal pellets were consistently lighter and smaller in rabbits fed muesli only, and increased 153 hay intake was associated with increased faecal pellet weight and size. There was a 154 statistically significant positive linear relationship between hay intake and both faecal pellet weight ($t_{1,30}$ =4.96, P<0.001, R²=45.0) and diameter ($t_{1,30}$ =4.25, P<0.001, R²=37.6, figure 1). 155 156 Faecal pellet weight was positively correlated with faecal pellet diameter at all timepoints (r>0.635, P<0.001). Rabbits fed muesli had the greatest reduction in faecal pellet size over 157 158 the course of the study. Significant changes in mean faecal pellet weight and diameter 159 occurred over the duration of the study (F3,25=3.24, P=0.039, Table 2), but this was most noticeable in the MH group (62% reduction in faecal pellet weight and 32% reduction in 160 diameter) and MO groups (47% reduction in faecal pellet weight and 14% reduction in 161 162 diameter). In contrast, smaller changes were observed in the HO (38% reduction in faecal pellet weight and 4% increase in diameter) and EH (26% reduction in faecal pellet weight 163 and 3% reduction in diameter) (Figures 1 and 2) groups. 164

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Significant differences were present in both faecal pellet weight and diameter at each time
point (F3,25=29.92, P<0.017, Figure 2). In week three faecal pellets produced by the HO
group were 26% heavier than those in the EH group and 23% heavier than the MH group,
while the MO group produced pellets that were 21% smaller than the MH group (P<0.028).
The EH and MH groups were not different from each other (P>0.127).

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In week 9 faecal pellets produced by the HO group ($0.34g\pm0.09$, $10.53mm\pm1.53$), continued to be significantly heavier and larger than all other groups (P<0.009) while those produced by the MO group (0.14 ± 0.05 , $8.52mm\pm0.85$) were both smaller (P<0.002) and lighter (P<0.035). There remained no difference between the EH ($0.2g\pm0.05$, $9.72mm\pm0.87$) and MH (0.23 ± 0.03 , $9.82mm\pm0.71$) groups (P=0.920).

- 177 The faecal pellets in the MH group reduced in size more than those produced by other
- groups and by week 21 they were 29% smaller than those produced by the EH group
- 179 (P=0.011) and by week 43 pellets produced by the MH group were 47% smaller than the EH
- group and were similar in size to those produced by MO group (P=0.90, Figure 3).
- 181

182 <u>Total faecal output</u>

- 183 Increased hay intake was associated with increased number of faecal pellets, with a positive
- linear relationship between hay intake and both the weight of faeces produced ($t_{1,13}$ =3.45,

185 P<0.004, R²=47.7) and the number of faecal pellets ($t_{1,13}$ =3.11, P<0.008,

- 186 R2=42.6).Significant differences in the number of pellets produced over a 24 hour period
- were present between groups at all time points ($F_{3,11}$ =18.89, P<0.003, Table 3).
- 188 The HO group produced 46 62% more faecal pellets over a 24-hour period than those in
- the MO group (P<0.001), 24-29% more than the EH group (P<0.07) and 32-43% more than
- the MH group (P<0.02). The EH produced (12- 25%) more faecal pellets than the MH group
- 191 although this difference was not significant (P>0.06). Likewise there was no difference in the
- number of faecal pellets produced by the MH and MO groups (P>0.13, Figure 4).
- 193
- 194 In week 52 a single rabbit in the MO group had signs of gastrointestinal stasis including
- 195 reduced food intake, hunched posture, tooth grinding, reduced faecal pellet production and

altered behaviour and was removed from the study for treatment.

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198 <u>Caecotrophs</u>

199 Uneaten caecotrophs were found in greatest frequency in rabbits fed muesli. No

200 caecotrophs were found in pens in the HO group for 11 of the 25 weeks where counts were

- 201 performed, and the caecotrophs found in the HO group in the remaining 14 weeks were only
- from one pen. Analysis was therefore only performed on the three remaining groups.
- 203 Overall, significant differences between groups were seen throughout the trial ($\chi^2_{3,15}$ =5.35,
- P<0.001, Figure 5). The MO group left more caecotrophs (18±2) than the EH (4±3) and MH

205 (7 ± 1) in week four (P<0.001), whilst no difference was present between the EH and MH 206 groups (P=0.273). However, by the end of the trial the reduction in the number of 207 caecotrophs left by the EH group (1±1) was significantly less than those left by the MH group 208 (5±2, P<0.02). While the number of caecotrophs left by the MO group had fallen (14±3) this 209 was still significantly more than all other groups (P<0.001).

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- 211

212 Discussion

213 This study demonstrates that diet has a statistically significant impact on faecal pellet weight 214 and size, total faecal output and caecotrophy. Significantly heavier and larger faecal pellets were produced by those rabbits consuming hay, in contrast to the significantly smaller, 215 lighter pellets produced by the MO group, which had no access to hay. Faecal pellets 216 217 produced by the MO group were similar in appearance to the small irregular pellets observed in the early stages of GI stasis (Lord 2012, Harcourt-Brown 2014). Although the mean faecal 218 diameter in the MO group was comparable with that reported in wild rabbits of 7.90mm, the 219 220 range included pellets as small as 5.80mm (Figure 3). As low fibre levels are frequently 221 implicated in GI stasis in pet rabbits (Varga 2014; Rees Davies and Rees Davies 2003; Lord 2012) the finding that low hay diets are associated with reduced faecal pellet diameter and 222 faecal output is of clinical relevance. 223

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225 The smaller faecal pellets produced by the MO group throughout the study and the MH group towards the end of the study are similar in size appearance to those observed by the 226 authors and described by Harcourt-Brown (2014) in early clinical cases of gastrointestinal 227 stasis. However, the low occurrence of GI stasis (one rabbit) in this study supports the 228 229 assertion that low fibre diets are only one contributing factor in the development of GI stasis (Harcourt- Brown 2002) and that low fibre diets are not directly causative. Dietary fibre has a 230 protective effect on the morbidity and mortality from digestive diseases observed in farmed 231 232 rabbits and at weaning (Bennegadi et al. 2001; Gidenne et al. 2001) this may also be true for 233 GI stasis. This protective effect may be a result of the effect of dietary fibre on volatile fatty 234 acid (VFA) production. Rabbits fed high fibre, low starch diets produce less VFA's than those 235 on low fibre, high starch diets (Carabaño et al, 1988; Gidenne et al, 2004). This would 236 suggest that rabbits fed the low fibre, high starch muesli diet would have higher VFA levels 237 which have been associated with reduced colon motility in in vitro studies (Squires et al. 238 1992; Dass et al. 2007). This reduction in motility may make rabbits fed on a muesli only 239 diet more susceptible to the development of GI stasis if other factors associated with 240 reduced motility such as pain or stress (Harcourt-Brown, 2014) are present.

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242 Faecal output over a 24 hour period was greater in rabbits consuming more hay (hay intake reported in Prebble and Meredith (2014)). As large fibre particles pass rapidly through the 243 colon and are excreted in faecal pellets (Jilge 1982; Carabaño et al. 1988), the differences in 244 245 hay (and therefore fibre) intake between the groups can explain these difference in faecal output. The number of faecal pellets produced by the EH and MH groups fall within the lower 246 end of the range (300-500 daily) generally reported in wild rabbits (Lockley 1962; Simonetti 247 248 1989; Gonzalez-Redondo 2009). The HO groups produced numbers closer to, or exceeding, 249 the top end of this range, but did not reach levels in wild rabbits reported by Taylor and 250 Williams (1956). The MO group had considerably lower faecal output, similar to the findings 251 of Arnold and Reynolds (1943) in Arizona and Antelope jack rabbits fed a highly digestible mash, which produced half as much as those fed grass. In addition, differences in faecal 252 253 pellet colour were observed between groups; the HO group produced faecal pellets that were lighter in colour, whilst those produced in the MO group were darker. The faecal pellets 254 produced by the EH and HO groups were similar in colour. Objective quantification of these 255 differences, for example using colorimetry, was outside the scope of this project. 256

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Uneaten caecotrophs were regularly found in all groups fed concentrates but infrequently in the HO group. Highest numbers were found in the MO group (up to 30), whilst the MH (up to 10) and EH (up to 5) groups that consumed hay left less caecotrophs, supporting findings 261 that caecotroph consumption is associated with forage based (Clauss et al. 2012) and high fibre, low protein diets (Fekete and Bokori 1985). Caecotrophy contributes to protein, energy 262 263 and vitamin requirements (Kulwich et al. 1953; Fraga et al. 1991; Kerti et al. 2005), and the 264 higher protein and energy content of concentrates allows nutritional requirements to be met 265 solely from the diet, resulting in increased numbers of uneaten caecotrophs. Other factors 266 implicated in reduced caecotroph consumption include obesity, perineal dermatitis, dental 267 and musculoskeletal diseases (Harcourt-Brown 2014). Obesity was observed in the MO group (Prebble et al. 2015), but was not sufficient to prevent grooming (observed frequently 268 269 by the researcher) and should not have impaired caecotrophy. No other conditions were 270 present in the rabbits before or during data collection. In the absence of other contributory factors, the differences in uneaten caecotrophs within the pens could be attributed to dietary 271 272 factors alone. The cause of the reduction in uneaten caecotrophs in the MO group by the 273 end of the trial is not known, but possibilities include an age-related effect, or changes in dry matter (DM) or water intake and would require further investigation... 274

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276 Smaller faecal pellet size and weight, lower total faecal output and greater number of 277 caecotrophs not consumed may indicate that muesli based diets contribute to the development of digestive conditions. In particular, feeding muesli in the absence of hay 278 cannot be recommended. Further study is required to assess the significance of diet in the 279 development of gastrointestinal stasis and the interaction of other factors. A higher fibre diet 280 281 promotes a larger faecal pellet size and weight and higher total faecal output, therefore feeding hay alone or alongside concentrate diets is recommended for pet rabbits and may 282 be beneficial in preventing protective against GI stasis. 283

284

286 <u>Acknowledgements</u>

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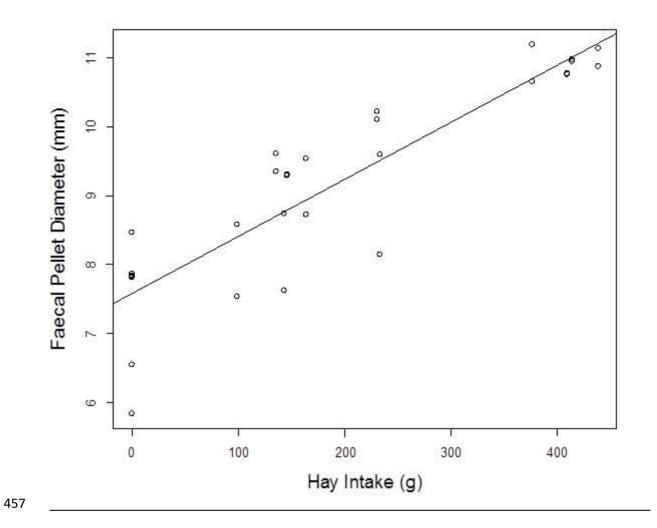
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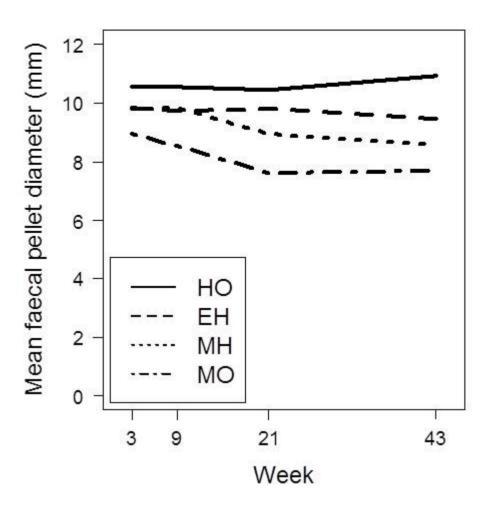
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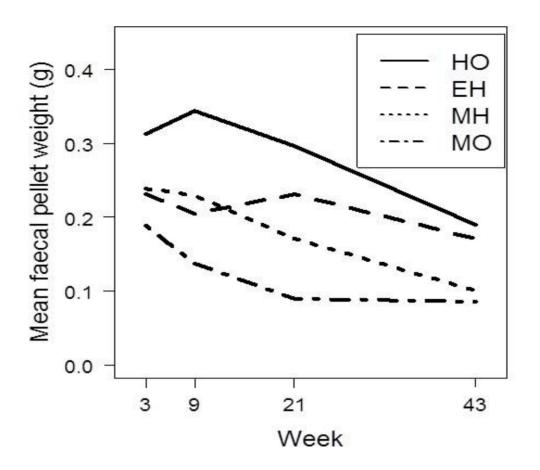
458 **Figure 1** Scatter plot of hay intake (g) by pen and faecal pellet diameter (mm) of Dutch

459 rabbits (n=30) fed four diets (HO, EH, MH and MO) in week 43 of the trial period with linear

460 regression line fitted to data.



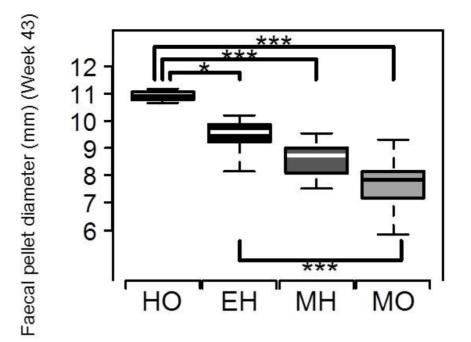
462 Figure 2 a) Mean faecal pellet weight (g) of Dutch rabbits (n=32) fed four diets (HO, EH, MH
463 and MO) measured at four points over a 40 week period.





b) Mean faecal pellet diameter (mm) of Dutch rabbits (n=32) fed four diets (HO, EH, MH and

466 MO) measured at four points over a 40 week period.

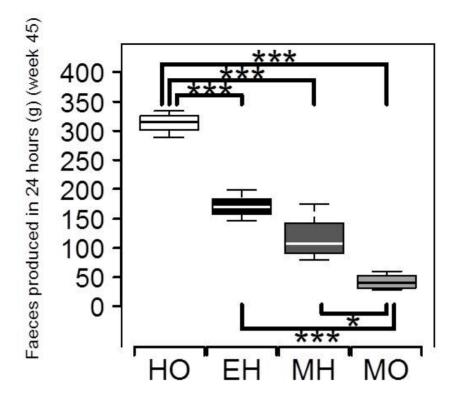




468 **Figure 3** Boxplot of faecal pellet diameter (mm) of Dutch rabbits (n=30) fed four diets (HO,

469 EH, MH and MO) in week 43 of the trial period. The horizontal bar represents the median,

- the box is the interquartile range and whiskers represent the range of values. *** significant
- 471 at P<0.001, * significant at P<0.05



- 473 **Figure 4** Boxplot of weight of faeces produced over a 24 hour period (g) by Dutch rabbits
- 474 (n=30) fed four diets (HO, EH, MH and MO) in week 45 of the trial period. Boxplots as for
- 475 Figure 3. *** significant at P<0.001, * significant at P<0.05

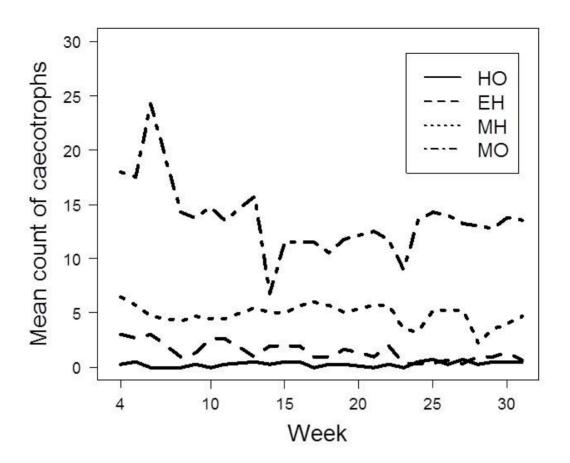


Figure 5 Mean number of caecotrophs found on a weekly basis in the pens of Dutch rabbits

478 (n=32) fed four diets (HO, EH, MH and MO) measured at over a 40 week period.

	Timothy Hay	Extruded Nugget ^a	Muesli ^b
Crude Protein (%)	9	13	14
Fat(%)	2	3	2.5
Crude Fibre(%)	29	19	14
Ash (%)	6.5	5.5	5
NDF ^c (%)	60	38	29
ADF ^d (%)	33	21	20
Calcium (%)	0.3	0.6	0.6
Phosphorus (%)	0.22	0.51	0.4
Ca:P Ratio	1.36	1.18	1.5

	Week 3	Week 9	Week 21	Week 43
HO	0.31g±0.07	0.34g±0.09	0.3g±0.04	0.19g±0.03
	10.53mm±0.71	10.53mm±1.53	10.43mm±0.78	10.91mm±0.18
EH	0.23g±0.03	0.2g±0.05	0.24g±0.05	0.17g±0.03
	9.82mm±0.58	9.72mm±0.87	9.83mm±0.8	9.44mm±0.69
MH	0.24g±0.06	0.23g±0.03	0.17g±0.03	0.09g±0.04
	9.80mm±0.7	9.82mm±0.71	8.93mm±0.8	8.67mm±0.75
MO	0.19g±0.04	0.14g±0.05	0.09g±0.02	0.1g±0.03
	8.93mm±0.78	8.52mm±0.85	7.61mm±0.96	7.67mm±0.91

 Table 2- Mean size and weight of faecal pellets produced in each group at each time point

	Week 10	Week 22	Week 45
НО	983 ±128	1040±37	1058±128
EH	751±99	784±49	753±70
MH	660±128	586±145	618±186
MO	534±114	472±76	402±65

Table 3- Mean number of faecal pellets produced by a pair of rabbits in each group at each time point