

1 **Effect of routine dentistry on faecal fibre length in Donkeys**

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6 **Abstract**

7 Many donkeys are kept as companions in the UK and are not ridden or work, therefore dental
8 pain can often go unnoticed by owners. Donkeys suffer from an increased frequency of dental
9 pathology compared to horses and require regular dental treatment (rasping) to optimise their
10 welfare. Faecal fibre length (FFL) has been suggested as a non-invasive method to assess when
11 *Equidae* require dental treatment. This study aimed to identify FFL pre-rasping in donkeys
12 requiring dental treatment and to evaluate how this changed over a 6-week period post-rasping.

13 Twenty adult donkeys of mixed sex and age, and subject to analogous management regimes
14 were selected from the Donkey Sanctuary. Faecal samples were taken for FFL analysis pre-
15 rasping (week 0) and post-rasping (weeks 1, 2, 3 and 6). Mean FFL, determined via laboratory
16 analysis, was recorded for each donkey and the cohort each week. Repeated measures ANOVA
17 with post-hoc Bonferroni analyses and a Bonferroni adjustment ($P \leq 0.01$) examined if
18 differences occurred in FFL between weeks.

19 The cohort's mean FFL was higher pre-rasping than for all weeks examined post-rasping.
20 Significant reductions in mean FFL for the cohort were reported pre- and post-rasping for week
21 0 to weeks 1, 2, 3 and 6, weeks 1 and 3, 1 and 6, weeks 2 and 3, and week 2 and 6 ($P < 0.0001$).
22 Pre-rasping FFLs > 3.3 mm were associated with the presence of dental elongations in adult,
23 companion donkeys. This suggest that FFL measurement is a useful non-invasive tool that
24 could be used to assess the dental health of donkeys.

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26 Key words: equine, rasping; prophylactic dentistry; welfare; dental pathologies

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28 **Highlights:**

- 29 1. Donkeys experience a higher incidence of dental pathologies than horses.
- 30 2. Dental pain can be hard to diagnose in unriden companion donkeys.
- 31 3. FFL>3.3mm were associated with dental pathology in the donkeys examined.
- 32 4. FFL reduced after rasping for the 6 weeks examined.
- 33 5. FFL could be used as a non-invasive indicator of dental pathology in donkeys.

34

35 **1.0 Introduction**

36 Modern management regimens [1] and diets of domesticated *Equidae* often restrict access to
37 forage and instead contain high concentrate rations [2]. These diets require reduced attrition
38 and do not cause sufficient wear of the occlusal surfaces needed to maintain hypsodont
39 dentition [3,4]. Subsequently, a higher prevalence of dental abnormalities is reported in
40 managed *Equidae* compared to their free-living peers [5,6]. Domesticated horses and donkeys
41 therefore require regular routine dental treatment (rasping) to facilitate functional mastication
42 and digestion [7,8].

43 There are approximately 44 million donkeys worldwide [9] the majority of which are working
44 animals [10]. In the UK, donkeys are often kept as companion animals (not ridden), which can
45 result in dental pain not being identified by their keepers and donkeys receiving minimal or no
46 regular dental treatment [11]. Dental pathologies are the second most common clinical
47 condition reported in the domestic donkey [12] and have been widely associated with impaction
48 colic cases [13,14]. Dental pathologies therefore represent a potential welfare issue in the
49 donkey.

50 To date, the majority of dental care protocols used in the donkey have been adapted from those
51 used in the horse [15]. Yet the assumption that the donkey and the horse are identical is an
52 incorrect with differences between digestive physiology and dental anatomy reported [16, 17].
53 Both species possess hypsodont dentition, with an annual eruption rate of 2-3mm reported
54 [18,19]. Donkeys possess between 36- 44 teeth dependent upon age, sex and presence of non-
55 functional wolf teeth [17], with the average adult animal presenting with 36 permanent teeth
56 [8]. Donkeys have a greater degree of anisognathia than horses, 27% compared to 24%
57 respectively [17] and a wider range of occlusal angles than the horse [20]. Changes to the
58 masticatory cycle due to either discomfort or an inappropriate diet can produce a more
59 pronounced vertical masticatory pattern resulting in increased occlusal surface angulation [14].
60 Therefore, the normal cheek teeth angulation and anisognathia found in donkeys, combined
61 with the impact of modern management regimes, predispose them to develop a higher
62 incidence of dental pathologies than the horse [15].

63 Faecal fibre length (FFL) can be used as an indicator of oral health and masticatory efficiency
64 in *Equidae* [21, 22] and could therefore be used to assess dental health status in donkeys. FFL
65 >3.6mm have been proposed as an indicator of the presence of dental abnormalities in horses
66 [18, 23]. Research in horses suggests that FFL does not significantly change after dental

67 treatment [24, 25]. However these studies used a technique (rubber ball to encourage fibre
68 separation, followed by dry sieving) which could cause excessive attrition of faecal fibres
69 producing measurements which are not representative of true FFL [22, 26]. The validation of
70 FFL as an indicator of masticatory efficiency and digestion in the donkey could provide a
71 monitoring tool informing frequency of routine rasping aiding in the maintenance of welfare
72 in donkeys. Therefore, this study aimed to identify FFL in donkeys requiring dental treatment
73 and to evaluate the effect of routine dental treatment on FFL in companion donkeys over a six-
74 week period. It was hypothesised that a reduction in FFL would occur after rasping.

75 **2.0 Materials and Methods**

76 Twenty donkeys of mixed sex (16 Jacks; 4 Jennys) and age (7.6 ± 2.8 years), subject to the same
77 management practices (group housed in a barn with turnout) and diet (haylage twice per day
78 and *ad libitum* oat straw), resident at The Donkey Sanctuary, Woods Farm, Devon, UK were
79 selected for inclusion in the study. All donkeys required routine dental treatment, as part of
80 their ongoing, yearly health care. The study was authorised by the site manager and the
81 management team. All procedures, including dental examinations and treatments were
82 approved as adhering to animal welfare guidelines by the University of the West of England
83 (Hartpury) Ethics Committee and were performed by a qualified equine dental technician
84 (EDT) adhering to British Equine Veterinary Association (BEVA) guidelines [27]. Data
85 collection took place from mid-October to the end of November 2013.

86 *2.1 Faecal sampling protocol*

87 An initial faecal sample was collected prior to any dental examination or treatment: week 0.
88 Individual donkeys were separated from the herd, but they were still in visual contact with the
89 rest of the herd to prevent putting them under undue stress, until they defecated. Faecal samples
90 were then collected from the naturally dropped faecal matter, fifty grams were weighed using
91 digital scales and placed into sealed plastic bags and frozen on the day of collection at -18°C ,
92 monitored using a digital thermometer. Each bag was labelled with the sample number and a
93 letter which represented the individual donkey. Once a sample had been successfully collected,
94 the donkey was moved back into the barn to prevent re-collection or sampling errors. The yard
95 where the donkeys were held was cleared of any existing faeces prior to and during sample
96 collection to avoid misidentification of the donkey the sample came from. Faecal sample
97 collection was repeated post-dental treatment for weeks 1, 2, 3 and 6 using the same procedure.

98 2.2 *Dental treatment*

99 Dental examination and treatment was performed over two days after the first (week 0) faecal
100 samples had been collected. All donkeys were treated by the same BEVA qualified EDT who
101 was a member of the British Association of Equine Dental Technicians.. The onsite veterinarian
102 assessed the donkeys and declared them fit to receive treatment and free from any pre-existing
103 clinical conditions other than dental elongations that could be corrected by rasping
104 accompanied by no further pathologies. The 20 donkeys were held in their normal yard whilst
105 receiving dental treatment to minimise stress.

106 A full oral examination was performed, visualising all dental surfaces/structures and assessing
107 all oral tissues. Donkey age, sex and dental diagnoses data were transcribed directly to a dental
108 chart; dental disorders noted included sharp enamel points, focal overgrowths, shear mouth,
109 step mouth, wave mouth, accentuated transverse ridges and diastema. Routine dental treatment
110 (rasping) was undertaken to reduce overgrowths, remove sharp enamel points, increase lateral
111 excursion, restore balance of the arcades and establish correct occlusal angles in accordance
112 with BEVA guidelines (2009).

113 2.3 *Laboratory analysis of faecal fibre length*

114 Prior to laboratory analysis, the sampling period individual samples came from was blinded
115 from the experimenter to prevent bias. Faecal samples were defrosted at room temperature (18-
116 24°C) until the sample reached 4°C. Five grams of faecal matter, taken from multiple sections
117 of the larger 50g sample to ensure a representative selection of fibre lengths, was weighed using
118 digital scales. Each 5g sample was added to a glass beaker filled with 500ml of distilled water.
119 The mixture was gently stirred to separate fibres from unwanted sediment. The mixture was
120 then poured through a 0.5mm sieve to eliminate all fibres under 0.5mm from analysis. The
121 remaining fibre mass was collected and gently spread over a foil square, labelled in indelible
122 marker with the sample's identification letter. All 20 samples were placed in the oven at 150°C
123 for 2 hours and once dried each sample was gently sieved through a 1cm sieve, using a soft
124 bristle brush to encourage fibre separation whilst attempting to prevent attrition to the fibre
125 length during the process. The separated dry fibres were re-sieved evenly over a 616 squared
126 grid, sub-divided into four labelled quadrants: A, B, C and D, each of which was subdivided
127 into 154 squares. One square from the 154 present in each quadrant was randomly selected for
128 analysis (e.g. Quadrant A, square 101). Ten faecal fibres were measured from each of the four
129 squares selected, providing a total of forty faecal fibres for each individual sample. Fibres were

130 removed from the grid using tweezers, placed on a separated white surface and were
131 individually measured using Mitutoyo Absolute Digimatic Digital Vernier Callipers (Mitutoyo
132 part number: 500 196-20, model: 500 196-20, accuracy $\pm 0.01\text{mm}$). The mean, standard
133 deviation, upper and lower and inter-quartile ranges were calculated for FFL of each sample
134 using Microsoft Excel™ Version 2010 prior to statistical analysis. The FFL analysis procedure
135 was repeated for each individual sample for weeks 0, 1, 2, 3 and 6.

136 *2.4 Statistical Analysis*

137 Data were analysed using Statistics Package for Social Scientists (SPSS, Version 20). Data
138 were parametric however whilst Pillai's Trace confirmed a highly significant difference in
139 mean FFL it could not provide specificity ($P=0.0001$) and Mauchly's test indicated that the
140 assumption of sphericity within the data had been violated ($P=0.002$). Therefore the degrees of
141 freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon=0.57$) and one-
142 tailed Repeated Measures ANOVA with a Greenhouse-Geisser correction was applied to
143 determine if significant differences were present in mean FFL across the cohort [28]. Post hoc
144 Bonferroni analyses were conducted with a Bonferroni correction applied, to adjust for
145 repeated measures, resulting in a revised significance level of $P \leq 0.01$. These tests were
146 performed to determine where statistical differences occurred in FFL between the data
147 collection weeks for the entirety of the study.

148 **3.0 Results**

149 The cohort's mean FFL pre-rasping was higher than all weeks examined post-rasping (Table
150 1). The majority of subjects recorded higher FFL (90%) pre-dental treatment compared with
151 their FFL recorded post-dental treatment; the magnitude of FFL changes varied between
152 individual donkeys as well as within the weeks evaluated (Table 2).

153 Significant changes in mean FFL (decreases) were found across the study period ($P < 0.0001$),
154 however after subsequent post-hoc analysis and Bonferroni adjustment for repeated measures,
155 this pattern was not repeated consistently for the entirety of the study period. Significant
156 reductions in mean FFL for the cohort were reported pre- and post-dentistry for week 0 to
157 weeks 1, 2, 3 and 6 ($P=0.0001$) with further reductions reported between weeks 1 and 3, 1 and
158 6, weeks 2 and 3, and week 2 and 6 ($P=0.0001$). No significant changes in FFL length occurred
159 between weeks 1 and 2, or between weeks 3 and 6 ($P > 0.05$).

160 Table 1: Faecal fibre lengths in millimetres (to 2 decimal places) across the cohort for the study
 161 period.

Faecal Fibre length (mm)	Pre- dentistry (week 0)	Post- dentistry (week 1)	Post- dentistry (week 2)	Post- dentistry (week 3)	Post- dentistry (week 6)
Mean	4.37	3.03	2.80	1.95	1.93
Standard deviation	0.65	0.40	0.25	0.27	0.30
Minimum	3.32	2.50	2.32	1.46	1.35
Lower quartile	4.02	2.60	2.66	1.80	1.74
Median	4.27	3.05	2.77	1.97	1.89
Upper quartile	4.79	3.41	2.98	2.13	2.16
Maximum	5.55	3.81	3.25	2.47	2.43

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163 Table 2: Individual faecal fibre length across the six weeks investigated in millimetres to 2
 164 decimal places

Donkey ID	Faecal fibre length (FFL) in millimetres (mm) post routine dental treatment				
	Week 0	Week 1	Week 2	Week 3	Week 6
1	4.01	2.52	2.32	1.80	1.86
2	4.82	2.93	2.54	1.96	2.13
3	4.51	2.59	2.98	1.80	2.41
4	4.93	2.56	2.93	2.24	2.23
5	4.11	2.66	2.90	1.63	1.85
6	5.46	2.60	3.20	1.46	2.33
7	4.62	2.50	2.64	1.51	2.31
8	4.29	2.91	2.43	1.89	1.75
9	5.34	3.19	2.86	2.40	1.66
10	3.32	3.47	2.51	1.98	1.63
11	3.58	3.01	2.77	2.05	1.91
12	4.65	3.26	2.93	2.18	1.94
13	3.38	3.46	2.70	1.66	1.75
14	4.04	3.50	2.76	1.82	1.35

15	4.20	2.61	3.25	2.12	1.65
16	4.71	3.81	2.74	2.47	1.70
17	4.04	3.15	2.75	1.99	1.78
18	4.24	3.08	3.03	2.01	2.08
19	3.64	3.22	3.09	1.88	1.93
20	5.55	3.56	2.73	2.13	2.43
Cohort					
Mean	4.37	3.03	2.80	1.95	1.93
Standard deviation	0.65	0.40	0.25	0.27	0.30

165

166 **4.0 Discussion**

167 At the start of the study, the majority of donkeys (90%) exceeded a FFL of >3.6mm the length
168 proposed to indicate the presence of dental abnormalities in horses [26, 29]. The presence of
169 dental pathologies were confirmed in these donkeys by EDT examination. However, EDT
170 examination confirmed a further two donkeys, who returned FFL <3.60mm (3.32 and 3.38mm
171 respectively), required dental treatment suggesting that the FFL level that is consistent with the
172 presence of dental abnormalities may be shorter in donkeys than that proposed in the horse,
173 however more research is required before this is confirmed. By week 3, the FFL for all donkeys
174 appeared to stabilise at lengths <2.50mm. Our results suggest that FFL measurement is a useful
175 non-invasive tool that could be used to assess the dental health of donkeys, with FFL >3.30mm
176 indicating the presence of dental elongation in adult donkeys.

177 The FFL length of the majority of donkeys (90%) reduced a week after rasping, but 5 (25%)
178 still presented with a FFL >3.3mm. However by week 2, all donkeys' FFL were >3.3mm and
179 further reductions in FFL occurred up to week 6. Routine rasping removes dental pathologies,
180 thus reducing restriction to occlusal contact allowing full excursion and improved attrition,
181 facilitating more efficient mastication [30]. The variation reported here suggests that the more
182 efficient attrition which occurs post rasping, generates a reduction in faecal particle size [31,
183 32]. Kinematic and electromyographic evaluation of how the mastication cycle in horses
184 changes post-rasping, suggests that the first week after dental treatment (rasping) represents a
185 period where fluctuations occurs in the mastication cycle demonstrated by changes in lateral
186 excursion and the power stroke [31] and masseter and temporalis muscle workloads [32]. This
187 adaptation could explain why there appears to be a transition period of 1 to 2 weeks for some

188 donkeys before FFL reduces below 3mm. Interestingly, donkeys that recorded FFL >3.3mm
189 presented with more severe dental elongations pre-rasping than their peers; therefore the rate
190 of FFL reduction post-rasping, may also be influenced by the incidence and severity of dental
191 pathologies present in the subject.

192 *4.1 Limitations and further research*

193 The results of this preliminary study are promising; however, further work incorporating larger
194 numbers of donkeys to confirm the results found here and to establish a standardised FFL
195 indicator of dental pathologies in donkeys is required. The current sample considered adult,
196 companion donkeys, therefore we would advocate repeating the study in working donkeys and
197 across wider age ranges to evaluate if differences in FFL present between adult and geriatric
198 samples.

199 **5.0 Conclusion**

200 Routine dental treatment resulted in significant reductions in FFL in donkeys, which suggests
201 that rasping has improved the efficiency of mastication. Our results suggest that faecal fibre
202 lengths of <3.3mm can be used as an indicator of the presence of dental pathologies in
203 companion, adult donkeys. If a standardised FFL length can signpost the presence of dental
204 pathologies, the measure has the potential to be implemented as a standard welfare indicator
205 particularly for working donkeys globally.

206

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210

211 **Conflict of interest**

212 No conflicts of interest apply to this work.

213

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217

218 **References**

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