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1     **The use of trivalent metal markers for estimating the individual feed**  
2                                     **intake of young pigs**

3

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12

13    **Abstract**

14       Twenty-four individually housed male pigs (6.6 ± 0.24 kg) were used in a pilot  
15    study to validate two trivalent metal markers, one in the feed and the other dosed  
16    orally to piglets, for the estimation of voluntary feed intake. Pigs were randomly  
17    assigned to one of three oral dosing treatments using 15 mg lanthanum oxide/day as  
18    the internal marker: once daily, twice daily, or 3 times daily. Piglets were offered a  
19    diet containing 1 g/kg of yttrium as the external marker. After a 7-day adaptation  
20    period, total faecal collection was made for the next 3 days. The first faecal sample  
21    voided after 1000 h was considered as the ‘grab sample’, to allow comparison of the  
22    technique with total collection. Intake of diets was recorded daily, and compared to  
23    feed intake using the ratio of the markers in the faeces. Daily samples were analysed  
24    for marker concentrations and a mean of the three-day data was used for regression  
25    analysis. Total collection data demonstrated that the accuracy of the estimation using  
26    the trivalent metals depended on the frequency of oral marker administration, as the

27 estimation principle relies on the continual flow of a known amount of marker in the  
28 gastrointestinal tract (GIT). Nevertheless and using total collection, dosing the oral  
29 marker 3 times a day estimated individual feed intake with reasonable accuracy ( $R^2 =$   
30 0.85). In contrast, the ‘grab sampling’ technique reduced the accuracy of estimation  
31 ( $R^2 = 0.74$ ), indicating that continual flow of the oral marker in the GIT is required for  
32 such a method. In conclusion, there is some potential in using trivalent metal markers  
33 to quantitatively estimate the feed intake of an individual pig, however the level of  
34 accuracy requires improvement.

35

36 *Keywords:* feed intake; Lanthanum; Yttrium; markers; Weaner pigs.

37

### 38 **1. Introduction:**

39 Pig producers traditionally offer a creep diet to sucking piglets during lactation to  
40 supplement their nutrition because the sow produces less milk as lactation advances.  
41 Our previous experiment (Pluske et al., 2007) qualitatively categorised piglets into  
42 “eaters” and “non-eaters” of creep feed using a colour marker and demonstrated that  
43 piglets eating more creep feed during lactation perform better after weaning, which is  
44 in agreement with Bruininx *et al.* (2002). A greater understanding of factors  
45 influencing creep feed consumption during lactation could have commercial  
46 implications, such as improved management of piglets after weaning. Greater  
47 understanding in this area, however, is hindered because it is not possible at the  
48 moment to determine how much creep feed an individual piglet in a litter consumes.

49 It is possible to estimate the herbage intake of an individual grazing sheep by  
50 chemically analysing natural (alkane) markers in the pasture and faeces (Dove and  
51 Mayes, 1991). Trivalent metal markers could be used in replacement of the alkane

52 markers but using the same method of calculation. Trivalent metal markers such as  
53 Lanthanum III oxide ( $\text{La}_2\text{O}_3$ ), Yttrium III oxide ( $\text{Y}_2\text{O}_3$ ) and Ytterbium III chloride  
54 hexahydrate ( $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$ ) have been successfully used as markers in fish (Austreng  
55 et al., 2000), ruminants (Sowell et al., 2003), dogs (Vhile et al., 2007) and pigs  
56 (Mavromichalis et al., 2001) for estimation of nutrient digestibility. In this pilot  
57 experiment, we tested the validity of trivalent metal markers to estimate the feed  
58 intake of young pigs that were housed individually, to verify whether the technique  
59 could be used to estimate the creep feed intake of an individual piglet in a litter.

60

## 61 **2. Materials and Methods**

### 62 2.1. Preparation of the oral marker solution

63 The oral marker, lanthanum oxide ( $\text{La}_2\text{O}_3$ , Sigma-Aldrich, Inc., St. Louis, Mo,  
64 USA), is 96% soluble in pH 3 solution and re-precipitates at pH 7 (Austreng et al.,  
65 2000). Therefore the lanthanum oxide was dissolved in diluted table vinegar (1:5 with  
66 distilled water, pH 2.8) at a rate of 5 mg/mL. Table sugar (5 mg/mL) was also  
67 dissolved in the solution to increase the palatability of the solution. The lanthanum  
68 oxide was dissolved with a magnetic stirrer under heat (110 °C) for 30 min. The  
69 solution was then filtered through filter paper (No. 541, Whatman) and stored at 4 °C  
70 until use.

71

### 72 2.2. Animal and experimental procedure

73 The experiment was approved by the Department of Agriculture and Food  
74 Western Australia Animal Ethics Committee. Twenty-four male weaner pigs weaned  
75 at  $21 \pm 2$  days of age (Large White  $\times$  Landrace,  $6.6 \pm 0.24$  kg) and provided creep

76 feed from 14 d were individually housed in a metal mesh-floored weaner crate with a  
77 space allowance of 1.6 m<sup>2</sup> and randomly allocated to one of 3 treatments (n=8). The  
78 three treatments were an oral dose (directly into mouth through a sterile injection  
79 tube) of 15 mg lanthanum (dissolved in 3 mL diluted table vinegar) either as a once  
80 daily dose of 3 mL (0800 h), a twice-per-day dose of 1.5 mL each (0800, 2000 h), or a  
81 dose of 1 mL three times per day (0800, 1600 and 2400 h). The solution was dosed  
82 into the oropharynx while restraining pigs in an up-right position until pigs had  
83 apparently ingested all of it. Piglets were fed a commercial weaner diet containing 1  
84 g/kg of a marker, yttrium (Y<sub>2</sub>O<sub>3</sub>, Sigma-Aldrich, Inc., St. Louis, Mo, USA). After 7  
85 days of adaptation, faecal samples were collected using the total collection method  
86 and “grab” sampling method daily for three days. The first faecal sample voided after  
87 1000 h was collected as “grab sample” and the amount of grab sample was  
88 numerically adjusted for the total collection data. Daily samples were analysed for  
89 marker concentrations and a mean of the three-day data was used for regression  
90 analysis. Voluntary feed intake (VFI) was recorded daily by measuring feed offered  
91 and residues each morning. The VFI estimation included any spillage on the floor.

92 Creep feed intake was calculated using the methodology of Dove and Mayes  
93 (1991),

94 Feed intake (g DM) = Faecal output (FO) / Dilution index (DI), where:

95 FO = Lanthanum marker consumed (g/day) / Lanthanum in faeces (g/g DM) and

96 DI = Yttrium marker in diet (g/g DM) / Yttrium in faeces (g/g DM).

97

### 98 2.3. Chemical analyses

99 The dry matter content of diet and faecal samples was determined by weighing  
100 each sample, oven drying for 2 h at 135 °C, then reweighing. The concentrations of

101 lanthanum and yttrium in diets, the oral marker solution and faeces were determined  
102 using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)  
103 (Austreng et al., 2000).

104

#### 105 2.4 Statistics

106 The feed intake and faecal output of pigs fluctuated day by day causing a large  
107 variation in the feed intake when it was estimated from the daily data. Therefore, the  
108 mean value for each pig for the 3 day collection period was taken and used for the  
109 regression analyses. Linear regression analysis was conducted to validate the accuracy  
110 of the marker method against reference measurement for estimation of individual feed  
111 intake (Minitab 13, Minitab Inc., State College, PA).

112

### 113 **3. Results and Discussion**

114 An important source of error can be an incomplete ingestion of orally-dosed  
115 lanthanum oxide. Pigs accepted the solution very well; however, some degree of  
116 regurgitation could have happened with the saliva. The oral marker lanthanum has a  
117 number of advantages than other trivalent metal markers, as it is known to completely  
118 dissolve in solution at pH 3 and re-precipitate in the duodenum (at pH 5-6), and flows  
119 with solid digesta (Austreng et al., 2000). Nevertheless, a critical aspect of this  
120 methodology is whether the steady flow of the oral marker lanthanum in the faeces  
121 occurs, and hence whether a once-daily faecal 'grab sample' is valid for the  
122 estimation of feed intake with reasonable accuracy compared to total collection of  
123 faeces. Therefore, the suitability of using an external marker procedure to estimate the  
124 intake of creep by individual piglets while remaining in group was tested in this study.  
125 Specifically, this study tested whether a fixed amount of lanthanum oxide given orally

126 as a La<sub>2</sub>O<sub>3</sub> solution in a single dose or split in two or three doses allows the estimation  
127 of creep intake with adequate accuracy.

128 Linear relationships between measured and estimated VFI using the three dosing  
129 frequencies and two collection methods are presented in Table 1. Results obtained  
130 from the total collection study demonstrated that trivalent metal markers (lanthanum  
131 and yttrium) are a valid method for estimating the individual feed intake of a pig,  
132 however the accuracy of the estimation depended on the number of times the oral  
133 marker was administered because an increasing number of doses significantly  
134 increased the accuracy of the estimation (Table 1). This, presumably, is because the  
135 estimation principle relies on the continual flow of a known amount of marker in the  
136 GIT, and more frequent dosing would have increased the stability of marker flow  
137 along the GIT. Nevertheless, by the total collection method, dosing the oral marker  
138 three times per day estimates individual feed intake with reasonable accuracy ( $R^2 =$   
139 0.85, Figure 1).

140 Since total collection of faeces would not be possible from an individual piglet in  
141 a litter, 'grab sampling' was also investigated in this study. Taking a 'grab sample'  
142 significantly reduced the accuracy of estimation and increased variability (RSD) of  
143 the estimated feed intake, indicating the continual flow of oral marker solution was  
144 most likely not achieved even by dosing the oral marker three times per day (Table  
145 1). However, our data showed that the higher variability was caused by those pigs  
146 eating more than 600 g diet per day (high standard error, see Fig. 1), most likely  
147 because these pigs may consumed larger meals before or after the dose of oral  
148 markers. This, in turn, would presumably alter the concentrations of oral and in-feed  
149 markers in the digesta. As sucking piglets most probably consume far less solid feed  
150 than this (Pluske *et al.*, 2007), we believe that the potential of the trivalent metal

151 marker method to estimate creep feed intake in a litter would be higher than that  
152 found in the present study. As found in this pilot study, further research is warranted  
153 to verify this hypothesis. Although this methodology can be considered an  
154 improvement to previous qualitative methods of estimating individual pig feed  
155 intake, the accuracy of the estimation should be improved using better methodology  
156 that can ensure quantitative ingestion and steady flow of the oral marker.

157 In conclusion, despite the accuracy of the 'grab sampling' method being  
158 significantly lower than total collection, a mean estimation of the three-day  
159 observations using the trivalent metal marker technique can be used with moderate  
160 accuracy for the estimation of individual feed intake in suckling piglets.

161

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167

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190

191 Table 1. Linear regression results for estimated (Y) and measured (X) feed intake in pigs given in-feed yttrium (Y<sup>+++</sup>) and oral lanthanum  
 192 (La<sup>+++</sup>)<sup>a</sup>.

Method	Oral marker dose <sup>b</sup>	Intercept	Slope	R <sup>2</sup>	RSD <sup>c</sup>	Number of observation <sup>d</sup>	P=
Total collection							
	1	69 (290)	1.19 (0.51)	0.47	127	8	0.06
	2	106 (88)	0.77 (0.14)	0.82	40	8	0.002
	3	-231 (156)	1.43 (0.25)	0.85	73	8	0.001
Grab sampling							
	1	-520 (347)	2.65 (0.67)	0.72	152	8	0.008
	2	-274 (310)	1.80 (0.53)	0.66	145	8	0.015
	3	-21 (160)	1.19 (0.29)	0.74	72	8	0.006

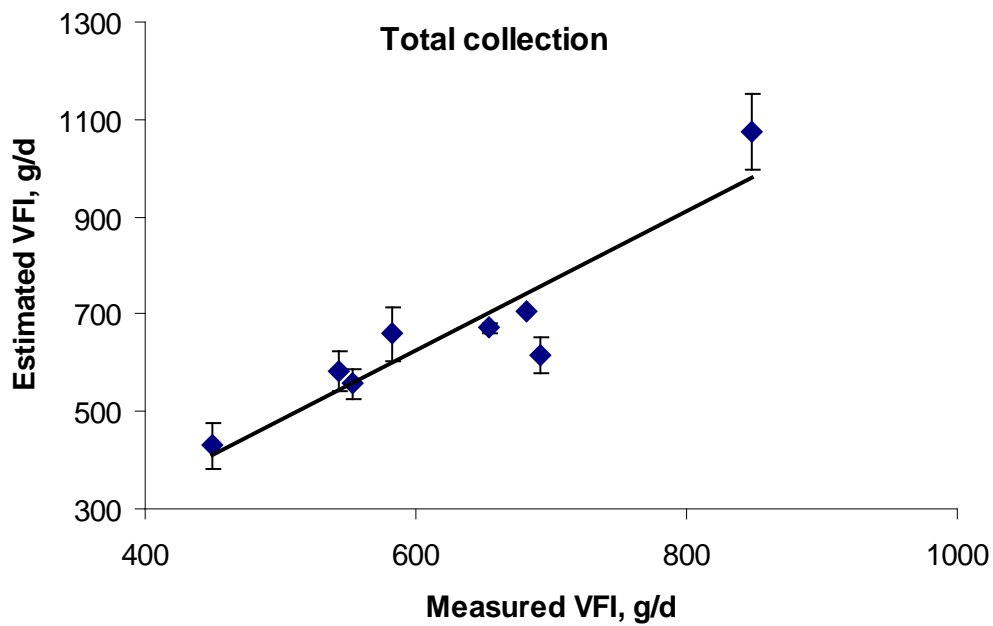
193 <sup>a</sup>Standard errors are reported in parentheses.

194 <sup>b</sup>Number of daily oral marker administration. The same amount of marker (15 mg of lanthanum oxide in 3 mL diluted vinegar solution) per day  
 195 was orally administered once, twice or three times.

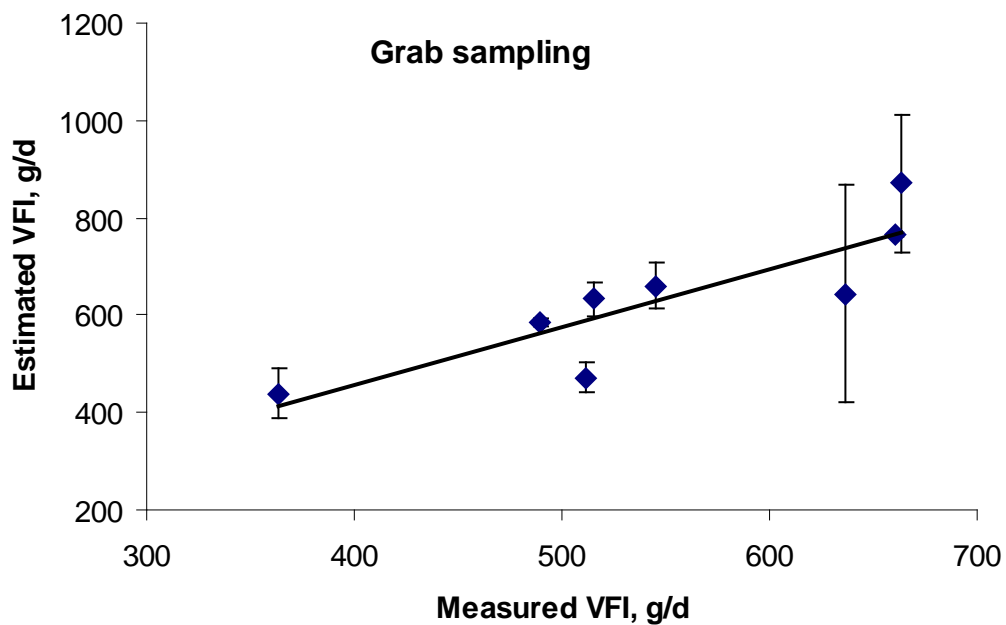
196 <sup>c</sup>Residual standard deviation.

197 <sup>d</sup>Mean of three daily observations.

198



199



200

201 Figure 1. Predictability of feed intake using the trivalent metal marker method. The  
202 oral marker was dosed three times per day. Total collection  $Y = -231 + 1.43X$   $R^2 = 0.85$   
203 (RSD = 73); grab sampling  $Y = -21 + 1.19X$ ,  $R^2 = 0.74$  (RSD = 72).