Effect of drying method on accuracy of n-alkane estimation of forage intake

D.L. BURNHAM, S.T. MORRIS and P.K. KENYON
Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North
D.Burnham@massey.ac.nz

Abstract

N-alkane marker techniques are commonly used in the measurement for forage intake in grazing animals. Traditionally sample preparation of feed for subsequent n-alkane analysis has been conducted on freeze-dried samples, however oven-drying is both less time consuming and less expensive. Ten mixed age rumen fistulated wethers were housed in individual metabolism crates in the Animal Physiology Unit of Massey University for 30 days. The wethers were randomly allocated to one of four treatments fed either 0.5, 1.0, 1.5 or 2.0x maintenance requirement based on individual liveweight, with 2, 3, 3 and 2 animals in each respectively. Fresh ryegrass/white clover pasture was offered twice-daily. Following a 10 day adaptation period, each animal had an n-alkane capsule inserted via rumen fistulae from d0-d20. Feed and faeces was sub-sampled for subsequent analysis of C_{31}, C_{32}, C_{33}, C_{35} and C_{36} alkane content on alternate days from d5-20. Capsules were removed temporarily on alternate days from d0, for measurement of the plunger travel, as an indirect measure of capsule release rate. All faecal samples were oven-dried at 60°C until a constant dry weight was achieved. Herbage sub-samples collected on d5, 9, 13 and 17 were stored at -5°C before freeze-drying, while sub-samples collected on d7, 11, 15 and 19 were oven dried at 60°C for 48 hours. The capsules achieved a constant release rate within four days post-insertion and remained at a steady rate up to day 20 post-insertion. No difference was found between estimated intakes using either C_{31}:C_{32} or C_{32}:C_{33} alkane ratios. Day had no significant effect on either actual or estimated intakes. Oven-drying the feed was found to produce a weak linear relationship whereas freeze-drying the feed samples produced a much stronger relationship when compared to *in vivo* intake. A significant difference ($P<0.05$) in relationship between the two methods of sample preparation was found.

Keywords: drying method, freeze-drying, herbage intake estimation, intra-ruminal capsule, n-alkane, oven-drying

Introduction

Measurement or estimation of intake is a core element in evaluation and definition of feeding standards for all grazing livestock. The introduction of the n-alkane marker indirect intake estimation technique (Dove & Mayes 1991) has improved the capability and accuracy in the measurement for forage intake in grazing animals. Dove *et al.* (2000) suggest that estimates of intake based on C_{32}:C_{33} alkanes are more accurate than those estimated using the chromium sesquioxide marker technique, and this methodology is increasingly being utilised by many pastoral researchers. Traditionally sample preparation of feed for subsequent n-alkane analysis has been conducted on freeze-dried samples, however oven-drying is less time-consuming and less expensive. Recent work by Scharch *et al.* (2002) has shown that for faeces, hay and beeswax marked cottonseed meal, oven-drying at 70°C for 48 hours can replace freeze-drying, however, Dove & Mayes (1991) found lowered n-alkane levels in oven-dried samples of fresh-cut lucerne. No previous work is known on comparing alkane concentrations of fresh herbage, or estimated intakes using the alkane marker technique, when herbage has been prepared via these two methods.

Materials and methods

Ten mixed age rumen fistulated wethers were housed in individual metabolism crates in the Animal Physiology Unit at Massey University for 30 days. The wethers were randomly allocated into one of four treatments fed either 0.5, 1.0, 1.5 or 2.0 times maintenance requirement based on individual liveweight, with 2, 3, 3 and 2 animals in each treatment respectively, to ensure a range of potential intakes were achieved. *Ad libitum* fresh water was provided. Fresh ryegrass/white clover pasture was cut on the day prior-to feeding using a sickle bar mower and sub-sampled for dry matter content determination and stored overnight at 3°C. Daily feed allocation was calculated and weighed, and offered twice-daily at 0800h and 1600h. Feed refusal was weighed and sub-sampled for dry matter content determination at 0800h the following day to calculate the *in vivo* intake of the sheep.

Following a 10 day adaptation period, each animal had an intra-ruminal controlled release capsule (Captec Alkane Capsule for Sheep, Captec (NZ) Ltd, Auckland, New Zealand) containing C_{32} and C_{36} alkanes inserted via their rumen fistulae (d0 of

capsule). Feed offered was sub-sampled for subsequent analysis for n-alkane content on alternate days from d5 (d5, 7….19). Faeces were sub-sampled on the following alternate days (d6, 8…d20 etc), on an individual basis. Each capsule was removed temporarily on alternate days (d0, 2, 4…20), for measurement of the plunger travel, as an indirect measure of capsule release rate.

All faecal samples were oven-dried at 60°C until constant dry weight was achieved. Herbage sub-samples collected on d5, 9, 13 and 17 were stored at -5°C before being freeze-dried, while sub-samples collected on d7, 11, 15 and 19 were oven dried at 60°C for 48 hours. All feed and faecal samples were analysed for C_{31}, C_{32}, C_{33}, C_{35} and C_{36}-alkane content using the method of Dove & Mayes (1991). Data was analysed using regression and GLM analysis in the statistical software package SAS (SAS 2000).

Results and discussion

The decrease of capsule matrix length over the duration of the present study is illustrated in Figure 1. The controlled release capsules achieved a constant release rate by d4 and remained at a steady rate up to d20. The calculated daily plunger travel was 1.50±0.24mm/day (mean±SD). Daily release of C_{32} and C_{36} alkanes during this study were calculated using the formula:

Mean release rate (mg/d)= daily plunger travel (mm) x linear density (mg/mm) where linear density =32.2mg/mm of both C_{32} and C_{36}.

The calculated release rate of 43.4mg/day was 4% lower than the published release rate of 44.9mg/d. This difference had no significant effect on resulting estimated intake values. Feeding level had no significant effect on capsule payout (data not shown). This supports the work of Mayes *et al.* (1986) who found that the validity of herbage intake estimates from C_{32}:C_{33} alkanes were unaffected by feeding level.

Three of the daily feed sub-samples had C_{36} levels below measurement limits, therefore C_{35}:C_{36} alkane analysis was not used. C_{31}:C_{32} alkane intake estimates were compared with C_{32}:C_{33} alkane intake estimates and were found to have a strong linear relationship (LR Y=0.981X + 0.024, r^2=0.90, P<0.001, where Y=C32:C33 and X=C31:C32). Therefore, comparison of the feed drying methods was conducted using C_{32}:C_{33} alkane intake estimates only. Dove *et al.* (2000) found that on three occasions, using pregnant and lactating grazing ewes, herbage intakes estimated using C_{31}:C_{32} alkanes were 0.838 (P<0.05), 0.881 (P<0.05) and 0.943 (NS) relative to C_{32}:C_{33} estimates.

Day had no significant effect on either actual (*in*

Figure 1 Alkane capsule matrix length as measured on alternate days in 10 rumen-fistulated sheep fed ryegrass-white clover pasture.

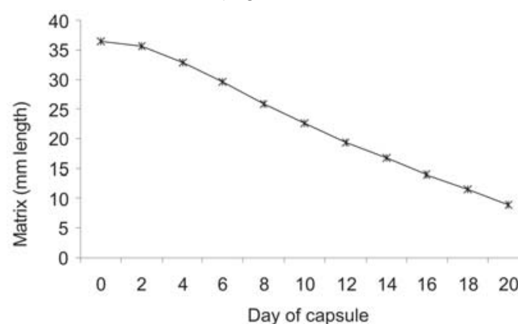
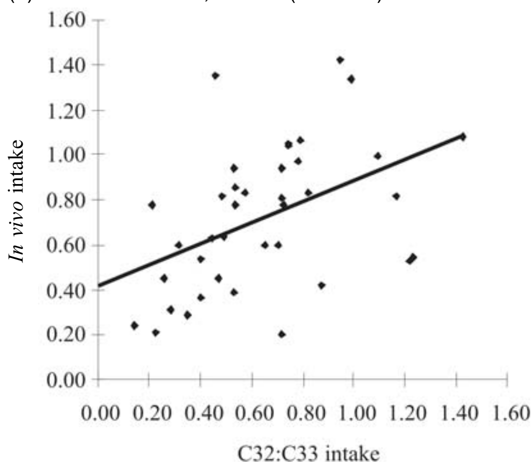


Figure 2 Relationship between intake estimated using C_{32}:C_{33} ratio and *in vivo* intake when herbage is (a) oven-dried and (b) freeze-dried.

(a) Y=0.473X+0.411, r^2=0.38 (P<0.005)



(b) Y=1.01X+0.139, r^2=0.54 (P<0.001)

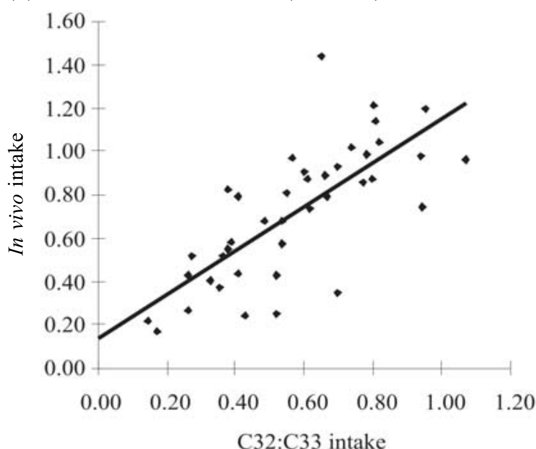


Table 1 Effect of drying treatment on the alkane concentrations found in ryegrass/white clover (present study) and lucerne (data source: Dove, H.; Archer, K.A.; Edwards, S.E.; Neutze, S.A.; Oddy, V.H., unpublished data cited by Dove & Mayes 1991).

Drying conditions	Alkane concentrations (mg/kg DM)					
	C_{27}	C_{28}	C_{29}	C_{31}	C_{32}	C_{33}
PRG/WC						
Freeze dry				234	13	103
Oven dry 60 °C/ constant wt achieved				211	11	91
	Alkane concentrations (mg/kg OM)					
Lucerne						
Freeze dry	63	21	386	687	21	48
Oven dry 70° C/ 48h	61	21	353	620	18	41
Oven dry 100°C/ 24h	45	22	262	505	16	39

vivo) or estimated intakes. The overall relationship between *in vivo* feed intake measured by DM eaten and estimated C_{32}:C_{33} alkane feed intake was:

Y=0.654X+0.318 (r^2=0.32, P<0.001) where Y = *in vivo* feed intake and X= C_{32}:C_{33} alkane estimate.

This relationship is relatively poor; however, it was determined from pooled data from both drying treatments. When the oven-dried and freeze-dried treatments were compared separately (Figure 2), oven-drying the feed was found to produce a relatively poor linear relationship (Y=0.473X+0.411, r^2=0.38, P<0.005), whereas, freeze-drying the feed samples produced a stronger relationship (Y=1.01X+0.139, r^2=0.54, P<0.001). Both the slopes and intercepts of these two regression equations were significantly different (P<0.001) from each other. This indicates a significant difference between the two methods of sample preparation and resulting estimated feed intake.

Table 1 shows the alkane content of the pasture (mg/kg DM) after freeze drying and oven drying at 60°C until a constant weight was achieved. Data from Dove *et al.* (unpublished data, cited by Dove & Mayes, 1991) as also shown in Table 1 indicates that drying fresh lucerne at 100°C for 24 hours resulted in reduced alkane concentration when compared to freeze dried samples. There were indications that drying at a lower temperature (70°C for 48 hours) resulted in intermediary reductions in alkane content measured (10 vs. 36%, 16 vs. 31% and 17 vs. 23% for C_{31}, C_{32} and C_{33} alkanes respectively). Our results showed oven drying at 60°C until constant dry weight was achieved resulted in decreases in alkane content of 5.6, 15.2 and 11.6% for C_{31}, C_{32} and C_{33} respectively. Lamb & Mayes (unpublished data cited by Dove & Mayes 1991) found that oven-drying did not affect alkane analysis of perennial ryegrass (*Lolium perenne*) however the alkane concentration of rush (*Juncus effuses*) exhibited a 50% reduction when oven-dried. They stated that the reduction did not appear to be due to evaporation as no loss was observed when alkane concentration was measured using a saponification method instead of solvent extraction. Further work on the time and temperature effects of oven drying is required.

Conclusion

Although previous studies have indicated that oven drying of samples at temperatures of 60-70°C for 48 hours is suitable preparation for drier feeds such as hay and meal, and for faecal samples, freeze-drying preparation of fresh herbages in this study produced a much close relationship to actual *in vivo* intake than oven-drying preparation. Therefore freeze-drying is recommended for fresh feeds, however further work is required to identify the relationship between time and temperature of oven drying and alkane content measurement. The results will be used as a basis for design of future grazing studies in sheep that will benefit the farming industries through improved knowledge of the feeding and nutrition of animals.

ACKNOWLEDGEMENTS

The authors would like to thank the McGeorge Research Fund of the Institute of Veterinary, Animal and Biomedical Sciences for funding this project. Thanks also must go to Ms Kim van de Put for her technical assistance.

REFERENCES

Dove, H.; Freer, M.; Foot, J.Z. 2000. The nutrition of grazing ewes during pregnancy and lactation: a comparison of alkane-based and chromium/*in vitro*-based estimates of herbage intake. *Australian Journal of Agricultural Research 51*: 765-777.

Dove, H.; Mayes, R.W. 1991. The use of plant wax alkanes as marker substances in studies of the

nutrition of herbivores: a review. *Australian Journal of Agricultural Research 42(6)*: 913-952.

Mayes, R.W.; Lamb, C.S.; Colgrove, P.M. 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. *Journal of Animal Science, Cambridge 107*: 161-170.

Scharch, C.; Dove, H.; Rodehutscord, M. 2002. Comparison of drying treatments in sample preparation for alkane analysis. *Proceedings of the Society of Nutrition and Physiology*: 11.

SAS 2000. SAS/STAT Version 8.1 SAS Institute, Cary, NC, USA.