# Use of *n*-alkane technique to estimate sheep dry matter intake

Glaucia A Amaral <sup>A</sup>, Teresa C M.Genro <sup>B</sup>, Armindo B Neto <sup>C</sup>, Jean V Savian <sup>D</sup>, Diego B de David <sup>A</sup>, Eduardo B Azevedo <sup>D</sup>, Felipe Jochims <sup>D</sup> and Paulo C de F. Carvalho <sup>D</sup>

<sup>A</sup> State Foundation of Agricultural Research (FEPAGRO), Rua Gonçalves Dias, 570, Bairro Menino Deus, Porto Alegre/RS, <u>www.fepagro.rs.gov.br</u>

<sup>B</sup> Embrapa Southern Region Animal Husbandry, <u>www.cppsul.embrapa.br</u>

<sup>c</sup> Federal University of Paraná, <u>www.ufpr.br</u>

<sup>D</sup>Federal University of Rio Grande do Sul, <u>www.ufrgs.br</u>

Contact email: glaucia-amaral@fepagro.rs.gov.br

Keywords: Annual ryegrass, herbage allowance, intake levels, metabolic cages, ruminants.

# Introduction

Given the complexity of evaluating intake on grazing, some compounds have been studied to promote qualitative and quantitative estimates of animal physiology. In this sense, the technique using n-alkanes as a marker has been used in several animal species, especially in grazing ruminants (Dove and Mayes 1996). By definition, validation under grazing or browsing conditions is not possible, because actual intakes are unknown (Dove and Mayes 2005). Thus, the aim of the study was to evaluate the methodology of nalkanes to estimate herbage intake by sheep in metabolic cages.

## Methods

Two experiments were conducted in metabolic cages using annual ryegrass (*Lolium multiflorum* Lam.) at different growth stages with the aim of evaluating the methodology of n-alkanes to estimate herbage intake. The experimental animals were 16 lambs with average live weight (LW) of  $27.1\pm4.10$  kg in the experiment where the pasture was in the pre-flowering stage, and  $32.2\pm3.97$  kg in the flowering stadium. The treatments consisted of four levels forage allowance: 1.5, 2, 2.5 % of dry matter of live weight or *ad libitum* that represented at least 20% of refusals. The experimental design was completely randomized with four treatments and four replicates per treatment. The forage offered to the animals on metabolic cages was cut right before feeding the animals and provided at 8 am and 6 pm. The animals were daily dosed with a pellet containing 100 mg of the external marker dotriacontane n-alkane ( $C_{32}$ ) for a period of twelve days and the total feces collection began on the seventh day. Feces were collected once a day from each animal till 12<sup>th</sup> day. The determination of n-alkanes in forage and in feces samples followed the methodology proposed by Dove and Mayes (2006) in the range of C-chain between 27 and 35. The dry matter intake (DMI) was then estimated according to the equation proposed by Dove and Mayes (1991). DMI were calculated from the ratio of alkanes homologous pairs  $C_{31:32}$  and  $C_{33:32}$ . For the intake measurement during collection days, forage offered and refusals were weighed and forage samples were collected daily for dry matter (DM) analysis. The intake was determined by the difference between the forage offered and refusals on DM basis.

#### Results

In both annual ryegrass growth stages, the n-alkane concentration was high at  $C_{31}$ : 243.27 and 301.36 mg/ kg DM, at pre-flowering and flowering respectively. The study of Laredo *et al.* (1991) showed that for good accuracy of the technique, the concentration of n-alkane present in the forage should be greater than 50. In our study the concentrate-ion of n-alkane  $C_{33}$  at pre-flowering and flower were 60.58 and 52.32 mg/ kg DM respectively.

The DMI data, estimated by the pairs of  $C_{31:32}$  and  $C_{32:33}$  are showed in Figure 1. At the pre-flowering period both pairs showed good accuracy between actual and predicted



Figure 1. Relation between actual dry matter intake (DMI) and predicted DMI for sheep fed fresh ryegrass using  $C_{31}$ :  $C_{32}$  (Figure A) or  $C_{32}$ :  $C_{33}$  (Figure B) in the pre-flowering stage.



Figure 2. Relation between actual dry matter intake (DMI) and predicted DMI for sheep fed fresh ryegrass using  $C_{31}$ :  $C_{32}$  (Figure A) or  $C_{32}$ :  $C_{33}$  (Figure B) in the flowering stage.

DMI. Indoor validation studies have shown that the alkane procedure provides reliable estimates of measured intake in sheep. The faecal recovery of longer alkanes adjacent in chain length is very similar so that intake can be estimated accurately using, for example, dosed  $C_{32}$  alkane and either  $C_{31}$  or  $C_{33}$  alkane from the plant (Dove and Mayes 2005).

At the flowering period neither pair showed good accuracy between actual and predicted DMI. The herbage offered to the animals during the flowering stage was characterized by a predominance of inflorescence and stem (48.6% and 34% of the total herbage, respectively). Probably we were not able to select the herbage selected by animals. According Dove and Mayes (2006), sheep frequently consume a diet which differs in terms of plant parts from the average of the available plant biomass. An accurate estimate of their nutrient intake requires an estimate of both the botanical composition of the diet and of total intake.

### Conclusion

 $C_{31}$  and  $C_{33}$  should be paired with  $C_{32}$  to estimate the dry matter intake in sheep eating annual ryegrass in preflowering stage. When dry matter intakes are estimated at the flowering stage, the herbage sample must to be taking carefully to represent the real amount of inflorescence, leaves and stems ingested by sheep.

## References

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