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Running title: N-alkanes as fecal markers of intake

Use of n-alkanes to estimate feed intake in ruminants: a meta-analysis

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ABSTRACT

Precise techniques to estimate feed intake by ruminants are critical to enhance feed efficiency and to reduce greenhouse gas emissions and nutrient losses to the environment. Using a meta-analysis, we evaluated the accuracy of the n-alkane technique to predict feed intake in cattle and sheep, and assessed the relationships between feed intake and fecal recovery of n-alkanes. The database was composed of 28 studies, including 129 treatments (87 and 42 for cattle and sheep, respectively) and 402 animals (232 cattle and 170 sheep) fed at troughs, from published studies. Relationships between observed (*in vivo* measurement) and predicted feed intake by C31:C32 and C32:C33 n-alkane pairs were evaluated by regression. Meta-regression addressed the relationships between the difference in fecal recovery of n-alkane pairs and the error in intake estimation, as well as the amount and duration of C32 n-alkane dosing. Regression of observed intake on n-alkane-based estimates revealed good relationships in cattle (adjusted $R^2 = 0.99$ for C31:C32, and adjusted $R^2 = 0.98$ for C32:C33; $P < 0.0001$) and in sheep (adjusted $R^2 = 0.94$ for C31:C32, and adjusted $R^2 = 0.96$ for C32:C33; $P < 0.0001$). Fecal recovery of natural n-alkanes showed a coefficient of variation about 15% and 16% for C31 and C33, respectively in cattle. In sheep, the coefficient of variation was 8% and 14% for C31 and C33, respectively. The relationships between the difference of fecal recovery of n-alkane pairs and the error in feed intake estimation in cattle were characterized by an adjusted $R^2 = 0.83$ for C31:C32 ($P < 0.0001$) and adjusted $R^2 = 0.93$ for C32:C33 ($P < 0.0001$). In sheep, they were characterized by an adjusted $R^2 = 0.69$ for C31:C32 ($P < 0.001$) and adjusted $R^2 = 0.76$ for C32:C33 ($P < 0.001$). The n-alkane technique provided the reliability for estimating feed intake in cattle and sheep in barn experiments. The present meta-analysis demonstrated that without correction for differences in fecal recovery of n-alkane pairs, deviation in feed intake prediction would occur. However, further research is necessary to determine the relationship between the n-alkane dosing procedure (daily amount and duration of dosing) and fecal recovery of n-alkane.

Key words: cattle, feed intake, markers, recovery, sheep.

List of Abbreviations: BW, body weight; DM, dry matterFR, fecal recovery; RMSE, root mean square error.

INTRODUCTION

Having accurate techniques to estimate feed intake is critical to evaluate the nutritive value of feed and the nutritional status of livestock. Additional benefit includes the selection for feed efficiency and reduction of greenhouse gas emissions by selecting more efficient livestock. However, individual feed intake is difficult to measure accurately in group-housed and grazing animals because of the lack of reliable methodologies (Penning, 2004). The n-alkane technique was used for estimating the herbage intake in grazing ruminants (Mayes et al., 1986). Errors due to incomplete recovery of n-alkanes would cancel out in intake calculations, when using consecutive pair of n-alkanes with similar fecal recovery rates. Nevertheless, the results from numerous studies were highly variable when the n-alkane techniques were used to estimate feed intake (Azevedo et al., 2014). Meta-analysis is a useful tool that can be used to both summarize the effects of treatment across studies and investigate factors explaining potential heterogeneity of response (Duffield et al., 2008). Mixed model regression methods allow for data from various experiments to be adjusted for random effects associated with trials and weighted for differences in variability associated with a particular study (St-Pierre, 2001; Arelovich et al., 2008). Very few studies have summarized the accuracy of intake estimation by n-alkanes in sheep and cattle, under variable experimental conditions. The hypothesis of the present study was that the accuracy of feed intake estimation is influenced by differences in fecal recovery of n-alkanes. The present study aimed to evaluate the accuracy of the n-alkane technique for estimating the feed intake in cattle and sheep fed at a trough, using a meta-analysis. The second objective was to analyze the relationships between the discrepancies of intake estimation and fecal recovery of n-alkanes, and the effect of the dosing procedure on fecal recovery of n-alkanes.

MATERIAL AND METHODS

Literature review and dataset construction

A literature search was carried out using two search engines: (1) the online databases Agricola (National Agricultural Library, U.S Department of Agriculture) and (2) CAB Abstracts and Global Health on Web of Science (Centre for Agriculture and Bioscience International). Candidate publications were selected using the following keywords: n-alkanes, intake, fecal recovery, ruminant, cattle, and sheep. The initial dataset had 62 studies focusing on feed intake estimation by n-alkane pairs C31:C32 and C32:C33. Thirty-four studies were excluded because they had no information about the observed feed intake (22 studies) and the dosing procedure (7 studies). Five studies were also rejected because they have been carried out with animal species other than cattle and sheep. After discarding these manuscripts, 28 studies were selected (19 for cattle and 9 for sheep), including 129 treatments (87 for cattle and 42 for sheep) and 402 animals (232 cattle and 170 sheep). Each study and treatment were coded by a number. The number of replicates within an experiment and standards errors of responses were included in the database. A template for data extraction was drafted, which included information about the number of replicates within an experiment, animals characteristics (species, sex, number of animals within the study, body weight [BW], age, and performance), diet characteristics (type, number of forage species, feeding levels, crude protein, digestibility, and n-alkane concentration), the procedure of synthetic n-alkane dosing (amount and duration) and fecal collection method. The trial number, literature references, and the number of treatments for the database were recorded (Table 1).

Data processing

For both animal species, the relationships between the observed feed intake (kg DM/d) and predicted feed intake from C31:C32 and C32:C33 n-alkane pairs (kg DM/d) were studied by linear regression. The relationship between the fecal recovery of n-alkanes and the error in feed intake estimation was also considered. For that purpose, the difference of fecal recovery for each pair of n-alkanes was calculated, as follows:

$$\mathrm{DFR}\ (\%) = \frac{(\mathrm{FR\ dosed} - \mathrm{FR\ natural})\ \mathrm{x}\ 100}{\mathrm{FR\ dosed}}$$

Where DFR = difference in fecal recovery (%); FR dosed = fecal recovery of dosed n-alkane; and FR natural = fecal recovery of naturally occurring odd-chain n-alkane.

The discrepancies between the observed and predicted feed intake were calculated, as follows:

$$\mathrm{DI}\ (\%) = \frac{(\mathrm{obs\ intake} - \mathrm{pred\ intake})\ \mathrm{x}\ 100}{\mathrm{obs\ intake}}$$

Where DI = discrepancies in intake estimation; obs intake = observed intake (kg DM/d); and pred intake = predicted intake (kg DM/d)

Statistical analyses

The meta-regression between the independent and dependent variables was analyzed using the Mixed procedure of Statistical Analysis System (SAS Inc., Cary, NC). The independent variable was the observed feed intake (kg DM/d) while the predicted feed intake by n-alkane pairs (kg DM/d) was the response variable. For other relationships, the fecal recovery of each n-alkane and the differences in fecal recovery between the n-alkane pairs (%) were used as independent variables. They were studied with other responses such as: (1) the discrepancies in observed vs. marker-predicted feed intake estimates (%); (2) the daily amount of C32 n-alkane dosing (mg·kg of $\mathrm{BW}^{-1}\cdot\mathrm{d}^{-1}$); and (3) the duration of the dosing (days). The effect of animal species on the fecal recovery of each n-alkane, the discrepancies in intake estimates and the differences in fecal recovery of n-alkane pairs were analyzed by analysis of variance. The average and standard error (SE) for each quantitative variable were recorded. If the SE was not reported, it was calculated by using the standard deviation and the number of replicates. In order to consider relative contributions of individual studies to the total effect estimate, each study was weight according to its number of replicates and SE (St-Pierre, 2001; Arelovich et al., 2008). Briefly, the optimal weight (w_2) was calculated as $w_1/\sqrt{}$ avg(w), where w_1 = 1/SE for a set of means in a study, and avg(w) = mean w_1 value. As described by St-Pierre (2001), this offers the advantage that the sum of $w_2 = 1$, and thus variance and covariance components are on the same scale as the original data.

First, trial-adjusted dependent variable means were computed, using a mixed model that included the random effect of study. Those variables were weighted using the "weight" statement in the Mixed procedure, as described above. Moreover, all independent variables were fitted to a model that first included a fixed slope and intercept and subsequently was corrected by a generated random slope and intercept clustered by study to yield trial-adjusted data. Once determined from mixed model analyses and weighted, trial-adjusted values of dependent variables were regressed between the considered independent variables.

RESULTS

Database characteristics

In the cattle database, each study included on average 11 animals with 5 replicates per treatment, a live BW that averaged 425 kg and a coefficient of variation (CV) of 45% (Table 2). On average, three different forages species were used for each trial, with average crude protein concentration and DM digestibility of 150 and 627 g/kg DM, respectively. The synthetic C32 n-alkane was dosed to animals during 1 to 20 d. The amount of dosed n-alkane averaged 540 mg/d and ranged between 177 and 1,122 mg/d with a CV of 52%. The n-alkane concentrations of the forage diet for cattle averaged 251, 10, and 91 mg/kg DM for C31, C32 and C33, respectively.

On average, 12sheep were used for each trial and five replicates per treatment, with a live BW that averaged 39 kg, and ranged from 30 to 65 kg, with a CV of 27% (Table 3). Two forage species were on average fed to sheep, with 169 and 590 g/kg DM of crude protein and

DM digestibility, respectively. Forage DM digestibility ranged from 573 to 608 mg/kg DM with a CV of 3%. The synthetic C32 n-alkane was administrated to sheep on average 116 mg/d for 14 d. In sheep, the forage diet contained on average 209, 8 and 68 mg/kg DM for C31, C32, and C33, respectively.

Accuracy of the n-alkanes techniques for estimating feed intake

In cattle, observed feed intakes were correlated with estimated feed intakes for both n-alkane pairs (Figure 1). The adjusted coefficient of determination (adjusted R^2) was 0.99 and the root mean square error (RMSE) was 0.65 kg DM/d ($P < 0.0001$) for C31:C32 while adjusted $R^2 = 0.98$ and RMSE = 0.77 kg DM/d ($P < 0.0001$) for C32:C33. In cattle, the observed feed intake ranged from 2.7 to 24.7 kg DM/d (6.3 to 58.1 g DM·kg of $BW^{-1}\cdot d^{-1}$) across various experiments. The estimated feed intake ranged from 2.6 to 24.7 kg DM/d (6.0 to 58.1 g DM·kg of $BW^{-1}\cdot d^{-1}$) and from 2.5 to 24.3 kg DM/d (5.9 to 57.2 g DM·kg of $BW^{-1}\cdot d^{-1}$), when using C31:C32 and C32:C33 ratios, respectively. In cattle, the intercept was estimated at 0.45±0.15 for C31:C32 which was different from 0 ($P < 0.01$). For C32:C33, the intercept was estimated at 0.11±0.15 which was not different from 0 ($P = 0.48$). The slope estimates of 0.90±0.01 and 0.97±0.01 for the n-alkane pairs C31:C32 and C32:C33, respectively were different from 1 ($P < 0.0001$).

In sheep, there were linear regressions between actual and estimated feed intakes (Figure 2) The adjusted R^2 and RMSE were respectively 0.94 and 0.13 kg DM/d for C31:C32 ($P < 0.0001$) while adjusted $R^2 = 0.96$ and RMSE = 0.14 kg DM/d for C32:C33 ($P < 0.0001$). In sheep, actual feed intake ranged from 0.4 to 2.4 kg DM/d (8.9 to 60.1 g DM·kg of $BW^{-1}\cdot d^{-1}$). The feed intake estimated by C31:C32 pairs ranged from 0.3 to 2.3 kg DM/d (8.5 to 58.5 g DM·kg of $BW^{-1}\cdot d^{-1}$), while the C32:C33 pairs ranged from 0.4 to 2.4 kg DM/d (9.0 to 60.8 g DM·kg of $BW^{-1}\cdot d^{-1}$). In sheep, the intercept for C31:C32 was estimated at 0.90±0.04 and had a $P < 0.05$ of being different from 0. Instead, the intercept was estimated at 0.04±0.04 for C32:C33 which was not different from 0 ($P = 0.29$). Likewise, the slope estimates of 0.92±0.04 and

0.98±0.03 for the n-alkane pairs C31:C32 and C32:C33, respectively also had a $P < 0.0001$ of being different from 1.

Fecal recovery of n-alkanes

There was no significant difference between animal species on the fecal recovery of C31 ($P = 0.09$), C32 ($P = 0.20$) and C33 ($P = 0.37$) n-alkanes (Table 4). In cattle, fecal recovery ranged from 0.60 to 1.04, from 0.80 to 1.06, and from 0.63 to 1.26, for C31, C32, and C33 n-alkanes, respectively. In cattle, the fecal recovery had a CV of 15 and 16% for C31 and C33, respectively. In sheep, fecal recovery ranged from 0.79 to 1.04, from 0.75 to 0.97, and from 0.72 to 1.16, for C31, C32, and C33 n-alkanes, respectively. In sheep, the fecal recovery had a CV of 8 and 14% for C31 and C33, respectively. The fecal recovery of synthetic C32 n-alkane showed a similar CV of about 6% among animal species. The difference in fecal recovery in n-alkane pairs was 30% ($P < 0.001$) and 32% ($P < 0.1$) lower in sheep than in cattle for C31:C32 and C32:C33, respectively.

Relationship between the fecal recovery and feed intake

The relationships between the difference of fecal recovery of n-alkane pairs and the error in feed intake estimation in cattle were characterized by an adjusted $R^2 = 0.83$ and adjusted $R^2 = 0.93$, $P < 0.0001$ for C31:C32 and C32:C33, respectively (Figure 3). The RMSE was about 2.74% and 1.75% for C31:C32 and C32:C33, respectively. In cattle, every percentage unit difference in fecal recovery between the n-alkane pair resulted in an error of 2.6% and 0.4% in estimated feed intake by C31:C32 and C32:C33, respectively. In sheep, the relationship had an adjusted $R^2 = 0.69$ ($P < 0.001$) and adjusted $R^2 = 0.76$ ($P < 0.0001$) for C31:C32 and C32:C33, respectively (Figure 4). In sheep, every percentage unit difference in fecal recovery between the n-alkane pair resulted in an error of 0.8% and 2.1% in estimated feed intake by C31:C32 and C32:C33, respectively.

Relationship between the fecal recovery and the procedure of dosing (amount and duration of dosing)

There was no relationship observed between the amount of C32 n-alkane dosing and the fecal recovery of C32 n-alkanes (*P* = 0.17) or between the day of dosing and the fecal recovery of C31 (*P* = 0.71), C32 (*P* = 0.15) and C33 (*P* = 0.12) in cattle (Table 5). The only exception occurred for the linear regression between the amount of C32 n-alkane dosed and the fecal recovery of C31 and C33 by cattle, which had an adjusted $R^2 = 0.69$ and adjusted $R^2 = 0.52$ ($P < 0.0001$), respectively. The relationships between the amount of C32 n-alkane dosed and the fecal recovery of C31 and C33 by cattle were negative. For each unit of C32 dosing (1 mg·kg of $BW^{-1}\cdot d^{-1}$), 85% and 92 % of fecal recovery of C31 and C33 were affected, respectively.

In sheep, there was no relationship between the day of dosing and the fecal recovery of C31 (*P* = 0.26), C32 (*P* = 0.08) and C33 (*P* = 0.81) n-alkanes (Table 6). The negative relationship between the amount of C32 n-alkane dosing and the fecal recovery in sheep had an adjusted $R^2 = 0.25$ for C31 ($P < 0.01$), an adjusted $R^2 = 0$ for C32 ($P = 0.95$) and an adjusted $R^2 = 0.34$ for C33 ($P < 0.001$). For each unit of C32 dosing (1 mg·kg of $BW^{-1}\cdot d\text{-}^{1}$), 92% and 97% of fecal recovery of C31 and C33 were affected, respectively.

DISCUSSION

The advantage of meta-analytic methods is the ability to integrate smaller studies using effect-size metrics and enhance the statistical power over that of any single study. Therefore, they provide the potential to explore new hypotheses (Rodney et al., 2015). This study was designed to explore the effects of fecal recovery of n-alkanes on the accuracy of feed intake estimation in cattle and sheep. The estimation of feed intake by using n-alkane techniques involved simultaneous computing of digestibility, from natural odd-chain n-alkane and fecal output, from dosed even-chain n-alkane, as follows:

$$\text{Feed intake (kg DM/day)} = \frac{\text{Dj}}{(\frac{\text{Fj} * \text{Ri}}{\text{Fi} * \text{Rj}}) * (\text{Hi} - \text{Hj})}$$

where Hi and Fi are the respective concentrations of natural odd-chain alkanes in diet and feces (mg/kg DM); Hj and Fj are the respective concentrations of even chain n-alkanes in diet and feces (mg/kg DM); Dj is the dose rate of even chain n-alkane to animals (mg/day); Ri and Rj are the respective fecal recoveries of natural odd-chain and even chain n-alkanes. In principle, errors arising from the incomplete fecal recovery of used n-alkanes are canceled out in the calculation, if the fecal recoveries of adjacent n-alkanes are similar. N-alkane pairs C31:C32 and C32:C33 were generally proposed to estimate feed intake because they had the lowest discrepancy in fecal recovery and gave better estimation of intake (Dove et al., 2002). Natural n-alkanes C31 and C33 can be found in greater quantities in the cuticular and epicuticular wax of plants, while n-alkane C32, a synthetic compound, is administrated to animals. The high n-alkane concentrations of natural odd-chain alkanes (C31 and C33) and low concentrations of even-chain alkanes (C32) in forage diet in our database are in agreements with the literature (Hu et al., 2014).

Fecal recovery of n-alkanes and accuracy of feed intake estimates

The significant linear regressions between the estimated and measured feed intake in cattle and sheep, indicated the accuracy of n-alkane techniques to estimate this parameter. All the models had adjusted coefficients of determination greater than 0.94 for both pairs of n-alkanes (C31:C32 and C32:C33). Azevedo et al. (2014) reported that there was a difference in feed intake estimation depending on the pair of n-alkane used and generally, n-alkane techniques overestimated the actual feed intake. The accuracy of feed intake estimation relies on the similarity in fecal n-alkane recovery of the dosed and herbage odd-chain alkanes. Our findings are in agreements with Dove and Mayes (1996) who reported a linear relationship between the difference in fecal recoveries of n-alkanes and errors in the estimated feed intake. Every percentage unit difference in fecal recovery between the alkane pair resulted in an error of 1.25% in estimated intake (Dove and Mayes, 1996). However, this proportion is slightly lower than our findings, which were on average 1.52 and 1.45% in cattle and sheep, respectively. Errors in intake estimates were dependent upon animal species and natural n-alkane used. In cattle, the n-alkane ratio C32:C33 seemed to be better while C31:C32 was better in sheep.

Fecal recovery and procedure of n-alkane dosing

The double n-alkane ratio technique involves dosing the known quantities of synthetic n-alkanes of a chain-length adjacent to natural n-alkanes present in plants. It requires an accurate administration method related to the amount and timing of n-alkane dosing. Several carrier matrices were used for synthetic n-alkanes administration to ruminants, such as paper pellets, controlled release capsule and devices, bolus, gelatin capsules, labeled feedstuffs (roughages or concentrates) or feed pellets, and alkane suspensions or oil-in-water. Paper pellets have been the most commonly used method in experiments with cattle and sheep (Giráldez et al., 2004). Smith et al. (2007) reported that the carrier material used, the frequency of dosing, and fecal sampling schedules are factors that may have an influence on the pattern of fecal concentration of dosed n-alkanes. The carrier matrices (method of administration of n-alkanes) were not quantitative variables, unlike the amount and duration of dosing. Therefore, in the present study, they were not analyzed by regression with the intake or fecal recovery of n-alkanes.

The average amount of dosed n-alkanes in this meta-analysis is relatively lower than the amount of C32 n-alkane reported by other studies. Indeed, to have a good accuracy of feed intake estimates, more than 700 to 800 mg/d must be dosed to cattle (Smit et al., 2005). Ferreira et al. (2007) reported a 5-day equilibrium period for n-alkane dosing involving paper pellets and controlled release devices to be adequate. After this period of equilibrium, feces collection has a reduced diurnal variation of n-alkanes in feces. In most cases, the animals were dosed once or twice daily for several days to achieve a steady state situation (Giráldez et al., 2004).

However, when using controlled release capsule and devices, a single administration of C32 n-alkane followed by repeated sampling of feces can be applied.

Fecal recovery and digestive kinetics of n-alkanes

Even as markers, long-chain n-alkanes are not totally recoverable in the feces but can be absorbed endogenously. It has been shown that they are probably taken up by the liver and metabolized mainly to phospholipids or broken down to carbon dioxide (Hargrove et al., 2004). Some authors have reported losses of n-alkanes along the gastro-intestinal tract (Hendricksen et al., 2003). However, the digestive kinetics of n-alkane and their loss through metabolism and absorption merits further study. The passage and kinetics of long-chain n-alkanes into the animal digestive tract are variable according to their source. Indeed, the dosed even-chain n-alkanes are linked with the liquid phase of digesta and pass more rapidly along the digestive tract while natural odd-chain n-alkanes are associated with the particulate phase of digesta (Dove and Mayes, 1991). Therefore, the dosed n-alkane C32 can be recovered in feces in a greater proportion than natural n-alkanes C31 or C33 (Elwert et al., 2008). These are in agreement with our observations in cattle, in which the fecal recovery of C32 n-alkanes had been greater than C31 and C33. Such was not the case in sheep when fecal recovery of alkanes tended to be similar. Many authors reported that the recovery of n-alkanes in sheep is greater and less variable compared with cattle (Dove and Mayes, 1991).

Validation of the models into grazing conditions

Models of feed intake estimation by n-alkane technique were developed in cattle and sheep under stall-feeding conditions. In grazing conditions, studies have been conducted using the n-alkane technique to measure feed intake but either actual feed intake had not been measured or fecal sampling had not been considered (Mann and Stewart, 2003). Indeed, most methods for measuring feed intake in grazing animals presented lack of precision (Smit et al., 2005). Moreover, as the total fecal collection is difficult to perform in grazing animals, literature values of n-alkane fecal recovery have been often used.

Despites these challenges, the developed models in barn experiments merit to be validated under grazing conditions. Some existing methods for measuring feed intake were proposed, such as the method of herbage mass difference before and after grazing, animal live weight difference, comparison of animal requirements and performance, empirical models, animal grazing behavior, and fecal near infrared reflectance spectrometry. However, the choice of the method depends on the objective and the duration of the study. Furthermore, the intra-ruminal controlled-release device technique can be used, which limits animal manipulation. This technique gives less diurnal variability of marker excretion allowing the validity of grab or spot fecal sampling in grazing animals. Grazing ruminants in tropical conditions have a diet based on heterogeneous grasslands, mostly with low nutritive value and large seasonal variations in quantity and quality. It is obvious that feed intake by grazing animals would be very different than in barn experiments. Before using the models to estimate the feed intake of animals on pasture, it is recommended to add more variability in data, such as animals breed, physiological status, body weight, forage species and climatic conditions (temperature and humidity) to be able to get closer to the reality in grazing conditions.

CONCLUSIONS

For more than 30 years, the n-alkane technique provided a valid alternative and reliable method for estimating feed intake in cattle and sheep in barn experiments. The feed intake estimated by both n-alkane pairs C31:C32 and C32:C33 were highly correlated with the observed feed intake. All the models were robust and the coefficients of determination (adjusted R^2) were similar for both pairs of n-alkanes (C31:C32 and C32:C33) and animal species. A linear relationship was observed between the difference in fecal recovery rates of n-alkane pairs used and the error in feed intake prediction. Without correction for differences in recovery of n-alkane pairs, deviation in feed intake prediction will be observed. Fecal recovery rates of n-alkanes were affected by animal species and types of n-alkanes. Effect of the dosing procedure, as the amount and the duration of n-alkane dosing on the fecal recovery rates of n-alkanes merits further study for validation. Validation of the method in grazing animals needs further reflection with the challenge that there is no reliable method as reference. The accurate measurement of feed intake enables to increase pasture management practices, which enhances the animal productivity and therefore, ensures a sustainable food security.

Conflict of interest statement

No actual or potential conflicts of interest which may affect the submission or reviewing this research article or its data were perceived.

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FIGURE LEGENDS

Figure 1. Relationships between observed and estimated feed intake in the cattle database. Regressions between observed intake and n-alkane-based estimates were expressed in kg DM/d in A and B for C31:C32 and C32:C33, respectively, and in g DM·kg of $BW^{-1}·d^{-1}$ in C and D for C31:C32 and C32:C33, respectively. As actual intake increased, n-alkane-based estimates of intake increased for A (Adjusted R^2 = 0.99, RMSE = 0.65, $P < 0.0001$), B (Adjusted R^2 = 0.98, RMSE = 0.77, $P < 0.0001$), C (Adjusted R^2 = 0.56, RMSE = 0.00, $P < 0.0001$) and D (Adjusted R^2 = 0.71, RMSE = 0.00, $P < 0.0001$). The continuous line represented the linear trend line of all data, the discontinuous line represented the y = x line.

Figure 2. Relationships between observed and estimated feed intake in the sheep database. Regressions between observed intake and n-alkane-based estimates were expressed in kg DM/d in A and B for C31:C32 and C32:C33, respectively, and in g DM·kg of BW-1·d-1 in C and D for C31:C32 and C32:C33, respectively. As actual intake increased, n-alkane-based estimates of intake increased for A (Adjusted R^2 = 0.94, RMSE = 0.13, P < 0.0001), B (Adjusted R^2 = 0.96, RMSE = 0.14, P < 0.0001), C (Adjusted R^2 = 0.90, RMSE = 0.01, P < 0.0001) and D (Adjusted R^2 = 0.89, RMSE = 0.01, P < 0.0001). The continuous line represented the linear trend line of all data, the discontinuous line represented the y = x line.

Figure 3. Relationships between the difference in fecal recovery and error in feed intake estimates in cattle for C31:C32 (A) and C32:C33 (B) n-alkane pairs. Every percentage unit difference in fecal recovery between the n-alkane pair resulted to an error of 2.6% in estimated feed intake by C31:C33 (Adjusted R^2 = 0.83, RMSE = 2.74, P < 0.0001) and 0.4% by C32:C33 (Adjusted R^2 = 0.93, RMSE = 1.75, P < 0.0001). The continuous line represented the linear trend line of all data, the discontinuous line represented the y = x line.

Figure 4. Relationships between the difference in fecal recovery and error in feed intake estimates in sheep for C31:C32 (A) and C32:C33 (B) n-alkane pairs. Every percentage unit difference in fecal recovery between the n-alkane pair resulted to an error of 0.8% in estimated feed intake by C31:C33 (Adjusted $R^2 = 0.69$, RMSE = 1.48, $P < 0.001$) and 2.1% by C32:C33 (Adjusted $R^2 = 0.76$, RMSE = 3.18, $P < 0.0001$). The continuous line represented the linear trend line of all data, the discontinuous line represented the $y = x$ line.

Table 1. Summary of the databases including the number of treatments

Database and trial	Reference	No. of treatments
Cattle		
1	Bani et al., 2014	5
2	Berry et al., 2000	4
3	Bezabih et al., 2012	7
4	Chavez et al., 2011	8
5	Chopa et al., 2012	2
6	Ferreira et al., 2004	4
7	Hameleers and Mayes, 1998	5
8	Hendricksen et al., 2003	5
9	Hofstetter et al., 2011	2
10	Molina et al., 2004	2
11	Morais et al., 2011	2
12	Moshtaghi Nia and Wittenberg, 2002	4
13	Oliván et al., 2007	2
14	Ouellet et al., 2004	8
15	Pérez-Ramírez et al., 2011	8
16	Premaratne et al., 2005	1
17	Richmond et al., 2015	8
18	Unal and Garnsworthy, 1999	6
19	Wright et al., 2018	4
Sheep		
1	Amaral et al., 2013	2
2	Charmley and Dove, 2007	4
3	Dove and Oliván, 1998	2
4	Keli et al., 2008	4
5	Lewis et al., 2003	7
6	Lin et al., 2007	6
7	Mayes et al., 1986	12
8	Sibbald et al., 2000	4
9	Vulich et al., 1991	1
	Total	129

Table 2. Number of replications and animals per treatment, animal body weight, forage nutritional and n-alkanes composition, procedure of dosing, observed and predicted intake in cattle

Item	Mean	Median	Mode	Range	SD
Replications/treatment	5	4	4	[2 – 16]	4
No. of animal/treatment	11	8	8	[3 – 32]	9
Animal BW[1], kg	424.9	422.0	422.0	[160.0 – 675.0]	191.3
No. of forage species	3	2	1	[1 - 12]	3
Crude protein, g/kg DM[2]	149.6	127.0	121.0	[68.0 – 254.0]	58.7
DM Digestibility, g/kg	627.4	610.0	596.0	[437.0 – 810.0]	104.4
N-alkanes concentrations in the forage					
C31, mg/kg DM	251.3	203.2	-	[28.1 – 625.5]	181.4
C32, mg/kg DM	9.95	8.25	-	[0.10 – 21.3]	6,64
C33, mg/kg DM	90.7	56.9	-	[3.20 – 422.2]	107.3
Procedure of dosing[3] (synthetic C32 n-alkane)					
Amount, mg/d	539.5	400.0	400.0	[176.8 – 1,122.0]	279.5
Duration, d	12	12	6	[1 - 20]	5
DMI[4], kg DM/d					
Observed	9.06	6.33	4.10	[2.69 – 24.7]	5.95
C31:C32	9.40	7.01	15.7	[2.55 – 24.7]	6.36
C32:C33	9.07	6.41	2.65	[2.52 – 24.3]	6.28

[1] BW: body weight

[2] DM: dry matter

[3] The daily amount of synthetic C32 n-alkanes administered to the animal during the experiment, expressed in mg/d, and the duration of administration, expressed in d.

[4] DMI: Dry matter intake

Table 3. Number of replications and animals per treatment, animal body weight, forage nutritional and n-alkanes composition, procedure of dosing, observed and predicted intake in sheep

Item	Mean	Median	Mode	Range	SD
Replicates/treatment	5	6	6	[2 – 12]	3
No. of animal/treatment	12	12	12	[4 – 24]	6
Animal BW[1], kg	39.3	38	30	[30.0 – 65.0]	10.8
No. of forage species	2	2	2	[1 - 4]	1
Crude protein, g/kg DM[2]	169.4	201	201	[93.0 – 201.0]	40.5
DM Digestibility, g/kg	590.0	598	573	[573.0 – 608.0]	15.1
N-alkanes concentrations in the forage					
C31, mg/kg DM	209.0	207.8	-	[137.0 – 272.3]	58.8
C32, mg/kg DM	7.66	8.03	-	[3.90 – 10.7]	2.92
C33, mg/kg DM	67.7	45.6	-	[26.7 – 142.3]	49.5
Procedure of dosing[3] (synthetic C32 n-alkane)					
Amount, mg/d	116.0	130	130	[50.0 – 200.0]	50.1
Duration, d	14	12	12	[6 – 21]	5
DMI[4], kg DM/d					
Observed	1.03	0.70	2.16	[0.35 – 2.36]	0.63
C31:C32	0.95	0.72	1.15	[0.34 – 2.30]	0.54
C32:C33	1.05	0.72	0.73	[0.35 – 2.39]	0.68

[1] BW: body weight

[2] DM: dry matter

[3] The daily amount of synthetic C32 n-alkanes administered to the animal during the experiment, expressed in mg/d, and the duration of administration, expressed in d.

[4] DMI: Dry matter intake

Table 4. Effect of animal species on fecal recovery[1] of C31, C32 and C33 n-alkanes and difference in fecal recovery of n-alkane pairs

Item	Mean	Range	SE	*P*-value
C31				
cattle	0.83	[0.60 - 1.04]	0.05	0.09
sheep	0.91	[0.79 - 1.04]		
C32				
cattle	0.93	[0.80 - 1.06]	0.02	0.20
sheep	0.90	[0.75 - 0.97]		
C33				
cattle	0.89	[0.63 - 1.26]	0.06	0.37
sheep	0.95	[0.72 - 1.16]		
Difference of fecal recovery in n-alkane pairs[2] (%)				
C31:C32				
cattle	16.10[a]		3.05	< 0.001
sheep	4.81[b]			
C32:C33				
cattle	10.12[a]		3.57	< 0.1
sheep	3.27[b]			

[a,b]The values of mean fecal recovery in the same column with different letters are significantly different ($P < 0.05$).

[1] The fecal recovery is the proportion of ingested n-alkanes recovered in feces

[2] The difference of fecal recovery for each pair of n-alkanes is the ratio between the difference of fecal recovery for dosed and naturally occurring odd-chain n-alkanes and the fecal recovery of dosed n-alkanes, as follows:

Difference in fecal recovery (%) = (FR dosed – FR natural) * 100 / FR dosed

Where FR dosed = fecal recovery of dosed n-alkane; FR natural = fecal recovery of naturally occurring odd-chain n-alkane.

Table 5. Regression relationships between fecal recovery of n-alkanes and procedure of dosing in cattle[1]

Alkane	Intercept	SE	Slope	SE	Adjusted R^2	*P*-value	RMSE[2]	CV (%)
C32 n-alkane dosing (mg·kg of $BW^{-1} \cdot d^{-1}$)[3]								
C31	0.95	0.01	-0.10	0.01	0.69	< 0.0001	0.04	5.36
C32	0.94	0.01	-0.10	0.01	0.02	0.17	0.04	4.47
C33	1.05	0.02	-0.14	0.02	0.52	< 0.0001	0.08	8.74
days of dosing								
C31	0.81	0.02	0.00	0.00	0.00	0.71	0.05	5.99
C32	0.91	0.01	0.00	0.00	0.02	0.15	0.04	4.47
C33	0.84	0.03	0.00	0.00	0.03	0.12	0.08	9.66

[1] The daily amount of synthetic C32 n-alkanes administered to the animal during the experiment, expressed in mg·kg of $BW^{-1} \cdot d^{-1}$, and the duration of administration, expressed in d.

[2] RMSE: root mean square error, which is the standard deviation of the residuals or prediction errors

[3] BW: body weight

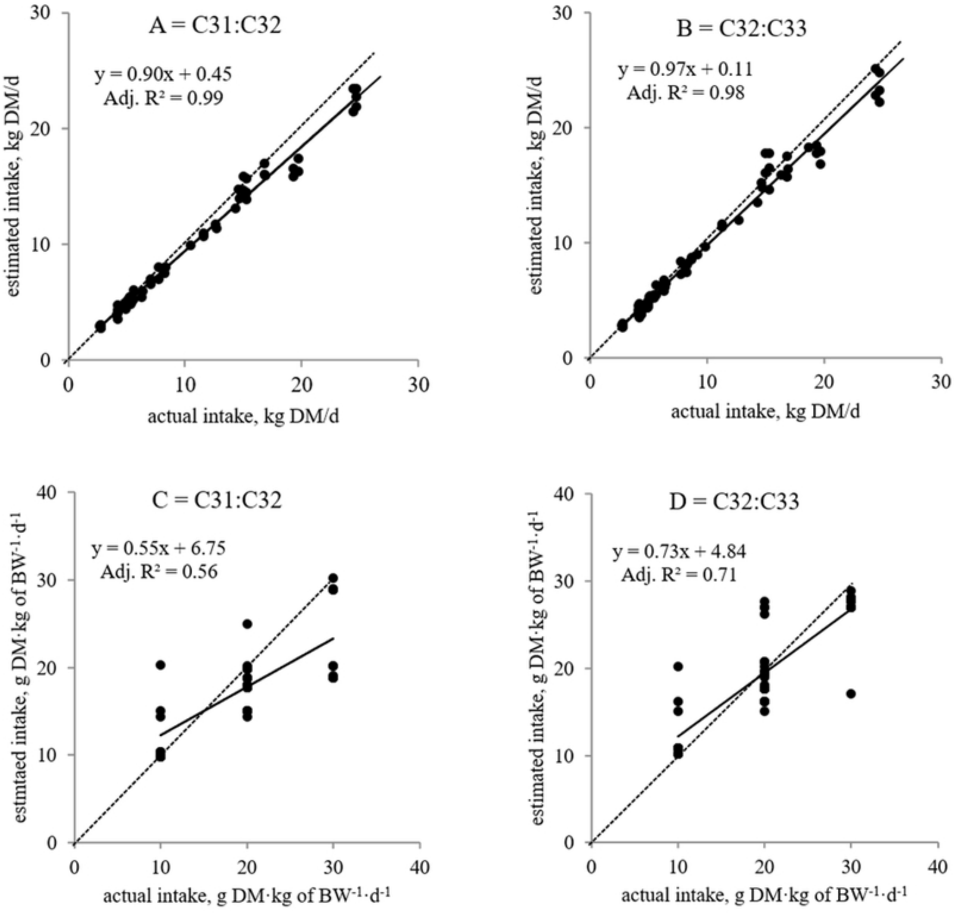
Table 6. Regression relationships between fecal recovery of n-alkanes and procedure of dosing in sheep[1]

Alkane	Intercept	SE	Slope	SE	Adjusted R^2	*P*-value	RMSE[2]	CV (%)
C32 n-alkane dosing (mg·kg of $BW^{-1} \cdot d^{-1}$)[3]								
C31	0.94	0.01	-0.01	0.00	0.25	<0.01	0.03	2.84
C32	0.90	0.02	0.00	0.01	0.00	0.95	0.04	4.37
C33	0.99	0.02	-0.02	0.01	0.34	<0.001	0.04	4.54
days of dosing								
C31	0.93	0.01	0.00	0.00	0.01	0.26	0.03	2.87
C32	0.93	0.02	0.00	0.00	0.07	0.08	0.04	4.62
C33	0.94	0.02	0.00	0.00	0.00	0.81	0.04	4.55

[1] The daily amount of synthetic C32 n-alkanes administered to the animal during the experiment, expressed in mg·kg of $BW^{-1} \cdot d^{-1}$, and the duration of administration, expressed in d.

[2] RMSE: root mean square error, which is the standard deviation of the residuals or prediction errors.

[3] BW: body weight



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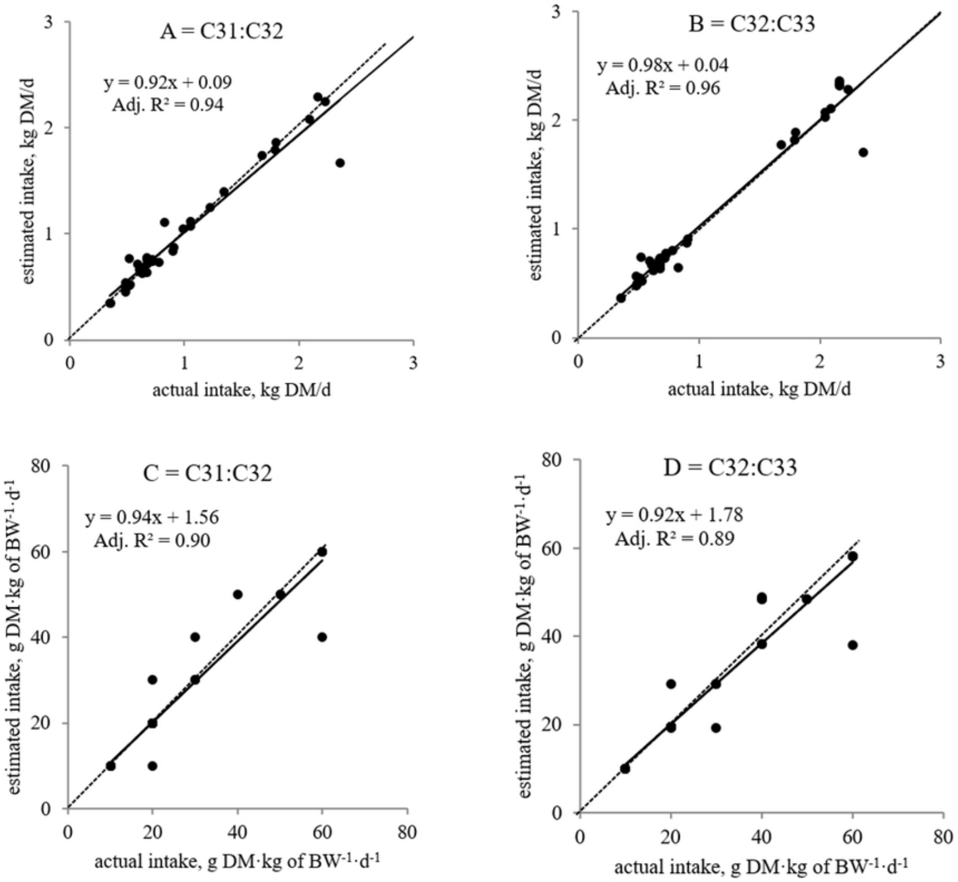

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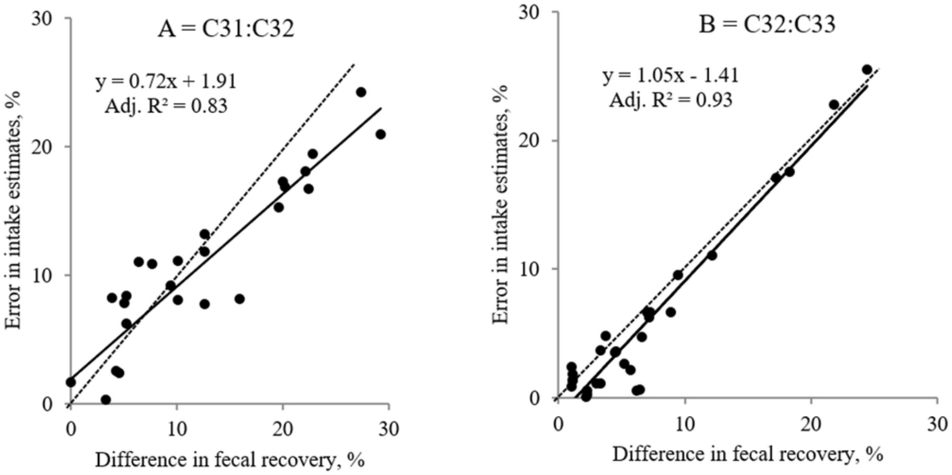

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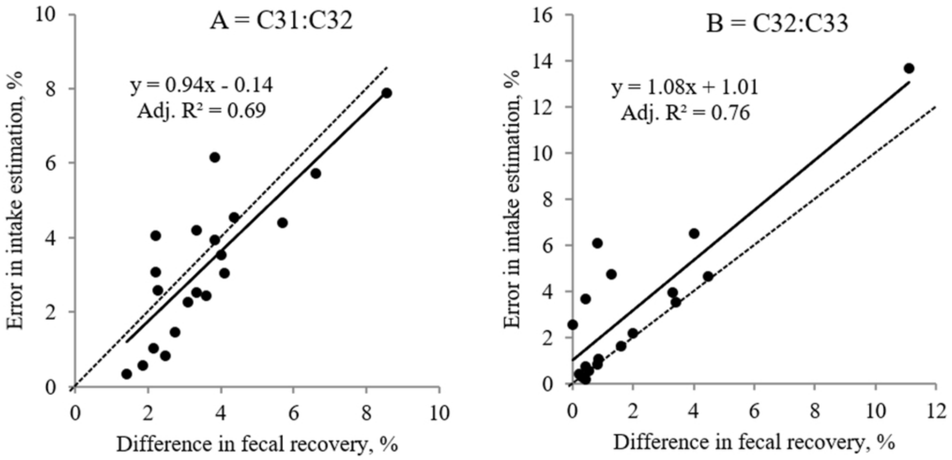

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