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Evaluating internal and external markers versus fecal sampling procedure interactions when estimating intake in dairy cows consuming a corn silage-based diet

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ABSTRACT

Feed intake assessment is a valuable tool for herd management decisions. The use of markers, either internal or external, is currently the most used technique for estimating feed intake in production animals. The experiment used 10 multiparous Holstein cows fed a corn silage-based diet, with 55:45 forage-to-concentrate ratio, the average fecal recovery (FR) of TiO_2 was higher than FR of Cr_2O_3, and both FR were more than unity. With internal markers, acetyl bromide lignin and cutin FR were lower than unity, and average FR for indigestible neutral detergent fiber (iNDF) and indigestible acid detergent fiber (iADF) was 1.5. The FR was unaffected by the fecal sampling procedure and appears to be an intrinsic property of each molecule and how it interacts with digesta. Of the 2 external markers, only Cr_2O_3 produced accurate fecal output (FO) estimates and the same happened to dry matter digestibility (DMD) when iNDF and iADF were used. Estimates for DMD and FO were affected by sampling procedure; 72-h bulk [sub-sample from total feces collection (TFC)] sampling consistently produced accurate results. The grab (sub-samples taken at specific times during the day) sampling procedures were accurate when using either of the indigestible fibers (iNDF or iADF) to estimate DMD. However, grab sampling procedures can only be recommended when concomitant TFC is performed on at least one animal per treatment to determine FR. Under these conditions, Cr_2O_3 is a suitable marker for estimating FO, and iNDF and iADF are adequate for estimating DMD. Moreover, the Cr_2O_3+iADF marker pair produces accurate dry matter intake estimates and deserves further attention in ruminant nutrition studies. The method of dosing the external markers is extremely important and greatly affects and determines results. Whichever the method, it must allow the animals to display normal feeding behavior and not affect performance. The grab sampling procedures can replace TFC (once FR is established), which may open new possibilities for pasture-based or collectively housed animals.
Key words: chromic oxide, intake, indigestible fiber, lignin, titanium dioxide

INTRODUCTION

Dry matter intake is a key determinant of nutrient intake and may be an index of the efficiency with which nutrients are metabolized; therefore, it is the major factor influencing animal performance. Many different strategies have been used over the years to estimate voluntary DMI in ruminants. Behavioral studies (time and rate of intake, mass of bite; Silva et al., 2004; Costa et al., 2011), before and after grazing pasture measurements (Poppi et al., 1987), and multiple regression equations that consider different variables believed to affect intake have been commonly used approaches.

The use of markers is currently the most widely used technique to estimate DMI in ruminants. Markers are indigestible substances that are not secreted or absorbed by the animal, have passage rates similar to feeds, can be recovered completely after ingestion, and allow for practical and precise chemical analysis (Fahey and Jung, 1983). According to Merchen (1993), none of the substances that are used as markers in ruminant digestion studies meet all of the requirements, but some are adequate enough to produce important data for the advancement of research.

The marker technique to estimate intake uses an external marker to estimate fecal output (**FO**) and an internal marker (naturally occurring in feedstuffs) to estimate dry matter digestibility (**DMD**). Intake is then calculated by dividing FO by the indigestibility (1 − DMD) of the feed. The main criticisms of this

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technique are the difficulty and labor of feeding the external markers twice a day, for several days; the need for excessive interaction between animals and people, which may affect intake; and the intensive labor and high cost of feces sample collection.

Total feces collection (**TFC**) is regularly used in intake studies as the standard to which estimates are compared and as the source of bulk samples for later analysis. The recommended number of days for TFC ranges from 7 to 10 d (Schneider and Flatt, 1975) to 3 to 5 d (Ferreira et al., 2008), with authors reporting no significant differences for digestibility estimates. Just as number of days for TFC can be reduced, different fecal sampling designs have been proposed to reduce the short-term bias of estimates (Langlands et al., 1963), which accounts for the inconsistencies of obtaining the feces samples. In this approach, grab samples collected at specific times during the day, either after spontaneous excretion or collected directly from the rectum, are composited to make up the daily samples. Sampaio et al. (2011a) compared 9 different fecal sampling designs and reported that a 4-d sampling period, in which 4 daily grab samples are taken, distributed along the whole day or just during the diurnal period, will produce accurate estimates.

From the above, it can be hypothesized that the combination of internal and external markers, with fecal sampling designs that produce composite grab samples over 3 to 4 d of collection period, should result in accurate estimates for DMD, FO, and DMI in dairy cows consuming a corn silage-based diet.

The objectives of the experiment were to test the accuracy of internal markers, acetyl bromide lignin (**ABL**), cutin, indigestible neutral detergent fiber (**iNDF**), and indigestible acid detergent fiber (**iADF**) for estimating DMD; the external markers Cr_2O_3 and TiO_2 for estimating FO; the combination of internal and external markers for estimating DMI; and to compare 3 different sampling designs against TFC.

MATERIALS AND METHODS

The experiment was conducted at the Dairy Cattle Research Laboratory of the Animal Nutrition and Production Department at the University of São Paulo. The experiment was approved by the Ethics Committee for Animal Use (CEUA) of the University of São Paulo (approval number: CEUA 24420603114). No animals were harmed or became ill during the experiment.

Animals, Diet, and Experimental Design

Ten multiparous Holstein cows (average ± SD; parity: 1.2 ± 0.4 lactations; DIM: 123 ± 53.7 d; milk yield; 25.5 ± 3.8 kg/d; BW: 616.7 ± 67.2 kg) were used for the study. The mouth, tongue, and teeth of all cows were examined before starting the experiment to guarantee absence of wounds or abnormalities that could compromise feed intake. Cows were housed in a covered, concrete floor freestall, divided by gates into individual stalls, each of which had a feed bunk, access to clean water, and a sand-covered bed. Cows were milked twice a day (0700 and 1500 h) and milk yield was recorded during the experimental period.

All cows received a corn silage-based diet, with 55:45 forage-to-concentrate ratio. Daily allowances of the TMR, plus 10% of calculated DMI (to guarantee orts), were fed in 2 equal portions at 0800 and 1600 h, to represent a natural diurnal feeding pattern of cattle. Subsamples of individual feeds and TMR were taken on Mondays, Wednesdays, and Fridays for DM determination [drying in a forced-draft oven (Solab Cientifica, Piracicaba, SP, Brazil) at 55°C for 3 d]. Orts were collected daily so that ad libitum intake could be determined as the difference between the DM offered and refused. The concentrate was composed of coarsely ground corn grains (50.9% of total grain mix DM), soybean meal (25% of total grain mix DM), and whole roasted soybeans (8.8% of total grain mix DM), cottonseed meal (10.2% of total grain mix DM), and mineral mix (4.4% of total grain mix DM). Cottonseed meal was added to replace part of the soybean meal, due to its high contents of cutin (from added cotton hulls). Increasing the cutin concentration of the diet was desirable to facilitate analysis and decrease variability of results. Composition of silage, concentrate, and TMR are shown in Table 1.

The experiment ran for 19 d (day regarded as the 24-h period from 0800 to 0800 h). The experimental period consisted of 3 phases: d 1 to 5 were allocated to adaptation of the animals to the diet; 10 d (d 5 to 15) of marker excretion stabilization and the final 4 d (d 16 to 19) were for sample collection. The animals were adapted to the experimental conditions during 14 d before the experimental period. The 10 cows were distributed into a split-plot design, with the main plot (markers) being completely randomized and the sampling designs as sub-plots.

Markers

Four internal markers (ABL, cutin, iNDF, and iADF) were used to estimate DMD and 2 external markers (Cr_2O_3 and TiO_2) were used to estimate FO. The 8 combinations (1 internal marker and 1 external marker) derived from these 6 markers were used to estimate voluntary DMI. Estimates of DMD, FO, and DMI were compared against their reciprocal total-tract apparent