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Letter to the Editor: Recovery test results as a prerequisite for publication of gaseous exchange measurements

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In the last decade, various applications of gaseous exchange measurements have been developed to quantify the production or consumption of particular gases by animals. Notably, booming research into methane emissions has led to an expansion of the number of facilities in which such measurements are made. Results of a ring test calibration of respiration chambers in the UK by Gardiner et al. (2015) confirmed our concern that not all research groups comply with the same standards of chamber operation. Three potential sources of experimental error (viz. analyzer error, ducting efficiency from chambers to analyzers including measurements of airflow, and chamber mixing) were evaluated by Gardiner et al. (2015) by testing the recovery of a calibrated reference source of ultra-high-purity methane standard (6 facilities, 22 individual chambers). It is alarming to read that full system recoveries of these respiration chamber facilities to vary between 59 and 115%. Ducting, including airflow measurement, was the largest source of variation within and between respiration chambers and facilities. If recoveries differ significantly from 100% or appear to vary, then it is good practice to identify the error. Use of correction factors to compensate for the over- or under-recovery is bad practice and may lead to incorrect interpretation of results (McLean and Tobin, 1988). Working with recoveries deviating from 100% would require

validation that this deviation is stable in time, volume and concentration, and requires assumptions on proportionality in the correction factor.

We are convinced that unacceptable recoveries such as reported by Gardiner et al. (2015) also occur elsewhere. From two courses on indirect calorimetry that were organized directly following the International Symposium on Energy and Protein Metabolism and Nutrition (ISEP) conferences in 2013 (Davis, California) and 2016 (Krakow, Poland), we learnt that recovery tests are not consistently performed around the world. Volume 99 and 100 of the *Journal of Dairy Science* include 26 publications in which methane production of ruminants was measured quantitatively, and 19 publications (73%) failed to report recoveries. The urge to perform such tests has also recently been emphasized by Hammond et al. (2016) in their review of in vivo measurement techniques (chamber and non-chamber techniques such as automated head chambers). Regardless of the method chosen, Hammond et al. (2016) argued that appropriate recovery tests are required for both method development and routine operation.

For all techniques aiming at quantification of gas exchange or production, notably indirect calorimetry, methane emissions by chamber technique, ventilated hood or head box techniques, we believe that full system recovery tests of the relevant gases should be performed. Such tests should be performed immediately prior to or following the measurement periods. Determination of recovery rates on a regular basis would allow the determination of recovery rate variability over time. The timing relative to the experiment and the results of these tests for each chamber or unit should be reported in the materials and methods section of the manuscript. Procedures for recovery tests are fairly simple and can easily be found in several textbooks (McLean and Tobin, 1988; Gerrits and Labussière, 2015).

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