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Technical note: Evaluating nuclear magnetic resonance spectroscopy for determining body composition in Holstein dairy calves using deuterium oxide dilution methods

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ABSTRACT

Deuterium oxide (D_2O) dilution methods have been used to assess body composition in live animals. Estimated body water content can be used to predict body fat and protein, and thus, the amount of energy reserves. It is an alternative method to direct chemical analysis and considered a noninvasive technique that is economical and repeatable. Deuterium oxide use is considered easy, safe, and accurate; however, the traditional methods of analyzing D_2O are expensive, tedious, and time consuming. The objective of this study was to evaluate the potential for using nuclear magnetic resonance spectroscopy (NMR) to determine body composition in Holstein dairy heifers. Nuclear magnetic resonance is less expensive and requires minutes to calculate the percentage of D_2O in the blood. This study used 24 newborn dairy heifer calves blocked by birth and randomly assigned to 1 of 3 treatments: (1) 446 g dry matter (DM) of a conventional milk replacer (MR) [CON; 20% crude protein (CP), 20% fat], (2) 669 g DM of a moderately high protein MR (MOD; 26% CP, 18% fat), or (3) 892 g DM of a moderately high protein MR (aggressive, AGG; 26% CP, 18% fat). All calves had free-choice access to starter and water. Both MR and starter were medicated with decoquinate. During weaning (d 43 to 49), the morning MR feeding ceased. On d 50, all MR feedings ended but starter and water intakes were continuously recorded until d 56. When calves were 50 d of age, a baseline blood sample was taken followed by injection of 300 mg of D_2O/kg of body weight in sterile physiological saline (0.9%). The syringes containing the D_2O in physiological saline were weighed before and after administration to record the actual dose of D_2O injected gravimetrically. After injection, the D_2O was allowed to equilibrate with body water for 1 h. Six blood samples were taken over 6 d (1/d) at 1630 h to estimate the dilution of the tracer. The plasma was aspirated and stored at −20°C until further D_2O analysis. This new method was validated using 4 calf plasma samples that were sent to an outside laboratory for measurement using an independent validation method. We detected no differences in total body water, protein, fat, or mineral content in calves fed CON, MOD, or AGG; however, results demonstrated that the D_2O dilution technique and analysis by NMR is an appropriate and easy method to estimate water, protein, ash, and fat in young heifers.
Key words: deuterium oxide, nuclear magnetic resonance, body composition, heifer

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The composition of lean body mass is relatively constant across species, and knowing body water content can be used to understand the composition of body fat, protein, and minerals (Byers, 1979). Water makes up about 73% of the lean body; it is the largest component and has become the choice for measuring body composition; however, measurement of the size and kinetics of aqueous pools in the digesta of ruminants is complicated by unknown contributions of variable magnitude from the digestive tract (Byers, 1979). Deuterium oxide (D_2O) methods have been used to differentiate these pools such that other body compartments can be estimated for growth (Byers, 1979).

Traditionally, the most accurate way of determining body composition was to slaughter the animal and then grind and analyze the entirety of the animal's tissues (Jesse et al., 1976). This method is precise but precludes measurement of further growth. In order to measure changes in composition over time, studies have conducted serial slaughter techniques where a large population of similar animals are allocated to treat-

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ments with a representative group on each treatment slaughtered at desired intervals of time or size (Byers, 1979). However, this method assumes that all animals are similar in body composition and that all respond similarly to treatments, making this method less accurate or valid. It is necessary to develop methods to precisely estimate body composition and composition of growth as it occurs in the live animal.

Numerous indirect methods have been used to determine total body water and are based on the dilution principle of water-soluble tracers such as antipyrine or urea, but they give rise to errors because tracers are bound to proteins, are rapidly metabolized and excreted, or are slowly and incompletely distributed to all the body water components (Novak, 1967; Schoeller et al., 1980). The best and most frequently used tracers have been water labeled with either tritium or deuterium (Novak, 1967; Schoeller et al., 1980); however, the use of deuterium has the disadvantage of tedious and time-consuming analysis, whereas the use of tritium involves a radiation hazard (Schoeller et al., 1980).

Deuterium oxide dilution methods can be used to predict estimated body water content, which can then be used to predict body fat and protein (Panaretto and Till, 1963). Deuterium oxide dilution methods for estimation of total body water have been used for assessment of body composition for many species including pigs, beef steers, dairy cows, dairy heifers, sheep, and goats (Novak, 1967; Shipley and Clark, 1972; Byers, 1979; Schoeller et al., 1980; Shields et al., 1983; Andrew et al., 1995; Auchtung et al., 2002; Rius et al., 2005) and are considered noninvasive techniques that are economical and repeatable. The D_2O dilution method measures the body water turnover rate and is safe, nonradioactive, and accurate. It was the method used to measure body water turnover in doubly labeled water studies of long-term metabolic rate (Schoeller et al., 1980). Estimated body water content can be used to predict body fat and protein, and thus, the amount of energy reserves (Panaretto and Till, 1963). Panaretto and Till (1963) found that total body fat and total body water were inversely correlated in mature castrated male goats ($r = -0.97$). Rius et al. (2005) compared D_2O methods and direct chemical analysis to assess body composition at 5 and 7 mo of age. In 5-mo-old heifers, correlations between estimates of body protein, water, and mineral contents as determined by D_2O dilution methods and direct chemical analysis of body tissue were 0.86, 0.85, and 0.76, respectively, but fat values were not correlated ($r = -0.068$). Those authors were unable to accurately estimate body composition using the D_2O dilution method for 7-mo-old heifers and attributed this to the gastrointestinal tract in young animals not being fully developed.

Studies have demonstrated that feeding calves a milk replacer (**MR**) with >25% CP at an increasing rate with age up to 900 to 1,200 g of DM/d can facilitate rapid and efficient early lean growth and allow for increased body protein deposition and growth rate without excessive fattening (Diaz et al., 2001; Tikofsky et al., 2001; Blome et al., 2003; Bartlett et al., 2006). However, in these studies, calves were slaughtered. Using D_2O might be an easy, inexpensive, and valid method for determining the composition of growth in calves fed using these aggressive MR feeding programs instead of direct chemical analysis. Deuterium oxide dilution methods will also allow for measurement of further growth. To date, nuclear magnetic resonance spectroscopy (**NMR**) has not been used to estimate body composition through D_2O dilution methods in animals. An NMR-based method has been used to determine total body water in humans (Khaled et al., 1987). Those authors validated the method against a standard infrared absorption procedure using a tracer dose of D_2O and concluded that the NMR method was fast, accurate, needed only a small dose of D_2O, and did not require any sample preparation. The objective of the current study was to evaluate the potential for using NMR to determine body composition in Holstein dairy heifers.

Twenty-four Holstein heifer calves (initial BW, mean ± SD) of 41.6 ± 4.8 kg were blocked by birth date and randomly assigned at birth to 1 of 3 treatments in a randomized complete block design. The treatments were (1) 446 g DM of a conventional MR (**CON**; 20% CP, 20% fat), (2) 669 g DM of a moderately high protein MR (**MOD**; 26% CP, 18% fat), or (3) 892 g DM of a moderately high protein MR (aggressive, **AGG**; 26% CP, 18% fat). Both MR products were provided by Provimi North America (Brookville, OH) and medicated with decoquinate. The 56-d study was conducted from August 2013 to March 2014. Calves were fed a 20.7% CP textured starter (Provimi North America) and water ad libitum starting at 2 d of age and continuing for the entire 56-d trial. Initial BW and skeletal measurements were taken before calves started MR treatment. Calves on CON were fed 227 g of 20:20 (CP:fat) MR reconstituted to 10.7% solids (at 0800 and 1700 h) from d 2 to 42 (1.89 L twice daily). Calves on MOD were fed 340 g of 26:18 (CP:fat) MR reconstituted to 13% solids (at 0800 and 1700 h) from d 2 to 42 (2.27 L twice daily). Calves on AGG were fed 340 g of 26:18 (CP:fat) MR reconstituted to 13% solids (at 0800 and 1700 h) from d 2 to 6 (2.27 L twice daily) and then 454 g of 26:18 (CP:fat) MR reconstituted to 13% solids (at 0800 and 1700 h) from d 7 to 42 (3.03 L twice daily). On d 43 to 49, calves on all treatments were fed once daily at 0800 h. Calves were fed MR and