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Validation of the ^{13}C-bicarbonate tracer technique for determination of CO_2 production and energy expenditure in ponies by indirect calorimetry

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A B S T R A C T

Four Shetland ponies were used to validate the ^{13}C-bicarbonate technique (^{13}C-BT) against indirect calorimetry (IC) for determination of CO_2 production and estimation of short term energy expenditure (EE), when a single bolus of ^{13}C-bicarbonate was given either as an oral or intravenous (IV) dose. The study was divided into two experiments. In experiment 1 the ponies were placed in respiration chambers making it possible to compare the ^{13}C-BT with IC, and to find a suitable respiratory quotient (RQ) and recovery factor (RF) of ^{13}C in breath CO_2 needed for the calculations of EE. In experiment 2 the ponies were measured in the stall and breath samples were collected with a mask and breath bags. There was no effect of the methods used in experiments 1 and 2 (IC, ^{13}C-BT$_{IC}$ or ^{13}C-BT$_{stall}$) on the measured CO_2 production ($P>0.05$) and the estimated EE ($P>0.05$). There was no effect ($P>0.05$) of administration route (IV or oral) on the RQ-value (RQ=0.794), but there was an effect ($P=0.026$) of route on the RF ($RF_{IV}=0.690$; $RF_{Oral}=0.760$). The average RQ and the respective RF for IV and oral administration of ^{13}C-bicarbonate were used for the calculations. This validation study against IC showed that the ^{13}C-BT can be used to determine CO_2 production for estimation of EE under resting conditions in ponies, independent of administration route of ^{13}C-bicarbonate. The results from IC were similar to measurements performed in the stall under normal resting conditions, where samples were taken with a mask and breath bags.

1. Introduction

Knowledge on the energy requirements of horses is essential for optimal feeding practice, and guidelines have been given for different types of horses in different physiological stages such as pregnant mares, growing or exercising horses (NRC, 2007; Martin-Rosset et al., 1994). However, energy expenditure (EE) and corresponding requirements are very complex and not easy to determine under field conditions. The gold standard for measuring EE is indirect calorimetry (IC) with measurements of O_2 consumption and CO_2 production making it possible to calculate EE according to Brouwer (1965). A disadvantage of this method is that the horse has to be acclimatized to and confined in a respiration chamber, and furthermore, advanced and expensive research facilities are needed. An alternative to IC is the use of stable isotope tracer techniques. The doubly labelled water (DLW) technique has been used widely in human studies for

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measuring EE (Speakman, 1998), and Fuller et al. (2004) validated the DLW technique against IC in ponies. However, due to the cost of the isotope ^{18}O the DLW method has limited application to experiments with large animals like the horse.

The ^{13}C-bicarbonate technique (^{13}C-BT) can, similar to the DLW technique, be used for indirect determination of the CO_2 production and hence estimate EE. Whereas the DLW method is expensive and measures long term EE (days to weeks), the ^{13}C-BT is relatively cheap and measures short term EE (hours). The ^{13}C-BT method is based on ^{13}C kinetics in breath or blood CO_2 after administration of ^{13}C labelled sodium bicarbonate ($NaH^{13}CO_3$), where the ratio between the ^{13}C and ^{12}C in samples of expired CO_2 can be used as a measure of the CO_2 production rate (Elia, 1991). The ^{13}C-BT has been used to determine EE in cattle (Junghans et al., 2007; Kaufmann et al., 2011), goats (Prieto et al., 2001), dogs (Larsson et al., 2014) and humans (Junghans et al., 2008) with success. Oral administration of ^{13}C-bicarbonate in a single bolus has been used by Junghans et al. (2008) and Larsson et al. (2014) in humans and dogs, respectively, making the ^{13}C-BT non-invasive. Urschel et al. (2009) used ^{13}C-bicarbonate to determine CO_2 production in horses for calculating the rate of amino acid oxidation, and found continuous intravenous infusion of ^{13}C-bicarbonate to give better estimates than frequent oral administration. However, the methods to be used in the present experiment differ from those of Urschel et al. (2009).

The ^{13}C-BT, contrary to IC, may be used under completely free living conditions with minimal interference with the natural life style of the horse. However, the ^{13}C-BT only assesses CO_2 production and an estimate of the respiratory quotient (RQ) is required for calculating EE. The recovery factor (RF) of the ^{13}C dose also needs to be determined, as the administered amount of ^{13}C is not recovered completely in breath $^{13}CO_2$ (Elia, 1991). Combining the ^{13}C-BT with IC makes it possible to determine both a RQ and a RF suitable for sedentary horses, and validate the accuracy of the ^{13}C-BT for determination of CO_2 production and estimation of EE.

The aim of the present experiment was to validate the ^{13}C-BT against IC, the gold standard method for determining EE. The main hypothesis was that the ^{13}C-BT, with oral or IV administration of the tracer in a single bolus, can be used for accurate determination of EE in equines, and hence with oral tracer administration and collection of breath samples provide a non-invasive alternative to other methods.

2. Materials and methods

2.1. Experimental design

The experimental procedures followed the Danish National Legislation and guidelines approved by the member States of the Council of Europe for the protection of vertebrate animals (Anonymous, 1986). The entire experiment lasted for 4 weeks and was divided into two experiments each lasting 2 weeks. The first experiment involved measurements in respiration chambers, and in experiment 2 the ponies were measured in the stall under normal resting conditions. In both experiments IV and oral administration routes of ^{13}C-bicarbonate were used, and each pony was measured twice in the respiration chamber and twice in the stall using both ^{13}C-bicarbonate administration routes.

2.2. Animals, housing and management

Four 3–4 years old Shetland ponies, one stallion and three mares, with a body weight (BW) of 178 ± 46 kg were borrowed from private owners. The four ponies arrived to the laboratory 1 week prior to the start of the experiment to acclimatize to the new location, and they were housed individually in an unheated barn (average temperature measured daily at 08:00 h: 10.6 ± 2.3 °C) in 3×3 m^2 stalls with wood shavings as bedding material. All ponies had a health control by a veterinarian prior to the start of the experiment. The ponies were hand walked or allowed access to a dirt paddock for 15 min 4 times a week during the experiment. Hay was purchased from a local farmer (chemical composition: dry matter: 85%; in % of dry matter: ash: 4%, NDF: 72%, ADF: 42%, lignin: 5%, crude protein: 8%, sugars: 12%, all analytical procedures are described by Jensen et al. (2010)), and the ponies were fed two daily equally sized meals at 08:00 h and 18:00 h ($\sim$16 g dry matter/kg BW). Water was available ad libitum at all time in the stall as well as in the respiration chambers.

2.3. Experiment 1, measurements in the respiration chambers

In experiment 1 the ponies were placed under resting conditions in open-air-circuit respiration chambers (volume: 3.5 m^3, air flow: 6000 l/h) for determination of CO_2 production and O_2 consumption. Construction and function of the chambers have been described previously by Chwalibog et al. (2004). The ponies were habituated to the respiration chambers, and they were placed in the chambers in the morning 1 h before measurements started. Either an oral or IV bolus dose (2.5 mg/kg BW) of $NaH^{13}CO_3$ (98 at% ^{13}C, Sigma Aldrich, St. Louis, USA) was given to the pony in the respiration chamber immediately prior to feeding the morning meal, allowing for comparison of the two methods (IC vs. ^{13}C-BT_{IC}). When the dose of ^{13}C-bicarbonate was given orally it was mixed in a 10 ml syringe with water ($\sim$5 ml) and syrup ($\sim$5 ml). The syringe was placed as far back in the mouth of the pony as possible before the dose was given. A sterile solution of ^{13}C-bicarbonate for IV administration was prepared at the University pharmacy (50 mg $NaH^{13}CO_3$/ml 0.9% saline), and it was injected into the jugular vein. The chamber was opened briefly when the oral dose was given, and with the IV dose the pony was quickly removed, injected and returned into the chamber. From the gas exchange measurements RQ and RF were determined to be used in the calculations. An infrared isotope analyser (IRIS, Wagner Analysen Technik GmbH, Bremen, Germany) was connected to the respiration chambers and the ^{13}C-enrichment of CO_2 in air leaving the chambers was