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Creatinine and pseudouridine in plasma and urine from Brahman-cross steers fed a low, medium or high plane of nutrition

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Abstract

Metabolic state as influenced by growth rate may influence meat toughness and can be estimated from metabolites excreted in urine. Urine collection over 24 h requires animals to be constrained in metabolism crates for many days. Single blood sampling to estimate metabolites in plasma would be less stressful on animals than collecting 24 h urine excretion. This study investigated the hypothesis that the plasma concentrations of pseudouridine and creatinine were representative of those found in 24 h urine excretions in steers fed different quality diets. Eleven Brahman-cross steers were fed a high ($n=3$), medium ($n=4$) or low ($n=4$) quality hay diet for three weeks. Steers were catheterized and housed in metabolism crates for 6 days. Urine was collected every 24 h and total volume sub-sampled for analyses of creatinine and pseudouridine. Jugular blood was collected from each steer every 3 h from 07:30 to 16:30 h. Plasma was separated from red blood cells by centrifugation and frozen for creatinine and pseudouridine analyses. No time of day effect was apparent for pseudouridine or creatinine so daily means were used to test for effect of diet and to relate to urinary concentrations. Nutritional restriction halted live weight gain but had no effect on urinary or plasma pseudouridine, suggesting that diet did not affect tRNA turnover. Increased plasma creatinine concentrations and reduced urinary creatinine concentration in steers experiencing nutritional restriction indicated that their renal clearance rate decreased. As a result, the ratio of plasma pseudouridine to creatinine concentration was not directly proportional to that of 24 h urinary excretion.

Keywords: Beef; Pseudouridine; Creatinine; Metabolism

Increased toughening of beef from cattle growing slowly prior to slaughter has been attributed to decreased post mortem muscle proteolysis due to reduced protein turnover in the live animal (Andersen et al., 2005). The balance between protein degradation and synthesis in the live animal determines the proteolytic state of muscle, and may be estimated in the live animal from metabolic indicators of protein turnover such as 3-methylhistidine expressed relative to creatinine. Creatinine is used as the denominator for between animal comparisons because creatinine excretion is indicative of renal filtration rate (Valtonen et al., 1982). It is also indicative of muscle mass because creatinine is formed when creatine spontaneously

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cyclizes after creatine phosphate is dephosphorylated by creatine kinase during short-term energy repletion (Borsook and Dubnoff, 1947). Creatinine is not metabolized once formed (Cowgill and Freeburg, 1957) and is therefore produced in a constant ratio to the amount of muscle present (Narayanan and Appleton, 1980).

Fractional protein synthesis can be quantified by measuring the incorporation of radiolabelled amino acids into muscle (Millward and Bates, 1983) but can also be estimated by measuring the 24 h urinary excretion of pseudouridine (Shingfield and Offer, 1998). Pseudouridine is a nucleoside isomer of uridine that exists in ribonucleic acids as a carbon–carbon linked ribonucleside on the TφC arm of transfer RNA (tRNA) (Lehninger, 1982) and it is released without further processing when tRNA is degraded (Weissman et al., 1962). Puchala et al. (1993) validated the use of pseudouridine as an indicator of tRNA degradation by showing that infusion of RNA into the rumen did not increase its excretion in the urine over 24 h. Although not a specific indicator of tRNA degradation in skeletal muscle, the amount of pseudouridine excreted in urine over 24 h is useful as a metabolic indicator of whole body protein synthesis.

Measurement of metabolites in urine often requires the use of metabolism crates to accurately collect and assess the volume of urine produced every 24 h over a few days. Measuring these metabolites in blood would be faster and more convenient than measuring them in urine but the validity of this estimation must be established because the concentrations of these metabolites could fluctuate diurnally or with feeding. No such validation has been performed for pseudouridine, however, and is required before confidence can be placed in plasma concentrations. To address this requirement, this study compared the concentrations of pseudouridine and creatinine in plasma to those in 24 h urinary excretions of Brahman-cross steers on three different diets in order to test the hypothesis that the concentrations of these metabolites in plasma in cattle experiencing different growth rates reflect those in 24 h urine excretions.

1. Materials and methods

1.1. Sample collection

All animal procedures were performed in accordance with the Commonwealth Scientific and Industrial Research Organisation animal ethics guidelines. Reverter et al. (2003) and Byrne et al. (2005) have presented details of the animals and diets used and the results from gene expression studies in this experiment. Briefly, eleven Brahman-cross steers with a mean live weight of 302 kg±31 kg (standard deviation (SD)) were identified for the experiment. The steers were grazed on lush pasture for 14 days prior to the beginning of the study, then weighed and randomly allocated to three diets such that the mean weight of the steers in each diet was approximately equal on study day 0. Of the eleven steers, three were assigned to receive a diet of high quality hay (control diet) consisting of *ad libitum* lucerne hay (*Medicago sativa*) that was designed to allow a body weight gain of 0.8 to 1.0 kg/day and represented a high plane of nutrition. A further four steers were assigned to a medium quality diet that consisted of restriction feeding of the same high quality lucerne hay (*M. sativa*) to produce a moderate weight gain of about 0.3 kg/day and represented a medium plane of nutrition. The final four steers were allocated to a low quality diet consisting of *ad libitum* Angleton grass hay (*Dichanthium aristatum*) to produce a weight loss of 0.3 kg/day, which represented a low plane of nutrition. Diets were fed to the steers for the duration of the experiment, which was 28 days. Steers were housed from days 0 to 21 in individual pens and from days 22 to 28 in metabolic crates, all under cover from sun and rain. Feed intake was estimated daily by subtracting feed remaining from feed supplied and the steers were weighed on study days 7, 14, 21 and 28.

On day 21 of the experiment, the steers were catheterized for serial blood sampling. Cattle were allowed to recover from catheterization overnight in their respective pens, and were then moved to individual metabolic crates the following day. Total urine produced over 24 h by each steer was collected from days 22 to 28 into plastic reservoirs of known weight with a small amount of 50% sulfuric acid included as a preservative. A 100 mL sub-sample of each 24 h collection was retained and stored at −20 °C until analysis for creatinine and pseudouridine. Blood was also collected from days 22 to 28 four times over each 24 h period at 07:30 (before feeding), 10:30, 13:30 and 16:30 h into evacuated, heparinized test tubes (Vacutainer, Beckman Scientific) and placed in ice until plasma was collected using centrifugation at 4 °C, 1200× *g*, for 25 min. During blood collection, each catheter was flushed with heparinized saline and the first 5 mL of blood drawn through the catheter were discarded. Plasma was frozen at −20 °C until analyzed for creatinine and pseudouridine.

1.2. Measurement of creatinine and pseudouridine

Creatinine and pseudouridine concentrations were estimated in the urine and blood samples according to the methods of Hewavitharana and Bruce (2003a,b).

1.3. Estimation of plasma clearance in kidneys

The ability of the kidneys to clear creatinine and pseudouridine from the plasma was estimated using the method outlined by Guyton (1982) where:

$$\text{Plasma clearance (mL/min)} = \frac{(\text{Quantity of urine mL/min} \times \text{Concentration in urine mg/mL})}{\text{Concentration in plasma mg/mL}}$$