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Technical note

Technical Note: Can tail arterial or tail venous blood represent external pudic arterial blood to measure amino acid uptake by mammary gland of cows?

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ABSTRACT

Sampling blood from the artery supply and venous drainage of the udder of dairy cows is essential for estimating the uptake of amino acids (AA) by the mammary gland using the arteriovenous difference approach. Since it is difficult to sample blood form the external pudic artery, finding a representative alternative was necessary. In this experiment, 13 lactating Holstein dairy cows were used to validate whether blood from the tail artery or tail vein could be used a substitute for external pudic arterial blood for calculation of amino acid (AA) extraction rate by the mammary gland. The results showed that no significant differences were noted in the individual AA concentration ($P > 0.10$) between the tail arterial, tail venous blood and external pudic artery, except for Asp, Thr and Pro. The calculated extraction rates based on the A-V difference between the external pudic artery, tail artery or tail vein and the subcutaneous vein were not different for individual AA ($P > 0.10$) except for those rates for Asp, Thr and Glu ($P < 0.10$). These results indicated that blood sample from the tail artery or tail vein could be used as an alternative to external pubic arterial blood for studying the uptake of most AA, but not for Asp, Thr and Glu, by the mammary gland of dairy cows.

Collection of arterial and venous blood samples is essential for estimating the uptake of precursors for synthesis of milk components by the mammary gland using the arteriovenous (A-V) difference method in studies on mammary gland metabolism. The blood samples must be representative of the artery supply and venous drainage of the bovine udder. Venous blood can be easily sampled from the subcutaneous abdominal veins in dairy cows (Graham et al., 1936; Cant et al., 1993). However, the external pudic artery that supplies blood to the udder is embedded deeply in the muscle, and it is hard to sample the arterial blood. There are a few of alternatives, however, for collecting representative arterial blood samples in researching on the mammary gland metabolism. The best approach for sampling blood is by implanting catheters in the carotid vessels, external pudic arterial vessels or external iliac arterial vessels (Peeters et al., 1979; Boisclair et al., 1993; Delamaire and Guinard-Flament, 2006), or by conducting punctures in the internal iliac arterial vessels (Graham et al., 1936). However, these techniques need professional sampling skills, and could cause possible contamination or infection of the peritoneal cavity and life endangering injury to the aorta (Nagy et al., 2002). The alternative approach is using the tail arterial or tail venous blood as representative of the arterial blood supply directly into the mammary gland (Emery et al., 1965). The coccygeal (median, caudal) arterial blood vessels, located on the ventral side of the tail, are easily accessible (West et al., 1991). Researchers usually take coccygeal venous blood or arterial blood, or mixing venous and arterial blood as sample blood in calculations for the A-V difference, mammary gland extraction rate and net uptake of nutrients (Lu et al., 2003; Doepel and Lapierre, 2010; Yang et al., 2012).

Opinions on the validity and representativeness of replacing external pudic arterial blood with tail arterial blood or venous blood is controversial in literature. Some authors believe that arterial blood is considered to be sufficiently mixed so that it may be obtained from any vessel location (Cant et al., 1993). The A-V differences in chemical compositions across the tail are assumed to be negligible, and thus the composition of either venous or arterial blood from the tail is assumed to be representative of that of the mammary arterial supply (Emery et al., 1965; Cant et al., 1993). This hypothesis has been widely accepted in studies on the

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mammary gland metabolism of milk precursors, such as amino acids (AA, Lykos and Varga, 1997; Doepel and Lapierre, 2010) and fatty acids (FA, Yang et al., 2012), and Moorby et al. (2002) reported that there was no significant difference between the arterial and tail vein in AA concentrations. Other authors, however, believe that tail arterial blood and tail venous blood must be interpreted with caution as the blood gas indexes (Tvedten et al., 2000) and ethanol metabolism are different (Levitt et al., 1994). To our knowledge, the difference of milk precursor such as AA in external pudic blood with those in tail arterial blood or tail venous blood has not been compared directly. This experiment was, therefore, carried out to compare AA compositions in the external pudic blood with those in the tail arterial or venous blood, and the mammary gland extraction rate of AA was derived upon examination. This was based on plasma AA differences between the external pudic artery, tail artery, or tail vein and the subcutaneous abdominal vein.

The experimental protocol was approved by the Animal Care Advisory Committee of the Chinese Academy of Agricultural Sciences. The health of the cows was monitored daily throughout the experimental period.

Thirteen lactating Holstein dairy cows with averaged milk yield of 27 ± 2 kg/d and 110 ± 20 days of lactation were used. All cows were fed and milked three times daily at 0700 h, 1400 h and 2100 h, and with *ad libitum* access to fresh water. The ingredients (dry matter basis) of the ration consisted of 17.3% alfalfa hay, 18.77% corn silage, 11.29% soybean meal, 4.19% rapeseed meal, 2.13% cottonseed meal, 2.06% puffed soybean, 4.16% beet pulp, 10.44% cotton seed, 25.55% ground corn, 1.14% fat supplement, 0.33% XP, 0.74% limestone, 0.46% salt, 0.92% sodium bicarbonate, 0.53% mineral-vitamin mix, and offered as a total mixed ration (TMR) for approximately 10% feed orts. The ration contained 18.12% CP, 47.85% NDF, 25.20% ADF, and 5.60% EE and with estimated NE_L of 1.55 Mcal/kg DM (NRC, 2001).

Tail arterial blood sample collection: the tail was lifted vertically with one hand up to horizontal position, a disposable blood collection needle (KDL®) was inserted, from the ventral side, perpendicularly into the tail artery located approximately 150 mm away from the root of the tail and a few mm deep into the skin, and blood samples were drawn into heparinized (or K_3EDTA) vacutainer tubes (VACUETTE®). The arterial origin of the blood was indicated by its light-red color (Graham et al., 1936), spontaneous outflow (or rhythmic squirts) of the blood (Tvedten et al., 2000). After blood collection, the puncture site was compressed manually for about 5 min to avoid possible bleeding and the occurrence of a hematoma.

Tail venous blood sample collection: the procedure was almost the same with that for collection of the tail arterial blood except that blood was drawn from the tail vein. The venous origin of the blood was indicated by its dark-red color.

External pudic arterial blood sample collection: the procedure described by Katamoto and Shimada (1990) was adopted in this experiment, where the authors injected the medicine directly into the external pudic artery. The procedure was as follows: One hand was gently inserted into the rectum to locate the external pudic artery that closes to the abdominal wall with obvious pulsation, and the other punctured a needle (20 gauge, 20 cm) into the artery to collect blood samples into vacutainer. Two operators were necessary for sampling the external pudic arterial blood. One operator was needed to place one hand firmly on the back of the cow to prevent the cow from arching her back. The other operator committed to blood sampling as described. The first 10 mL of the arterial blood was discarded to prevent contamination of the sample (Graham et al., 1936). The arterial blood samples were successfully collected from all the cows without causing any serious disturbances to the animals.

Subcutaneous abdominal venous blood sample was collected from the subcutaneous abdominal veins following the procedure for collection of tail arterial blood samples.

Blood samples from the external pudic artery, tail artery, tail vein and subcutaneous abdominal vein were collected from all cows 1 h after the morning feeding. Each sample was divided into two aliquots. One aliquot of the whole blood was kept on ice, and sent for measurement of PCO_2 (partial pressure of carbon dioxide) and SO_2 (oxygen saturation) within 4 h after collection, and the other aliquot of blood was centrifuged at $3000 \times g$ for 15 min at 4 °C to harvest plasma, and the plasma sample was stored at −20 °C until analysis.

PCO_2 and SO_2, reported as indicators separating arterial blood from venous blood (Slanina et al., 1992), were determined using an auto-analyzer for direct-reading of blood gases (Roche Diagnostics GmbH, Germany), and both the indexes were used to identify the representativeness of the blood from arterial and venous blood vessels.

Plasma samples for AA analysis were deproteinized by addition of 8% (wt/vol) sulfosalicylic acid for 12 h at 4 °C followed by centrifugation at $12{,}000 \times g$ at 4 °C for 20 min. The supernatant was used to determine amino acid concentrations in a Hitachi L-8900 AA analyzer as described by Noguchi et al. (2006).

The extraction rate (ER) of nutrients by the mammary gland was calculated using the formula as followings:

$$\text{ER, \%} = \frac{([A] - [V])}{[A]} \times 100$$

where [A] represents nutrient concentrations in blood of the external pudic artery, tail artery or tail vein, and [V] is the corresponding nutrient concentration in blood of the subcutaneous abdominal vein. This extraction rate reflects the fraction of arterial supply net removed by transport and metabolism within the gland (Lemosquet et al., 2009).

The data of the variables in the blood samples collected from the various vessels were analyzed using the GLM procedure of SAS (SAS 8.2; SAS Institute Inc., Cary, NC). The model used for the blood

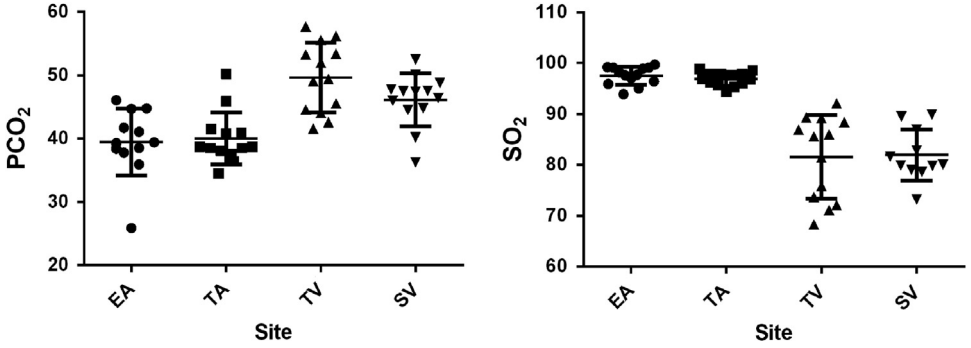

Fig. 1. Blood PCO_2 and SO_2 indexes of the external pudic artery (EA), tail artery (TA), tail vein (TV) and subcutaneous abdominal vein (SV) of 13 dairy cows.