



Isotopic ratio analysis of cattle tail hair: A potential tool in building the database for cow milk geographical traceability



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ABSTRACT

The potential for the isotopic ratio analysis of cattle tail hair in determining the geographical origin of raw cow milk in Peninsular Malaysia had been investigated in this research using exploratory visualization. A significant positive correlation ($p < 0.0001$) ($n = 54$) was noticed between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in milk with that of hair which indicated that these matrices could be used in tracing the geographical origin of animal produce and tissues, and there is a possibility that hair could be used as a substitute in building the database for the geographical traceability of milk. It was also observed that both hair and milk isotopic ratio correlations exhibited separation between the northern and southern regions. The accuracy of using isotopic ratio in determining geographical discrimination had been clearly demonstrated when several commercial milk samples from the same regions under the study were correctly assigned to the appropriate geographical clusters.

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1. Introduction

As adulteration and mislabeling are important factors in the food industry, studying the geographical origin of food is imperative (Camin, Bontempo, Perini, & Piasentier, 2016; Nietner, Haughey, Ogle, Fahl-Hassek, & Elliott, 2014; Wu et al., 2015). Hence, it is certainly sensible for dairy companies to seek regional information of the raw milk used in their products as an effort to maintain their quality. Malaysia is a country that has uniform equatorial marine climate but there are some differences in the geographical parameters, such as temperature and latitude that can influence isotopic ratio information similar to a study carried out in New Zealand (Ehtesham, Hayman, McComb, Van Hale, & Frew, 2013).

Various analytical methods are used for the determination of geographical origin and traceability. Among them, IRMS is the most reliable (Bontempo et al., 2014; Camin et al., 2016; Longobardi et al., 2015). Stable isotopic ratios of C, N and O have

been used to determine food traceability and origin (Camin et al., 2016; Crittenden et al., 2007; Rees et al., 2016). The isotopic ratios of animal tissue and produce reflect the animal's diet and could be used for tracing its geographical origin (Chesson, Valenzuela, O'Grady, Cerling, & Ehleringer, 2010; Erasmus, Muller, van der Rijst, & Hoffman, 2016). Each isotope reflects different information. Among the isotopes, $\delta^{15}\text{N}$ is related to the nutrition of soil, consumption of leguminous plants, fertilizers, grazing condition of the cow and cultivation of the plants (Erasmus et al., 2016; Sandberg, Loudon, & Sponheimer, 2012). $\delta^{13}\text{C}$ is related to the carbon fixation cycle such as C_3 , C_4 or CAM (Erasmus et al., 2016; Smith, 1971). Researchers have found strong correlations between the produce (meat) and tissue (hair) of cattle (Guo, Wei, Pan, & Li, 2010; Yanagi et al., 2012). They have mentioned that the isotopic composition of hair records the animal's diet history (Cerling et al., 2004) as does the isotopic information of milk (Molkentin & Giesemann, 2010). Due to milk's short shelf life, it was thought that hair could be used, and would be a better choice instead of animal produce, which in this case is milk, in building the database for the geographical traceability of milk. This is because hair is resilient to external factors such as humidity, and temperature change, it is easier to collect and it offers permanence to isotopic records (Yanagi et al., 2012). Furthermore, hair is a keratinized tissue grown regularly, with stable properties which its keratinized structure and constituents doesn't change after growing (Liu, Guo, Wei,

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Shi, & Sun, 2013; Schwertl, Auerswald, & Schnyder, 2003). Thus, it can also be used to record the diet behavior of cattle over time (Schwertl, Auerswald, Schäufele, & Schnyder, 2005; Zazzo et al., 2007). Moreover, the analysis of hair is preferred for monitoring the diet change in wild animals (Cerling et al., 2004; Mizukami et al., 2005). Therefore, in this research, we have attempted to determine the geographical origin of cattle produce and tissue and determine the feasibility of using the isotopic ratios of C and N of cattle tail hair in the development of milk geographical traceability database. Although there are studies that have used isotopic data to determine the geographical origin of milk, but to the best of the authors' knowledge, there is no reported information on the isotopic ratio of tropical milk and its correlation with cattle hair.

2. Materials and methods

2.1. Milk sampling

Overall, 69 raw and factory milk samples were collected directly from farms or supermarkets in the states of Perlis, Kedah, Terengganu, Perak, Pahang, Pulau Pinang, Kuala Selangor, Johor and Melaka in Peninsular Malaysia. These are reported in Table S1 (Supplementary) where the sampling followed two monsoon wind seasons of the southwest (May-Sep) and northeast (Nov-March) as shown in Fig. 1. The 1st sampling (S1) was carried out during the northeast monsoon while the 2nd (S2) and 3rd (S3) samplings were carried out during the southwest monsoon season. The breeds of cows varied from farm to farm and are reported in Table S1 (Supplementary). Milk samples were transferred to the laboratory in an icebox and then, for each sample, 5.0 mL of the milk was poured into polyethylene bottles which had been soaked in 10% nitric acid overnight and rinsed with ultra-pure water. The samples were then transferred to the freezer until the following day. Consequently, the samples were freeze dried in a freeze dryer (CHRIST, Germany) for 24 h in order to be dried.

2.2. Hair sampling

Overall, 54 tail hair samples in triplicates were obtained from 7 farms located in Perlis, Pulau Pinang, Terengganu, Perak, Kuala

Selangor, Johor and Melaka as shown in Fig. 1. These were from the same cows from which milk samples had been collected for S1, S2 and S3. Hair samples were collected from the tail of the cattle. They were cut 50.0 mm to the distal of the hair. The hair samples were soaked in deionised water, then rinsed and washed with acetone and then rinsed three times with deionized water to remove/decrease any contamination possibility.

2.3. Determination of analytes using isotopic ratio elemental analyses (EA-IRMS)

For the analysis of carbon and nitrogen, 1.5 mg of freeze dried milk samples and 1.5 mg of hair samples were weighed and placed in tin capsules sealed tightly using the one step method, ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured at the same time (Wu et al., 2015). Each sample was analysed three times.

In this research, all isotopic analyses were performed at the Malaysian Nuclear agency using the IRMS SECRON GEO 20-20 (U. K.) combined with the SECRON elemental analyzer. The determination of isotopic ratios of C and N were carried out on homogenized freeze dried milk samples and cattle hair. The fluctuation in stable isotope ratio were calculated as parts per thousand (‰) deviation from the internationally accepted standards of Vienna Pee Dee Belemnite (VPDB) for the atmospheric carbon and atmospheric nitrogen (AIR) for nitrogen. The relative deviation values were obtained using:

$$\delta(\text{‰}) = (R_{\text{sample}} - R_{\text{standard}}/R_{\text{standard}}) \times 1000$$

In the above equation, R stands for the ratio of heavy to light isotope; hence, the larger the δ , the more the sample is enriched in heavy isotope. Moreover, the accuracy of analysis was examined using in home standards such as R001 which was used for the analysis of carbon and nitrogen via combustion ($\delta^{13}\text{C} = -26.43\text{‰} \pm 0.2$, $\delta^{15}\text{N} = +2.55\text{‰} \pm 0.1$).

2.4. Statistical analyses

Two statistical analyses were carried out for the milk and hair samples obtained in this work. The first analysis involves the correlation analyses between the isotopic ratios amongst the hair

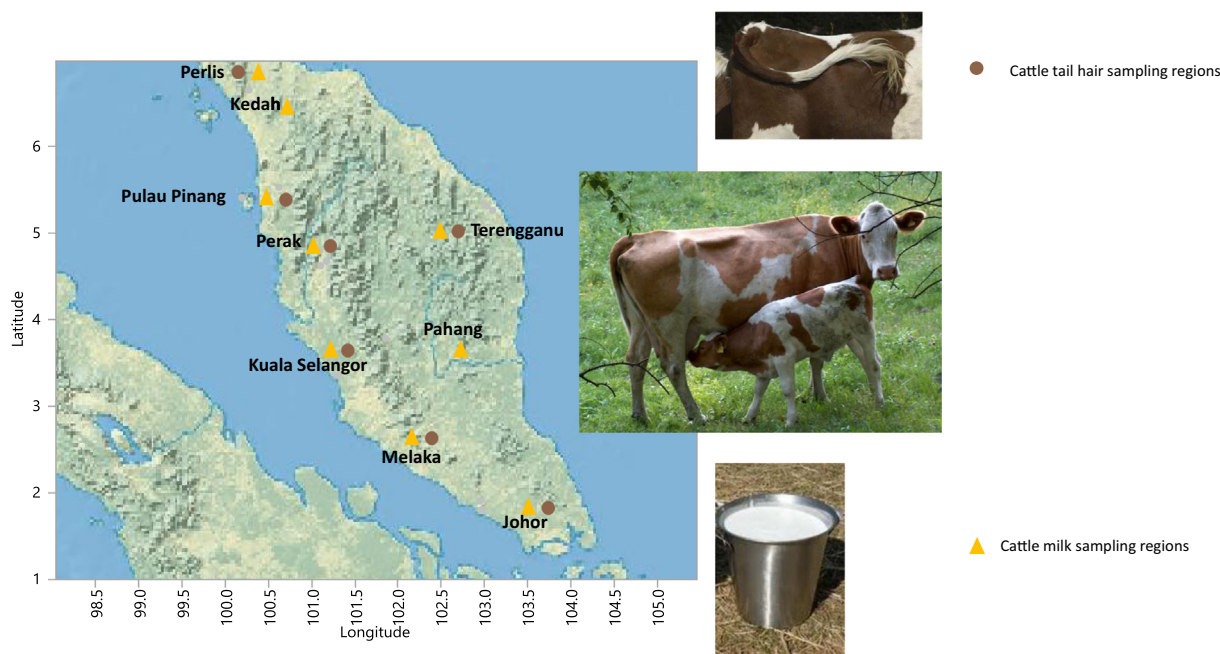


Fig. 1. Milk and hair sampling stations.

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