Animal Feed Science and Technology 215 (2016) 37–46

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

True digestibility of protein and amino acids in goats using plants naturally enriched in ^{13}C as a label to determine endogenous amino acid excretion

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ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form 22 February 2016
Accepted 23 February 2016

Keywords:
Digestibility
Protein
Amino acid
Carbon
Isotope labeling
Goats

ABSTRACT

The objective of this study was to determine whether ^{13}C labeling occurs in goats fed diets with different natural levels of ^{13}C enrichment and, if so, to use ^{13}C as a label to determine true amino acid (AA) digestibility. Thirty-six *Xiangdong* black goats with initial body weights of 7.5 ± 0.5 kg were selected as experimental animals and fed dietary treatments consisting of wheat, barley, and soybeans (C3 diet) or corn, sorghum grain, and sugar cane (C4 diet). The ^{13}C abundance of the amino acid fraction (AAF) of the C3 and C4 diets had average delta ^{13}C values of −28.57‰ and −12.93‰, respectively. Three goats/treatment were slaughtered on days 1, 28, 56 and 84 during the labeling phase, and the AAFs of organs were analyzed for ^{13}C abundance. ^{13}C in the blood AAF increased (−21.70‰, −22.65‰, −23.59‰, and −24.43‰, respectively) with increasing feeding durations in goats fed the C3 diet and decreased (−21.13‰, −16.01‰, −13.78‰, and −12.03‰, respectively) in goats fed the C4 diet. Longissimus dorsi, liver and wool AAFs showed similar trends to those observed in the blood. Each tissue showed a significant dietary treatment effect ($P < 0.05$) and a dietary treatment × labeling phase interaction ($P < 0.01$). After the labeling phase (day 85), six goats from each treatment group were placed in metabolism cages and fed at 0700 and 1900. Three of the goats from each treatment group were implanted with the ruminal cannula and duodenal fistulae and given a 14-day recovery period. From days 15 to 21, a total of 4 g chromic oxide, used as an indigestible marker, was administered daily via the ruminal fistulae. At 1900 on day 18 of the collection phase, all goats were switched to the opposite diet type. Thereafter, the duodenal fluids and total feces were collected from days 19 to 21. Additionally, one goat that had not been implanted with a ruminal cannula and duodenal fistulae from each treatment group was killed at 0700 (before feeding) on days 19, 20 and 21, and the AAFs of organs were analyzed for ^{13}C abundance. ^{13}C abundance of the blood AAF was used as an index of endogenous protein labeling. Apparent and true protein digestibilities in the forestomach, whole intestine and whole digestive tract were not different ($P > 0.05$) between the diet treatment groups. The levels of true protein digestibility in the forestomach, whole intestine and whole digestive tract in goats fed the C4 diet

Abbreviation: AA, amino acid; N, nitrogen; ME, metabolizable energy; DM, dry matter; Ca, calcium; P, Phosphorus; NDFom, neutral detergent fiber; AAF, amino acid fraction; CP, crude protein; EAAs, essential amino acids; NEAAs, nonessential amino acids; VFA, volatile fatty acid; Arg, arginine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine; Ala, alanine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; Tyr, tyrosine.

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http://dx.doi.org/10.1016/j.anifeedsci.2016.02.019

tended to be higher than for those in goats fed the C3 diet. On average, the true digestibilities of protein, essential AAs and nonessential AAs were 5.30%, 4.28% 3.17% (forestomach), 5.20%, 5.39% 3.75% (whole intestine) and 3.01%, 4.97%, 4.17% (whole digestive tract) higher, respectively, than the apparent digestibilities of these components in goats fed the C3 diet. In summary, goat tissue protein was labeled with ^{13}C using different diets, and the results show that ^{13}C can be used to determine true AA digestibility.

1. Introduction

Apparent amino acid (AA) digestibility is the preferred method for the estimation of AA availability in feedstuffs for livestock. However, apparent AA digestibility usually underestimates the amount of AAs absorbed during digestion because of the presence of endogenous AAs in the digesta. For traditional methods used to calculate true AA digestibility, it is assumed that the amount of endogenous AAs are not affected by the amount of protein and other factors in the diet. However, many studies have indicated that this assumption is not valid (Reynolds and Kristensen, 2014).

Stable-carbon isotope analysis has been increasingly used as a tool to delineate dietary patterns in terrestrial and marine ecosystems (Peterson and Fry, 1987) because the stable-isotopic compositions of the tissues of the consumer can often be predictably related to the stable-isotopic compositions of the diet (Deniro and Epstein, 1978; Smith, 1989). In dietary studies of ruminants, stable-carbon isotope analysis has been used to determine lipid digestion and metabolism, the isotopic relationship between the whole body and the animal's diets, the dependence on diet of the carbon-isotope contents in dairy cows, and the potential for the use of stable-carbon isotope ratios of single fatty acids as tracers for the transformation of fatty acids obtained from the diet into milk (Arentson and Zimmerman, 1995; Deniro and Epstein, 1978; Metges et al., 1990; Richter et al., 2012a,b).

Atmospheric carbon dioxide (CO_2) contains approximately 0.011 of the heavier ^{13}C isotope and 0.989 of the lighter ^{12}C isotope. Plants discriminate against ^{13}C during photosynthesis in ways that reflect the plant's metabolism (O'Leary, 1981). Plants that fix CO_2 via the dicarboxylic acid pathway (C4 pathway) have less isotopic discrimination than plants that fix CO_2 via the Calvin cycle (C3 pathway) (Minson et al., 1975). Absolute values of ^{13}C abundances are difficult to obtain, and for most purposes it is adequate to give delta ^{13}C values relative to carbon from a standard source. Commonly, the standard used is Pee Dee belemnite. The delta ^{13}C for C4 plants is $-13.5 \pm 1.5‰$, and for C3 plants it is $-28.1 \pm 2.5‰$ (O'Leary, 1981). Among the commonly used feedstuffs for ruminants, wheat, barley and soybeans are C3 plants, and corn, sorghum grain and sugar cane are C4 plants.

The stable-isotope technique is useful in situations where two isotopically distinct dietary sources are available to animals. In such cases, isotopic analysis of tissues provides quantitative information on the relative contributions of each source to the diet. The isotope approach cannot typically replace conventional techniques where detailed (*i.e.*, taxonomic) dietary information is needed, particularly when several dietary options are available (Arentson and Zimmerman, 1995). However, important advantages of using naturally occurring stable isotopes as dietary indicators over the use of conventional techniques include the following: (1) isotopic dietary estimates are based on assimilated and not just ingested food and (2) comparatively long-term dietary information can be obtained.

The period over which tissue isotopic concentrations will reflect the isotopic signature of a particular diet will depend, in part, on the stable labeling of the isotope in that tissue. Tissues with rapid isotopic turnover will reflect the animal's recent diet, whereas those with slow turnover will reflect longer-term dietary averages. The choice of tissue type used for an isotopic analysis will depend, therefore, on the ecological question of interest. Tieszen et al. (1983) suggested that by analyzing combinations of tissues, greater information concerning an animal's diet might be obtained.

Currently, there are two major limitations to the application of stable-isotope analysis in dietary studies of ruminant. First, it is not well understood how stable isotopes fractionate or change once they are incorporated into tissues. Knowledge of diet-tissue fractionation factors is critical to studies concerned with predicting the isotopic compositions of diets based on the isotopic compositions of tissues. Second, the precise isotopic labeling rates in tissues of ruminants are poorly known. Therefore, as a first step in determining the rates of stable isotopic labeling in various tissues of ruminants, we designed controlled tests to feed *Xiangdong* black goats (a local breed in the south of China). By designing diets consisting of different stable-carbon isotopic signatures using C3 and C4 plants fed to goats, we determined the stable isotopic labeling rates of different tissues (blood, liver, muscle and wool). We then determined the true digestibility of nitrogen (N) and AAs using feeds naturally enriched in ^{13}C as a label to determine endogenous AA excretion in goats. To the best of our knowledge, this is the first attempt to determine true N digestibility and endogenous N losses in ruminants based on the stable-carbon isotopic composition of diets.

2. Materials and methods

This experiment was approved by the Animal Care Committee, Institute of Subtropical Agriculture (ISA), Chinese Academy of Sciences, Changsha, P.R. China (No. KYNEAAM-2006-0015).