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Digestible nutrients and available (ATP) energy contents of two varieties of kiwifruit (*Actinidia deliciosa* and *Actinidia chinensis*)

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ABSTRACT

A model, which combines a dual *in vivo–in vitro* digestibility assay and stoichiometric relationships describing nutrient catabolism, has been recently developed to allow prediction of the available energy (AE) content of a food in terms of its ATP yield. The model uses the growing pig as an *in vivo* model for upper gastrointestinal tract digestion in humans. Terminal ileal digesta from the pig are incubated with human faecal inocula (*in vitro* fermentation model) to simulate human hindgut fermentation. The respective *in vivo* and *in vitro* digestibility assays provide predictions of the ileal absorbed and hindgut-fermented nutrient contents of a food which are then used to predict ATP production post-absorption, based on known stoichiometric relationships. In this study, the model was used to determine the AE contents of fresh, ripe Hayward (*Actinidia deliciosa var Hayward*) and Hort16A (*Actinidia chinensis var Hort16A*) kiwifruit. Kiwifruit pulp, containing 3 g kg^{-1} of titanium dioxide, included as an indigestible marker, was fed to growing pigs and terminal ileal digesta were collected. Ileal nutrient digestibilities were determined. A sample of digesta was incubated *in vitro* with a fresh human faecal inoculum and the fermentable organic matter determined. The predicted available (ATP) energy contents of the Hayward and Hort16A kiwifruits were 5.9 and 6.2 kJ g^{-1} dry matter, respectively, approximately 44–47% of the determined apparent digestible energy (ADE) content. The AE contents of the kiwifruit, expressed relative to the AE content of dextrin (a highly digestible source of glucose) were 0.57 and 0.61 for Hayward and Hort16A, respectively. Comparable ratios for metabolisable energy (ME) were 0.74 and 0.73. The predicted AE from kiwifruit was much lower than the predicted ME from kiwifruit when compared to dextrin. The ME values overestimate the energy content of kiwifruit that is available to the cell. AE was not only lower than ME but the two energy systems ranked the kiwifruit types differently in terms of energy supply to the body. The relatively low energy content per unit of dry matter and high water content of kiwifruit make kiwifruit an ideal weight loss food.

1. Introduction

Determining the energy content of foods accurately is important and has direct application for weight control in humans. There are several measures of dietary energy that are currently used in practice, including apparent digestible energy (ADE) and metabolisable energy (ME) (Livesey et al., 2000). ADE is determined as the difference between dietary energy intake and faecal energy output while ME is determined as the difference between energy intake and the sum of faecal and urinary energy outputs. In practice, the ME content of a food is usually estimated using factorial or empirical models, such as the Atwater system or modified versions of the Atwater system. Estimation of food energy values by the Atwater and similar systems is based on several assumptions which are not always tenable and recent evidence suggests that models which estimate the digestible or metabolisable energy content of foods may not be accurate, particularly in foods that are low in fat or high in fibre (Baer, Rumpler, Miles, & Fahey, 1997; Brown et al., 1998; Kruskall, Campbell, & Evans, 2003; Livesey, 1990; Zou, Moughan, Awati & Livesey, 2007). The energy values assigned in predictive ME models to dietary fibre and protein, in particular, are difficult to define generically, due to the diversity in chemical composition and digestibility (Ferrer-Lorente, Fernandez-Lopez, & Alemany, 2007; Livesey, 1990).

Calculating the available energy (AE) content of a food in the form of ATP delivered to the cell (Livesey, 1984) may be a more accurate measure of the energy value of a food than digestible or metabolisable energy (Coles, 2010). The prediction of ATP yields

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relies on being able to quantify the nutrients available for metabolism, based on uptake from the digestive tract, which should be determined separately in the upper tract and the large intestine (Coles, Moughan, Awati, Darragh, & Zou, 2010). Ideally, the most appropriate method for determining energy in foods for humans involves conducting *in vivo* trials with humans; however, it is not possible to obtain separate upper tract and hindgut measures *in vivo* in humans (Darragh & Hodgkinson, 2000). One approach is to use animal models to give the predicted upper tract absorption of each nutrient and to use *in vitro* fermentation assays (based on human faecal inocula) to predict short chain fatty acid production. This approach would allow the determination of energy uptake from different parts of the digestive tract, thus allowing consideration of differential efficiencies of nutrient utilisation (Coles et al., 2010). Such refinements are likely to become important when evaluating AE in foods containing high amounts of protein, resistant starch or fibre. The dual *in vivo–in vitro* digestibility approach was suggested by McBurney and Thompson (1987) and was subsequently validated by McBurney and Sauer (1993) and more recently by Coles et al. (2010), who compared organic matter digestibility for diverse diets determined using either the dual assay (rat, *in vitro*) or a human balance study.

Recently, Coles (2010) developed an AE model that extends the method of McBurney and Sauer (1993) to allow determination of ATP yield at the cellular level. This new model estimates the ATP available to the cell, based on the ileal digestible nutrient content determined, using a rat or pig (*in vivo*) model, hindgut-fermentable nutrient content, determined by the incubation of ileal digesta with human faecal inoculum (*in vitro* model) to simulate hindgut fermentation in humans, and the known stoichiometric relationships between absorbed nutrients and the ATP produced in the cell (Coles, 2010).

Kiwifruit is a fruit commonly consumed in western countries and its nutrient composition has been well characterised (Ferguson & Ferguson, 2003; Harman & McDonald, 1989; MacRae, Bowen, & Stec, 1989; MacRae, Lallu, Searle, & Bowen, 1989). Despite this, little is known about the digestibility of nutrients in kiwifruit or the AE derived from those nutrients. The nutrient composition of kiwifruit and its high fibre content suggest that kiwifruit should be a good weight loss food. In this study we applied the new assay, as described by Coles (2010), to determine the AE content of both Hayward (*Actinidia deliciosa var Hayward*) and Hort16A kiwifruit (*Actinidia chinensis var Hort16A*).

2. Materials and methods

2.1. Diet preparation

Fresh Hayward and Hort16A kiwifruit, pre-ripened to a similar degree of firmness (firmness RTE (ready to eat) of 0.5–0.8 kgf and 0.5–1.0 kgf, respectively), were prepared as follows: the skins of the Hayward kiwifruit were removed by hand and the flesh pulped using a kitchen food processor. The Hort16A kiwifruit was deskinned and the flesh pulped by cutting the kiwifruit into halves and putting the halves through a grinder (mesh size approximately 4 cm). The kiwifruit pulp was prepared fresh and stored at 4 °C for no more than 2 days.

2.2. In vivo studies

Ethics approval for the study was granted by the Massey University Animal Ethics Committee, Massey University, Palmerston North, New Zealand (application 08/43). Twelve Large White × (Large White × Landrace) entire male pigs of approximately 22.9 ± 0.1 kg body weight were obtained from a commercial farm and housed individually in metabolism crates, in a temperature-controlled room at 22 ± 1 °C. The pigs had access to water at all times.

2.2.1. Determination of apparent ileal nutrient digestibility in kiwifruit

During a 14 day acclimatisation period, all pigs were fed a commercial cereal-based pig-grower diet at 10% of their metabolic body weight. At the beginning of each week, each pig was weighed and the dietary intake adjusted accordingly. The pigs received their daily ration as nine equal meals, one meal fed each hour between 08.30 and 16.30 h. At the end of the acclimation, the pigs were allocated, at random, to one of two treatments; Hayward kiwifruit or Hort16A kiwifruit. The kiwifruit flesh was gradually incorporated into the daily diet so that, by the end of the 14 day experimental period, each pig was consuming a diet that consisted of either 100% Hayward or Hort16A kiwifruit. The daily ration of kiwifruit dry matter was 10% metabolic body weight. Titanium dioxide (3 g kg^{-1} of kiwifruit dry matter) was added to the diet on the final day (day 28) of the trial as an indigestible marker.

On day 28, 5–7 h after the start of feeding, each animal was sedated with midazolam (0.1 mg kg^{-1}) and ketamine (15 mg kg^{-1}) by intramuscular injection 20 min before commencement of general anaesthesia. The general anaesthesia was induced and maintained with isofluorane inhalation. The abdomen was opened by an incision along the mid-ventral line and the skin and musculature were folded back to expose the viscera. The section of the terminal ileum 20 cm anterior to the ileocaecal valve was dissected out. Blood was washed off the outside of the ileal section with deionised water and digesta were carefully flushed out into plastic bags using deionised water. The collected digesta were then frozen and stored at −20 °C prior to chemical analysis. The animal was euthanised while unconscious by severing of the portal vein and diaphragm.

2.2.2. Determination of in vitro dry matter and organic matter fermentability of kiwifruit

Fresh human faeces were collected from three healthy volunteers under anaerobic conditions. The volunteers had been eating an unspecified western diet and had received no antibiotic treatment for 3 months. Faeces (80 g) were immediately homogenised for 3 min with 250 ml of phosphate buffer (0.1 M at pH 7), filtered through six layers of cheesecloth to remove particulate matter and the material used immediately. The phosphate buffer was preboiled and then cooled under a stream of oxygen-free nitrogen and kept at 37 °C. Inoculum preparation was performed under a constant flow of CO_2.

Aliquots (5 ml) of inoculum were transferred to 50 ml McCartney bottles containing either 5 ml of phosphate buffer alone (blanks) or phosphate buffer with 100 mg of finely ground, homogenised terminal ileal digesta, obtained as described above. Each bottle was flushed with CO_2, capped and incubated at 37 °C for 24 h. After 24 h, the bottles were placed in an autoclave to stop fermentation. The dry matter and organic matter of the unfermented residue were then determined.

2.3. Chemical analysis

All analyses were carried out in duplicate. Dry matter, ash and total lipid were determined according to the methods described by AOAC (1995). Briefly, dry matter was determined gravimetrically after oven-drying overnight at 105 °C, while ash was determined gravimetrically after ashing at 500 °C overnight. Total lipid was determined gravimetrically after extraction in petroleum ether using a Soxtec solvent extraction system (Foss, Hillerød, Denmark). The total nitrogen content was determined on a LECO analyser (LECO Corporation, St. Joseph, Michigan, USA), using the