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# Microbial digestion in the koala (*Phascolarctos cinereus*, Marsupialia), an arboreal folivore

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**Summary.** The rate of volatile fatty acid (VFA) production in the hindgut of three koalas (*Phascolarctos cinereus*) maintained on *Eucalyptus punctata* foliage was measured by in vitro incubation.

VFA production in both the caecum and proximal colon was slow (11 μmole $ml^{-1}$ $h^{-1}$) and the contribution made by total daily VFA production was estimated to be only 9% of digestible energy intake. This is attributed primarily to the indigestible nature of the eucalypt-derived digesta entering the hindgut. Estimates of cell-wall digestion by koalas in associated experiments explained only 60–70% of the VFA production in vitro suggesting that some fermentation of other substrate occurred.

It is concluded that, despite its extreme development, the hindgut in koalas plays a relatively minor role in the extraction of energy from eucalypt foliage.

## Introduction

The koala, *Phascolarctos cinereus*, is a moderately small (5–13 kg adult body weight), arboreal marsupial which utilizes a foliage diet high in cell-wall (fibre) content (Cork et al. 1983). Mammals rely for the digestion of cell-wall carbohydrate upon symbiotic micro-organisms inhabiting the digestive tract (Parra 1978) but it is postulated that, owing to physiological constraints, small species are incapable of deriving a large proportion of their energy requirements from this source (see Cork et al. 1983).

The koala is an apparent exception to this generalization. It has long been considered that its caecum and proximal colon, which are among the most highly development in mammals (Mackenzie 1918), are major sites of microbial activity and are important in the utilization of eucalypt foliage by this animal (e.g. Hill and Rewell 1954; Harrop and Degabriele 1976). This notion is consistent with both the appearance of digesta in these organs and the pH therein of 6–7 (Cork et al. 1983). However, cell-wall digestion in koalas fed mature *Eucalyptus punctata* foliage is relatively inextensive ($\bar{x}=25\%$), probably owing to the high lignin content of this diet (Cork et al. 1983). It has therefore been concluded that non cell-wall constituents are the koala's chief dietary source of energy (Cork et al. 1983) but neither the site of their digestion nor the extent, if any, to which they undergo microbial fermentation has been determined with certainty.

This paper investigates using an in vitro incubation technique the production of volatile fatty acids (VFA), the principal non-gaseous end-products of microbial fermentation in other mammals, in three koalas to assess the contribution of microbial digestion to the energy economy of this species.

## Materials and methods

*Animals and housing.* Three adult male koalas of 7.30, 6.75 and 7.70 kg body weight were used. Their husbandary prior to the experiment was described by Cork and Warner (1983).

*Digestible energy intake.* Intakes of digestible energy (DE) were not measured specifically for this experiment. Earlier, Cork et al. (1983) measured the DE intakes of koalas in six feeding trials throughout all seasons of the year. In those trials there were no significant differences in DE intake between the three animals used in the present experiment and the other five animals. Therefore the overall mean DE intake of

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*Abbreviations:* *DE* digestible energy, *VFA* volatile fatty acids

**Table 1.** Total concentrations and molar proportions of volatile fatty acids (*VFA*) along the gut in three koalas. Values are means ± SE *n* negligible (less than 0.05% of total VFA)

| | Total VFA mmol $l^{-1}$ | Acetic | Molar proportion (%) | | |
|---|---|---|---|---|---|
| | | | Propionic | Butyric[a] | Valeric[b] |
| Forestomach | 25.8±2.6 | 93.8±0.9 | 5.7±0.3 | 0.5±0.3 | n |
| Hindstomach | 4.3±1.2 | 92.4±2.5 | 7.3±2.7 | 0.1±0.1 | 0.2±0.1 |
| Small intestine | 12.6±1.8 | 93.0±2.1 | 1.9±0.8 | 4.7±0.9 | 0.4±0.4 |
| Caecum | | | | | |
| distal | 18.5±2.1 | 88.1±1.0 | 7.8±0.5 | 3.9±0.9 | 0.2±0.1 |
| proximal | 33.2±5.2 | 84.5±1.7 | 9.8±1.4 | 5.5±2.0 | 0.1±0.1 |
| Proximal colon | | | | | |
| proximal | 29.3±10.5 | 85.6±1.7 | 10.1±0.9 | 4.2±0.8 | 0.1±0.1 |
| distal | 26.8± 3.9 | 86.6±1.8 | 10.4±2.4 | 3.1±0.7 | n |
| Distal colon | 36.3±14.7 | 91.7±1.6 | 5.6±1.3 | 2.5±0.4 | 0.2±0.2 |
| Faeces | 62.1±22.1 | 95.7±1.6 | 2.8±0.6 | 1.6±1.2 | n |

[a] In all cases iso-butyric acid was less than 0.1% of the total VFA
[b] In all cases iso-valeric acid was less than 0.2% of the total VFA

0.50 MJ $kg^{-0.75}$ $d^{-1}$ (Cork et al. 1983) was used in this experiment when assessing the contribution of VFA production in the hindgut to the energy economy of the koala.

*Volatile fatty acid production.* These experiments were conducted in conjunction with the experiments described by Cork and Warner (1983) on the size distribution of particulate digesta in the gut, and by Cork et al. (1983) on changes in relative concentrations of chemical constituents of *E. punctata* along the gut of the koala. The three animals were killed as described by Cork and Warner (1983) between 1100 and 1400 h on separate days; the peak period of feeding preceded this by 4–8 h (Cork and Warner 1983) but feeding had been intermittent up to the time of death.

Immediately after death the body cavity was opened and the digestive tract divided into seven segments and ligated (Cork and Warner 1983). The techniques of Hume (1977) were used to sample digesta from the proximal (fore-) and distal (hind-) extremes of the stomach, the small intestine (total contents removed and mixed), the distal colon and faecal nodules in the rectum and to measure the in vitro rate of volatile fatty acid production in the contents of caecal (C1, C2) and proximal colonic (PC1, PC2) segments.

Caecal and colonic segments were processed in the order C1, C2, PC1, PC2. The average time between death of the animal and commencement of the first incubation was 15 min, and a further 15 min elapsed before the final incubation was commenced. While awaiting processing the segments were left in the body cavity to avoid undue changes in temperature. Following addition of mercuric chloride, samples were frozen on dry ice and stored at −15 °C prior to analysis.

Each sample was shaken with an equal volume of distilled water to extract VFA. High speed centrifugation was used to separate digesta fluid from most particulate matter. A dry matter determination on the supernatant was necessary to correct for remaining suspended solids. Samples of the supernatant were analysed for total VFA concentration by steam distillation by a modification of the procedure of Friedmann (1938) and correction made for distilled water added in extraction. The molar proportions of individual VFA were determined by gas-liquid chromatography (Erwin et al. 1961).

Mean production rates of VFA were calculated as described by Hume (1977) and were compared statistically by analysis of variance (Snedecor and Cochran 1961).

## Results

### *VFA concentrations along the gut*

The concentration of total VFA in digesta fluid (Table 1) was higher in the forestomach (25.8 mmole $l^{-1}$) than in the hindstomach (4.3 mmole $l^{-1}$). The VFA concentration in the small intestine was intermediate between these values (12.6 mmole $l^{-1}$) and in the hindgut increased to values similar to those in the forestomach. The higher concentration in the faeces (62.1 mmole $l^{-1}$) can probably be attributed to their lower water content.

The molar proportion of acetate was highest in the stomach and small intestine, and lowest in the caecum and proximal colon ($P < 0.05$). In contrast, the proportion of propionate was higher in the caecum and proximal colon than in the stomach and small intestine ($P < 0.05$). The proportion of n-butyrate tended to be lower in the stomach than anywhere else in the gut. The proportions of iso-butyric and iso-valeric acids were negligible throughout the gut and those of n-valeric acid were too low for any trends to be evident.

### *Rates of production of individual VFA*

A representative sample of the change in concentration with time of acetate, propionate and butyrate in an incubation vessel is shown in Fig. 1. Since there was no apparent change in the rate of production of any of the three acids during the incubation, linear equations were fitted to the data using the least squares method. There were no significant differences in production rates between the

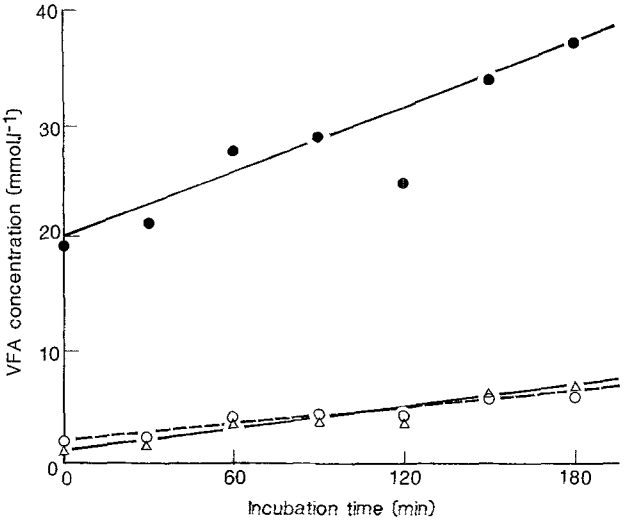


**Fig. 1.** The change in concentration with time of acetic acid (●), propionic acid (○) and butyric acid (△) in caecal digesta fluid during incubation under $CO_2$ at 37 °C

**Table 2.** Rates of production (in μmol $ml^{-1}$ digesta fluid $h^{-1}$) of individual volatile fatty acids (*VFA*) in the caecum and proximal colon in three koalas estimated in vitro. Values are means ± SE

| | Caecum | Proximal colon | Mean | % of total |
|---|---|---|---|---|
| Acetic | 7.5 ±1.0 | 6.0 ±0.4 | 6.7 ±0.6 | 61.9 |
| Propionic | 2.1 ±0.2 | 2.4 ±0.5 | 2.2 ±0.2 | 20.3 |
| Iso-butyric | 0.01±0.01 | 0.02±0.01 | 0.02±0.00 | 0.2 |
| Butyric | 1.7 ±0.2 | 2.0 ±0.3 | 1.8 ±0.2 | 16.6 |
| Iso-valeric | 0.1 ±0.0 | 0.1 ±0.0 | 0.1 ±0.0 | 0.9 |
| Valeric | 0.02±0.02 | 0.01±0.00 | 0.01±0.01 | 0.1 |

caecum and proximal colon (Table 2). Acetic acid accounted for 62% of the total production of VFA while propionic and butyric acids accounted for 20% and 17%, respectively. These proportions are 0.7, 2.1 and 4.0 times respectively the average molar proportions of these acids in zero-time digesta samples (Table 1).

### *Total daily production of VFA*

Total daily production of VFA (Table 3) was calculated on the assumption that the hourly rates of production measured were representative of the rates attaining throughout the day. Total VFA production in the caecum was not significantly different from that in the proximal colon. Total production of VFA in the caecum plus proximal colon accounted for 9% of the mean intake of digestible energy in all three animals. Faecal excretion of VFA, estimated from the proportions of individual VFA in the faeces (Table 1) and the mean faecal excretion of dry matter in the feeding trials of Cork et al. (1983), amounted to only 2.5% of the VFA produced in the caecum plus proximal colon.

**Table 3.** Daily production of total volatile fatty acids (VFA) in the caecum and proximal colon in three koalas estimated in vitro

| | Koala | | | Mean ± SD |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Body weight (kg) | 7.30 | 6.75 | 7.70 | 7.25±0.48 |
| Wet weight of digesta (g) | | | | |
| Caecum | 290 | 335 | 419 | 348±65 |
| Proximal colon | 501 | 459 | 268 | 409±124 |
| Production of total VFA mmol·$d^{-1}$ | | | | |
| Caecum | 53.2 | 76.8 | 104.0 | 78.0±25.4 |
| Proximal colon | 110.2 | 76.4 | 60.8 | 82.5±25.3 |
| kJ·$d^{-1}$ | | | | |
| Caecum | 66.9 | 90.5 | 127.8 | 95.1±30.7 |
| Proximal colon | 140.5 | 98.7 | 80.1 | 106.4±30.9 |
| % intake of apparently digestible energy | | | | |
| Caecum | 3 | 4 | 6 | 4.3±1.5 |
| Proximal colon | 6 | 5 | 3 | 5.0±1.0 |
| Total | 9 | 9 | 9 | 9.0±0.0 |

## Discussion

The concentrations of total VFA found in the stomach of the koala in this study are within the range reported for that of other simple-stomached herbivores such as the pig, horse and raccoon (Stevens et al. 1980). The noticeable fall in concentration between the fore- and hindstomach indicates incomplete mixing of gastric digesta, and differences in rates of fermentation in, or absorption from, the two stomach regions. The low pH found in both the fore- and hindstomach by Cork et al. (1983) suggests that fermentation is probably limited in both gastric regions. Differential rates of absorption of total VFA between different regions of the stomach have been observed in horses (Argenzio et al. 1974) and pigs (Argenzio and Southworth 1974), but whether this is so in the koala is not known.

Digesta fluid from the forestomach and distal colon of the koala contained a similar concentration of total VFA to that from the caecum/proximal colon (Table 1). However, the much larger fluid volume of the latter region (Cork and Warner 1983) makes it probable that the nutritional significance of VFA absorption from the forestomach or distal colon is small in comparison.

The concentration of total VFA in the hindgut of the koala was similar to that in the caecum of another marsupial eucalypt feeder, the greater glider (*Petauroides volans*) (Cork and Hume 1978), but markedly lower than that in the hindgut of the dog, pig, horse, raccoon (Stevens et al. 1980), porcupine (Johnson and McBee 1967), sheep and wallabies (Hume 1977). Studies on the absorptive epithelia of the gut in both foregut- and hindgut-fermenting eutherians indicate little difference between species in the potential rate of VFA absorption per unit surface area (Stevens et al. 1980). Thus the low concentration of VFA in the hindgut of the koala (and greater glider) suggests not a faster rate of absorption but a slower rate of fermentation there than in the hindgut of these other species.

The mean rate of VFA production measured in the hindgut of the koala (11 μmole $ml^{-1}$ $h^{-1}$) was indeed slower than that estimated using similar techniques in the hindgut of a number of other species, fed forage diets, such as the pig (40 to 50 μmole $ml^{-1}$; Farrell and Johnson 1970), sheep (18 μmole $ml^{-1}$ $h^{-1}$; Hume 1977) and in two species of wallabies (28 μmole $ml^{-1}$ $h^{-1}$; Hume 1977). This may have been due to either a slower intrinsic fermentation rate by microorganisms in the koala hindgut or to some aspect of the eucalypt-derived digesta therein. No information on the first alternative is available. However, the fibre of *Eucalyptus* foliage is highly lignified and this, together with the presence of tannins (Cork et al. 1983), suggests that the substrate available to the microorganisms in the hindgut of the koala (and greater glider) is not readily fermentable.

Concentrations and rates of production of VFA in the caecum of rabbits (Hoover and Heitmann 1972) are also lower than in the hindgut of the species listed above. Both the rabbit and the koala selectively retain solutes and fine particles in the hindgut and this results in the koala in the exclusion of much of the dietary structural carbohydrate from the caecum/proximal colon (Cork et al. 1983). This may help to reduce fermentation rates by lowering the fermentable substrate per unit of digesta.

Digestion of cell-wall carbohydrate by koalas in the associated experiments (Cork et al. 1983) accounted for 9–11% of the DE intake. Allowing for a loss as gas of approximately 30% of this energy (2–3% of DE intake) in the production of VFA's in the proportions observed here (Hungate 1966), this leaves 30–40% of the in vitro VFA production not accounted for by fermentation of cell-walls suggesting that some other substrate is also fermented in the hindgut.

The difference between the proportional production rates of acetate, propionate and butyrate in the hindgut (62:20:17) and their molar proportions in zero time digesta samples (86:10:4) suggests that selective absorption of individual VFA may occur in vivo in the order butyrate > propionate > acetate. A similar trend has been found using in vitro techniques in the greater glider (Cork and Hume 1978) and in the rabbit (Hoover and Heitmann 1972). In vivo measurements by Glinsky et al. (1976) in the caecum of the horse exhibit a similar trend, although to a lesser degree. In both the ruminant forestomach and the equine hindgut the relative rates of absorption of individual VFA appear to depend on not only the concentration of the acids in the lumen but also their relative rates of metabolism in the gut wall (Stevens and Stettler 1966; Argenzio et al. 1974). Selective absorption of butyrate and propionate would seem to be of some benefit, especially to species in which VFA production is slow, since the calorific values of these acids are higher than that of acetate, but further verification of its occurrence is required.

Another possible explanation for the difference between proportional production rates and initial molar proportions of VFA's is end-product inhibition of acetate production in vitro. However, this is unlikely to have been a significant factor in view of the slow and linear rates of production observed (Fig. 1).

Despite the relatively slow rate of VFA production in the hindgut, the contribution made by total VFA production in the koala (9% of DE intake) was similar to contributions made by VFA production measured in vitro in the porcupine (8%) (Johnson and McBee 1967) and in the pig (4 to 9%) (Imoto and Namioka 1978). This is explained by the large capacity of the koala's hindgut and the very slow turnover of digesta therein (Cork and Warner 1983).

In vitro incubation is thought to underestimate VFA production in the rumen of sheep and cattle by up to 50% (Whitelaw et al. 1970). However, the same may not be true in the hindgut where conditions are likely to be more stable owing to considerably lower concentrations of rapidly-fermentable rapidly-exhaustible substrates. Thus, Faichney (1969) obtained similar estimates of VFA production in the ovine caecum using in vitro incubation and in vivo isotope dilution. Isotope dilution was not used in the present study because of the risk involved in surgical procedures and because near-instantaneous mixing of injected isotope in the caecum and proximal colon could not be assumed. Thus in vitro incubation was used

with the knowledge that some underestimate of VFA production rates may occur.

Error in the estimation of total daily VFA production may also arise from the assumption that rates of production measured at one point in time are representative of rates over 24 h. The slow rates of digesta passage and fermentation in koalas and the fact that they fed intermittently throughout each 24 h preceding these experiments (Cork and Warner 1983) suggest that fluctuations in substrate availability in the hindgut would be small. Domestic rabbits with a similar feeding pattern have been shown to maintain relatively constant rates of VFA production, measured in vivo, throughout the day and night (Parker and McMillan 1976).

It must be concluded that, unless large amounts of unusual fermentation end-products are produced, the hindgut of koalas plays a minor role in the extraction of energy from eucalypt foliage. It seems improbable that such a highly developed characteristic has persisted without an important function and further research is needed to identify that function.

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