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ORIGINAL PAPER

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Administration of a $5HT_3$ receptor antagonist increases the intake of diets containing *Eucalyptus* secondary metabolites by marsupials

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Abstract The effect of a naturally occurring plant phenolic constituent (the acylphloroglucinol derivative, jensenone, derived from *Eucalyptus jensenii*) on the food intake of two folivorous marsupials, the common ringtail (*Pseudocheirus peregrinus*) and the common brushtail possum (*Trichosurus vulpecula*) was studied. When fed diets containing varying concentrations of jensenone, both species regulated their intake of jensenone so as not to exceed a ceiling intake. This ceiling was about twice as high for common ringtails as for common brushtails from northern Australia. Southern populations of common ringtails showed greatly reduced capacities to tolerate jensenone. When common brushtails were injected ($0.5\ mg \cdot kg^{-0.75}$ body mass) with ondansetron (a selective antagonist of serotonin $5HT_3$ receptors), they ate significantly more jensenone than animals injected with physiological saline. The same pattern was observed when common ringtails were fed diets containing both jensenone and ondansetron ($0.0035\ mg \cdot g^{-1}$ wet mass of diet). Ondansetron injection had no effect on food intake when the food did not contain jensenone while the addition of higher doses of ondansetron to diets of common ringtails very slightly reduced food intakes of a non-jensenone diet. When common brushtails were given 50 mg of jensenone by gastric lavage, their average subsequent intake of dietary jensenone matched the difference between the daily threshold and the dose given, although the response of individuals was highly variable. Lavage with water alone had no effect on subsequent jensenone intake compared with the pre-dose period. We interpret these results as evidence that the antifeedant effects of jensenone and related compounds are partly mediated by serotonin action on $5HT_3$ receptors most likely via "nausea" to condition a food aversion.

Key words Serotonin · Emesis · Conditioned food aversion · *Pseudocheirus* · *Trichosurus*

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Introduction

Many vertebrate herbivores feed to avoid exceeding a threshold dose of particular plant secondary metabolites (Meyer and Karasov 1989; Jakubas et al. 1993; Pass et al. 1998). In effect, feeding is regulated so that the animal's intake of dry matter is reduced as the concentration of plant secondary metabolite in the diet in increased. In most cases, these patterns are very tightly controlled and there may be no significant increase in the intake of the plant secondary metabolite even when the dietary concentration is increased five to ten fold (e.g. Jakubas et al. 1993)

How is this impressive regulatory feat achieved? Many authors have speculated on the effect of plant secondary metabolites on mammalian herbivores but there have been few demonstrations of the effects of particular compounds on animal metabolism that could result in specific feedback signals. Foley (1992) and Foley et al. (1995) argued that the effects of many plant secondary metabolites could be ascribed to disturbances in acid-base metabolism, but they could find no evidence for specific regulatory mechanisms. Guglielmo et al. (1996) suggested that dilution of useable nutrients by plant secondary metabolites was responsible for the se-

lective foraging of ruffed grouse (*Bonasa umbellus*), but again it is not clear how this dilution effect could function as a feedback control.

In contrast, Provenza and co-workers (e.g. Provenza 1995, 1996; Provenza et al. 1990, 1992) have argued that animals feeding on diets rich in plant secondary metabolites develop conditioned food aversions mediated by feedback from the "emetic centre" of the medulla. They hypothesized that animals should adjust their feeding so as not to exceed some threshold intake on the basis of this feedback. Provenza et al. (1992) argued that this feedback does not have to result in overt illness and that the animal need not even be conscious of the event that acts as a powerful trigger to provide sufficient signal for the animal to modulate its intake. Thus Provenza and co-workers have identified a possible regulatory mechanism that could explain why animals feeding on food containing plant secondary metabolites do, in fact, limit their food intake.

There are difficulties with interpreting feedback from plant secondary metabolites as emesis or vomiting *per se*. Many herbivores do not vomit and, even in those species that do, the degree to which we can attribute our perception of nausea to animals remains uncertain (Andrews et al. 1988; Veyrat-Follet et al. 1997). Secondly, emesis can result from many different pathways, all mediated by different neuroactive agents (Veyrat-Follet et al. 1997). Finally, previous work in this area has been conducted with rats and sheep given LiCl (Provenza 1995, 1996; Provenza et al. 1992, 1994) rather than naturally occurring plant secondary metabolites. Consequently it is difficult to interpret physiologically Provenza's concept of "emetic stimulation" in the context of herbivores eating natural plant diets.

In this study we address these difficulties by examining the response of the marsupials *Trichosurus vulpecula* (common brushtail possum) and *Pseudocheirus peregrinus* (common ringtail possum) to dietary jensenone. Jensenone (Fig. 1) is an acylphloroglucinol derivative found in the foliage of *Eucalyptus jensenii* (Boland et al. 1992; Ghisalberti 1996) and is a simple form of the complex phloroglucinol-terpene adducts (e.g. macrocarpal G; Fig. 1) that are responsible for the selective feeding of these marsupial folivores (Lawler et al. 1998, Pass et al. 1998).

We sought first to establish whether the animals regulated their intake of food according to the concentration of jensenone in the diet and secondly, whether we could increase voluntary food intake by injecting the animals with a potent serotonin $5HT_3$ receptor antagonist, ondansetron. Serotonin at the $5HT_3$ receptor mediates part of the emetic response in humans and laboratory animals. Ondansetron is widely used to control nausea and vomiting during some cancer therapies (Butler et al. 1998) and aids in restoration of normal food intake of chemotherapy patients (Beck 1992). We expected that if serotonin acting at the $5HT_3$ receptor mediated the antifeedant effects of jensenone, then administration of a drug such as ondansetron would lead to a significant increase in food intake relative to controls.

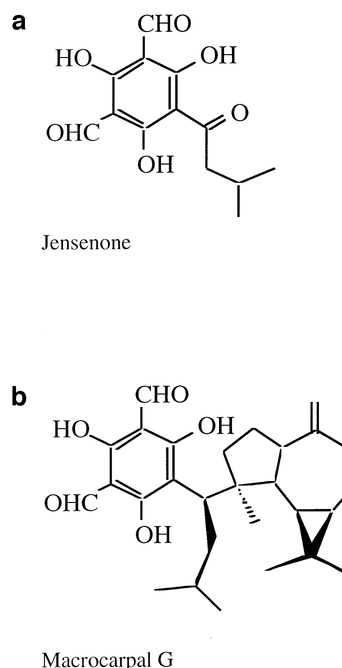


Fig. 1 Planar structures of **a** jensenone and **b** macrocarpal G, acylphloroglucinol derivatives of *Eucalyptus*

Material and methods

Animals and basal diets

Twelve common brushtail possums (*T. vulpecula*) and 12 common ringtail possums (*P. peregrinus*) were caught by hand in woodland near Townsville (northern Australia). A further 12 common ringtail possums were collected near Canberra (southern Australia) for later experiments. Animals were maintained in individual metabolism cages in an air-conditioned room maintained at 21 ± 2 °C. The room was maintained on a 12:12 light:dark regime and the lights were connected to a dimmer that allowed gradual changes in the light intensity to simulate dawn and dusk. Both species are nocturnal feeders in nature but in captivity the common brushtail may feed irregularly during the day.

All animals were fed a palatable basal diet that consisted of (percentage wet matter) 55.5% grated apple, 28.3% banana pulp, 4.7% lucerne hay ground to pass through a 2 mm sieve, 5.5% ground rice hulls, 4.7% ground Weetbix (a wheat-based breakfast cereal) and 1.6% acid casein. All animals maintained body mass on this basal diet. This diet contained 32% dry matter and this dry matter contained 97% organic matter, 1.9% N, 6.0% cellulose, 6.4% hemicellulose and 9.9% acid lignin. These analyses followed methods as described in Foley (1992) and Lawler et al. (1997). To determine the dry matter intake (DMI) of the animals, the dry matter content of the diet offered was determined by subsampling the food offered and the dry weight of food refusals was determined by drying all refusals for 24 h at 80 °C. Free drinking water was always available ad libitum.

Experiment 1: the effect of jensenone on DMI of common ringtail and brushtail possums

Common ringtail possums

We measured the DMI of six adult (four male, two female) common ringtail possums (mean body mass 0.74 kg) fed six concentrations of jensenone in a basal diet. The protocol used was a no-choice experiment as a 6 × 6 Latin square. The concentrations used were: 0, 0.06, 0.12, 0.24, 0.36% and 0.48% of the wet weight of the diet and the diets were prepared as described above. Treated food was provided ad libitum at 1700 hours (1 h before the dark period). Urine was collected into plastic bottles set in a slurry of solid CO_2 and kept frozen at −20 °C until assayed for glucuronic acid (Blumenkrantz and Asboe-Hansen 1973) as a potential index of biotransformation of jensenone (Foley et al. 1995).

The treated food was replaced with untreated basal diet at 0500 hours (1 h before the end of the dark period) because animals offered high concentrations of jensenone had eaten very little. Common ringtail possums are very reluctant to feed outside of their usual nocturnal period and this ensured they had some time to feed. However, they typically ate only a small amount during the remaining period of darkness ($<$35% of normal intake), so treatment days were alternated with days when only untreated basal diet was offered, to ensure the welfare of the animals and reduce carry-over effects between periods.

Common brushtail possums

Experiments carried out with brushtail possums followed the same design as for ringtails with six adult males (mean body mass 2.28 kg) and the same jensenone concentrations used. Food was offered at 1630 hours and removed at 0830 hours, when ad libitum amounts of untreated basal diet were provided; this was removed at 1200 hours. As brushtail possums are more inclined to feed outside of their normal nocturnal period, their intakes of the untreated basal diet were consequently higher and it was not considered necessary to alternate treatment days with non-treatment days for these animals.

Experiment 2: effect of ondansetron on DMI and jensenone intake in common brushtail and common ringtail possums

Intraperitoneal injection of ondansetron

Eight common brushtails (mean body mass 2.20 kg) and, in a separate experiment, eight common ringtails (mean body mass 0.74 kg) were allocated among four treatments in double Latin square designs in which we measured DMI and jensenone intake. Jensenone was added to the diets at a concentration of 0.2% (wet weight) (brushtail) or 0.35% (wet weight) (ringtail) of basal diet. Treatments were: (1) dietary jensenone plus an injection of 0.5 ml of 0.9% sterile saline, (2) dietary jensenone plus an intraperitoneal injection of $0.5\ mg \cdot kg^{-0.75} \cdot day^{-1}$ of ondansetron, (3) basal diet plus an injection of ondansetron, and, (4) basal diet plus saline. Protocols for feeding the animals were as for experiment 1, except that for the common ringtail possums, the beginning of the dark period was changed to an hour earlier a week prior to the commencement of the experiment. This was to ensure that the ondansetron injection was given as close to the beginning of the feeding period as possible. We purchased ondansetron ("Zoffran": Glaxo) as an aqueous solution ($2\ mg \cdot ml^{-1}$) and used the contents of a single 2 ml vial within 30 min.

Dietary administration of ondansetron

In common ringtail possums there was no effect of intraperitoneal ondansetron on jensenone intake, which contrasted with the results for common brushtail possums (see below). We reasoned that this was due to different feeding behaviours after injection, which resulted in the common ringtail possums not feeding until much of the injected ondansetron had been metabolized (see Discussion). The experiment was therefore repeated at a later date (with common ringtail possums collected in southern Australia) with ondansetron mixed with the diet to maintain higher plasma levels of ondansetron while feeding on the jensenone-treated diet. Ondansetron was purchased as 8-mg tablets ("Zoffran": Glaxo), crushed in a mortar and pestle and added at $0.035\ mg \cdot g^{-1}$ (wet weight) of the diet. The experimental design was as described above with a jensenone concentration reduced to 0.12% (wet weight), due to an apparently lower threshold for jensenone in common ringtail possums from southern Australia.

Experiment 3: effect of jensenone given by gastric lavage on intake of dry matter and jensenone in common brushtail possums

Eight male brushtail possums (mean body mass 2.27 kg) were randomly assigned to two groups, test and control. All animals were fed the basal diet with 0.1% (wet weight) jensenone ad libitum. The four animals in the test group were given 30 mg of jensenone by gastric lavage so they could not taste it. Jensenone was administered dissolved in 1 ml of 2.0% $NaHCO_3$. Immediately after dosing, 2 ml of water was flushed through the lavage tube before it was removed from the animal to ensure that complete dose was received. This amount was approximately 30% of the total amount of jensenone the animals were voluntarily ingesting (see results for experiment 2). Therefore, if jensenone was a metabolic deterrent and caused post-ingestive effects, DMI should be reduced by approximately 30% relative to controls values. The four possums in the control group were dosed in the same way with 0.9% saline. This experiment was repeated with a second group of eight male brushtail possums (means body mass 2.32 kg), because the results of the first experiment were equivocal. In this experiment the animals were dosed with 50 mg of jensenone but all other procedures were the same.

Extraction and purification of jensenone

Eucalyptus jensenii foliage was collected from natural stands at Mount Bundy in the Northern Territory of Australia and from a small number of cultivated trees at Gympie, SE Queensland. The foliage was air-dried and ground to pass a 2 mm screen; 1 kg lots were extracted in 20% acetone:light petroleum for 6 h in a Soxhlet apparatus. The extracts were concentrated and combined with 1 l of di-ethyl ether and then washed 2–3 times with 0.3 M NaOH. These washes were acidified (12 M HCl) and the precipitate washed with ethanol and re-crystallized from acetone to give jensenone (98% by ^1H NMR) in a yield of about 2.8% (dry matter).

Statistical analysis

In all experiments, possible differences in mean DMI and jensenone intake were analysed by analysis of variance. Terms accounting for the main effects of possum, day, treatment and possible carry-over effects of treatments were included in this analysis. Where carry-over effects were found to be highly non-significant ($P > 0.35$) they were dropped from the model and the data re-analysed (Ratkowsky et al. 1993). Where jensenone intake was compared between treatments, the treatment with no jensenone was omitted from the analysis. In experiment 2 we were less interested in overall differences between treatments, but focused on the differences between specific combinations of treatments. These comparisons have been made using *t*-tests.

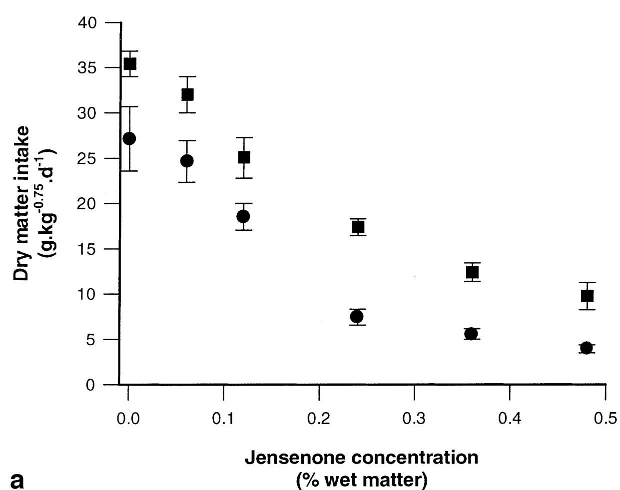
Measurements of intake of plant secondary metabolites and excretion of metabolites have been scaled to $M^{0.75}$ because several studies (Walker 1978; Freeland 1991) have shown that interspecific detoxification capacity scales to this exponent. Body mass in each experiment is provided to allow alternative scaling factors to be calculated.

Results

Dose response experiments in common brushtail and ringtail possums

Increasing the concentration of jensenone in the diet of both common brushtail and common ringtail possums led to significant reductions in DMI ($P < 0.0001$ for both species, Fig. 2a). In both species, at jensenone concentrations above approximately 0.12% (wet weight), DMI tended to decrease with increasing jensenone concentrations. Both species appeared to regulate food intake to remain below a ceiling dose of jensenone (Fig. 2b). Brushtail possums were unwilling to ingest more than approximately 70 mg · $kg^{-0.75}$ · d^{-1} and this was constant across all concentrations of jensenone ($P = 0.108$). The amount of jensenone eaten by common ringtails varied significantly ($P = 0.002$), but this was because the amount eaten was lower at 0.06% jensenone, perhaps due to partial satiation reducing their willingness to take in the compound. If this concentration was omitted, jensenone intake was shown to be regulated at a constant level ($P = 0.124$), much higher than the common brushtails, of approximately 140 mg · $kg^{-0.75}$ · d^{-1}.

Only trace amounts of glucuronic acid were excreted by both species and this was independent of the jensenone intake (brushtails $P = 0.349$, ringtails $P = 0.521$). The mean excretion rates were 111 μmol · $kg^{-0.75}$ · d^{-1} in the common ringtails and 102 μmol · $kg^{-0.75}$ · d^{-1} in the common brushtails.



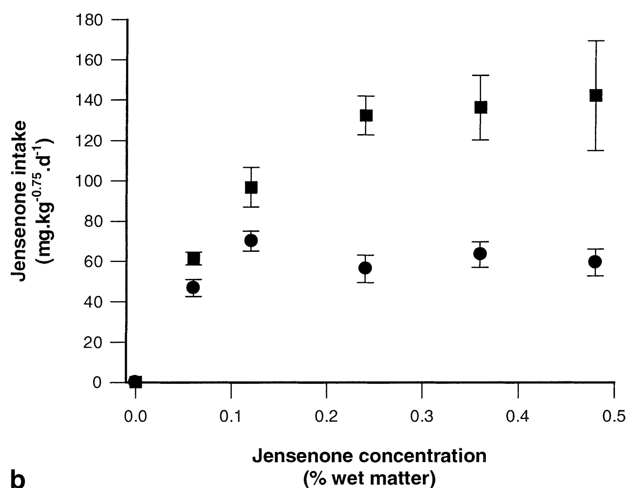


Fig. 2a The effect of jensenone on the dry matter intake (DMI) of common ringtail and brushtail possums offered food containing jensenone at one of six concentrations. **b** The effect of varying concentrations of dietary jensenone on the intake of jensenone in common ringtail and brushtail possums. *Squares* are ringtail possums, *circles* are brushtail possums

Effect of ondansetron on the intake of jensenone by common ringtail and brushtail possums

Intraperitoneal injection of ondansetron

Brushtail possums that received injections of ondansetron ate significantly more jensenone than those that received injections of saline solution ($P < 0.001$, Fig. 3). Ondansetron alone had no effect on DMI in the brushtails ($P = 0.652$). Nonetheless, ondansetron did not restore food intake of animals fed jensenone in the diet to the levels seen in the control groups ($P < 0.001$).

In contrast in ringtail possums, injections of ondansetron had no effect on the intake of jensenone ($P = 0.696$, Fig. 3). Again, ondansetron itself did not affect DMI ($P = 0.837$) but DMI of the jensenone diet remained at about 30% of that observed in controls ($P < 0.001$).

Dietary administration of ondansetron

When ondansetron was added to the diet, thereby increasing internal levels of ondansetron simultaneously with jensenone ingestion, intakes of jensenone were substantially increased ($P < 0.001$, Fig. 3) but again not fully restored to the level of intakes on control diets ($P = 0.001$). Ringtail possums very slightly reduced their intake of diets treated with ondansetron, but only relative to untreated controls ($P = 0.020$).

Effect of gastric lavage of jensenone on intake of jensenone-containing diet by brushtail possums

When the animals were dosed with 30 mg of jensenone (about 30% of their average voluntary intake of jensenone per unit metabolic body mass), there was a trend towards reduction in subsequent dry matter and jensenone intake, relative to controls (Fig. 4a). However, there was no significant difference between either the

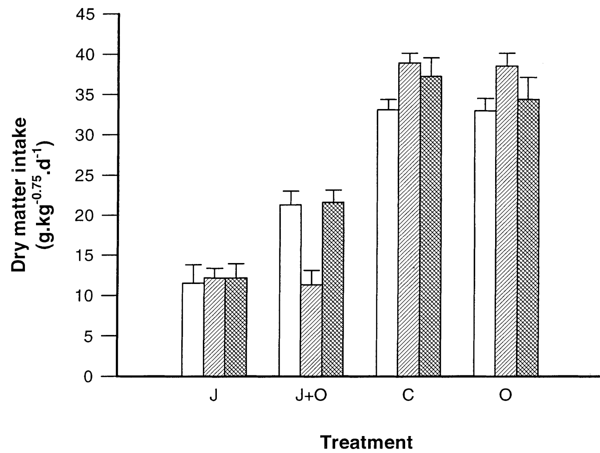
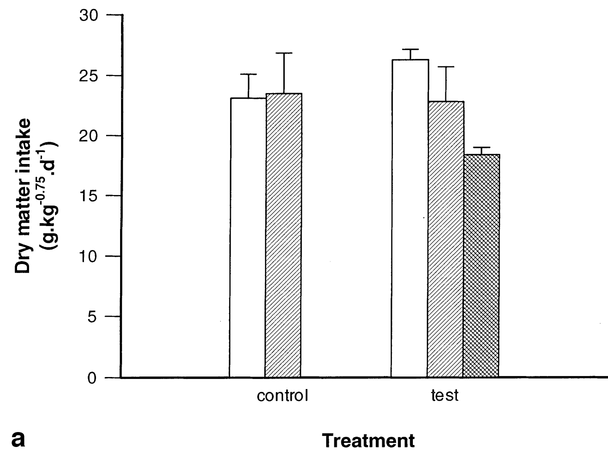


Fig. 3 The effect of administration of ondansetron (*O*) or water (*C*) on DMI of common ringtail possums and brushtail possums fed dietary jensenone (*J*). *Unshaded bars* are brushtail possums injected intraperitoneally and fed 0.2% (wet weight) jensenone, *diagonally shaded bars* are northern Australian ringtail possums injected intraperitoneally and fed 0.35% jensenone, *crosshatched bars* are southern Australian ringtail possums administered ondansetron orally and fed 0.12% jensenone



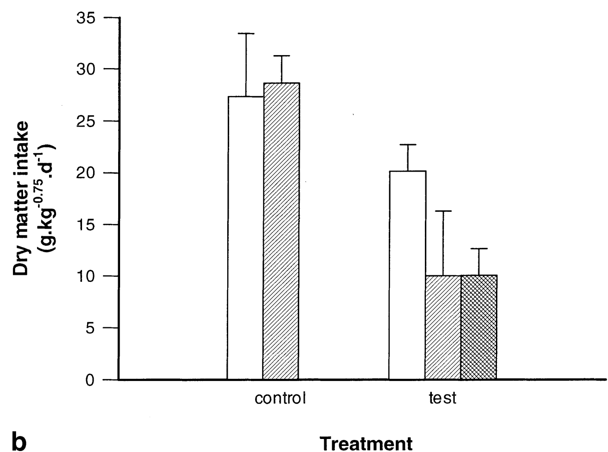


Fig. 4a, b The effect of jensenone given by gastric lavage to common brushtail possums fed 0.1% (wet weight) dietary jensenone. The expected DMI is calculated assuming that jensenone causes a dose-dependent decrease in food intake as shown in Fig. 2. (**a**) 30 mg dose of jensenone, and (**b**) 50 mg dose of jensenone. *Unshaded bars* are pre-dose intakes, *diagonally shaded bars* are post-dose intakes, and *crosshatched bar* represents expected post-dose intakes

pre- and post-dose intakes of the treated animals ($P = 0.424$) or the post-dose and expected intakes for the treated animals ($P = 0.295$). This was due to the highly variable responses of the animals: two animals reduced their intakes to close to the expected levels while the other two slightly increased their intakes. The intakes of the control animals in this experiment did not change ($P = 0.853$).

Increasing the oral dose to 50 mg (about 50% of the average voluntary intake per unit metabolic body mass) resulted in a greater reduction in subsequent intake of jensenone, but again the data were sufficiently variable so that this reduction was not significantly different from pre-dose values ($P = 0.176$). Much of the variability resulted from two of the animals vomiting within 3 h of the lavage. Nonetheless, the average intake of jensenone was almost identical to that expected if jensenone acted in a dose-dependent fashion ($P = 0.992$) (Fig. 4b). Again, animals lavaged with water as a control did not reduce their intake of jensenone compared with pre-dose values ($P = 0.869$).

Discussion

Regulation of toxin intake is mediated by serotonin

This study has provided clear evidence that both common ringtail (*P. peregrinus*) and common brushtail possums (*T. vulpecula*) regulated their feeding so as not to exceed a threshold intake of jensenone, and that at least part of this regulatory ability is mediated by serotonin. Because ondansetron is such a selective antagonist of serotonin $5HT_3$ receptors (Butler et al. 1988), we can be certain that part of the antifeedant action of jensenone is mediated via serotonin through this receptor site, but it remains uncertain exactly where the receptors are located. Jensenone may cause the release of serotonin from enterochromaffin cells in the small intestine (Veyrat-Follet et al. 1997). Serotonin receptors may thus be found in the gut on visceral afferent fibres which in turn carry signals to the brain or, less likely, the toxins may be carried in the systemic circulation and exert a central effect at the chemoreceptor trigger zone.

Other related research has used less selective anti-emetic agents, or mixtures of agents, which make interpretation of results more difficult. Two earlier studies used either high doses of metoclopromide (Aldrich et al. 1993) or cocktails of metoclopromide, dexamethazone (which enhances the effects of metoclopromide) and diphenhydramine (Provenza et al. 1994) to attenuate the effects of either LiCl or alkaloid-infected grass on food

intake of sheep (Aldrich et al. 1993). Although both studies showed an elevation of food intake in response to the drugs, the less selective nature of the agents makes it difficult to explain the results in terms of specific physiological pathways. Metoclopromide in particular, is primarily a dopamine (D_2) receptor antagonist but at high doses also acts as an antagonist of $5HT_3$ receptors (Andrews et al. 1988; Veyrat-Follet et al. 1997). High doses of metoclopromide can also have significant effects on gastrointestinal motility (Veyrat-Follet et al. 1997), and although the same is true for ondansetron (Butler et al. 1988), the lower doses of this agent necessary to attenuate emesis mean that these side effects are less likely to occur and did not occur in this study. Accordingly, we conclude that ondansetron affected food intake through its action as a $5HT_3$ receptor antagonist rather than through any action on gut motility.

Although it is valuable to be able to specifically identify receptor sites that are involved in modulating feeding, we can still not be certain whether the feedback that is used as a regulatory point is due to nauseous sensations. Nausea remains a subjective sensation (Andrews et al. 1988; Veyrat-Follet et al. 1997) and we do not know whether animals perceive the same sensations that we do. In the case of the brushtail possums, a bolus dose of jensenone given by lavage caused vomiting in two animals, so we could cautiously interpret the effects of ondansetron in terms of a reduction in the nauseous sensations induced by ingestion of jensenone (Veyrat-Follet et al. 1997). However, to an unsatisfactory extent, we can only say with certainty that the feedback which allows marsupials to regulate their intake of jensenone is mediated in part through $5HT_3$ receptors.

Because of these uncertainties, it seems best to discontinue use of the general term ''emetic stimulation'' when referring to the mechanism by which herbivores regulate their intakes of plant secondary metabolites. Several groups of herbivores appear not to have the ability to vomit and this makes it difficult to attribute to them feelings of nausea. While it may be possible in some circumstances to make objective measurements of physiological traits related to nausea, such as plasma vasopressin (Andrews et al. 1988), difficulty will still be encountered in calibrating these measures against the animals' perceptions. A further problem may be encountered if these measurements are not appropriate for the particular emetic stimulus. For example, while plasma vasopressin may increase in response to an emetic stimulus, other stimuli do not increase vasopressin (Andrews et al. 1988). In contrast, describing the feedback processes as envisaged by Provenza and coworkers in terms of specific receptors draws attention to the many other effects mediated by these pathways. This raises the possibility that receptor-based differences between herbivore species and individuals may be consistent with different tolerances for specific toxins, for example those that we have observed between common ringtail and brushtail possums.

Although we have identified a partial control mechanism that explains how jensenone intake is regulated, we still do not know whether jensenone has more widespread effects or what its metabolic fate is within the animals. The increase in jensenone intake when animals were administered ondansetron, together with the effect of gastric lavage on jensenone intake of brushtails, confirms that jensenone exerts its effects, not through taste or smell, but by causing an 'internal malaise' (*sensu* Provenza 1995). Making the link between the regulatory mechanism and toxicological processes is an important goal in understanding the effects of plant secondary metabolites on mammalian foraging.

Jensenone is a diformylphloroglucinol derivative that shares its core phenolic moiety with a range of other compounds that have been identified as natural feeding deterrents in *Eucalyptus* such as Macrocarpal G (Fig. 1; Lawler et al. 1998; Pass et al. 1998). All members of this family of compounds contain a phenolic moiety linked to a terpene, but in the case of jensenone the full mono- or sesqui-terpene found in most of the compounds is replaced by an isoprene unit. This lipid-soluble side chain is presumed to facilitate the passage of the compound across membranes and so carry the reactive phenol and aldehyde groups to sites where they may damage cells (Pass et al. 1998). We have not been able to recover jensenone, or its metabolites, from the faeces or urine and thus assume that the whole dose has been absorbed (S. McLean, S. Brandon and W. Foley personal observation).

Inter- and intra-specific differences in response to jensenone and ondansetron

In our initial experiments, where results are directly comparable between species, common brushtails consumed only half the amount of jensenone eaten by common ringtails. Common brushtails are perceived as generalist browsers whereas the greater reliance of the smaller-sized common ringtails on *Eucalyptus* foliage has led to them being labelled as specialists (e.g. McArthur and Sanson 1993). Although we corrected jensenone intakes for metabolic body size, the difference between the two species persisted. Irrespective of the size of organs such as liver, smaller species with their higher mass-specific metabolic weight should be able to biotransform and clear toxic components from the body more rapidly than larger species (Freeland 1991). Since detoxification capacity scales to about the same exponent as does metabolic rate (Walker 1978; Freeland 1991), we could expect that expressing intake in this way should have eliminated the differences between the species. That it did not suggests that in the northern Australian populations of animals used, the common ringtails possess mechanisms that allow them to tolerate and/or excrete jensenone and its metabolites over and above the advantages conferred by body size alone.

It is perplexing then, that in our later experiment with ringtail possums from Southern Australia the threshold jensenone intake was substantially reduced. We propose two hypotheses. The first is that perhaps there is a geographic difference in the ability of this species to tolerate jensenone. The initial experiments were conducted in Northern Australia while the latter experiment (oral dosing of ondansetron) was conducted with possums collected some 2500 km south. Whether there are genetic differences between populations in their capacity to detoxify these compounds, or perhaps phenotypic differences, due to different levels of exposure to diformylphloroglucinol derivatives is uncertain at this time. The latter seems unlikely as these compounds are found in most *Eucalyptus* species and there is no reason to suspect they are in higher concentrations in northern species (D.M. Pass, W.J. Foley, B.M. Eschler personal observation).

The second explanation is a component of learned avoidance of jensenone or reduced physiological capacity due to repeated exposure to this and related compounds. The animals used in the second experiment had previously been fed leaf diets, that were occasionally high in sideroxylonals (dimers of jensenone – Ghisalberti 1996) and had also been used in two other experiments using jensenone (I.R. Lawler, W.J. Foley, B.M. Eschler personal observation). Over these last three experiments there was also a smaller but notable decrease in their jensenone threshold from approximately $80 \text{ mg} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$ to $50 \text{ mg} \cdot \text{kg}^{-0.75} \cdot \text{d}^{-1}$ (I.R. Lawler personal observation). This suggests some reduction in threshold due to repeated exposure, but the difference between northern animals and southern animals at their first captive exposure to jensenone was nevertheless significant.

We do not believe differences in the purity of jensenone between experiments were important. Our data show that all jensenone samples were highly pure and any differences certainly could not account for a threefold decrease in the threshold. Experiments with southern populations of brushtail possums conducted concurrently with these later ringtail possum experiments, and using the same batch of jensenone, did not show a corresponding decrease in threshold (threshold approx. $60 \text{ mg} \cdot \text{kg}^{-0.75} \cdot \text{day}^{-1}$; J. Stapley personal observation). This reinforces our impression that there are differences between populations of ringtail possums in their capacity to tolerate or detoxify jensenone. Controlled experiments with possums of both regions tested simultaneously, to avoid various confounding factors, are required to unequivocally demonstrate interpopulation differences in jensenone tolerance.

Differences in the feeding patterns of the two species are most likely responsible for differences in their response to injected ondansetron. Brushtail possums commenced feeding immediately after the injection of ondansetron and presumably fed when plasma concentrations of the drug were highest. However, the food intake in animals injected with ondansetron was still less than that of controls, suggesting either that there is some other feedback that is limiting the intake, or more likely that the drug was cleared from the plasma rapidly enough to render it ineffective in the latter part of the night. In humans, ondansetron must be re-injected every 8 h or oral supplements taken to maintain effective plasma concentrations (Butler et al. 1998; Rudd and Naylor 1996). Even though the dose we gave both possums was about ten times the normal human dose, it must have still been cleared rapidly enough to reduce its effectiveness, so may not have been effective for the duration of the 12 h feeding period (F. Mitchelson, personal observation).

We believe that a similar effect explains the lack of an effect of injected ondansetron in the common ringtails. In contrast to the brushtails, the ringtails retreated to their nest boxes immediately after the injection and showed little inclination to feed in the first 4 h afterwards. This coupled with their smaller size (which should lead to a more rapid clearance of the drug), meant that the dose that we were able to give them was ineffective when their feeding was at its peak. Oral dosing via the addition of ondansetron to their diet, which presumably raised plasma ondansetron levels to correspond with jensenone intakes, confirmed that the drug could at least partly ameliorate the effects of jensenone.

In conclusion, understanding how plant secondary metabolites affect animal food choice is an important step in quantifying the metabolic costs for certain diets and foraging patterns. Other mechanisms which might control the ingestion of plant secondary metabolites have been proposed (e.g. acid-base regulation: Foley et al. 1995) but these experiments are the first to demonstrate a specific regulatory mechanism. It remains now to demonstrate what the effects of jensenone are in the animals and to determine whether the intakes of other plant secondary metabolites are regulated in a similar fashion; clearly however, serotonin acting at the $5HT_3$ receptor is a major factor regulating feeding in these marsupials.

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