J Comp Physiol B (2000) 170: 211–218 

ORIGINAL PAPER

J. Stapley · W. J. Foley · R. Cunningham · B. Eschler

# How well can common brushtail possums regulate their intake of *Eucalyptus* toxins?

Accepted: 4 January 2000

**Abstract** We studied factors affecting the ability of common brushtail possums (*Trichosurus vulpecula*) to regulate their intake of a dietary toxin, jensenone, extracted from *Eucalyptus* leaves. Increasing concentrations of jensenone in the diet led to a dose-dependent decrease in food intake best described as an exponential decay. Animals that had not previously been exposed to jensenone ate significantly more when first offered food containing the compound than on subsequent days. However, when offered the same amount of food in a number of portions throughout the night, naïve animals ate significantly less than animals offered the total meal at once. When offered food containing jensenone over a 13-day period, the animals' intake varied cyclically with relatively high food intakes followed by relatively low intakes. Furthermore, animals that were exposed to cold conditions (4 °C) ate more than those maintained at 18 °C but this difference was abolished when jensenone was included in the diet. We interpret these results as showing that regulation of toxin intake by common brushtail possums depends on learned responses that can override other important influences on feeding.

**Key words** Marsupial · Detoxification · Antifeedant · Conditioned food aversion · Cold exposure

Communicated by I. D. Hume

J. Stapley · W. J. Foley (✉) · B. Eschler
Division of Botany and Zoology, Australian National University
Canberra 0200, Australia
e-mail: william.foley@anu.edu.au
Fax: + 61-264-92535

R. Cunningham
Statistical Consulting Unit, Australian National University
Canberra 0200, Australia

B. Eschler
Research School of Chemistry
Australian National University
Canberra 0200, Australia

**Abbreviations** *DM* dry matter · *DMD* dry matter digestibility · *DMI* dry matter intake · *PSM* plant secondary metabolites

## Introduction

Browsing mammals, including folivorous marsupials, encounter a diverse range of plant secondary metabolites (PSMs) in most, if not all, foods on which they feed (Palo and Robbins 1991). Therefore, they cannot hope to avoid ingesting PSMs and so must regulate their intake of any potentially toxic constituents (Foley et al. 1999). An animal's ability to regulate the intake of a PSM depends partly on whether it can detect the compound, but also on its physiological capacity to detoxify or biotransform it. This in turn must be coupled with some sort of feedback mechanism that leads to a rapid change in feeding rate.

Feedback can arise from cues developed before, during or after ingestion of PSMs and may be affected by many factors such as the nature of the feedback signal (Lawler et al. 1998a, b) and delays between ingestion of the toxin and the development of the effect. For example, Provenza et al. (1993) found that the longer a negative post-ingestive feedback is delayed, the weaker the aversion to a novel food. Accordingly, experiments in which this period is manipulated may shed light on the regulatory processes. One source of feedback is via $5HT_3$ (serotonin) receptors, probably in conjunction with nauseous sensations, that lead to conditioned food aversions (Provenza 1995, 1996; Lawler et al. 1998a, b). Nonetheless, provision of a range of drugs that antagonise some of the receptors likely to be involved in nausea have only partially ameliorated the depression in food intake caused by dietary toxins suggesting that there are multiple feedback signals.

When toxins are added to basal or artificial diets, the mean intake of toxins by groups of animals remains relatively constant even when toxin concentration

in the diet changes five- to tenfold (Jakubas et al. 1993; Lawler et al. 1998a, b). Although this suggests precise regulatory mechanisms, patterns in individual animals or in herbivores fed natural plant diets are not so clear-cut. For example, Pfister et al. (1997) showed that cattle ate alkaloid-rich, tall larkspur (*Delphinium barbeyi*) more on some days than on others. This resulted in a cyclical pattern of food intake over time and implies that plasma levels of the toxic alkaloids varied substantially over time. Alternatively, when more than one toxin is present, as may occur with natural plant diets, there may be competing regulatory signals to be integrated. In addition, extra energy demands such as those incurred during exposure to cold conditions or during lactation may result in animals receiving powerful stimuli promoting ingestion at the same time that there are stimuli to limit intake. Food deprivation has been suggested to limit an animal's ability to ingest toxins because of reduced nutritional resources to devote to detoxification (Harju 1996; Wang and Provenza 1997) but the responses seen may depend on the nature of the toxic stimuli (Foley et al. 1999).

These areas of uncertainty suggest a broad approach is needed to understanding the regulation of the intake of PSMs by herbivores. Therefore, we investigated the regulatory pattern of a herbivorous marsupial, the common brushtail possum (*Trichosurus vulpecula*), when fed a diet containing a well-characterised plant toxin (jensenone – a diformylated phloroglucinol derivative) isolated from *Eucalyptus* leaves. In particular, we wanted to know how precisely brushtail possums could regulate their intake of this toxin and whether manipulating the timing of the feedback signal could affect feeding rates. Finally, we wanted to know whether animals could maintain higher intakes of jensenone when energy requirements were elevated by exposure to cold.

## Materials and methods

### Animals and diets

We trapped common brushtail possums (*T. vulpecula*) in woodland near Canberra, in south-eastern Australia and held them in individual metabolism cages in a room maintained at 18–20 °C on a 12 h:12 h light:dark cycle. We changed the diet of the animals slowly until they were eating a basal diet of fruit and cereals as described by Lawler et al. (1998a, b). This diet consisted of (% wet matter): grated apple (55.5); grated banana and carrot (15.0); sugar (5.35); ground Weetbix (a commercial breakfast cereal) (3.0); ground lucerne (1.0); ground rice-hulls (5.0) and acid casein (0.15) and contained, on average, 26% dry matter (DM) and 4.7% crude protein. Water was supplied ad libitum at all times.

We used jensenone, a formylated phloroglucinol derivative found in *Eucalyptus jensenii* leaves as a model toxin in these experiments because previous work (Lawler et al. 1998a, b, 1999a, b) had given us some understanding of its mode of action on marsupials and because we could extract sufficient quantities from natural sources (Lawler et al. 1998b, 1999a). We dissolved jensenone in a minimum volume of acetone and added this solution to the dry components of the diet and allowed the solvent to evaporate. This material was then thoroughly mixed with the mashed fruit and the diet was presented to animals as a wet mash. Control diets were treated with acetone alone.

### Experiment 1: the effect of different dietary jensenone concentrations on DM intake of brushtail possums

We fed 12 common brushtail possums, (mean body mass 2.8 kg) that had not previously encountered jensenone (hereafter referred to as "naive" possums), the basal diet into which we added one of 12 concentrations of jensenone over 12 nights in a Latin-square design. We used concentrations of 0%, 0.18%, 0.2%, 0.37%, 0.41%, 0.48%, 0.5%, 0.6%, 0.74%, 1.1%, 1.5%, and 1.7% (DM). We offered food ad libitum at 1700 hours and dried a subsample of the diet at 80 °C for 24 h to measure the DM content of the diet. Uneaten food (hereafter referred to as "food refusals") was collected the following morning at 0800 hours and was dried in an oven at 80 °C for 24 h to determine its DM content. The DM intake (DMI) was then calculated by subtracting the total DM refused from the DM offered. Animals were offered 30 g DM of basal diet between 0800 hours and 1200 hours (hereafter referred to as "morning feed") to ensure their welfare and to ensure that all had a similar motivation to feed on the next night. The effect of jensenone on DMI was tested using a mixed model ANOVA appropriate for the design described above. Differences in the daily intake of jensenone were tested using least significant differences (lsd).

### Experiment 2: the effect of providing food in several timed portions on jensenone intake

In experiment 1 we observed that animals ate much more jensenone when it was first offered than on subsequent nights. We hypothesised that this was because the animals had no experience to guide them in their feeding and that a delay between feeding and post-ingestive feedback meant that they were unaware of the toxin before they had eaten too much. On subsequent exposure to jensenone-rich diets, their previous experience conditioned a more cautious pattern of feeding.

We tested this hypothesis by feeding jensenone-rich diets at different rates throughout the night to two groups of naive common brushtail possums. We assigned ten brushtail possums (mean body mass 2.85 kg) randomly to one of two groups. We fed one group a diet treated with 0.4% (DM) of jensenone as normal at 1700 hours. We offered the second group the same quantity of this diet divided in four equal portions at 1700 hours, 2000 hours, 2300 hours and 0200 hours on the first night of the experiment. We ensured that we removed and replaced the food containers of both groups throughout the night so that any confounding effect of disturbance was controlled. Food refusals were collected at 0800 hours and a morning feed was offered to both groups as described earlier. We measured DMI and jensenone intake in both groups on all three nights of the experiment. Comparisons of jensenone intake for each experimental night were made using ANOVA.

### Experiment 3: effect of cold exposure on food intake, jensenone intake and DM digestibility

We wanted to know whether animals regulate their intake of jensenone even when their motivation to feed was greater through exposure to cold conditions. We randomly assigned 16 common brushtail possums (mean body mass 2.8 kg) to one of two groups. We housed 8 animals in a room maintained at 4 ± 1 °C (the "cold-treated group") and the other 8 in a room maintained at 18 ± 1 °C (the "control group"). We fed both groups of animals the basal diet for 6 weeks and then measured the intake and DM digestibility (DMD) of that diet by animals in each group over 6 days. We then

fed both groups the basal diet supplemented with 0.4% (DM) jensenone for 6 days and again measured DMI and DMD. The mean DMI and DMD over the 6-day period were compared between rooms using a mixed-model ANOVA. Data were transformed using a natural logarithmic function to meet assumptions of homoscedasticity. Only one room was available that could be set to 4 °C and so the experiment could strictly be described as pseudoreplicated. This constraint must be considered when examining the results.

## Results

### Experiment 1: the effect of jensenone concentrations on DMI of brushtail possums

There was a significant decline in DMI as the concentration of jensenone in the diet was increased ($P < 0.001$; Fig. 1). The relationship is best described by the exponential decay function (Fig. 1). There was no evidence of residual effects of different treatments ($F_{1,11} > 0.1$). There were no changes in body mass or other outward signs of toxicosis apart from the loss of 15% of body mass in one animal, but this mass was recovered after 2 days of eating the basal diet alone.

Mean jensenone intake reached a plateau with increasing jensenone concentration, with an overall mean jensenone intake of 114 mg day$^{-1}$ (Fig. 2). The inverse of the exponential decay function fitted in Fig. 1 and replotted in Fig. 2, suggests a plateau of jensenone intake of 125 mg day$^{-1}$, which was reached at a jensenone concentration of 0.48% (DM). There was considerable variation in observed values from the fitted equation of mean jensenone intake as jensenone concentration increased (Fig. 2). Although the variation is not significantly different over the different jensenone concentrations (represented by the lsd bar in Fig. 2) variation appears to be greater at the mid-range jensenone concentrations.

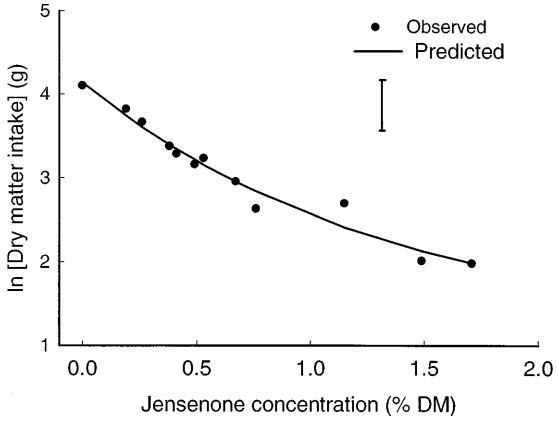


**Fig. 1** Log of mean dry matter intake (DMI) of common brushtail possums fed a basal diet containing 12 concentrations of jensenone. Observed points refer to mean ln (DMI), the line represents the exponential decay function fitted to the data: ln (DMI) = 1.203 + 2.934 (0.462) $^{[jensenone]}$, ($r^2 = 0.95$). Bar represents least significant difference (lsd) = 0.3, for comparing means based on experimental error estimated by ANOVA

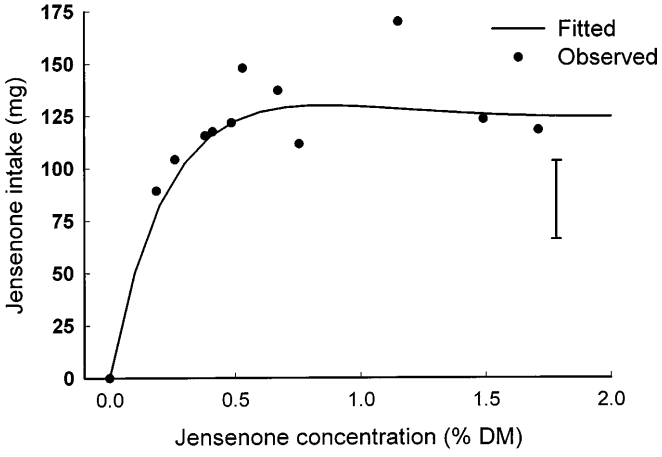


**Fig. 2** Mean jensenone intake of common brushtail possums fed a basal diet containing 12 different concentrations of jensenone. Observed points represent the observed mean jensenone intake, the line represents the inverse of the fitted equation described in the legend of Fig. 1. Bar represents lsd = 19.2 for comparing means based on experimental error estimated by ANOVA

### *Daily jensenone intake*

Several features concerning the mean daily intake of jensenone are apparent in Fig. 3: (1) high jensenone intake on day 1 compared to subsequent days, (2) the fluctuations in daily intake on days 2–12, and (3) the overall decrease in jensenone intake over the 12 days of the experiment.

#### *High jensenone intake on day 1*

Mean jensenone intake on day 1 was significantly greater than the mean jensenone intake on subsequent days (Fig. 3; lsd = 30.1). This could represent an increased ability of brushtail possums to ingest jensenone on first exposure, or an "accidental" overingestion of jensenone. These data show that the jensenone intake was 1.4 times the jensenone intake predicted by the equation in Fig. 1 (167 mg day$^{-1}$ compared to predicted threshold of 125 mg day$^{-1}$). The intake on day 1 was further investigated in experiment 2.

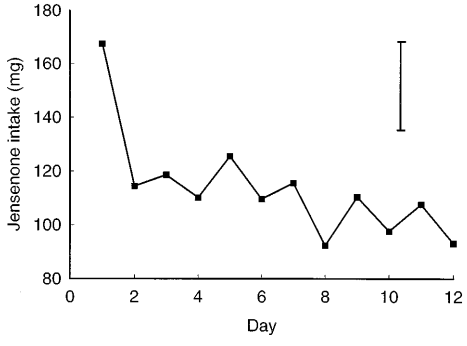


**Fig. 3** Mean daily jensenone intake of common brushtail possums fed a basal diet containing 12 different concentrations of jensenone. Bar represents lsd = 30.1 estimated by ANOVA

*Fluctuating jensenone intake on days 2–12*
The mean daily jensenone intake fluctuated between day 2 and day 12 and a day of higher mean jensenone intake was followed by a day of lower jensenone intake, suggesting a cyclical pattern of intake.

*Overall decrease in jensenone intake over the treatment period*
There was a weak trend of a decrease in mean jensenone intake over the treatment period. Mean jensenone intake was reduced by 45% between day 1 and day 12 (167 mg $day^{-1}$ compared with 93 mg $day^{-1}$). Even when day 1 was excluded, a marked reduction was still observed; 20% reduction between day 2 and day 12 (114 mg $day^{-1}$ compared with 93 mg $day^{-1}$). This suggests that exposure of animals to dietary jensenone over the longer term may have some cumulative effects.

## Experiment 2: the effect of providing food in several timed portions on jensenone intake

Brushtail possums that were provided with food on day 1 in four equal portions throughout the night ate less ($P < 0.01$) of a jensenone-treated diet than animals that were given their whole ration at the beginning of the night. There was no difference in DMI and jensenone intake on the 2nd and 3rd days when both groups were provided with all their food at the beginning of the night as normal (Fig. 4).

## Experiment 3: the effect of cold exposure on food intake and jensenone intake of common brushtail possums

Mean body mass (± standard error of the mean) of cold-treated animals was 2.7 ± 0.2 kg and control animals 2.9 ± 0.2 kg. Body mass did not change throughout the experimental period. Animals that were held at 4 °C ate more DM than those held at 18 °C ($F_{1,14} = 4.83$, $P = 0.04$; Fig. 5). DMD did not differ in the two groups ($F_{1,14} = 1.99$, $P = 0.18$; mean of cold-treated = 74.1%; control = 76.3%). The increased food consumption without a change in DMD of the cold-treated group confirms that brushtail possums have increased energy requirements at 4 °C compared to 18 °C. However, addition of jensenone to the diet abolished the differences in DMI between animals in the cold-treated group and the control group (Fig. 5; $F_{1,14} = 1.12$, $P = 0.308$) and the mean jensenone intake was not different between the two treatments ($F_{1,14} = 0.82$, $P = 0.380$).

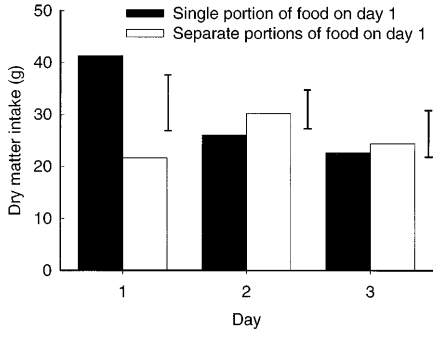


**Fig. 4** The effect of a basal diet containing 0.4% (dry mass) jensenone on DMI in common brushtail possums. Half of the animals were fed four separate portions of food through the night on day 1, the other half were fed the entire meal at the beginning of the night. Bars represent lsd as estimated by ANOVA, day 1 = 10.5, on day 2 = 7.2 and on day 3 = 8.6

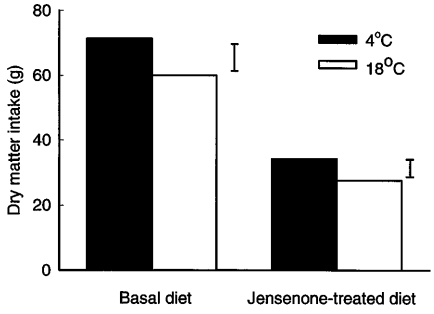


**Fig. 5** Mean DMI of brushtail possum housed in a 4 °C or 18 °C room fed a basal diet or the basal diet + 0.4% (dry mass) of jensenone. Bars represent lsd = 10.26 for the basal diet and lsd = 6.11 for the jensenone-treated diet estimated from ANOVA

## Discussion

Brushtail possums can regulate their food intake in response to large variations in dietary jensenone concentration. This is one of few studies which has shown such a strong dose-dependent relationship that spans a ten-fold increase in dietary toxin concentration. Harju (1996) has shown a change in DMI over a 20-fold increase in PSM concentrations, but only four different concentrations of birch bark powder were used in those studies and the shape of the relationship between DMI and dietary PSM concentration was not identified. Understanding the shape of this relationship should allow us to make more accurate predictions of the effects of PSM on food intake.

One of the most important aspects of regulation of PSM intake is the need to continually sample the diet to assess the palatability (Provenza et al. 1992; Cassini 1994). The exponential decay function derived from this study is the best model of this process because it suggests that, as concentration of PSM increases in the diet, food intake approaches, but does not equal zero. In other words, animals should continuously sample foods to assess their palatability and so improve their ability to regulate their intake of PSMs. A previous study of brushtail possums and jensenone described the relationship between DMI and dietary jensenone concentration as a straight line (Lawler et al. 1998a). However, this would suggest that DMI should be zero at some

arbitrary concentration of dietary jensenone and that a herbivore may refuse to ingest the diet at higher concentrations. This would subsequently restrict the sampling behaviour that is vital to effective regulation. We believe that for most animals that regulation of PSM intake was more important than outright avoidance and so it is unlikely that animals would simply refuse to sample the food. Continual sampling of the diet is essential to assessing the palatability of the diet and utilising the various food sources within a habitat. An exponential relationship was also observed when common ringtail possums were fed leaves from different *E. polyanthemos* trees that varied in their concentration of sideroxylonal (Lawler et al. 2000).

## Jensenone intake over varying jensenone concentrations

Mean jensenone intake was regulated around a threshold despite substantial variation in the concentration of jensenone in the diet. The predicted threshold from the inverse of the exponential decay function is about 125 mg $day^{-1}$. The ability of brushtail possums to regulate jensenone intake appeared to be affected by the jensenone concentration of the diet (Fig. 2). Variation of the observed mean jensenone intake from the predicted equation appeared to be higher at jensenone concentrations between 0.53% (DM) and 1.15% (DM), but the variation was lower at low jensenone concentrations [0–0.49% (DM)] and at the two highest jensenone concentrations [1.5 and 1.71% (DM); Fig. 4].

The time taken to reach this threshold can affect the ability of brushtail possums to regulate jensenone intake. Therefore, at higher concentrations of jensenone, brushtail possums should be able to eat quickly and exceed this threshold simply because the time taken to reach the threshold is shorter. However, the mean intake of jensenone at the two highest concentrations was closer to that predicted than the mid-range concentrations [0.53–1.15% (DM)]. There are two possible explanations for this: (1) the flavour of jensenone at high concentrations is strong enough to induce an aversion or cautious sampling which reduces the possibility of overingestion, or (2) higher concentrations of jensenone induce post-ingestive feedback mechanisms faster. The first of these explanations is quite straightforward and implies that the use of pre-ingestive cues for regulation improves the ability of common brushtail possums to regulate their intake of jensenone more precisely. This has been shown in other studies, where flavour cues can reduce the likelihood of overingestion and improve regulation of toxin intake (Ralphs et al. 1995; Lawler et al. 1999b). However, it seems unlikely to us that the animals cannot detect the flavour of jensenone below concentrations of 1.15% DM.

The second explanation relies on assumptions about our understanding of the post-ingestive effects of jensenone. Following experiments with a selective $5HT_3$ (serotonin) antagonist, Lawler et al. (1998a, b) argued that jensenone damages enterochromaffin cells in the small intestine releasing serotonin, which triggers a nauseous response which serves to modify feeding. However, high concentrations of jensenone may induce this feedback more rapidly especially when food intake is low. This argument implies that the effect of jensenone on intestinal cells may be affected by the presence of other compounds (i.e. nutrients) in the gut. At mid-range jensenone concentrations [0.53–1.15% (DM)] the emetic response may have been delayed by a slower absorption of jensenone from the gut. At low concentrations of jensenone [<0.53% (DM)], the concentrations were either too low to be a limiting factor, or the rate of food intake was reduced over the course of the night as animals approached satiety and consequently, subtle feedback effects were more easily detected. To substantiate these suggestions, studies using more concentrations of jensenone above 1.15% (DM), and extending beyond 1.71% (DM) are required. A similar example of a non-linear relationship between toxin concentration and the onset of the emetic response was shown with cisplatin injections in ferrets (Andrews et al. 1988).

## Daily jensenone intake

There were three interesting features in the pattern of daily jensenone intake: (1) high jensenone intake on day 1, (2) fluctuating intake on subsequent days, and (3) an overall decrease in jensenone intake over the experimental period.

### *High jensenone intake on day 1*

Limiting the rate at which the animals could ingest the jensenone-treated diet (Fig. 4) reduced the excessive jensenone intake seen on day 1 (Fig. 3). This suggests that on the first exposure to jensenone, the brushtail possums ate so quickly that they rapidly passed the threshold that triggered feedback but that they were not able to modify their feeding. Previous studies have shown that cautious sampling of novel diets enables herbivores to assess diet palatability, and acquire an aversion or a preference to it based on feedback (Provenza et al. 1992). However, in the present study, the brushtail possums did not appear to treat the diet as novel. We argue that the subtle flavour of a diet treated with jensenone was not sufficient to induce cautious sampling, so brushtail possums assumed the jensenone-treated diet was the same as the basal diet they had become accustomed to, and subsequently they ate quickly. This suggests that the rate of ingestion relative to the rate of feedback is important to the regulation of jensenone intake when strong flavour cues are absent. Further, by restricting the rate at which brushtail possums eat (by providing the food in separate portions

throughout the night), this excessive intake of jensenone can be avoided.

It is important to note that the rate of intake of a highly digestible basal diet may be unrealistically high when compared to free-ranging animals eating a natural foliage diet. When consuming *Eucalyptus* leaves, brushtail possums may be limited in their rate of consumption by structural components of the foliage. Leaf toughness and subsequent longer mastication times may limit the rate of food intake and the spatial distribution of leaves within a tree (and even trees within a habitat) may also serve to slow the rate of feeding. Consequently, it is difficult to relate the overingestion observed in captive animals fed a basal diet to free-ranging animals, as a variety of other features of leaf chemistry, such as nitrogen, water and fibre concentrations could also enhance or diminish the ability of brushtail possums to regulate PSM intake.

On subsequent days the animals do not overeat to the same extent and this could be due to a reduced rate of intake, as the animals take a more cautious approach to the basal diet following a bad experience associated with nausea (Provenza 1995). Alternatively, an increased rate of feedback may be induced by the flavour of the jensenone-treated diet (Fedorchak and Bolles 1988). The presence of a flavour previously associated with negative feedback can cause the animal to sample the diet more cautiously as they have developed an aversion to the diet (Provenza 1995; Lawler et al. 1999b). This could in turn reduce the rate of intake and reduce the possibility of overingestion, as shown in experiment 2. Alternatively, the flavour previously associated with negative consequences can prematurely induce feedback systems that were present in the previous feeding occasion. Fedorchak and Bolles (1988) have shown that the flavour of the diet can induce the release of the neuropeptide cholecystokinin (CCK), which can lead to reduced food intakes in ruminants. It may be that the flavour of jensenone can induce the premature release of serotonin within the gut, so that as ingestion proceeds, the threshold required to elicit a response in food intake is reached more quickly.

### *Fluctuating daily jensenone intake*

The fluctuating daily intake of jensenone (Fig. 3) demonstrates the inability of the possums to maintain a steady rate of intake from day to day. This may reflect a fluctuation in body concentrations of jensenone, which is a reasonable assumption given what we know about the pharmacokinetics of other drugs in small mammals (S. McLean, personal communication). Pfister et al. (1997) noted that cattle given a choice of foods showed a cyclical intake of tall larkspur. The amount of tall larkspur eaten (as a proportion of the total diet) was reduced for one to three days following a day on which a large proportion of the diet was larkspur. Tall larkspur contains toxic alkaloids, and Pfister et al. (1997) suggested that a period of intoxication was followed by a period of detoxification, which induced the cyclical pattern of intake.

The cyclical intake in brushtail possums was on a much smaller scale to that observed in cattle. In the possums, intake fluctuated from day to day and the maximum difference between subsequent days was approximately 20%. This may be the result of the rapid mass-specific metabolic rate of small mammals compared to larger animals, suggesting that metabolism of the compound is important. The cycles could be due to either a change in detoxification capabilities between days or may be related to the nauseous impact of jensenone. Jensenone may cause imbalances in body stores of some compounds or specific substrates needed for its detoxification and elimination from the body, and this could induce a cyclical pattern of food intake. Nutrient imbalances have been found to induce cycling in food intake in rats (Wallwork et al. 1981) and it is possible that deficiencies in substrates needed for detoxification reactions are inducing the cycles. A diminishing availability of substrates could be causing the brushtail possums to eat less on alternative days.

The fate of jensenone in the body is poorly understood and it is difficult to make predictions about what substrates may limit intake. Nonetheless, it is interesting to note that the overingestion observed on day 1 did not reduce the ability of possums to eat jensenone on day 2 any more than subsequent cycles. If the reduced intake is due to a reduction in substrate availability, one would expect to see either a lower intake on day 2 (compared to the pattern on subsequent days) or a longer detoxification period following such a high intake. A slightly smaller change in jensenone intake between days 2, 3 and 4, could suggest some carry-over effects of the high ingestion on day 1.

Alternatively, fluctuating intakes may be induced by a delayed component to the emetic response. Rudd and Naylor (1996) observed that ondansetron does not abolish the delayed component (nausea on days 2 and 3 following treatment) of the emetic response in ferrets or humans given the cytotoxic drug cisplatin. It is unclear what causes the delayed response. One explanation is that once activated by serotonin, the afferent neurones could remain activated or sensitised for a prolonged period (Andrews et al. 1988). The delayed feedback associated with the emetic system may cause an increased strength in sensations at lower total amounts of ingested jensenone.

### An overall reduction in mean jensenone intake over the experimental period

The final interesting feature of daily intake shown in Fig. 2 is reduction in mean intake of jensenone over the course of the experiment. This could be due to either a reduced willingness to consume the jensenone-rich diet or a lowered tolerance to ingest jensenone. It is possible

that the brushtail possums changed their feeding behaviour because the morning feeds were always free of jensenone. In using morning feeds in these experiments, we assumed that the hunger of brushtail possums during the night is strong enough to persuade them to consume as much of the diet offered as possible. Due to their high mass-specific metabolic rate and their relatively high-energy requirements, food deprivation during the night should provide enough motivation to consume the diet offered.

Alternatively, the tolerance of the animals to jensenone may be reduced as a result of a reduced nutrient status decreasing detoxification capacities, or the possible saturation of storage sites (if jensenone is sequestered in body lipids). A reduced tolerance to the consumption of PSM in animals having a low nutritional status has been identified in previous studies (Harju 1996; Wang and Provenza 1996). However, without knowing the fate of jensenone in the body of brushtail possums, it is difficult to say what nutrients may be limiting. Sequestration remains a possibility because metabolites of jensenone have not been found in either faeces or urine (S. McLean, S. Brandon and W. J. Foley, unpublished data) but it is difficult to accept that jensenone could be sequestered indefinitely.

### The effect of cold exposure on the intake of jensenone-rich diets

The results of this experiment suggest that although animals may have increased energy requirements they are unlikely to ingest more jensenone. Previous studies such as Harju (1996) and Wang and Provenza (1996) have shown that overingestion of a toxin is unlikely under conditions of food deprivation. Wang and Provenza (1996) found that intake of LiCl was lower in those lambs that were given the least food, compared to those moderately deprived and animals fed ad libitum. They concluded that the animals were more susceptible to the ingestion of toxins because of a reduced nutrient status. In other studies, cold exposure has been shown to increase food intake in common brushtail possums, and the increase in DMI was attributed a 12% increase in heat production at 3 °C (Van den Ord et al. 1995). The ability of animals to increase their intake of food when it is supplied ad libitum was thought to be limited by the gut size, and an increased gut size under increased energy demands has been reported in many studies over the past 10 years (e.g. Hammond and Wunder 1991; Bozinovic 1995). Although we did not specifically measure an increase in gut size in the animals held at 4 °C, the increased DMI, without changes in DMD suggests that the animals are either increasing the passage rate of digesta or have larger guts and we can conclude that either an increased gut size and or increased passage rate of digesta in brushtail possums will not increase jensenone intake, even if energy requirements have been substantially increased. It is possible that animals in these situations (e.g. a female marsupial in late lactation and exposed to cold conditions) will have to eat foods containing fewer or different toxins if they are to meet their energy requirements.

Overall these studies have shown that dietary PSMs have wide-ranging effects on food intake that may override other homeostatic mechanism that are believed to be important in allowing wild mammals to maintain themselves through seasonal changes in energy requirements and food quality (Hammond and Wunder 1991; Guglielmo 1996; Harju 1996). The key to the effects that we have observed is likely due to the powerful reinforcement of nausea as a feedback signal (Provenza 1995). Other regulatory feedbacks such as those induced by stimulation of trigeminal pathways may not override energy acquisition in the same way and studies directed to this question would be valuable.

**Acknowledgements** We thank Dr Ivan Lawler, Mr Ben Moore and Ms Michelle Watson for help in these experiments and Mr Bruce Barry and Mr Alan Muir for their assistance in constructing experimental apparatus. We are grateful to Ms Christine Donnelly for help in computing statistical tests. The research described in this paper was approved by the Animal Experimentation Ethics Committee of the Australian National University and conforms to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The work was supported by a grant from the Australian Research Council to WJF.

## References

Andrews PLR, Rapeport WG, Sanger GJ (1988) Neuropharmacology of emesis induced by anti-cancer therapy. Trends Pharmacol Sci 9: 334–341

Bozinovic F (1995) Nutritional energetics and digestive responses of an herbivorous rodent (*Octodon degus*) to different levels of dietary fiber. J Mammal 76: 627–637

Cassini MH (1994) Behavioural mechanisms of selection of diet components and their ecological implications in herbivorous mammals. J Mammal 75: 734–740

Fedorchak PM, Bolles RC (1988) Nutritive expectancies mediate cholecystokinin's suppression-of-intake effect. Behav Neurosci 102: 451–455

Foley WJ, Iason G, McArthur C (1999) Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores – how far have we come in 25 years? In: H-J.G. Jung, G.C. Fahey Jr (eds) Fifth International Symposium on the Nutrition of Herbivores. American Society of Animal Science, Savoy, Ill., pp 203–274

Guglielmo CG, Karasov WH, Jakubas WJ (1996) Nutritional costs of a plant secondary metabolite explain selective foraging in ruffed grouse. Ecology 77: 1103–1115

Hammond KA, Wunder BA (1991) The role of diet quality and energy need in the nutritional ecology of a small herbivore, *Microtus ochrogaster*. Physiol Zool 64: 541–567

Harju A (1996) Food selection and performance of *Microtus* voles in relation to dietary protein and woody bark. Published PhD Thesis, Science University of Joensuu, Joensuu, Finland

Jakubas WJ, Karasov WH, Guglielmo CG (1993) Coniferyl benzoate in quaking aspen (*Populus tremuloides*) – its effect on energy and nitrogen digestion and retention in ruffed grouse (*Bonasa umbellus*). Physiol Zool 66: 580–601

Lawler IR, Foley WJ, Eschler B, Pass DM, Handasyde K (1998a) Intraspecific variation in *Eucalyptus* secondary metabolites determines food intake by folivorous marsupials. Oecologia 116: 160–169

Lawler IR, Foley WJ, Pass G, Eschler BM (1998b) Administration of a 5-$HT_3$ receptor antagonist increases the intake of diets containing *Eucalyptus* secondary metabolites by marsupials. J Comp Physiol B 168: 611–618

Lawler IR, Eschler, BM, Schliebs DM, Foley WJ (1999a) Relationship between chemical functional groups on *Eucalyptus* secondary metabolites and their effectiveness as marsupial antifeedants. J Chem Ecol 25: 2561–2573

Lawler IR, Stapley J, Foley WJ, Eschler BM (1999b) Ecological example of a conditioned food aversion in plant-herbivore interactions: the effect of terpenes of *Eucalyptus* leaves on feeding by common ringtail and brushtail possums. J Chem Ecol 25: 401–415

Lawler IR, Foley WJ, Eschler BM (2000) Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. Ecology (in press)

Palo RT, Robins CT (1991) Plant defences against mammalian herbivory. CRC Press, Boca Raton, Florida

Pfister JA, Provenza FD, Manners GD, Gardner DR, Ralphs MH (1997) Tall larkspur ingestion: can cattle regulate intake below toxic levels? J Chem Ecol 23: 759–777

Provenza FD (1995) Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J Range Manage 48: 2–17

Provenza FD (1996) Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. J Anim Sci 74: 2010–2020

Provenza FD, Pfister JA, Cheney CD (1992) Mechanisms of learning in diet selection with reference to phytotoxicosis in herbivores. J Range Manage 45: 36–45

Provenza FD, Nolan JV, Lynch JJ (1993) Temporal contiguity between food ingestion and toxicosis affects the acquisition of food aversions in sheep. Appl Anim Behav Sci 38: 269–281

Ralphs MH, Provenza FD, Wiedmeier RD, Bunderson FB (1995) Effects of energy source and food flavor on conditioned preferences in sheep. J Anim Sci 73: 1651–1657

Rudd JA, Naylor RJ (1996) An interaction of ondansetron and dexamethasone antagonizing cisplatin-induced acute and delayed emesis in the ferret. Br J Pharmacol 118: 209–214

Van den Oord QGW, Wijk EJA van, Lugton IJ, Morris RS, Holmes CW (1995) Effects of air temperature, air movement and artificial rain on the heat production of brushtail possums (*Trichosurus vulpecula*): an exploratory study. NZ Vet J 43: 328–332

Wallwork JC, Forsmire GJ, Sandstead HH (1981) Effect of zinc deficiency on appetite and plasma amino acid concentrations in the rat. Br J Nutr 45: 127–136

Wang J, Provenza FD (1996) Food deprivation affects preference of sheep for foods varying in nutrients and toxins. J Chem Ecol 22: 2011–2021

Wang J, Provenza FD (1997) Dynamics of preference by sheep offered foods varying in flavours, nutrients, and a toxin. J Chem Ecol 23: 275–288