

## NUTRITIONAL ECOLOGY

# Nutritional toxicology of mammals: regulated intake of plant secondary compounds

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## Summary

1. Many mammalian herbivores continually face the possibility of being poisoned by the natural toxins in the plants they consume. A recent key discovery in this area is that mammalian herbivores are capable of regulating the dose of plant secondary compounds (PSCs) ingested.
2. The 'regulation model' describes the factors driving ingestion of PSCs by mammals and can be dissected into two separate hypotheses related to meal size and inter-meal interval (IMI). Testing these hypotheses independently yields a more thorough understanding of the underlying and potentially interconnected mechanisms.
3. Three mechanisms could influence the size of meals that contain PSCs. These are the plasma concentration of PSCs, conditioned learning, and activation of bitter receptors in the intestine.
4. Two mechanisms are proposed to govern the IMI. The first predicts that IMI is dependent on the concentration of PSC metabolites in the plasma; feeding will not resume until metabolite concentrations are acceptable for further ingestion of PSCs. The second hypothesis proposes that the intestinal bitter receptors modulate IMI through release of satiety compounds.

**Key-words:** aversions, bitter receptors, meal size, plant secondary compounds, regulation

## Introduction

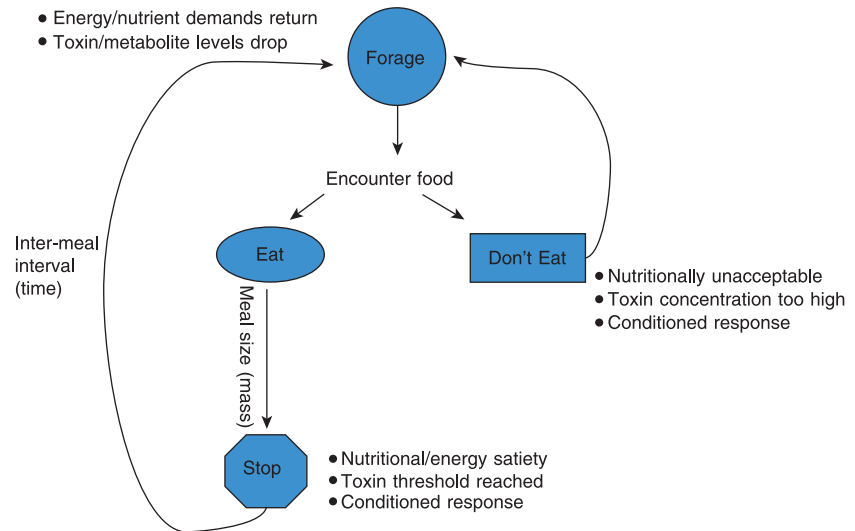
The majority of wild mammalian herbivores confront food items which contain a myriad of chemical compounds that are potentially poisonous. Plant secondary compounds (PSCs) are arguably some of the most abundant and diverse naturally occurring toxins on earth. Although some herbivores behaviourally circumvent ingestion of marked quantities of PSCs either through food manipulation or avoidance (Dearing 1997), many herbivorous mammals regularly ingest foods with PSCs that if over-ingested could have serious consequences including death (Aldrich *et al.* 1993; Foley & McArthur 1994; Iason & Murray 1996; Bozinovic & Novoa 1997; Dearing 1997; Dearing *et al.* 2001; Mangione *et al.* 2004; McLister *et al.* 2004; Dearing *et al.* 2005; Sorensen *et al.* 2005c). Yet wild herbivores rarely voluntarily ingest PSCs in doses that elicit pharmacological effects (Fowler 1983). In fact, many mammalian herbivores are capable of carefully regulating intake of PSCs (Lawler *et al.* 1998a,b; Lawler *et al.* 2000; Mangione *et al.* 2000; Boyle & McLean 2004; Sorensen *et al.* 2005a). Thus, herbivores have evolved physiological mechanisms for processing PSCs as well as behavioural feedback mechanisms to permit feeding on plants with toxins while avoiding ill effects.

In the several decades since Freeland and Janzen's seminal paper regarding the effects of PSCs on the foraging behaviour of mammalian herbivores (Freeland & Janzen 1974), ecologists and physiologists have only begun to identify the mechanisms mammals use to process and regulate intake of dietary toxins. In this review, we focus on the mechanisms that herbivores may employ to regulate intake of absorbed PSCs to avoid toxicosis. We primarily focus on the behavioural mechanisms and associated physiological cues that herbivores may use to regulate intake of toxins. To understand how food consumption is affected by PSCs, we draw strongly on work from the field of ingestive behaviour as this discipline is more advanced in understanding the physiological regulators of ingestion in the absence of PSCs and has much to offer the field of plant–mammal interactions. Wherever possible we point out the areas that we find as the frontiers warranting future investigation.

## The constraints of herbivory

The specialist's constraint: Few mammalian herbivores have evolved the ability to forage nearly exclusively from a single species of plant (Freeland & Janzen 1974). Surprisingly, the plant species consumed by specialists tend to be low in nutrients and well-defended by PSCs (Shipley *et al.* 2006). Specialist herbivores are extraordinary because they are capable of

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**Fig. 1.** Model of ingestive behaviour of mammalian herbivores. Discrete phases of feeding are depicted in shaded shapes. Factors controlling phase change at each stage are bulleted.

taking in large doses of plant toxins with no obvious ill effects. The biotransformation enzymes permitting a diet rich in PSCs are just being discovered (Ngo *et al.* 2000; Ngo *et al.* 2006; Haley *et al.* 2007a,b). Not surprisingly many of these enzymes are in the diverse superfamily of the cytochrome P450 enzymes. However, there appear to be consequences of specialization as recent work suggests that the ability to ingest high levels of PSCs from one plant species limits a specialist's ability to process novel toxins (Sorensen *et al.* 2005b).

**A generalist's quandary:** The vast majority of mammalian herbivores are generalists and as such, they select and ingest several different plant species each day from a multitude of options. However, generalists have preferences for a limited number of items from the available choices (Dial 1988; Plumptre 1995; Dearing 1996; Shipley *et al.* 1998). Although preferred items often represent the least toxic options, many of the plants consumed have PSC concentrations that could exceed the physiological capacity of the herbivore if ingested exclusively. Thus, generalists are confronted with a delicate balancing act of selecting food items in appropriate amounts to avoid exceeding their physiological capacities for processing PSCs.

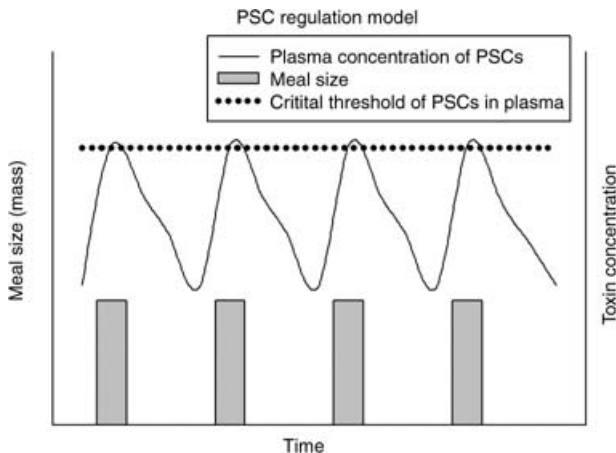
Regardless of whether an herbivore is a specialist or a generalist, all mammalian herbivores are subjected to the same general scenario (Fig. 1). While foraging, herbivores encounter numerous potential food items and must decide whether or not to ingest them. If an herbivore chooses to consume an item, two critical decisions follow. First, herbivores must decide how much food to ingest (i.e. when to cease feeding during a meal). Second, once the meal is completed, they need to know when it is physiologically safe to resume feeding. For a non-toxic food, these decisions are based on nutritional and/or energetic balance (e.g., Parker *et al.* 2009). In contrast, the occurrence of PSCs in food necessitates the metabolism of these compounds by biotransformation enzymes to reduce their harmful effects, and thus, animals may need to halt food intake prior to meeting energy demands (see also Raubenheimer *et al.* 2009). A thorough understanding of how PSCs impact the feeding behaviour of

herbivores requires knowledge of not only the biotransformation enzymes involved in metabolism of PSCs but also the cues (e.g. hormones and neuropeptides) that provide essential feedback to the herbivore on its physiological status (Fig. 1).

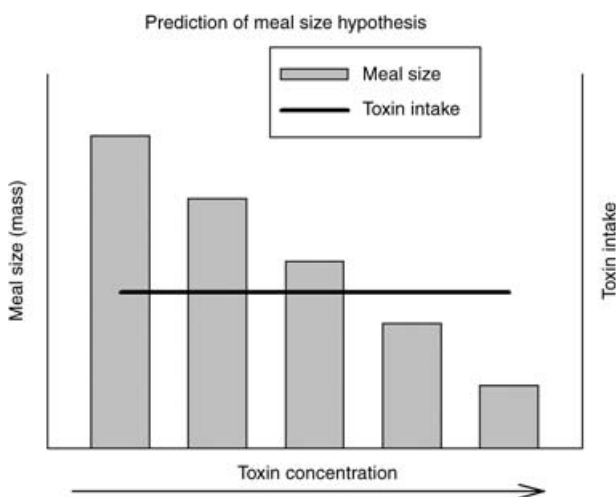
### The regulation model

A recent key discovery in the field of nutritional toxicology is that mammalian herbivores are capable of controlling intake of PSCs on a daily basis. The general pattern that has emerged from studies on a variety of herbivores and PSCs is herbivores with free access to food (not force fed) ingest a constant amount of PSCs over the course of a day by modulating intake to accommodate changes in the dietary concentration of PSCs (Lawler *et al.* 1998a,b, 2000; Mangione *et al.* 2000; Boyle & McLean 2004; Sorensen *et al.* 2005a). As the concentration of PSCs in the diet increases, herbivores maintain a constant intake of PSCs by decreasing daily food intake, often below intake levels necessary to sustain body mass. For example, brushtail possums (*Trichosurus vulpecula*) ingested the same amount of cineole even when the concentration doubled in the diet (Boyle *et al.* 2004). These original studies generated interest in how such daily regulation was achieved and on what temporal scale (Foley *et al.* 1999). Results of recent studies revealed that regulation of PSC intake occurs on a much finer scale than originally predicted. That is, herbivores appear to control PSC ingestion within a meal as opposed to longer time scales such as a day (Wiggins *et al.* 2003, 2006b; Boyle *et al.* 2005; Marsh *et al.* 2007; Sorensen *et al.* 2007).

The observation that mammals can regulate daily PSC intake has resulted in the generation of a series of models that we collectively refer to as the 'regulation model' (Provenza 1995; Foley *et al.* 1999; Marsh *et al.* 2006a; McLean & Duncan 2006). This model made a significant impact on the field of plant-mammal interactions by providing a framework for testing hypotheses. The regulation model predicts that herbivores regulate the dose of PSCs such that the toxin concentration in the blood is kept below a physiological threshold (Fig. 2). The regulation model can be dissected into two distinct



**Fig. 2.** Hypothetical figure depicting the 'regulation hypothesis,' recreated from Foley *et al.* 1999. The model predicts that herbivores cease intake such that the toxin concentration in the blood is kept below a physiological threshold and do not reinitiate intake until plasma concentration decreases. The threshold level of PSC in the plasma is represented as a constant (dotted line) whereas the actual concentration (solid line) fluctuates with meal ingestion (gray bars).



**Fig. 3.** Hypothetical figure depicting the predicted decrease in meal size (gray bars) necessary to maintain intake a threshold dose of PSCs (solid line) as toxin concentration increases in the diet.

hypotheses depicting how an animal could behaviourally regulate the dose of PSCs in its diet. The first hypothesis is that meal size dictates the dose of the PSCs ingested. This hypothesis predicts that an animal consuming a toxic food should halt food intake before surpassing some critical threshold of circulating PSCs (Fig. 3). We refer to this as the 'meal size' hypothesis. The second hypothesis of the regulation model is concerned with the time between meals and predicts that feeding will not resume until the concentration of PSCs in the general circulation is reduced from the previous meal. This reduction is necessary to permit ingestion of more PSCs without physiological damage. An implication of this hypothesis is that the reduction of plasma PSCs will actively lead to, or passively allow, meal initiation. We refer to this as the 'inter-meal interval' (IMI) hypothesis.

The regulation model focuses mainly on meal size and IMI, which are only two of five discrete phases of feeding behaviour. The five phases can be categorized as: foraging, meal initiation, food consumption, meal termination and postprandial satiety as measured by IMI (Fig. 1, Smith 1998; Collier & Johnson 2004; Geary 2004; Day *et al.* 2005). These phases form a cycle, as postprandial satiety comes to a close, foraging and meal initiation begin. In subsequent sections we will be focusing exclusively on the predictions of the regulation model, which leads to the exclusion of foraging and meal initiation. This is in no way meant to trivialize their role in feeding behaviour. It is clear that diet selection and food acceptance play an extremely important role in herbivory, however, these behaviours are outside of the scope of this review.

### The meal size hypothesis

Briefly stated, the meal size hypothesis argues that an herbivore presented with variable PSC concentrations in its diet should maintain its PSC dose below its critical threshold by adjusting meal size (Figure 3). Five studies on herbivores and two on omnivores investigated the influence of variable concentrations of PSCs on the microstructure of daily food intake (Table 1). Uniformly across studies, the response to increased dietary PSC concentration was a reduction in meal size. Thus, herbivores actively controlled the dose of toxin ingested during a meal. These studies examined five herbivore species and six different compounds; therefore, this behaviour appears to be a general mammalian response to fluctuating PSC concentrations. Furthermore, these data overwhelmingly support the hypothesis that PSCs are behaviourally regulated via reduction in meal size. The mechanisms proposed to regulate meal size are speculative and as such represent a frontier in need of future research. We review three leading mechanisms for the regulation of PSCs via meal size regulation: plasma concentration control, conditioned learning and bitter receptor mediated satiety. The latter is a novel mechanism from the field of ingestive behaviour that has not previously been examined in herbivores. These mechanisms are not mutually exclusive and likely act in concert to control PSC intake.

#### PLASMA CONCENTRATION CONTROL

The plasma concentration control hypothesis suggests that meal size is mediated by the plasma concentration of PSCs (Boyle *et al.* 2005; McLean & Duncan 2006; McLean *et al.* 2007). This hypothesis predicts that meal size is related to the herbivore's rate and capacity of biotransformation and that increased capacity facilitates greater PSC intake and therefore larger meals. This hypothesis has been directly tested once. Boyle *et al.* (2005) demonstrated that brushtail possums (*T. vulpecula*) rapidly absorb cineole, a terpenoid PSC. Consistent with the hypothesis, peak plasma concentrations of cineole coincided with meal termination (Boyle *et al.* 2005). Additional, but indirect, support exists for the idea that plasma concentration affects intake. Stephen's woodrat (*Neotoma stephensi*), a specialist on juniper, has greater

**Table 1.** Mammalian meal patterns on PSC and non-PSC containing diets

Treatment	Animal model	Total intake	Meal size	Inter-meal interval	Meal/bout number	Intake rate	Notes	Reference
Cineole	Brush-tail possum	↓	↓	Unreported	↑ (trend)	↓	Compared to lower doses of cineole	Wiggins <i>et al.</i> (2003)
Cineole	Brush-tail possum	↓	↓	Unreported	↑ (trend)	=	Compared to lower doses of cineole	Boyle <i>et al.</i> (2005)
Phenolic resin	Desert woodrat	↓	↓	=	=	↓	Compared to control diet	Sorenson <i>et al.</i> (2005a)
Sideroxylonal	Ring-tail possum	↓	↓	Unreported	=	↓	Compared to lower doses of Sideroxylonal	Wiggins <i>et al.</i> (2006a)
Formylated phloroglucinol compounds	Koala	↓	↓	=	=	↓	Compared to lower doses of phloroglucinol compounds	Marsh <i>et al.</i> (2007)
Simmondsin	Wistar rat	↓	↓	Unreported	=	↓	Data presented here is from second treatment with Simmondsin compared to control	Lievens <i>et al.</i> (2003)
Quinine	Lister rat	↓	Unreported	Unreported	↑	↓	1-h test post-deprivation, compared to control	Blundell <i>et al.</i> (1985)
Quinine	Long Evans rat	Unreported	↓	Unreported	Unreported	Unreported	Animals were sham fed, compared to control	Weingarten & Watson (1982)
*Threonine deficient diet	Wistar rat	↓	↓	=	=	↓	Compared to control	Feurte <i>et al.</i> (2002)
*LiCl infusion	Long Evans rat	↓	=	Unreported	↓	↓	Infusion was paired with feeding, compared to saline control	West <i>et al.</i> (1987)
*Fat infusion	Wistar rat	Unreported	↓	↑	Unreported	Unreported	Effect listed here is on first meal post-infusion only, compared to saline control	Burton-Freeman <i>et al.</i> (1997)
*Protein infusion	Wistar rat	Unreported	↓	↑	Unreported	Unreported	Effect listed here is on first meal post-infusion only, compared to saline control	Burton-Freeman <i>et al.</i> (1997)

Meal patterns of animals consuming PSCs are very similar to each other regardless of species or diet. \*Diet treatments that are not PSCs but are included as physiological references for deficiency, emetic stimulation and satiety.

daily intakes of juniper and greater biotransformation capabilities compared to the sympatric generalist, the whitethroat woodrat (*N. albigula*, Haley *et al.* 2007b). The greater biotransformation capacities of the specialist may facilitate its greater intake of juniper. In addition, brushtail possums have a higher daily intake when offered a variety of foods processed by different biotransformation pathways (Dearing & Cork 1999; Wiggins *et al.* 2003, 2006b; Marsh *et al.* 2006b). These studies suggest that biotransformation capacity governs intake of PSCs but none measured meal size as a function of PSC plasma concentrations. Clearly more studies are warranted to establish a link between PSC plasma concentrations and meal size.

#### CONDITIONED LEARNING

The second mechanism proposed to regulate meal size is conditioned learning, which includes conditioned aversion. There are many types of conditioned learning, and it could indirectly modulate meal size (Smith 1996). Conditioned learning has received considerable attention in the field of plant–mammal interactions. There is evidence that animals can learn to reduce meal size if experience reveals the food is either satiating (Davis *et al.* 2001) or causes discomfort (Provenza 1995).

The most commonly studied type of conditioned learning related to food is called a ‘conditioned taste (or flavor) aversion’ (CTA). In the strictest sense, a CTA is a marked reduction in the ingestion of a food item caused either by nausea or gastrointestinal illness (Garcia 1989). CTAs are a conditioned learning task that occurs rapidly, i.e., within a single exposure to a negative post-ingestive stimulus (Riley & Freeman 2004). It has been argued that learning about the repercussions of food happens quickly because it may be a requirement for survival. A central objective of a CTA experiment is to determine whether an animal can associate a taste with a negative post-ingestive stimulus; thus, experimental demonstration of a CTA relies on the separation of taste from the negative post-ingestive stimulus. The typical experimental protocol for a CTA experiment involves pairing a flavour, for example, grape, with a negative stimulus, typically lithium chloride (LiCl) administered via injection to circumvent oro-sensory rejection of the negative stimulus. The animal’s response to the flavour paired with the post-ingestive consequence is tested in a later experiment where the negative stimulus is removed but the flavour remains. The animal is said to have developed a CTA if after the pairing the animal reduces its intake of the flavoured food in the absence of the post-ingestive negative stimulus. The reduction in intake of the associated flavour can persist for months in the absence of the negative stimulus. That is, repeated aversive stimulus in subsequent meals is not required for conditioned reductions.

CTAs have been proposed as a primary mechanism used by herbivores to regulate intake of PSCs during a meal (Provenza 1995; Lawler *et al.* 1998b; Marsh *et al.* 2006a). There is evidence that many herbivores can form classical CTAs. Numerous studies have demonstrated that a variety of mammalian

herbivores decrease subsequent food intake of a target food when that food is paired with a dose of a compound that either stimulates the emetic system in the area postrema of the brain or produces overt symptoms of toxicosis (Du Toit *et al.* 1991; Lawler *et al.* 1999; Dziba *et al.* 2006). These studies overwhelmingly demonstrate that herbivores are capable of conditioned learning as evidenced by the reduction in future consumption of a food that has previously resulted in a significant pharmacological effect. In these scenarios, animals learned that there was a negative consequence to their original intake and reduced later intake in the absence of the negative stimulus. The reduction in intake is proportional to the discomfort from or dose of the negative stimulant ingested at the original pairing (Du Toit *et al.* 1991).

The same term, CTA, has also been used to describe a transitory effect of nausea that is predicted to reoccur at each exposure to a food item associated with negative post-ingestive stimulus (Lawler *et al.* 1999; Marsh *et al.* 2006a). In this context, the CTA is proposed to be transitory such that it extinguishes quickly to permit re-ingestion of the food within hours or days. In this second paradigm, nausea is the proposed negative feedback signal registered by the herbivore that causes it to terminate each meal (Marsh *et al.* 2006a). The evidence for this paradigm is that herbivores increase intake of select PSCs when the emetic system is blocked with pharmacological agents (Aldrich *et al.* 1993; Lawler *et al.* 1998b). Although this association is termed a CTA in the herbivory literature, we argue that in these are two very different paradigms and as such we prefer to use the more general term, aversion, for the transitory phenomenon described in this paragraph.

To further address the conditioned learning hypothesis, we believe it is critical to understand the types of compounds and the dosages required to produce conditioned learning responses especially in the context of the doses of PSCs ingested by herbivores in nature. Although there is evidence for conditioned aversion, its ecological role remains unclear. Several studies have used LiCl (not a PSC) or high doses of PSCs to generate the conditioned response (Pfister *et al.* 1997; Dziba *et al.* 2006; Dziba & Provenza 2008). In some of the studies conducted on herbivores, the doses of PSCs administered were far greater than what an animal could ingest in a meal. Moreover, lower, more realistic doses often did not result in a conditioned aversion (Du Toit *et al.* 1991; Dziba *et al.* 2006; Dziba & Provenza 2008). Furthermore, aversions are inadequate predictors of food intake in more natural foraging situations where herbivores have access to multiple food types (Duncan & Young 2002). This result suggests mechanisms other than aversions provide feedback to an animal to terminate feeding. The significance of conditioned learning under natural foraging conditions requires further attention.

#### ACTIVATION OF INTESTINAL BITTER RECEPTORS

The third mechanism that could facilitate PSC regulation is meal termination mediated through activation of bitter receptors, particularly those in the intestine. Before intro-

ducing this hypothesis, we briefly address its obvious corollary, oro-sensory bitter taste rejection. Bitter taste rejection plays a critical role in diet selection. There is convincing evidence from laboratory studies that the bitter compound quinine causes a reduction in intake in a dose dependent manner in rats (Weingarten & Watson 1982). However, in nature it is not clear that plant toxicity is related to bitter rejection by herbivores (Molyneux & Ralphs 1992; Glendinning 1994). Furthermore, there is evidence that herbivores have substantially higher thresholds for bitter tastants than other trophic levels, (e.g. carnivores or omnivores) and that they are not deterred by all bitter tastants, with quinine being a notable exception (Nolte *et al.* 1994). It has been hypothesized that many herbivores cannot 'afford' to reject bitter tastants, as this would restrict their diet too greatly (Glendinning 1994). It is clear that more work is required to explore the role of oro-sensory contributions to bitter taste rejection in meal size control. We suggest that oro-sensory mechanisms likely work in concert with the post-ingestive mechanisms proposed here to produce meal size effects. To date the relative contributions of oro-sensory and post-ingestive mechanisms have yet to be distinguished and this question lies on the frontier of this research.

We propose post-ingestive feedback from intestinal bitter receptors as a new mechanism of meal size regulation. There is a family of approximately 30 types of bitter receptors in the mouth and components of these receptors are also present in the gastrointestinal tract (Rozenfurt 2006). The bitter receptors in the gut are hypothesized to contribute to the regulation of dietary toxins (Rozenfurt 2006; Glendinning *et al.* 2008). Activation of intestinal bitter receptors is correlated with the secretion of satiety signals (i.e. peptides linked to meal termination) and stimulation of hindbrain areas associated with satiety (Hao *et al.* 2008). The ability of the gut to detect molecules that are bitter and to initiate the release of satiety peptides from such stimulation has interesting implications for ingestive behaviour of herbivores given that many PSCs are bitter (Harborne 1991). Furthermore, activation of the intestinal bitter receptors slows gastric emptying, which has been attributed to the need of an animal to decrease the rate of toxin absorption to allow for biotransformation (Glendinning *et al.* 2008). Lastly, bitter receptors in the gut do not require a coordinated stimulus from those in the mouth to generate a response to a bitter compound (Glendinning *et al.* 2008).

We propose that PSCs could activate the intestinal bitter receptors by triggering release of the same satiety signals used by herbivores for meal termination in the absence of PSCs. Early meal termination would then alter meal size and control PSC dose. Activation of bitter receptors is postulated to halt food intake via the release of any of the three satiety signals associated with bitter taste receptors to date and with known effects on meal size, that is, cholecystokinin, peptide YY (PYY) or glucagon like peptide-1 (GLP-1, Rozenfurt *et al.* 2006; Hao *et al.* 2008). Cholecystokinin (CCK) is the most extensively studied satiety signal. It is released from the small intestine after stimulation by ingested nutrients including dietary fat, protein, carbohydrates (Moran & Kinzig 2004) and alcohol

(Kulkosky *et al.* 1998, 2004). Administration of CCK reduces meal size in an array of vertebrates including herbivores (Morley 1995). PYY and GLP-1 are also released in the gut and in response to caloric intake. Like CCK, these peptides reduce food intake when administered peripherally and are considered satiety signals (Cummings & Overduin 2007).

The hypothesis that PSC regulation is achieved via activation of intestinal bitter receptors and subsequent release of satiety signals could be addressed in experimental studies with mammalian herbivores. Experimental protocols for such investigations exist for studies of ingestive behaviour (Glendinning *et al.* 2008; Hao *et al.* 2008). However, to date there has been no research on PSCs and differential potencies of the satiety compounds or the relationship of these PSCs to the intestinal bitter receptors. As a note, the modulation of PSCs via intestinal bitter receptors does not require that an herbivore be made ill daily from its diet or suffer toxicosis from novel plants. We see the role of the intestinal bitter receptors as a new frontier for the field of plant-herbivore interactions.

#### SYNTHESIS OF MEAL SIZE REGULATION MECHANISMS

The three meal termination mechanisms described above are not mutually exclusive and could function in concert to regulate intake of PSCs. For example, it has been hypothesized that bitter receptors could facilitate a protective response such as meal termination or the formation of aversions (Sternini 2007). In support of this idea, large doses of bitter compounds administered directly into the intestine cause conditioned aversions in laboratory rats (Glendinning *et al.* 2008). However, bitter receptors do not seem to be co-localized with receptors containing serotonin (Rozenfurt *et al.* 2006) thus, some of the commonly used anti-emetics are inappropriate for testing bitter receptor mediated aversion. In addition, the conditioned learning mechanism and the plasma concentration mechanism are likely inter-related. The conditioned aversion mechanism could depend on circulating PSCs or their metabolites as negative feedback to halt feeding.

#### The IMI hypothesis

Any decrease in meal size to reduce the dose of PSCs also results in a reduction in energy intake. Thus, for an animal to maintain daily energy intake, it must increase the number of meals ingested. If the animal has a limited amount of foraging time per day, the required increase in intake must be accomplished by decreasing the amount of time between meals or the IMI (Foley *et al.* 1999). If PSCs reduce meal size but have no direct effect on the IMI, then the null hypothesis is that IMIs should be shorter on high PSC diets compared to diets low in PSCs. However, the regulation model predicts that PSCs will have a direct effect on IMI as herbivores could regulate blood concentrations of PSCs by adjusting the IMI to coincide with the levels of PSCs in their diet. Extending the IMI allows the animal to clear toxins from the previous meal before adding more to the system. There are two predictions of the IMI hypothesis. If PSCs directly affect IMI, then with a reduction

in meal size, the time between meals should be the same or longer as PSCs increase. Second, as PSCs in the diet increase, the IMI will increase in a dose dependent fashion to allow for the necessary increase in clearance time.

The IMI hypothesis has not been directly tested to date and can only be addressed through inference from the data available. Of seven studies that monitored microstructure of feeding with respect to PSC concentration in the diet, only two reported IMI data (Table 1). In the first study, *Neotoma lepida* reduced meal size when fed increasing levels of phenolic resin; however, the dose of phenolics (mg phenolic/meal) increased with concentration despite decreases in daily food intake, i.e., the regulation was imperfect (Sorensen *et al.* 2005a). A study on koalas reported similar results of limited regulation (Marsh *et al.* 2007). These results are consistent with the idea that PSCs affect IMI; there was no decrease in IMI to compensate for the reduction in energy intake during each meal. However, these data are not consistent with the second prediction that IMI should be extended to compensate for clearance of a larger dose of toxin because IMI did not change in either case. The impact of PSCs on IMI research is fertile ground for future research.

Two mechanisms may impact IMI with respect to PSCs. The pharmacokinetics of PSCs, that is, levels of circulating PSCs or their metabolites, have been suggested to impact IMI. Secondly, activation of intestinal bitter receptors, likewise may extend IMI through the release of the satiety compounds described earlier, some of which have been documented to play a direct role in extending IMI. Each of these is discussed in more detail below.

#### PHARMACOKINETIC CONTROL OF IMI

The pharmacokinetic concept predicts that an herbivore should regulate the dose of PSCs ingested in a meal to match the body's capacity for processing PSCs and that further ingestion of similar PSCs should be delayed until current xenobiotics are processed and plasma levels are below the concentration of physiological impact (Boyle *et al.* 2005; McLean *et al.* 2006).

Biotransformation enzymes are the primary mechanism herbivores use to process absorbed PSCs. These enzymes are primarily located in the liver; however, some occur in the small intestine and work in tandem with efflux transporters that eject toxins or their metabolites out of the body and back to the gut (Dearing *et al.* 2005; Sorensen & Dearing 2006). Despite the putative importance of biotransformation enzymes in dictating diet composition and intake of PSCs, studies on the specific enzymes used by herbivores and the differences between specialist and generalist mammalian herbivores are in the incipient stages. The few investigations published indicate that PSCs induce the activities of biotransformation enzymes (Pass *et al.* 1999; Ngo *et al.* 2000, 2006; Pass & McLean 2002; Pass *et al.* 2002; Ngo *et al.* 2003; Sorensen *et al.* 2007) and that herbivores consuming large quantities of PSCs have higher activities of some biotransformation enzymes (Ngo *et al.* 2003; Haley *et al.* 2007a,b). More recently, Boyle *et al.* (2005) suggested that the metabolites of

biotransformed PSCs mediate IMI (Boyle *et al.* 2005). They found that brushtail possums fed a diet of cineole had high levels of metabolites long after plasma levels of cineole had declined. Although circulating metabolites have undergone the biotransformation process they are not necessarily 'detoxified' as some metabolites are more reactive than the initial PSCs. In addition, metabolites of PSCs are often acidic, which could impact acid-base homeostasis until the metabolites have been eliminated (Foley, McLean & Cork 1992). Thus, levels of PSC metabolites could dictate the length of the IMI.

#### BITTER RECEPTION AND IMI

The intestinal bitter taste receptors may also mediate IMI. PSCs could activate intestinal bitter receptors, causing the release of peptides that affect IMI. Two of the satiety signals described earlier (GLP-1 and PYY) extend IMI as well as influence meal termination (Naslund *et al.* 1998; Chelikani *et al.* 2005). Although there are no experiments to date documenting the relationship between PSCs and satiety compounds mediated through the intestinal bitter receptors, evidence from other areas suggests that this scenario is plausible. Proteins have been proposed to interact with the bitter receptors (Cummings & Overduin 2007) and protein ingestion increases IMI in a non-dose dependant manner (Burton-Freeman *et al.* 1997). Thus, it is possible that PSCs interact with intestinal bitter receptors to alter IMI in a manner similar to protein.

Addressing the IMI hypothesis directly would yield critical insight into the mechanisms herbivores employ in avoiding toxicosis. Not only would it be important to address IMIs in laboratory experiments with increasing concentrations of a PSC, but it may be more ecologically relevant to see if the IMI is adjusted under more natural settings such as those described in Wiggins *et al.* (2006a) where herbivores expend energy to forage plants with different PSC profiles.

#### Summary and future directions

In summary, the field of plant–mammal interactions is at a nexus between behaviour and physiology where exciting new directions and paradigms for understanding how mammals regulate PSC intake are beginning to emerge. Although herbivores clearly regulate PSCs by adjusting meal size to control dose, the mechanisms underlying the detection of dose and regulation of meal size remain somewhat speculative. Finding the 'counter' that tracks ingestion of PSCs lies on the frontier of this research. We have outlined several possible paradigms for understanding mechanisms governing meal size including conditioned learning, PSC plasma concentrations, and the newly discovered intestinal bitter receptors. Understanding the role of intestinal bitter receptors in PSC regulation has the potential to markedly transform our understanding of herbivore foraging.

The IMI has received less attention than meal size in regulating PSC intake. However, IMI is a critical component of daily PSC intake and requires further consideration. It would be useful to have more studies that measure plasma

concentrations of PSCs and metabolites in other species of herbivores to correlate PSC clearance with feeding patterns. This approach would also help to illuminate the relationship that the rate and capacity of liver enzymes play on the animal's ability to ingest particular doses and concentrations of PSCs. The hypotheses on meal size and IMI also apply to comparisons of specialist and generalist herbivores. If meal size is dictated by biotransformation capacity, we predict that specialists with their putatively higher capacity, would not regulate meal size or IMI in the same way as generalists. We encourage more work on biotransformation enzymes as much of Freeland and Janzen's (Freeland *et al.* 1974) original hypothesis was based on the idea that generalists consume different foods to efficiently utilize various biotransformation pathways. Classification of compounds into different detoxification pathways would permit testing this hypothesis by comparing feeding patterns when an animal faces foods containing multiple PSCs that either compete for a single pathway or that use different pathways.

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