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Responses of lambs to camphor highlight opportunities for management of savannas

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Abstract. Herbivores ingest large quantities of plant secondary metabolites (PSMs) due to their abundance in plants. While the PSMs woody plants produce can act as defenses against mammalian herbivory, herbivores have behavioural and physiological means for coping with PSMs. Understanding the relationship between physiology and behavior in mitigating toxicity from PSMs is a critical step toward enhancing productivity of savannas. We hypothesized that the monoterpene camphor influences food intake in sheep, and that rate of elimination of camphor from the body influences food intake. We used monoterpenes as an example of herbivore response to PSM. We infused one group of lambs intravenously with 40 mg/kg BW camphor dissolved in methanol and intralipid. We dosed another group of lambs intraruminally with a single-bolus dose of 125 mg/kg BW camphor dissolved in vegetable oil. We monitored feeding behavior. During intravenous infusions, dosed lambs stopped feeding sooner than control lambs ($P < 0.05$). Lambs began feeding again soon after the infusions stopped, which was consistent with the rapid rates at which camphor was eliminated from the body. Ruminally dosed lambs stopped feeding sooner than control lambs ($P < 0.05$). We conclude that systemic concentrations and rates of elimination influenced feeding behaviour. Thus, lambs expressed physiological and behavioural responses that enabled them to cope better with the potentially toxic effects of camphor. We draw inferences from this example based on known characteristics of chemically defended plants and generalize to chemically defended plants in wooded rangelands.

Keywords. Kinetics – Monoterpenes – Camphor – Woody plant encroachment – Sheep.

La réponse des agneaux au camphre : Un outil de gestion des savanes

Résumé. *Les herbivores ingèrent de grandes quantités de métabolites secondaires qui sont abondants dans les plantes (PSM). Alors que les plantes ligneuses produisent et utilisent les PSM pour se défendre contre les herbivores mammifères, ces derniers sont capables grâce à des moyens comportemental et physique de s'adapter aux PSM. La compréhension de la relation entre la physiologie et le comportement pour éviter l'intoxication par les PSM est une étape primordiale permettant d'améliorer la productivité des savanes. Notre hypothèse est que le camphre du monoterpène affecte l'ingestion chez le mouton et que la vitesse d'élimination du camphre de l'organisme agit sur l'ingestion des aliments. On a utilisé des monoterpènes comme un exemple de réponse de l'herbivore aux PSM. On a injecté par voie intraveineuse 40 mg de camphre/kg de poids vif d'un groupe d'agneaux. Le camphre a été dissous dans le méthanol et l'intra-lipide. On a dosé par voie intra-ruminale un autre groupe d'agneaux avec une dose unique de 125 mg/kg poids vif de camphre dissous dans une huile végétale. On a contrôlé le comportement alimentaire. Durant les injections intraveineuses, les agneaux ayant été dosés avec le camphre ont arrêté la consommation des aliments plus tôt que les agneaux témoins ($P < 0,05$). L'ingestion a repris chez les agneaux expérimentaux plus rapidement que chez les agneaux témoins lorsque l'infusion a été stoppée. Ce résultat s'aligne avec les vitesses rapides avec lesquelles le camphre a été éliminé de l'organisme. Les agneaux recevant le camphre par voie ruminale ont stoppé de s'alimenter plus tôt que les agneaux témoins ($P < 0,05$). En conclusion, il apparaît que les concentrations systémiques et les vitesses d'élimination du camphre affectent le comportement alimentaire. Par conséquent, les agneaux ont exprimé des réponses comportementales et physiologiques qui leur ont permis de mieux s'adapter aux effets potentiellement toxiques du camphre. On déduit de cet exemple basé sur des caractéristiques connues des plantes se défendant chimiquement que les plantes ligneuses utilisent aussi la voie chimique pour s'auto-défendre.*

Mots-clés. *Cinétiques – Monoterpènes – Camphre – Plante ligneuse – Mouton.*

I – Introduction

Monoterpenes such as camphor decrease the preference of herbivores for plants such as sagebrush (Welch *et al.*, 1983; Personius *et al.*, 1987; Bray *et al.*, 1991; Ngugi *et al.*, 1995; Dziba and Provenza, 2007). Herbivores limit the amount of food with monoterpenes they ingest through feedback-mediated interactions that link physiology and behavior (McArthur *et al.*, 1991; Provenza, 1995). Understanding these relationships is vital for understanding how sheep mitigate the toxic effects of monoterpenes and how they cope under such stress.

The objective of this study was to determine the effect of elimination rates of camphor, which occurs in sagebrush, on the feeding behavior of lambs dosed intravenously or into the rumen with camphor. We hypothesized systemic concentrations of camphor lead to centrally mediated satiation and the ensuing feedbacks, in turn, lead to regulation of intake of foods high in monoterpenes (Foley *et al.*, 1995; Provenza, 1995). Detoxification processes complement behavioral responses by mediating the negative effects of monoterpenes, resulting in higher food intake (Gugliemo and Karasov, 1996; Foley *et al.*, 1999). We draw inferences from this study and generalize the principles to savanna ecosystems encroached by chemically woody plants. We focus on how small ruminants can be used to enhance and maintain productive savanna and/or grassland-shrubland ecosystems.

II – Material and methods

Eight lambs weighing 44 kg (SE = 0.5) were used for the intravenous infusion experiment and another set of 8 lambs weighing 36 kg (SE = 1.2) were used for the rumen dosing experiment. Lambs were habituated to experimental conditions and the experimental diet, which was formulated from a mixture of beet pulp, grape pomace, and soybean meal at the ratio of 49:43:8 to provide 2.44 Mcal/kg digestible energy, 8.77% crude protein and 52% total digestible nutrients (NRC, 1985). This diet is similar in nutrient content to sagebrush in the fall (Welch and Pederson, 1981; Kelsey *et al.*, 1982; NRC, 1985). Lambs had free access to water and trace mineralized salt blocks. Intralipid (20% emulsion) was purchased sterile from Sigma-Aldrich, St Louis, MO. Camphor (95% purity; CAS# 76-22-2) was purchased from Sigma-Aldrich (Milwaukee, WI). High Performance Liquid Chromatographic (HPLC) grade ethyl acetate and HPLC grade water were purchased from Fisher Scientific (Fair Lawn, NJ). Camphor is relatively insoluble in water, but soluble in alcohol, ether, and benzene (Merck Index, 2001). Thus, for the intravenous infusion experiment, 3.0 g of camphor were dissolved in 3 ml methanol, placed in 75 ml intralipid, and thoroughly mixed using a vortex mixer. This maintained the camphor in solution, while attempts to make camphor soluble directly into intralipid did not. For rumen administration, 5.0 g of camphor was mixed thoroughly in 20 ml of vegetable oil. The dose for each experiment was determined in preliminary dosing trials with a separate group of lambs of similar body size.

Eight lambs were randomly assigned to the two treatments for the intravenous infusion experiment: (i) camphor in methanol and intralipid; and (ii) control (methanol and intralipid only). The solution of camphor and intralipid was delivered at a constant rate infusion of 1 ml/min and 1 min/kg BW using a VWR variable flow peristaltic pump. This provided a total dose of 40 mg/kg over a period of 40 to 50 min. The infusion line was attached to an Abbocath® -T, 18 G × 2" indwelling intravenous jugular catheter and secured to the back and ear of the lamb to ensure the line stayed in place. The catheter was fitted with a lock surflo injection plug for repeated blood collection. During rumen dosing, eight lambs were randomly assigned to two treatments: (i) 4 dosed lambs (camphor dissolved in vegetable oil); and (ii) 4 control lambs (no dosing, as previous studies had verified that no effect occurred from vegetable oil only). The dose was delivered at a rate of 0.625 ml/kg BW using an Abbocath® - T 16 G 2'' catheter, which amounted to 125 mg/kg BW.

An Abbocath®-T, 18 G × 2" indwelling catheter was placed in the jugular vein for sample collection and a control blood sample was collected before dosing in each experiment. Post-infusion sampling started at the time the infusion stopped and after 2 minutes following rumen dosing. Blood samples

(10 ml) were collected into EDTA coated blood collection tubes (Vacutainer ®). After the intravenous infusions, blood samples were taken at 0, 1, 3, 6, 12, 18, 30 and 45 min and at 1, 1 ½, 2, 3, 4, 6 and 8 h. After rumen dosing, blood samples were collected at 2, 4, 6, 12, 18, 30 and 45 min and at 1, 1 ½, 2, 3, 5 and 7 h. Heparinized 0.9% NaCl injectable solution (3 ml) was used to flush the catheter to prevent clotting. Samples were then centrifuged for 10 minutes at 3000 rev/min using an IEC HN-SII series centrifuge. Plasma was separated, frozen immediately, and stored at -18°C until analysis. The method for analyzing camphor from plasma is described in detail in Kimball *et al.* (2004).

Lambs were offered the experimental diet and feeding behavior (time to stop feeding) was monitored during and following dosing. Enough food was offered to ensure all lambs had food until dosing and observation were completed in 1 h. The criterion for "stopping feeding" was based on 60 seconds of continuous non-feeding activity. The time it took lambs to return to feeding after dosing stopped was recorded. Feeding behavior data were analyzed using repeated measures Mixed ANOVA (Littell *et al.*, 1996) with 2 treatments (dosed lambs and controls). Feeding time was the response variable. The plasma concentration-time data for both experiments were analyzed using PK Solutions 2.0.

III – Results and discussion

During intravenous infusion lambs fed for only 29 min (SE = 3.3), while control lambs fed for 60 min (SE = 0.8) ($P < 0.05$). All control lambs ate until they finished their food. Dosed lambs began feeding again in 7 min (SE = 2.03) after the infusion was stopped. Lambs eliminated camphor rapidly during the primary (α) elimination-distribution phase and more slowly during the terminal (β) elimination phase (Fig. 1). We hypothesized systemic concentrations of camphor mediate feeding behavior. Our results are consistent with this notion, as the relationships between systemic concentrations of camphor, feeding time, and time to resume feeding following infusion were all significant. Lambs stopped feeding during camphor infusions, and they resumed feeding within minutes after the intravenous infusions stopped. Camphor was quickly metabolized, eliminated, and/or distributed to peripheral tissues (Merck Index, 2001) as shown by its rapid decline from central circulation, which can explain the rapid return to feeding shortly after the infusion. These patterns of feeding behavior support earlier observations that lambs regulate food intake in response to dietary and, presumably, systemic concentrations of sagebrush monoterpenes (Dziba *et al.*, 2006), a phenomenon also demonstrated with tall larkspur in cattle (Pfister *et al.*, 1997), jensenone in possums (Stapley *et al.*, 2000), resins in woodrats (Sorensen *et al.*, 2005), and LiCl in sheep (Wang and Provenza, 1997).

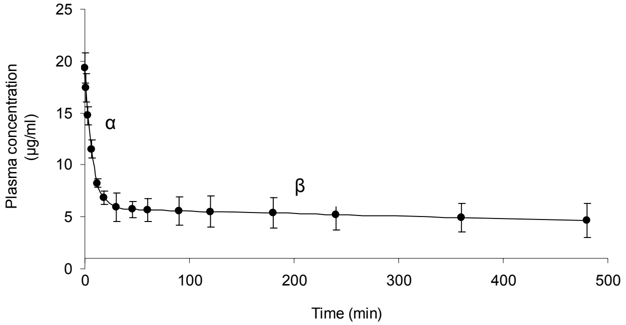

Fig. 1. Plasma concentration time curve for intravenous infusion with camphor.

Regulating meal frequency in response to toxin load is an important behavioural response of herbivores to reduce the likelihood of consuming toxic quantities of plant secondary compounds (Pfister *et al.*, 1997; Foley *et al.*, 1999; Dziba and Provenza, 2007). The meal-frequency model is based on interactions between behavioural and physiological responses to toxins, whereby physiological thresholds influence feeding behaviour to maintain systemic concentrations of toxins below satiation thresholds. The ability of these systems is seldom overcome as feedback mechanisms cause animals to limit intake before toxicosis ensues (Provenza, 1995). Though lambs were dosed intravenously, with no influence of taste or effects on rumen microbes, they still consumed less food compared to controls, which suggests flavour-postingestive effects mediated by the central nervous system. During eating, neurally mediated interactions between the senses of taste and smell and postingestive feedback from toxins change the affective value of foods, which in our study caused lambs to limit food intake (Smith and Margolis, 1954; Provenza, 1995). As a result, behaviour (food intake) is adjusted according to physiological effects (camphor) (Provenza *et al.*, 1992).

During rumen dosing lambs spent 40 min feeding (SE = 4.1), while control lambs fed for the maximum allocated time of 60 min (SE = 2.2) ($P < 0.05$). Absorption of camphor was very rapid. Camphor was eliminated relatively fast during the primary elimination-distribution, while camphor concentrations declined more slowly during the terminal elimination-distribution phase (Fig. 2).

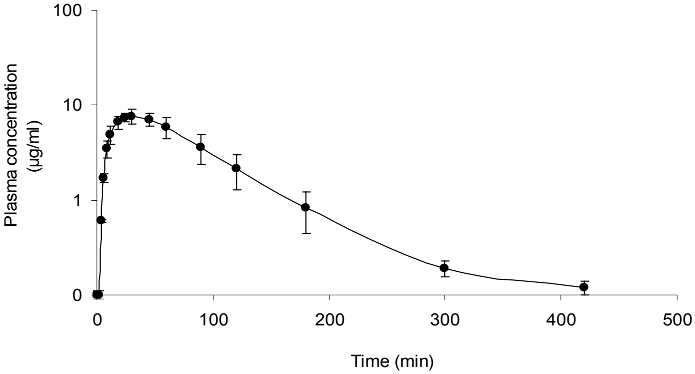

Fig. 2. Plasma concentration time curve following rumen dosing with camphor.

Systemic concentrations of camphor played a significant role in influencing feeding behavior of lambs. Higher systemic concentrations were the result of fast absorption and slower rates of elimination, which together negatively effected intake. In the longer term, this may be of special significance in ecosystems dominated by plants that contain secondary compounds. We argue that managing for diversity in an ecosystem improves options for animals to choose among alternatives that are detoxified by varied pathways (Freeland and Janzen, 1974) and meet specific requirements for enhanced detoxification (Provenza *et al.*, 2003). Animals with access to nutritious alternatives should be able to find a diet that not only meets nutrient requirements, but one that also satisfies the need for specific nutrients required for enzyme production, thereby enhancing detoxification and reducing systemic concentrations of plant secondary compounds below satiation thresholds (Foley *et al.*, 1999). Thus, animals can cope well with the challenges posed by plant secondary compounds if physiological mechanisms are complemented by behavioural responses that affect intake regulation.

The physical and biological complexity inherent in savanna ecosystems increases the productivity, diversity and stability of these systems. This complexity is not only important for ecosystem function, but it has profound influence on the productivity of animal populations that depend on these systems. Where chemically defended woody plants dominate, the plant-herbivore principles outlined in this example highlight opportunities for using small ruminants to manage savanna ecosystems for enhanced production or diversity. For example, small ruminants that forage on chemically defended woody plants can be strategically supplemented to enhance elimination of toxins and reduce their deleterious effects (see Dziba *et al.*, 2007). This can increase their consumption and may lead to enhanced diversity taking into account other important factors that influence the dynamics of savannas or steppes.

IV – Conclusion

We conclude that systemic concentrations and rates of elimination influenced feeding behaviour. Lambs expressed physiological and behavioural responses that enabled them to cope better with the potentially toxic effects of camphor. The inferences we draw from this example suggests this work presents a unique opportunity for managing rangelands dominated by chemically defended encroaching woody plants where less palatable and chemically defended plants are more likely to dominate.

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