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Transmission of self-medicative behaviour from mother to offspring in sheep

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Herbivores challenged by diets with high concentrations of tannins learn by individual experience to self-select medicinal compounds such as polyethylene glycol (PEG), which neutralizes the negative postingestive effects of tannins. We investigated the transmission of this acquired self-medicative behaviour from mother to offspring. One group of ewes (experienced, $N = 8$) was conditioned to associate the beneficial effects of PEG after consuming a tannin-rich diet. Ewes ingested a meal of high-tannin food and were then offered PEG. Subsequently, ewes ingested the same tannin-rich meal and were then offered a food (grape pomace; control) that did not have the medicinal effects of PEG. After conditioning, the experienced group and a naïve group of ewes ($N = 8$) were given a choice between the high-tannin food, PEG and grape pomace. Experienced ewes showed higher intake and preference for PEG than did naïve ewes ($P < 0.05$). Subsequently, experienced and naïve ewes with their naïve lambs, as well as a group of naïve lambs without their mothers ($N = 8$), were exposed to the tannin-rich diet, PEG and grape pomace. Lambs were then tested for their ability to self-medicate with PEG by offering them a choice between the tannin-rich diet, PEG and grape pomace. Lambs from experienced and naïve mothers showed a higher preference for PEG than did lambs exposed without their mothers ($P = 0.05$). Thus, the presence of the mother (experienced or naïve) was important for naïve lambs to learn about the medicinal benefits of PEG. We conclude that the mother's presence per se may increase the efficiency of creating new knowledge, such as preference for a medicine, within a group, beyond transmitting and maintaining this knowledge across generations.

Social models play an important role in diet selection and food preferences of young animals (Thorhallsdottir et al. 1987; Galef 1991; Sclafani 1995). Socializing enhances learning efficiency because each animal no longer has to discover everything through trial and error (Thorhallsdottir et al. 1987; Provenza 1995; Provenza et al., in press) and this foraging information is passed transgenerationally from the experienced mother to the offspring (Key & MacIver 1980; Lynch et al. 1983; Green et al. 1984; Thorhallsdottir et al. 1990; Provenza & Cincotta 1993). A mother's influence on the diet of her offspring begins in utero (Nolte et al. 1992; Mennella et al. 2001), continues through the chemical composition and flavour of her milk (Nolte & Provenza 1991), and is particularly important as a young animal begins to forage prior to weaning (Chapple & Lynch 1986; Mirza & Provenza 1990, 1992, 1994; Thorhallsdottir et al. 1990).

While herbivores avoid toxic foods or prefer nutritious foods as a function of their mothers' avoidances or preferences (Mirza & Provenza 1990, 1992), no information is available regarding social transmission of other types of behaviours that are equally important for the fitness of the individual. Animals use plant secondary compounds and other non-nutritional substances to combat or control disease (Huffman 1997; Klein et al. 2008). Sheep self-select medicinal substances such as polyethylene glycol (PEG), a non-nutritive polymer that attenuates the aversive effects of tannins, and they increase intake of PEG as concentrations of tannins increase in the diet (Provenza et al. 2000; Villalba & Provenza 2001; Villalba et al. 2006).

In this study, we sought to determine the influence of the mother and the mother's level of experience with the medicinal effects of PEG after a tannin challenge on the transmission of self-medicative behaviour to her offspring. We predicted that (1) mothers trained to associate the beneficial effects of PEG while consuming tannins would transmit this information to their offspring, which would learn at a faster rate than the offspring of naïve mothers, and (2) lambs with social models would be more efficient at using PEG, even in the presence of a naïve social model, than would lambs without a social model.

METHODS

We conducted the study at the Green Canyon Ecology Center, Utah State University, Logan, UT, U.S.A. We housed 16 St Croix ewes

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(approximately 3–6 years of age) with their suckling lambs and eight St Croix lambs (approximately 2 months of age) in outdoor pens under a protective roof. Ewe–lamb pairs were penned in single, adjacent pens measuring 6 × 5 m, while the remaining eight lambs were penned in separate and adjacent individual pens (2.4 × 3.6 m). The animals were fed 1.5 kg of alfalfa pellets and 300 g of grain following daily trials. All animals had free access to sodium chloride with trace mineral blocks and fresh water throughout the study. Research protocols for the study were approved by an Institutional Animal Care and Use Committee (IACUC Approval 1409, Utah State University).

The ewes, each with their single lamb, were randomly assigned to a treatment group ($N = 8$ ewes and lambs) or a control group ($N = 8$ ewes and lambs). The third group included lambs without their mothers ($N = 8$ lambs).

The study was conducted in four phases. In the first phase, ewes from the treatment group were given experience to recognize the benefits of ingesting medicinal substances such as PEG when consuming a tannin-rich diet. In the second phase, we tested whether ewes from the treatment group learned about the benefits of PEG while consuming tannins by offering them choices between PEG and a nonmedicinal food (grape pomace). In the third phase, lambs with their mothers (experienced or inexperienced) or alone were offered a tannin-rich diet, PEG and grape pomace. After this exposure, we tested lambs' preference for PEG in a fourth phase.

Phase 1: Sequential Conditioning of Treatment Ewes

Conditioning with a medicine (PEG)

During conditioning with PEG, ewes from the treatment group were conditioned to experience the beneficial effects of PEG (medicine) at attenuating the postingestive stress caused by a diet high in tannins. Ewes were first fed a tannin-rich food and then offered PEG. Animals are more likely to learn about the benefits of a medicine when they experience illness and then ingest a medicine that leads to recovery (Provenza et al. 2000).

From day 1 to day 15 at 0845 hours, ewes were separated from their lambs with a panel that split the pen into two compartments. Lambs and ewes could still see, hear and smell each other. Thus, this sensory interaction added to the close proximity between mothers and their young, minimizing the negative effects of temporary separation (Price et al. 2003). Subsequently, ewes were offered a high-tannin diet (15% tannin (Tannin Corporation, Peabody, MA, U.S.A.), 55% alfalfa hay and 30% barley) from 0900 to 1000 hours and then offered PEG (MW, 3,350; Spectrum Chemical, Los Angeles, CA, U.S.A.) from 1000 to 1100 hours. Immediately after being offered PEG for 1 h, ewes were again offered the high-tannin diet for 1 h. After this procedure, each treatment ewe was reunited with her lamb and all animals were fed a basal diet of 1.5 kg alfalfa pellets mixed with 300 g barley.

Animals are initially reluctant to eat PEG, which has no nutritional value (Villalba & Provenza 2001). Hence, ewes were offered a 60:40 mixture of PEG:barley on day 1, with decreasing proportions of barley: 70:30 on day 2 and 80:20 on day 3. Thereafter, the proportion of barley was either increased or decreased based on the individual intake of each ewe in the group. If ewes ate more than 75 g of the PEG–barley mix, the proportion of barley was reduced to 10% for 1 day, and then eliminated (100% PEG) the next day. If ewes ate less than 75 g of the PEG–barley mix, the proportion of barley in the mix was maintained at 20%.

As the intake of PEG by ewes was low after 15 days of exposure, we increased the time of exposure to PEG. From day 16 to day 33, ewes were separated from their lambs and fed 1 kg of high-tannin diet from 0900 to 1000 hours, and were then offered 300 g of 100% PEG for 7 h. Refusals were collected at 1700 hours, the ewes were reunited with their lambs and all animals were fed the basal diet of 1.5 kg of alfalfa pellets mixed with 300 g of barley.

Conditioning with a nonmedicinal food (grape pomace)

In the conditioning phase with a nonmedicinal food, ewes from the treatment group were offered a low-quality feed (grape pomace) while experiencing the postingestive stress caused by a diet high in tannins. Treatment ewes were separated from their lambs, as described above, and fed 1 kg of high-tannin diet from 0900 to 1000 hours. Subsequently, we offered ewes 300 g of grape pomace (a novel food) mixed with 50 g of barley (a familiar food) to encourage them to sample the novel food. After day 8, the animals were fed 100% grape pomace. Refusals were collected and weighed at 1700 hours, the ewes were reunited with their lambs and all animals were fed the basal diet of 1.5 kg of alfalfa pellets mixed with 300 g of barley. Conditioning with grape pomace was conducted for 17 days until intake of grape pomace stabilized over time.

During phase 1, control ewes and their lambs were fed a basal diet of 1.5 kg of alfalfa pellets mixed with 300 g of barley.

Phase 2: Preference for PEG by Ewes

Ewes from the treatment and control groups were separated from their lambs, as described above, offered the high-tannin diet from 0900 to 1000 hours, and then offered a choice among the tannin-rich food, PEG and grape pomace until 1700 hours. Ewes were then reunited with their lambs. Refusals were collected and weighed, and the amount of medicinal/nonmedicinal food and tannin-rich diet consumed by the ewes was measured for 2 consecutive days.

Phase 3: Transmission of Self-medicative Behaviour

We conducted two consecutive trials, one of 7 days duration (period 1) and another of 5 days duration (period 2). Preference tests (see below) were conducted for all lambs after each period.

Ewe–lamb pairs and single lambs were offered a choice of the high-tannin food, PEG and grape pomace from 0900 to 1700 hours. Each ewe and her lamb ate together during this phase and, as opposed to single lambs without their mothers, daily intake of each food represented the combined consumption of the pair.

As an index for the ingestive behaviour of lambs and ewes during this phase, an observer recorded the foraging behaviour of each ewe and lamb at 5 min intervals from 0900 to 1030 hours (Altman 1974). We recorded incidences of feeding on each of the alternatives available as well as bouts of inactivity. Frequency of feeding on each alternative was calculated as a percentage of the total number of scans in which ewes and lambs were feeding. Total number of scans of eating events and noneating events (bouts of inactivity such as not eating or resting) was also recorded.

We collected and weighed refusals at 1700 hours and determined the intake of each feed at the end of each day. Ewe–lamb pairs received the basal diet of 1.5 kg of alfalfa pellets mixed with 300 g of barley; single lambs received 1 kg of alfalfa mixed with 300 g of barley.

Phase 4: Preference for PEG by Lambs

We determined lambs' preference for a medicine (PEG), a nonmedicinal food (grape pomace) and a tannin-rich food after exposure to these foods during phase 3. The day after each period of 'transmission of self-medicative behaviour', lambs were separated from their mothers, as described above, and offered a choice between the tannin-rich food, PEG and grape pomace from 0900 to

1700 hours. Ewes were also fed the tannin-rich food to minimize distraction caused by separation from their lambs. We collected and weighed refusals and recorded each individual's intake of each food at 1700 hours. Subsequently, the ewes were reunited with their lambs and ewe–lamb pairs were given the basal diet of 1.5 kg of alfalfa pellets mixed with 300 g of barley; single lambs were fed 1 kg of alfalfa mixed with 300 g of barley.

Statistical Analyses

The statistical design for the ANOVA in all phases of the study was a split-plot with animals (random factor) nested within groups. Group (1, 2 and 3) was the between-subject factor and day was the repeated measure in the analysis (fixed factors). The response variables for the study were the amount of tannin-rich food, medicine (PEG) and grape pomace consumed by animals, percentage preference for medicine and grape pomace ((intake of individual feed/total intake of medicine and grape pomace) $\times$ 100) during preference tests, and proportion of scans ((scans on individual feed/total number of scans in which animals were feeding) $\times$ 100). Separate analyses were conducted for ewes and lambs, except for phase 3 ('transmission of self-medicative behaviour') where food intake represented the combined consumption of ewe–lamb pairs for groups 1 and 2.

To explore the influence of mothers' initial preference for PEG on the subsequent ingestion of PEG by their offspring, we determined the linear relationship (measured as Pearson correlation) between intake and preference for PEG manifested by ewes and lambs during preference tests (phase 2 for ewes; phase 4 for lambs). We used an analysis of covariance with group (experienced, naïve) as a categorical scale explanatory factor and intake (or preference) as a continuous-scale explanatory factor. The model also included the interaction between group and PEG intake (or preference). Because both intake and preference values were standardized ((individual value−mean)/standard deviation) within each group prior to fitting the model, the slope estimated by the model for each group was equivalent to the correlation coefficient for intake and preference values, and the interaction term (group $\times$ intake; group $\times$ preference) tested for equality of the correlation coefficients between groups.

Analyses were computed using a mixed model (MIXED procedure; SAS Institute, Inc., Cary, NC, U.S.A.; Version 9.1 for Windows). The model diagnostics included testing for a normal distribution of the error residuals and homogeneity of variance. Means were analysed using pairwise differences (DIFF) of least squares means (LSMEANS).

RESULTS

Phase 1

Conditioning ewes with a medicine (PEG)

Intake of PEG fluctuated when treatment ewes received PEG with varying proportions of grain on days 1–12. On day 13, there was a noticeable decrease in the intake of PEG, as all treatment ewes were given 100% PEG. After day 15, as the time of exposure to PEG and tannin-rich food increased from 3 h to 7 h, mean $\pm$ SE intake of PEG gradually increased and stabilized at 78 $\pm$ 6 g/day, $N = 8$ (Fig. 1). Intake of the tannin-rich food also increased gradually and stabilized at 1000 $\pm$ 21 g/day, $N = 8$ (Fig. 1).

Conditioning ewes with a nonmedicinal food (grape pomace)

Intake of grape pomace and of the tannin-rich food decreased over time (Fig. 1). From day 1 to day 8, ewes were enticed to sample grape pomace by adding 50 g of barley to 300 g of grape pomace. The mean $\pm$ SE intake of grape pomace from days 1 to 8 was 101 $\pm$ 24 g/day ($N = 8$) and that of tannin-rich food was 849 $\pm$ 24 g/day ($N = 8$). From day 9, when animals were offered 100% grape pomace, the amount of grape pomace eaten dropped considerably, even when offered for 7 h/day. Intake of the tannin-rich food also decreased (Fig. 1) to approximately 400 g lower than that for the period of conditioning with PEG.

Phase 2: Preference for PEG by Ewes

When given a choice between the tannin-rich diet, PEG and grape pomace, ewes conditioned to experience the medicinal effects of PEG ate more (106 versus 23 g; SE = 26 g; $F_{1,14} = 5.42$, $P = 0.04$) and had higher preference for PEG (71% versus 19%, SE = 10.28; $F_{1,14} = 12.57$, $P = 0.003$) than naïve ewes (Fig. 2). Intake

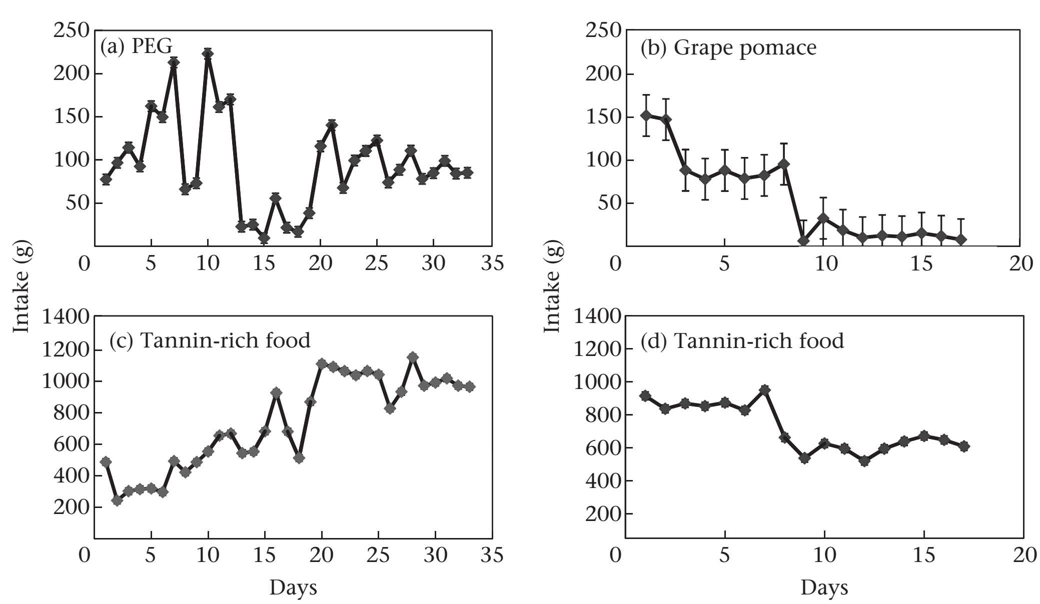

Figure 1. Daily intake of (a) polyethylene glycol (PEG) and (c) tannin-rich food by ewes during conditioning with a medicine, and of (b) grape pomace and (d) tannin-rich food by the same group of ewes during conditioning with a nonmedicinal food (phase 1 of the study). Values are means $\pm$ SE.

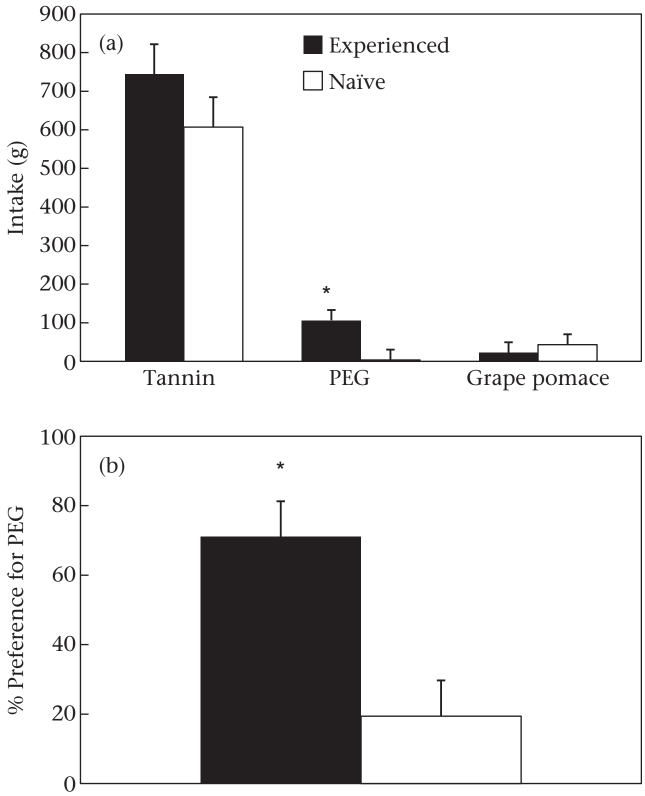

Figure 2. (a) Intake of tannin-rich food (tannin), polyethylene glycol (PEG) and grape pomace during preference tests and (b) preference for PEG by ewes that were either conditioned to experience the beneficial effects of PEG on tannins (experienced), or not conditioned to experience the beneficial effects of PEG on tannins (naïve) (phase 2 of the study). $^*P < 0.05$. Values are means ± SE.

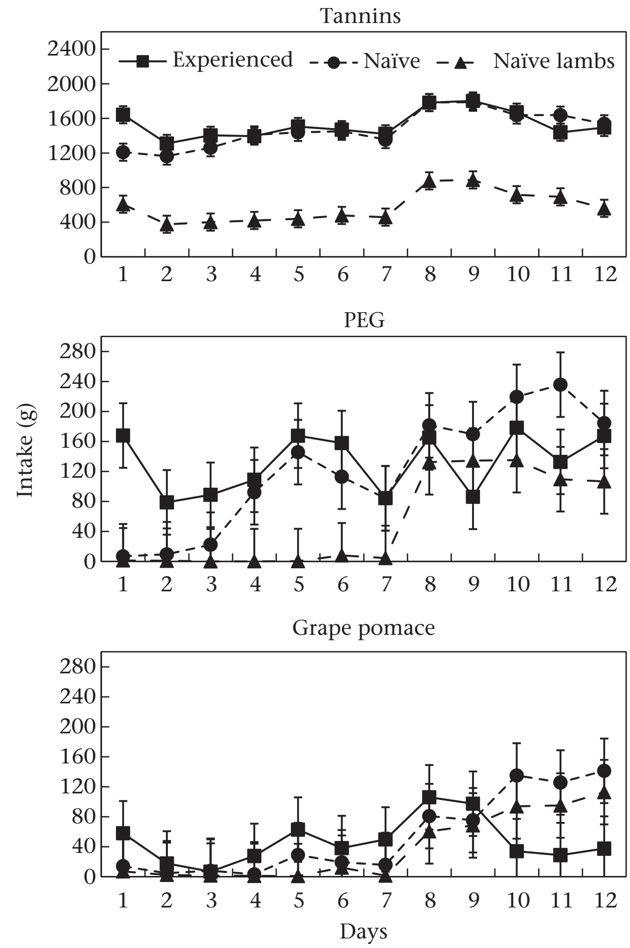

Figure 3. Intake of tannin-rich food (tannin), medicine (polyethylene glycol; PEG) and a nonmedicinal food (grape pomace) by two groups of ewe–lamb pairs and a group of naïve lambs without their mothers (naïve lambs). Mothers in one group were conditioned to experience the beneficial effects of PEG on tannins (experienced), whereas mothers in the other group were naïve to the beneficial effects of PEG on tannins (naïve) (phase 3 of the study). Values are means ± SE.

of grape pomace did not differ between groups ($P = 0.58$). No differences in intake of tannin-rich food were found between experienced and naïve ewes during preference tests (744 versus 607 g; SE = 77 g; $F_{1,14} = 1.57$, $P = 0.23$; Fig. 2).

Phase 3: Transmission of Self-medicative Behaviour

Intake of test feeds

PEG. During days 1–7 (period 1), ewe–lamb pairs with experienced mothers ate more PEG than lambs without their mothers (122 versus 2 g; SE = 34 g; $F_{2,21} = 3.14$, $P = 0.02$). No differences in consumption of PEG were detected among groups of animals on days 8–12 (period 2) ($F_{2,21} = 0.48$, $P = 0.62$; Fig. 3).

Grape pomace. During days 1–7 (period 1), ewe–lamb pairs with experienced mothers ate more grape pomace than pairs with naïve mothers and lambs without their mothers (37 versus 13 and 4 g, respectively; SE = 6 g; $F_{2,21} = 8.61$, $P = 0.002$). No differences in consumption of grape pomace occurred on days 8–12 (period 2) ($F_{2,21} = 0.51$, $P = 0.61$; Fig. 3).

Tannin-rich diet. Lambs without their mothers ate the least amount of tannin-rich food in both periods (period 1: $F_{2,21} = 39.95$; period 2: $F_{2,21} = 66.89$, $P < 0.0001$; Fig. 3). Ewe–lamb pairs did not differ in intake of tannin-rich food ($P > 0.10$), except that, for the first day of period 1, ewe–lamb pairs with naïve mothers ate more tannin-rich food than pairs with experienced mothers (treatment × day: $F_{12,124} = 2.51$, $P = 0.006$; Fig. 3).

Scan sampling

PEG. Experienced ewes had twice the proportion of scans on PEG relative to inexperienced mothers (6% versus 3% of the ingestive events, respectively; SE = 3%), but the difference was not significant ($F_{1,14} = 0.57$, $P = 0.46$; Fig. 4). Likewise, no differences in scans on PEG occurred among groups of lambs (4.4% versus 3.3% of the ingestive events for lambs with experienced and inexperienced mothers, respectively; SE = 1.5; $F_{1,14} = 0.26$, $P = 0.62$; Fig. 4).

Grape pomace. No differences between groups of ewes ($F_{1,14} = 1.30$, $P = 0.27$) or lambs ($F_{1,14} = 0.06$, $P = 0.81$) were detected in scans for grape pomace (Fig. 4).

Tannin-rich diet. No differences between groups of ewes ($F_{1,14} = 1.00$, $P = 0.33$) or lambs ($F_{1,14} = 0.18$, $P = 0.68$) were found in the proportion of scans on the tannin-rich food (Fig. 4).

Phase 4: Preference Tests (Lambs)

Intake of test feeds

PEG. Lambs exposed with their mothers (experienced and naïve) to PEG and grape pomace while being offered a high-tannin diet showed a higher preference for PEG than lambs exposed without their mothers (74%, 74% and 44%, respectively, SE = 10%; $F_{2,21} = 2.78$,

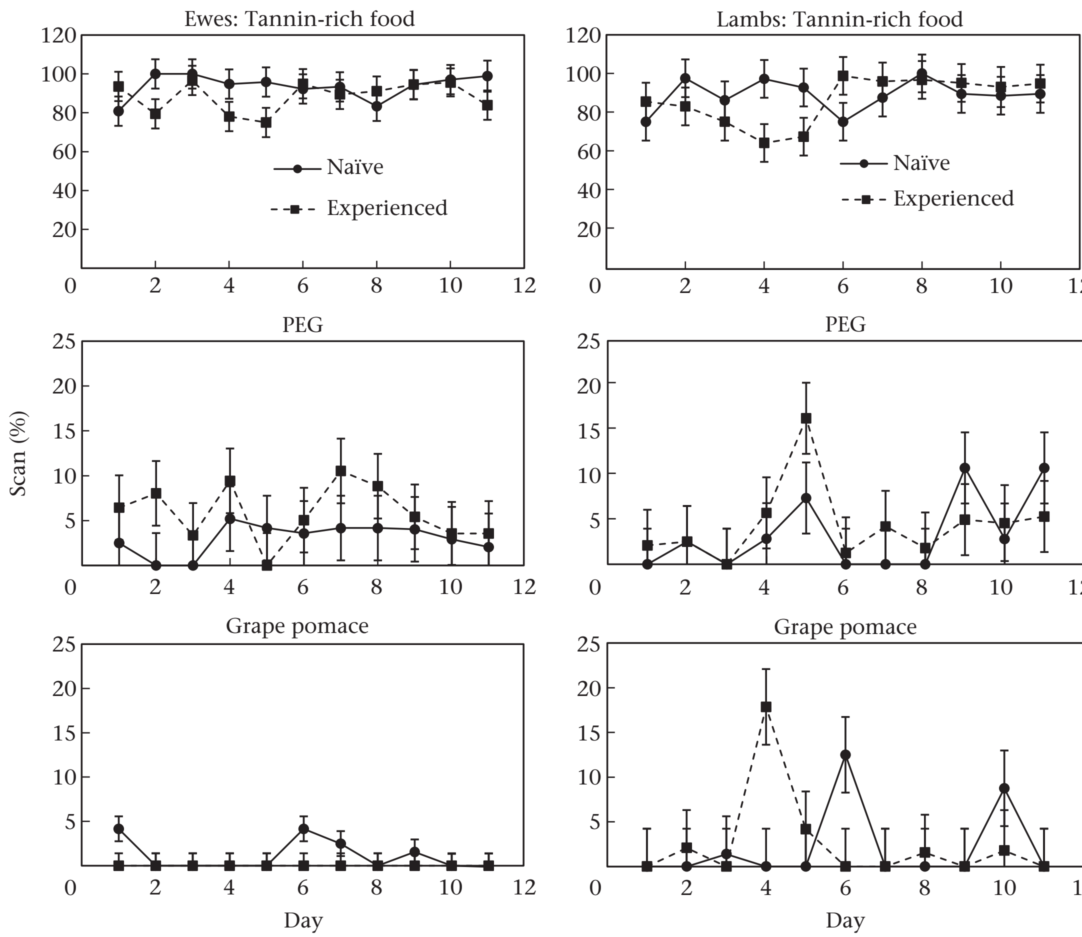

Figure 4. Ingestive events (represented as a percentage of total scans recorded) by two groups of ewe–lamb pairs offered a tannin-rich food (tannin), a medicine (polyethylene glycol; PEG) and a nonmedicinal food (grape pomace). Mothers in one group were conditioned to experience the beneficial effects of PEG on tannins (experienced), whereas mothers in the other group were naïve to the beneficial effects of PEG on tannins (naïve) (phase 3 of the study). Values are means ± SE.

$P = 0.05$; Fig. 5). During the first preference test, lambs without their mothers consumed only 2 g of PEG, whereas lambs exposed to PEG with their mothers (experienced and naïve) consumed 32 g and 29 g, respectively, but differences were not significant (SE = 9 g; $F_{2,21} = 1.83$, $P = 0.18$). No differences in intake of PEG were detected among groups during preference test 2 ($F_{2,21} = 0.16$, $P = 0.85$; Fig. 5).

Grape pomace. Preference for grape pomace did not differ between lambs exposed with (experienced and naïve) or without their mothers to PEG and grape pomace while offered a high-tannin diet: 56% versus 26% and 27%, respectively (SE = 12%; $F_{2,21} = 1.97$, $P = 0.16$). The same was true for intake of grape pomace: 71 g versus 19 g and 26 g, respectively (SE = 19 g; $F_{2,21} = 2.16$, $P = 0.14$; Fig. 5).

Tannin-rich food. No differences in intake of tannin-rich food were detected between groups during preference test 1 ($F_{2,21} = 1.03$, $P = 0.37$). However, during preference test 2, lambs exposed with their mothers (experienced and naïve) showed a higher intake of tannin-rich food than lambs exposed without their mothers (773 g and 791 g versus 484 g, respectively, SE = 51 g; $F_{2,21} = 11.36$, $P = 0.001$; Fig. 5).

Relationships between Intake and Preference for PEG by Ewes and Their Offspring

Correlation coefficients between intake of PEG by ewes and their lambs during preference tests did not differ between groups (Pearson correlation: experienced ewes and their lambs: $r_{14} = 0.05$, $P = 0.87$; naïve ewes and their lambs: $r_{14} = 0.17$, $P = 0.54$; group × intake of PEG: $F_{1,28} = 0.10$, $P = 0.75$). In contrast, the hypothesis of equality of correlation coefficients between the two groups was rejected when the continuous-scale explanatory factor was preference for PEG instead of intake of PEG (group × preference for PEG: $F_{1,28} = 9.38$, $P = 0.005$). While the association was positive for experienced ewes and their lambs (Pearson correlation: $r_{14} = 0.35$, $P < 0.20$), the relationship was negative for naïve ewes and their offspring ($r_{14} = -0.65$, $P < 0.001$; Fig. 6).

DISCUSSION

Ewes that experienced the beneficial effects of PEG while consuming tannins developed a preference for PEG over grape pomace. In contrast, naïve ewes did not recognize the medicinal effect of PEG, as their intake of PEG was low, and they ate similar and low amounts of grape pomace (nonmedicinal) and PEG (medicinal). These results are consistent with previous findings that lambs fed a high-tannin food prefer PEG to a nonmedicinal food (Villalba & Provenza 2001, 2002), and that experienced animals regulate the amount of PEG consumed in proportion to the amount of condensed tannin in their diet (Provenza et al. 2000). Experienced ewes ate more tannin-rich food during conditioning with PEG than during conditioning with the nonmedicinal food. These results suggest that PEG effectively attenuated the negative

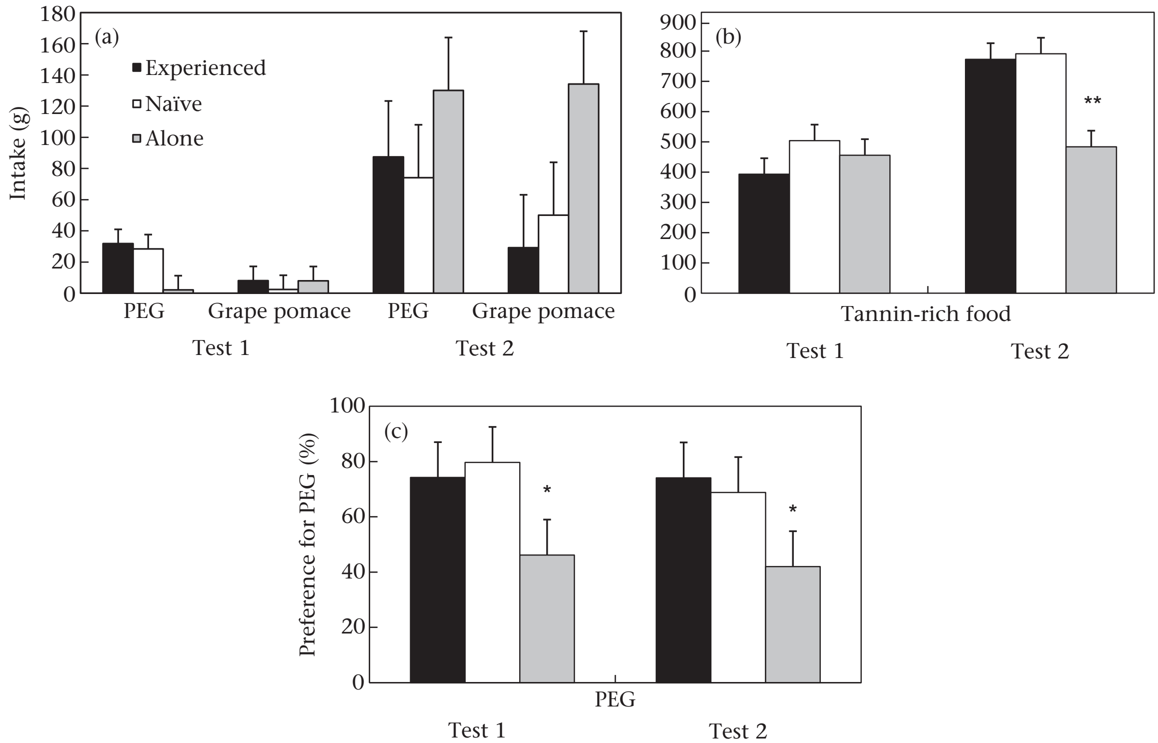

Figure 5. Intake of polyethylene glycol (PEG), grape pomace and tannin-rich food in (a) preference test 1 and (b) preference test 2, and (c) preference for PEG during preference tests by three groups of lambs. One group of lambs was exposed to the three foods with mothers that were previously conditioned to experience the beneficial effects of PEG on tannins (experienced). Another group of lambs was exposed to the three foods with mothers that were naïve to the beneficial effects of PEG on tannins (naïve). Finally, the third group of lambs was exposed to the same three foods without their mothers (alone). *$P = 0.05$; **$P = 0.001$. Values are means ± SE. (Phase 4 of the study.)

postingestive effects of tannins, allowing experienced ewes to eat more basal diet.

The interaction between PEG and tannins apparently occurs by hydrogen bonding between oxygen through an ether linkage of the PEG chain and the phenolic hydroxyl group of the tannin (Silanikove et al. 1996). This interaction is irreversible over a wide range of pH, and renders tannins unavailable to form protein–tannin complexes (Jones & Mangan 1977) that adversely affect animal tissues and nutrient absorption (Foley et al. 1999). A PEG-condensed tannin ratio of 1:2 totally neutralizes the negative effects of condensed tannins (Silanikove et al. 1994). The intake of

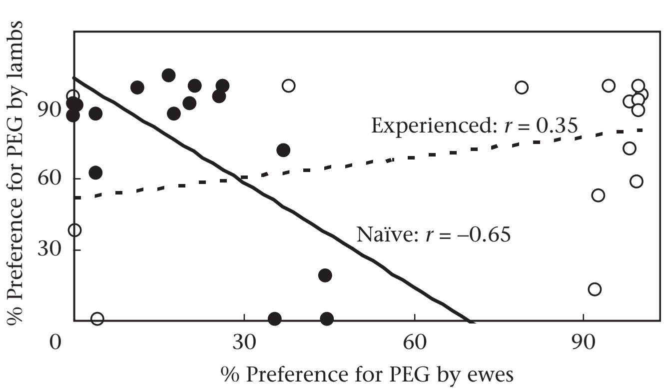

Figure 6. Preference for PEG by lambs and by their mothers during preference tests. One group of lambs was exposed to a tannin-rich food, a medicine (polyethylene glycol; PEG) and a nonmedicinal food (grape pomace) with mothers that were previously conditioned to experience the beneficial effects of PEG on tannins (experienced; open circles). Another group of lambs was exposed to the three foods with mothers that were naïve to the beneficial effects of PEG on tannins (naïve; solid circles).

the tannin-rich food (15% tannin) stabilized at 1000 g/day, while the intake of PEG stabilized at 78 g/day. Hence, the ewes were consuming an average of 150 g/day of condensed tannins, which presumably were completely neutralized by the amount of PEG they ingested. During preference tests, lambs previously exposed with their mothers ate 68 g (day 1) and 113 g (day 2) of tannin food, and 30 g (day 1) and 75 g (day 2) of PEG during preference tests (Fig. 5), representing quantities of PEG that closely (day 1) or completely (day 2) neutralized the negative effects of the condensed tannins ingested, assuming the proposed 1:2 PEG:tannin ratio.

During conditioning, naïve ewes' initial reluctance to eat PEG was probably due to cues that signalled PEG's lack of nutritional value and lack of attractive orosensory properties, so it was not interpreted as food. We enticed ewes to sample PEG by mixing familiar, nutritious barley with PEG and gradually decreasing the ratio of barley until they started consuming 100% PEG (Villalba et al. 2006). The daily exposure to PEG and tannin-rich food was also lengthened to enhance the acceptance of the food (Zajonc 1968; Pliner 1982; Villalba & Provenza 2001). During conditioning with PEG, intake of tannin-rich food increased as the ewes experienced the beneficial effects of PEG (Fig. 1).

During conditioning with grape pomace (nonmedicinal food), intake of the tannin-rich food and grape pomace decreased as ewes experienced the negative postingestive effects of the high-tannin food in the absence of PEG. Tannins interact with the mucosal and salivary proteins in the mouth and hence decrease the palatability and intake of the food (Kumar & Singh 1984). Tannins also decrease the permeability of the outer cellular layer of the gut and reduce the passage of nutrients into the body (Kumar & Singh 1984). Tannins bind to the digestive enzymes in the rumen and inhibit digestibility of the food (Kumar & Singh 1984).

Results from this study provide evidence that preference for PEG by lambs depended on the presence of mother. Lambs exposed with either experienced or inexperienced mothers showed a much higher preference for PEG than lambs without their mothers. The presence of a lamb's mother per se, whether experienced or naïve, was thus consequential for enhancing the use of PEG by lambs. In contrast, the mother's experience is important for young lambs to learn which foods to eat (e.g. nutritious) and which foods to avoid (e.g. toxic) (Mirza & Provenza 1990). Rat pups also eat the same diet as experienced social models do (Galef & Clark 1971a) and are reluctant to eat foods that adults avoid (Galef & Clark 1971b). Leaf swallowing in chimpanzees to physically expel intestinal parasites appears to originate from opportunistic feeding by some individuals, which is later passed down in the form of a behavioural tradition (Huffman & Hirata 2004). Even behaviours without adaptive values such as stone-play behaviour can be passed on from generation to generation (Huffman 1996; Huffman et al. 2010). In our study, the presence of the mother per se may have better enabled ewe–lamb pairs to learn about the medicinal properties of PEG, compensating for the lack of experience by naïve ewes.

In contrast, lambs without their mothers had a higher preference for grape pomace. During the second preference test they increased their intake of PEG and grape pomace to a similar extent. This finding reinforces the idea that lambs without mothers did not learn to discriminate the beneficial effects of PEG from those of grape pomace when experiencing a tannin challenge. The nonselective increase in intake of PEG and grape pomace during the second preference test is consistent with this hypothesis. In contrast, lambs exposed to the test foods with their mothers (experienced and naïve) selectively increased preference for PEG, suggesting that they learned to discriminate the effects of PEG from grape pomace. Moreover, a higher preference for PEG than grape pomace by these lambs led to greater intakes of the tannin-rich diet, and thus a greater intake of nutrients, than lambs exposed to the same feeds but without their mothers.

The close proximity of a lamb to its mother enhances learning by the lamb (Provenza & Balph 1987, 1988; Mirza & Provenza 1990), and the mother is more effective than an unrelated adult ewe at influencing food preference in lambs (Thorhallsdottir et al. 1990; Saint-Dizier et al. 2007). Thus, pairs of lambs or pairs of ewes probably could have enhanced learning about the medicinal properties of PEG, as social facilitation influences cattle to graze on poisonous plants (Ralphs et al. 1994), although the presence of a lamb's mother may strengthen even further the learned response. The transmission of adaptive maternal effects on the feeding behaviours of mammals is reinforced through sensory stimuli (visual, olfactory and auditory), physical stimuli such as scent or physical alteration, and activity stimuli (movement or interaction with objects in the environment) (Coussi-Korbel & Fragaszy 1995). The presence of the mother encourages offspring to sample foods and gives young animals social cues to eat the particular food that their mother just ate (Mirza & Provenza 1990; Visalberghi & Addessi 2000; Shie & Huber 2006).

Experience with flavoured milk may influence subsequent preference for flavoured feeds by lambs (Nolte & Provenza 1991). However, it is not likely that tannins, PEG or grape pomace flavoured the milk after ewes consumed these feeds in the present study. Condensed tannins are large molecules, flavonoid polymers, not susceptible to hydrolysis (Porter et al. 1986). They typically remain in the gastrointestinal tract and have been regarded as digestibility reducers through their ability to form complexes with dietary and other proteins in the gut (Robbins et al. 1991). Polyethylene glycol is not absorbed by ruminants and serves as an inert marker in digestion trials (Bauman et al. 1971). Grape pomace is a low-quality food poorly digested in the digestive tract of ruminants (Van Soest 1994). Thus, it is unlikely that lambs experienced these feeds through their mothers' milk. Alternatively, recently consumed foods could have been detected by lambs in the ewes' mouth or breath. The breath of rats carries odours of recently ingested feeds and influences preferences in conspecifics identifying such signals (reviewed by Galef 2001).

Continued exposure to the test foods by naïve lambs without their mothers may have eventually led to discrimination about the effects of PEG and grape pomace, as single tested animals can learn to distinguish the effects of simultaneously offered foods (Drucker et al. 1993; Zafra et al. 2007). However, in view of the present results, such discrimination, if possible, would have required a longer period of exposure to the test feeds than that required by lambs with their mothers.

The presence of the mother may have contributed to the emergence of a new behaviour (i.e. selection of the medicine PEG) within the ewe–lamb pair. An innovation is likely to arise when an individual or group is faced with a new challenge (i.e. a tannin-rich diet) for which it currently has no workable solution in its existing behavioural repertoire (Huffman 1996). Many animals respond to environmental stressors by creating a new behaviour or using existing behaviours in a novel context (Kummer & Goodall 1985). Exploration has been regarded as a precursor to innovative behaviour as it may enable an animal to gather information and develop new behaviours or novel means of exploiting the environment (Huffman & Quiatt 1986; Kendal et al. 2005). Thus, it is likely that, in our study, exploratory behaviour was enhanced in the ewe–lamb pair relative to the group of lambs without their mothers. Such enhanced exploratory behaviour probably increased consumption of PEG by lambs (and ewes), which primed individual learning through experience of the postingestive medicinal effects of PEG. For instance, once lambs sampled PEG while consuming a high-tannin basal diet, their individual experience with the beneficial postingestive effects of PEG probably reinforced their preference towards PEG (Villalba et al. 2006). Indeed, lambs from all groups showed a higher intake of PEG on the second preference test than on the first preference test (See Fig. 5).

The positive association between the preference for PEG by experienced mothers and offspring suggests that mothers' prior experience and preference for PEG influenced offspring's food choices. In contrast, naïve mothers' had a low initial preference for PEG and we found a negative association between naïve mothers' initial preference for PEG and lambs' preference for PEG. The low initial preferences for PEG recorded for naïve mothers compounded with such negative association is at odds with the high preferences for PEG displayed by lambs from naïve mothers during phase 4.

Perhaps older models do not readily learn new behaviours unless they are in the presence of young animals that sample novel foods readily. In primates, as well as in ruminants, the diffusion of behavioural innovation often occurs from younger to older animals (Kummer & Goodall 1985; Thorhallsdottir et al. 1990; Huffman 1996), which suggests that ewes may have benefited by being exposed to a medicine and a tannin-rich food while they were with their offspring. Although 33 days of enticing were required for ewes to eat PEG (Fig. 1), only 4 days were necessary for naïve ewe–lamb pairs to eat substantial amounts of PEG (Fig. 3). Thus, ewes' prior experience with PEG was important, but so was sampling of PEG by naïve ewe–lamb pairs.

Even if young animals are more innovative than adults, older animals may play an important role in exploration and innovation, as innovation typically builds upon other skills and may require a certain degree of experience (Reader & Laland 2001). Thus, ewes may have initiated self-medicative behaviour in the naïve group and lambs followed the pattern as imitative processes can generate new behaviours (Russon & Galdikas 1995). Whatever the direction of diffusion of the new behaviour within the pair, the occurrence of the pair enhanced sampling and selection of PEG relative to lambs exposed alone to PEG.

Conclusion

Our study shows that apart from influencing ingestion of nutritious and toxic foods, the mother also influences the ability of offspring to self-medicate. The presence of the mother per se was as important in the emergence of lambs' self-medicative behaviour as the mother's experience with the beneficial effects of the medicine. Thus, mother and offspring may contribute to creating new knowledge within a social group, as well as to improving the transmission and maintenance of this knowledge across generations.

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