MATERNAL EFFECTS IN TRANSMISSION OF SELF-MEDICATIVE BEHAVIOR FROM MOTHER TO OFFSPRING IN SHEEP

by

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ABSTRACT

Maternal Effects of Transmission of Self-medicative Behavior from Mother to Offspring in Sheep

by

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Mammals begin learning food preferences *in utero* and maternally mediated influences early in life help offspring develop their feeding habits. Mammals also learn by individual experience to ingest medicinal compounds such as polyethylene glycol (PEG), which attenuates the negative post-ingestive effects of tannins, a group of potentially toxic plant secondary compounds. The objective of this study was to investigate the transmission of acquired self-medicative behavior from mother to offspring using polyethylene glycol (PEG) as a medicine to relieve malaise caused by tannins. I hypothesized that: 1) mothers trained to associate the beneficial effects of PEG while consuming tannins will pass this information to their offspring, and 2) lambs will be more efficient at utilizing PEG as a medicine against tannins in the presence of mother than lambs which learn without the influence of the mother.

This hypothesis was evaluated in four phases: in the first phase, a group of ewes (Experienced) was conditioned to associate the beneficial effects of PEG after consuming a tannin-containing diet. Ewes were offered a meal of high-tannin food and PEG and subsequently, the high-tannin food and grape pomace (GP) with little nutritional and no "medicinal" effects. In the second phase, the experienced and a naïve group of ewes (Inexperienced) were given a choice between the high-tannin food, PEG, and GP. In the third phase, experienced and inexperienced ewes with their naïve lambs, and the group of naïve lambs without their mothers, were exposed to the tannin-containing diet, PEG, and GP. Finally, in the fourth phase, lambs were separated from their mothers, and lambs from all groups were offered a choice between the tannin-containing diet, PEG, and GP.

Lambs from experienced and inexperienced mothers showed a higher preference for PEG than lambs exposed without their mothers who tended to show a higher preference for GP. Thus, the presence of mother (experienced/inexperienced) was important for naïve lambs to learn about the medicinal benefits of PEG.

This source of trans-generational knowledge could aid in maintaining the information in the herd, increasing the efficiency and reducing the risk of learning about foods and environments exclusively by individual experience.

(77 pages)

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For my parents

Niral and Kanti Sanga

CONTENTS

Page

ABSTRACT.....2

AKNOWLEDGMENTS.....4

DEDICATION.....5

LIST OF TABLES.....7

LIST OF FIGURES.....9

CHAPTER

1. LITERATURE REVIEW..... 11
2. INTRODUCTION..... 19
3. MATERIALS AND METHODS..... 22
4. RESULTS.....29
5. DISCUSSION..... 43
6. CONCLUSION 50

REFERENCES 57

APPENDICES..... 66

LIST OF TABLES

Table Page

A1. SAS Output: Intake of PEG by ewes 67

A2. SAS Output: Preference of PEG by ewes 68

A3. SAS Output:

Intake of PEG by ewe-lamb pair during day 1-7 69

A4. SAS Output:

Intake of PEG by ewe-lamb pair during day 8-12 70

A5. SAS Output:

Intake of grape pomace by ewe-lamb pair during day 1-7 71

A6. SAS Output:

Intake of grape pomace by ewe-lamb pair during day 8-12 73

A7. SAS Output:

Intake of tannin by ewe-lamb pair during day 1-7 74

A8. SAS Output:

Intake of tannin by ewe-lamb pair during day 8-1 76

A9. SAS Output: Scan sampling of ewes for PEG 77

A10. SAS Output: Scan sampling of lambs for PEG 78

A11. SAS Output: Scan sampling of ewes for Grape Pomace 80

A12. SAS Output: Scan sampling of lambs for Grape Pomace 82

A13. SAS Output: Scan sampling of ewes for tannin 83

A14. SAS Output: Scan sampling of lambs for tannin 85

A15. SAS Output:

Preference of PEG in lambs during Preference test 1.....86

A16. SAS Output:

Preference of PEG in lambs during Preference test 2.....87

LIST OF FIGURES

Figure Page

4-1 Daily intake of PEG and tannin-containing food by ewe during conditioning..... 30

4-2 Intake of grape pomace and tannin-containing food by ewes during conditioning 32

4-3 Intake of tannin-containing food, PEG and Placebo (Grape Pomace) and preference for PEG by a group of ewes conditioned with the medicinal effects of PEG on tannins (C) and by a naïve group of ewes (NC) 34

4-4 Intake of tannin-containing food, medicine (PEG), and placebo (grape pomace) by two groups of ewe-lamb pairs (C: naïve; NC: treatment) and a group of naïve lambs without mothers(Lambs alone) 36

4-5. Behaviors recorded during scan sampling between ewes and lambs consuming PEG, GP and Tannin when offered the choices 38

4-6 Preference for PEG: Intake of PEG and grape pomace (GP) and intake of tannin-containing food by lambs with conditioned mothers (C), lambs with unconditioned mothers (NC) and lambs without mothers (ALONE) 40

4-7 Linear regression plot estimating the relationship between consumption of PEG by mothers and by lambs during preference tests 1 and 2 42

CHAPTER 1

LITERATURE REVIEW

Mammals have been known to ingest medicinal compounds (i.e., self-medicate) by consuming leaves of certain plants containing potentially toxic secondary compounds (Huffman 1997, 2009). Herbivores have also been known to transfer their individual dietary choices and preferences to their young through pre-natal and post-natal care (Launchbaugh et al. 1999, Mirza and Provenza 1992, 1994). This study was conducted to investigate if herbivores also transmit information on their medicinal choices to their offspring through the maternal influences of the mother.

Self-medication

Herbivores adapt to the variability of the external environment and to their changing internal needs not only by generating homeostatic physiological responses, but also by operating in the external environment (i.e., by selecting appropriate feeds). Under this view, food selection is interpreted as the quest for substances in the external environment that provide homeostatic utility to the internal environment (Villalba and Provenza 2007). In the co-evolution of plant-animal relationships, species from arthropods to humans use plant secondary compounds to self-medicate (Huffman 2009). Interactions among plants, herbivores and their predators (e.g., parasites) play an important part in

the study of self-medication behavior in animals (Price et al. 1980, Clayton and Wolfe 1993). Plant secondary compounds, apart from being functional in helping plants attract pollinators, recover from injury, protect against UV radiation, defend against excessive herbivory, also act as medicines against pathogens when consumed by herbivores in moderate amounts (Janzen 1978, Rhoades et al. 1979, Villalba and Provenza 2007).

Most of the studies on self-medication of herbivores through plant secondary compounds have been anecdotal and equivocal (Clayton and Wolfe 1993, Lozano 1998). The preference or avoidance of a certain food by the herbivore is a function of its overall fitness and herbivores learn to ingest toxic secondary compounds found in plants as medicines to increase their fitness (Janzen 1978, Villalba and Provenza 2007). To date, scientific studies of self-medication have been made on the African great apes (Huffman 1997) and sheep (Villalba and Provenza, 2007). Studies relating to secondary compounds and other non-nutritional compounds for treating or preventing diseases have emerged into a new field called 'zoo-pharmacognosy' (Rodriguez and Wrangham, 1993).

Self medication behavior have been categorized by Clayton and Wolfe into the following categories (1993):

1. Ingestion: Some herbivores ingest plants containing chemicals with medicinal values to combat intestinal parasites For example, Chimpanzees *(Pan troglodytes)* consume juice of *Vernonia*

amygdalina (Huffman 1997) and Tobacco hornworms ingest nicotine to reduce the growth of *Bacillus thuringiensis* (Krischik et al. 1991).

2. Absorption: Some animals self medicate through absorption of medicinal chemicals across skin or mucous membrane. For example, Chimpanzees self-medicate by massaging *Aspilia* leaves across their tongue to absorb Thiarubrine A., an antibiotic in *Aspilia*, through the buccal membrane (Huffman 1997, Lozano 1998).

3. Topical application: Some animals rub medicinal compounds on their fur/bodies to medicate against ectoparasites. For example, White-faced monkeys *(Cebus capucinus)* rub *Dieffenbachia* leaves into their fur (Clayton and Wolfe 1993).

4. Proximity: Some animals use medicinal substances without coming in direct contact. Birds medicate against ectoparasites by weaving plants with antibacterial and insecticidal properties into their nests (Clayton and Wolfe 1993).

According to the 'phytochemical co-evolution' theory, as the concentration and toxicity of secondary compounds in plants increases, the taxons become more phylogenetically diversified. In turn, herbivores responded by either becoming specialists or generalists (Cornell and Hawkins 2003). As herbivores become specialists, they developed adaptations to cope with higher concentrations of plant secondary compounds (Cornell and Hawkins 2003).

Maternal effect

Maternal effects are predominantly the effect of the environment provided by the mother on the phenotype of the offspring (Cheverud and Moore 1994). Typically, the phenotype of an organism is measured as the sum of the heritable effects of genes (H) and the environmental effects (E) (Cheverud and Moore 1994).

$$P = H + E.$$

While considering maternal effects, the environmental effect is further divided into additive maternal effects (M) and the individual environment effect of the organism (E_i)

Thus,

$$P = H + M + E_i$$

The additive maternal effects, in turn, are measured as the sum of the heritable effects of the mother's genes (H_m) and the environmental effects experienced by the mother (E_m).

$$M = H_m + E_m$$

Mothers contribute to their offspring's phenotype in various forms:

(i) Cytoplasmic inheritance where the inheritance of organelles in zygote occurs through the cytoplasm of the female egg. There is no transfer of genes in the offspring via meiosis and mitosis.

(ii) Maternal nutrition either via the egg or via pre- and post-natal care

(iii) Transmission of pathogens and antibodies through the pre-natal blood supply or by post-natal feeding

(iv) Imitative behavior, and

(v) Interactions between conspecifics and social models. Mothers respond to environment cues and transmit the knowledge to their offspring (phenotype) to enhance offspring fitness (Mousseau and Dingle 1991, Bernardo 1996).

Plant-herbivore studies show that self-medication behaviors can be adaptive and that they are heritable (Clayton and Wolfe 1993). Maternally mediated influences in *utero* and early in life enable sheep and cattle to use forages of poor nutritional quality and those high in secondary compounds (Thorhallsdittor et al. 1990, Mirza and Provenza 1992, Launchbaugh 1999). Mothers form the social model under the influence of which the offspring develops its feeding habits (Green et al. 1984). Young animals learn which foods to eat and which to avoid from interactions with mother. The influence of the mother on the offspring's dietary behavior begins *in utero* and continues even after weaning (Provenza et al. 1992). Food ingested by the mother influences the flavor of her milk (Bassette et al. 1986), which in turn affects food preferences in the offspring. As animals begin to forage, the presence of mother enhances the acceptance of novel foods by lambs (Green et al. 1984, Thorhallsdottir et al. 1990). The food preferences of lambs exposed with their mothers are more persistent than with lambs exposed alone

(Thorhallsdottir et al. 1987). Consumption of poisonous food made ewes more neophobic to novel food as compared to lambs (Thorhallsdittor et al. 1987). Further, the age of the lambs at exposure to the novel food with the mother effects learning to select or avoid food from their mothers (Mirza and Provenza 1990, 1992; Thorhallsdottir et al. 1990). Maternal effect was stronger when lambs were exposed at 6 weeks of age than when they were exposed at 12 weeks of age (Mirza and Provenza 1990).

Background on Tannins and

Polyethylene Glycol

The complexities of plant chemistry and animal metabolism and extrapolation of laboratory results to the field make it difficult to provide substantial evidence for the benefits of secondary compounds as medicines when consumed in moderate amounts (Foley and Moore 2005). Previous studies on self-medication in sheep have used tannin-PEG association as a model for studying the effect of non-toxic polyethylene glycol (PEG) as medicine to the adverse effects of tannin, a secondary compound found in many plant species. In my study, I have replicated the tannin-PEG model to study the transmission of self-medication in the mother and their offspring.

Tannins are high molecular weight polyphenolic compounds that at high concentrations can reduce digestive efficiency in the rumen by

forming complexes with proteins and other molecules (Goldstein and Swain 1965). Previous studies with steers (Donnelly 1954) grazing *Sericea lespedeza* show that animals consume more of low- than high-tannin containing plants. At low levels, tannins can be beneficial for herbivores as they enable more efficient use of protein by reducing protein degradation in the rumen, greater nitrogen retention by increasing the flow of protein and essential amino acids to the intestine, prevent bloat and increase microbial efficiency (Launchbaugh et al. 1999, Priolo et al. 2000). At high levels of tannins in the diet, however, herbivores experience anti-nutritional effects such as lower feed intake due to decreased palatability, decreased nitrogen absorption, reduced availability of minerals and damage to the mucosal lining of the gastrointestinal tract, thus reducing the digestibility of food in the rumen (Reed 1995). The physiological activities of tannins result from either direct inhibition of digestive tract enzymes or from the absorption of dietary proteins (Silanikove et al. 2001). Tannins either reduce the solubility of enzyme protein by forming insoluble protein-phenolic complexes (Williams 1963) or they inhibit digestive enzyme activity by forming soluble but inactive enzyme–inhibitor complex (Kumar and Singh 1984). Tannins also decrease the palatability of plants through their “astringent” nature by precipitating the salivary protein or by immobilizing enzymes in the mouth (Kumar and Singh 1984).

Polyethylene glycol (PEG) is an inert and unabsorbed molecule, which binds with tannins to form a stable, insoluble complex that prevents tannins from binding to protein in the rumen (Decandia 2000). Polyethylene glycol has been used to counteract the adverse effects of tannins and can be added to the feed to improve digestibility, palatability and intake of tannins in ruminants (Titus et al. 2001).

The theory of post-ingestive feedback holds that animals do not avoid or prefer food based only on flavor; rather, they form preferences and aversions by integrating the flavor of food with its post-ingestive consequences (Provenza 1995). One prediction emerging from this theory is that herbivores supplemented with PEG should consume more tannin-containing feed as the negative effects of tannins are attenuated by the presence of PEG in the gastrointestinal tract. Indeed, steers in pen trials supplemented with PEG markedly increase their intake and preference for fresh-cut *sericea* – a legume with concentrations of condensed tannins of about 15% (Mantz et al. 2009). In other studies, lambs increase intake of PEG when the concentration of quebracho tannin in their diet is increased (Provenza et al. 2000). Lambs also learned to differentiate the medicinal effects of PEG from other non-medicinal foods such as wheat straw by selective intake of PEG after consumption of tannin-containing feeds (Villalba and Provenza 2001,2002).

CHAPTER 2

INTRODUCTION

Social models play an important role in diet selection and food preferences of a young animal (Thorhallsdottir et al. 1987, 1990). Socializing enhances learning efficiency because each animal no longer has to discover everything through trial and error (Provenza 1995; Thorhallsdottir et al. 1987) and this foraging information is passed trans-generationally from the experienced mother to the offspring (Key and MacIver 1980, Lynch et al. 1983, Thorhallsdottir et al. 1990, Green et al. 1984, Provenza and Cincotta 1993). Mother's influence begins *in utero,* continues after birth as the flavors of foods mother eats are transmitted in her milk, and continues as offspring learn what to eat from mother (Provenza and Cincotta 1993).

While herbivores learn to prefer nutritious foods as a function of mother's preferences (Mirza and Provenza, 1990; 1992), no information is available regarding social transmission of other types of behaviors, equally important for the fitness of the individual. For instance, animals use plant secondary compounds and other non-nutritional substances to combat disease (Huffman 1997). Sheep self-select medicinal substances such as polyethylene glycol (PEG), a non-nutritive polymer that attenuates the aversive effects of plant secondary compounds such as

tannins, as concentrations of these illness-inducing compounds increase in the diet (Provenza et al. 2000; Villalba and Provenza 2001).

While the medicinal effects of PEG are known, as well as the ability of sheep to self-select PEG when challenged with a tannin-containing diet (Villalba and Provenza 2001), little is known about the influence of mother on the ability of her offspring to self-medicate with PEG. More generally, critical information regarding transmission of self-medicative behavior from mother to offspring is lacking.

My objectives were to determine whether:

1. The presence of mother, experienced with the medicinal effects of PEG when eating a high-tannin basal diet, enhances use of PEG in her naïve lambs relative to naïve lambs with their naïve mothers, or to naïve lambs without their mothers.

2. The presence of mother *per se* (experienced or naïve) influences the ability of lambs to self-medicate.

My hypothesis is that the presence of experienced mothers enhances learning efficiency by offspring because each animal no longer has to discover everything through trial and error. It can be difficult for animals to learn through trial and error about the medicinal effects of substances, especially if behavior and consequences are not paired consistently or closely in time. Thus, I predict lambs observing experienced mothers and challenged with a tannin-containing diet will learn to self-medicate with PEG at a faster rate than lambs with mothers

unfamiliar to the medicinal benefits of PEG, or than lambs without the possibility of observing an experienced or naive model (i.e., lambs without their mothers).

CHAPTER 3

MATERIALS AND METHODS

The study was conducted at the Green Canyon Ecology Center, located at Utah State University in Logan. St. Croix ewes (n = 16, approximately 3-6 yr of age) with their lambs and 8 St. Croix lambs (approximately 2 mo of age) were penned outdoors under a protective roof. Ewe-lamb pairs were penned in single, adjacent pens measuring 6 x 5 m while the remaining 8 lambs were penned in separate and adjacent individual pens (2.4 x 3.6 m). The animals were fed 1.5kg of alfalfa pellets and 300 g of grain following daily trials. They had *ad libitum* access to mineralized salt blocks and fresh water throughout the study. The study was done in accordance with procedures approved by the Utah State University Institutional Animal Care and Use Committee (IACUC Approval 1409).

The study was conducted in four phases in which ewes, each with 1 lamb, were randomly assigned to either a treatment group (n = 8 ewes and lambs) or to a control group (n = 8 ewes and lambs); the third group of 8 lambs was without their mothers. In the first phase ewes from the treatment group ingested PEG when consuming a tannin-containing diet. In the second phase, we tested whether ewes from the treatment group preferred PEG over GP while consuming a tannin-containing food by offering choices between PEG and GP. In the third phase, lambs with

their experienced mothers, inexperienced mothers, or alone were offered a tannin-containing food, PEG, and GP. After this exposure, in a fourth phase I determined lambs' preference for PEG over GP when offered a high-tannin food.

Phase 1: Sequential Conditioning of Treatment Ewes

a) With PEG

Ewes from the treatment group were offered PEG (medicine) to attenuate the effects a diet high in tannins. Ewes were first fed a tannin-containing 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 15, ewes were separated from their lambs by dividing the pen into two compartments with a panel. Subsequently, ewes were offered a high-tannin food (15% tannin [Tannin Corporation, Peabody, MA], 55% alfalfa hay and 30% barley) from 0900 to 1000 and then offered PEG (MW 3,350; Spectrum Chemical, Los Angeles, CA) from 1000 to 1100. Immediately after ingesting PEG for 1 h, ewes were again offered the high-tannin diet for 1 h. After this procedure, each treatment ewe was re-united with her lamb and fed a basal diet of 1.5 kg alfalfa pellets mixed with 300 g barley.

Animals are reluctant to eat PEG during initial conditioning due to its nil nutritional value (Villalba and Provenza 2001). Hence, ewes were

first 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 > 75 g of the PEG-barley mix, the proportion of barley was reduced to 10%, and then eliminated (100% PEG) the next day. If not, the proportion of barley in the mix was maintained at 20%.

As intake of PEG by ewes was low even after 15 d of exposure, we increased the time of exposure to PEG. From d 16 to 33, all treatment ewes were separated from their lambs and fed 1 kg of high-tannin diet from 0900 to 1000. Subsequently, ewes were offered 300 g of 100% PEG. Refusals were collected at 1700 hrs, the ewes re-united with their lambs and all animals were given the basal diet of 1.5 kg of alfalfa pellets mixed with 300 g of barley.

b) With GP

Ewes from the treatment group were conditioned to consume GP (a low-quality feed) after eating a food high in tannins. Treatment ewes were separated from their lambs as described before, and fed 1 kg of high-tannin food from 0900 to 1000. Subsequently, ewes were offered 300 g of GP mixed with 25 g of barley GP to encourage the animals to sample the GP. After day 8, the animals were fed 100% GP. Refusals were collected at 1700, the ewes re-united 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 GP was carried out for 17 d until intake of GP stabilized over time.

Phase 2: Preference for PEG by Ewes

Ewes from the treatment and control groups were separated from their lambs as described before, offered the high-tannin diet from 0900 to 1000, and then offered a choice between the same tannin-containing diet, PEG and GP until 1700 hrs, when ewes were re-united with their lambs. Refusals were collected and weighed, and the amount of medicine/GP and tannin-containing diet consumed by the ewes was measured for 2 consecutive d.

Phase 3. Transmission of Self-Medicative Behavior

Ewe-lamb pairs and single lambs from all three groups received a simultaneous offering of the high-tannin containing food, PEG and GP from 0900 hrs to 1700 hrs. Thus, each ewe with its lamb ate together during this phase and, different from single lambs without their mothers, daily intake of each feed represented the combined consumption of the pair. In order to discriminate between the ingestive behavior of lambs and ewes, one observer recorded the behavior of ewes and their lambs while they ate, using scan sampling (Altman, 1974),at 5-min intervals from 0900 to 1030. I recorded incidence of feeding on each of the

alternatives available, or 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. I also recorded the total number of scans of eating events and non-eating events (bouts of inactivity such as not eating or resting).

Two periods, of 7 d (Period 1) and 5 d (Period 2), respectively, were carried out during this phase. The same procedure was followed in both the periods. Preference tests (see below) were conducted for all lambs after each of these periods.

Refusals were collected at 1700, weighed, and intake of each feed was determined at the end of each day. Ewe-lamb pairs were given the basal diet of 1.5 kg of alfalfa pellets mixed with 300 g of barley, and single lambs 1 kg of alfalfa mixed with 300 g of barley.

Phase 4. Preference for PEG by Lambs

The objective of this phase was to determine lambs' preference for medicine (PEG), GP, and tannin-containing diet after exposure to these feeds with experienced mothers, inexperienced mothers, or alone during Phase 3. The day after Periods 1 and 2 of Phase 3, lambs were separated from their mothers as described before, and lambs from all groups were offered a choice between the tannin-containing diet, PEG and GP from 0900 to 1700.

During this phase, ewes were also fed the tannin-containing diet in order to minimize distraction of the lambs. Refusals were collected and weighed and individual intake of each feed was recorded at 1700 hrs. Subsequently, the ewes were re-united 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, and single lambs 1 kg of alfalfa mixed with 300 g of barley.

Statistical Analysis

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 (fixed factors). The response variables were the amount of tannin-containing food, medicine (PEG) and GP consumed by animals, preference for those foods ([intake of individual feed/total intake] x 100) during preference tests, and proportion of scans ([scans on individual feed/total number of scans in which animals were feeding] x 100). Separate analyses were conducted for ewes and lambs, except for Phase 3 ("Transmission of Self-Medicative Behavior") where food intake represented the combined consumption of ewe-lamb pairs for Groups 1 and 2. Linear regression analysis was used to estimate the relationship between consumption of PEG by mothers and by lambs during preference tests.

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

CHAPTER 4

RESULTS

Phase 1: a) Conditioning of ewes with PEG (Medicine)

Intake of PEG by treatment ewes fluctuated from d 1- 12, while ewes received PEG with varying proportions of grain. On d 13, there was a noticeable decrease in the intake of PEG when all treatment ewes were given 100% PEG. From d 15 to 33, as the time of exposure to PEG and tannin-containing food was increased from 3 h to 8 h, intake of PEG gradually stabilized to an average of 78 g/d (Figure 4-1). Intake of the tannin-containing food also increased gradually and stabilized at 1000 g/d (Figure 4-1).

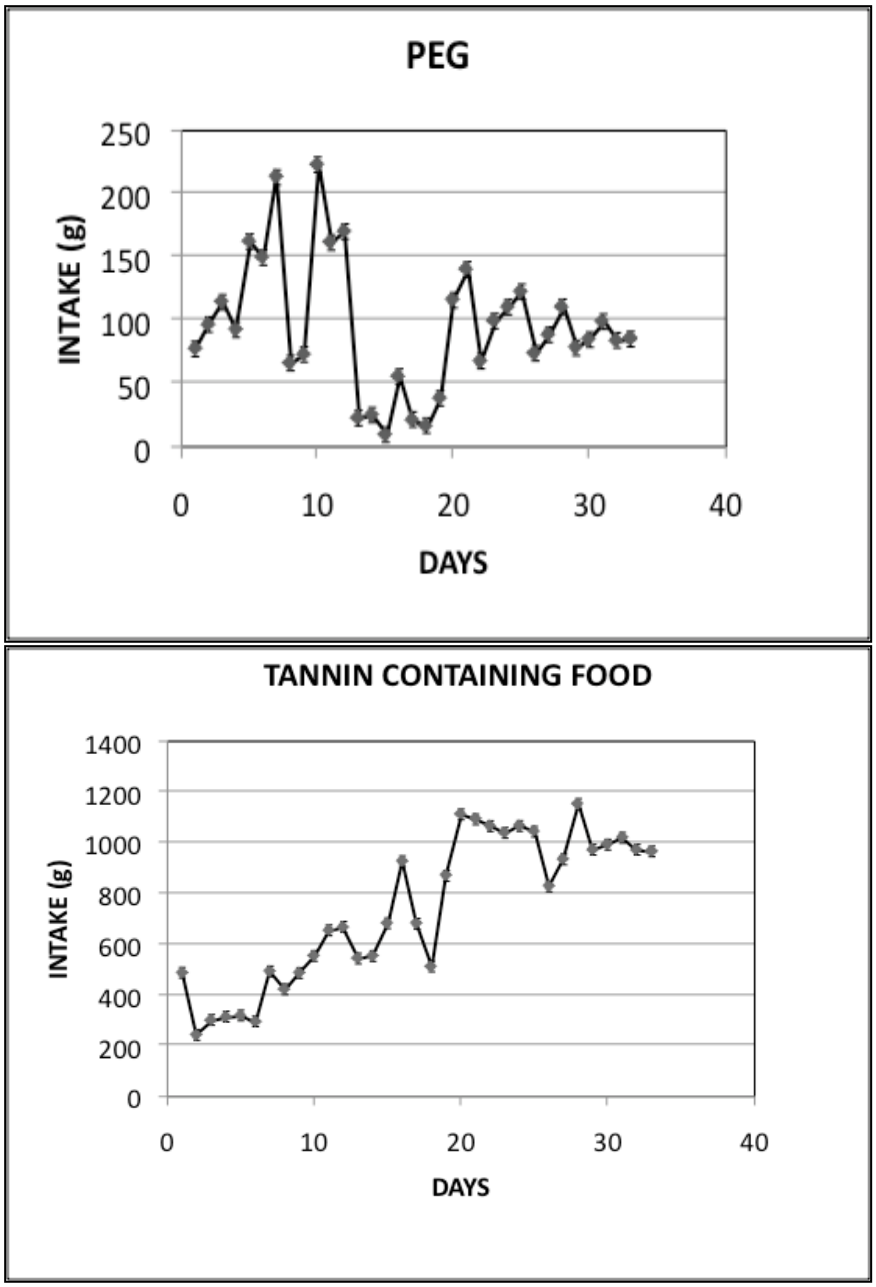


Figure 4-1. Daily intake of PEG and tannin-containing food by ewes during conditioning.

Phase1: b) Conditioning of ewes with GP

Intake of GP, as well as intake of the tannin-containing food, decreased over time (Figure 4-2). From day 1 to 8, ewes were enticed to sample GP by adding 50 g of barley to 300 g of GP. The average intake of GP from day 1 to 8 was 101 g/d and the average intake of tannin-containing food was 849 g/d. Starting from day 9, animals were offered 100% GP, which resulted in a considerable drop in the amount of GP ingested, even when exposure to GP was 8 h/d. In contrast to the response observed during conditioning with PEG, intake of the tannin-containing food decreased over time (Figure 4-2). The average intake of GP from d 9 to 8 for the treatment ewes was 14 g/d and the average intake of tannin-containing food was 605 g/d (Figure 4-2), about 400 g lower than the period of conditioning with PEG.

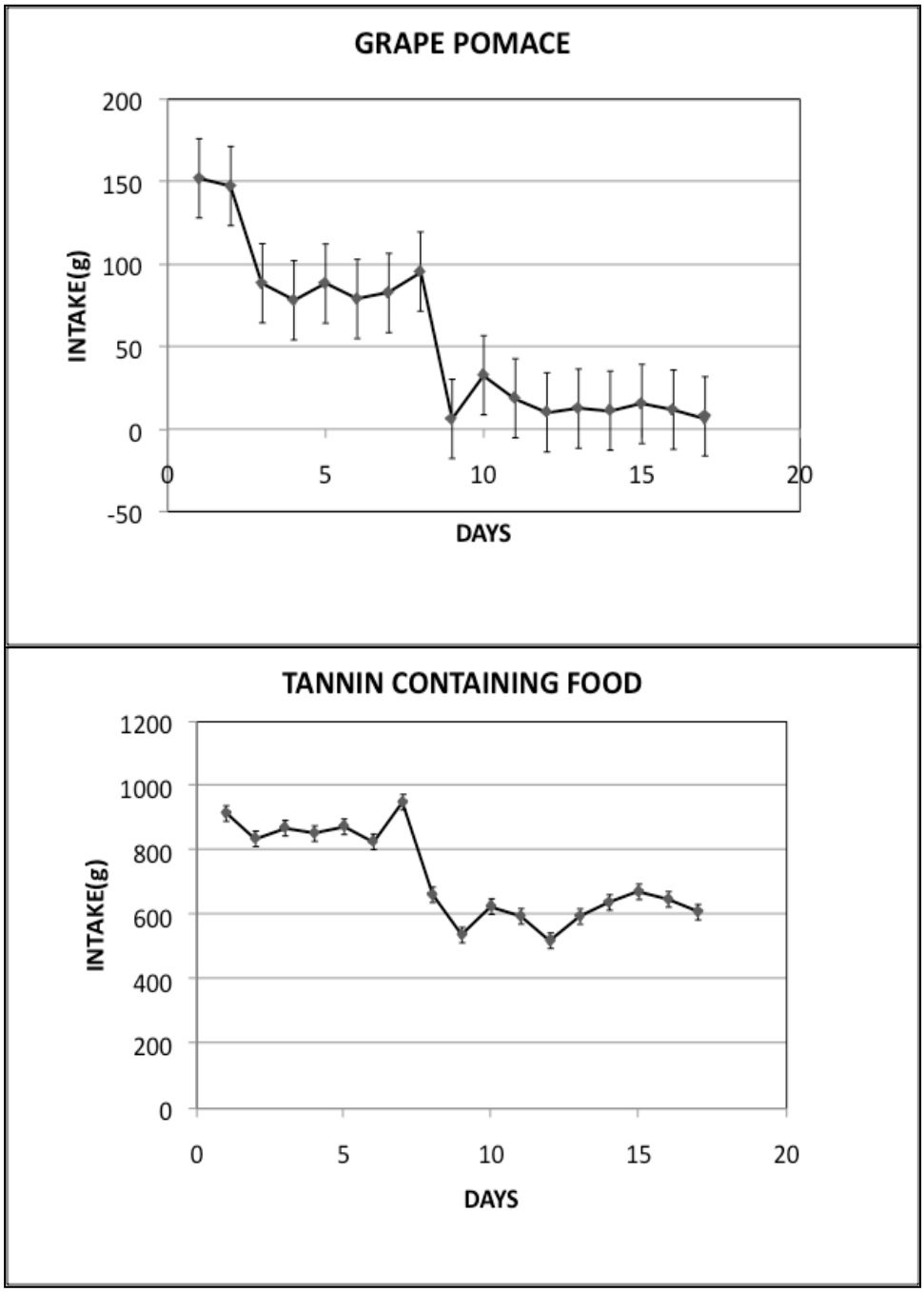
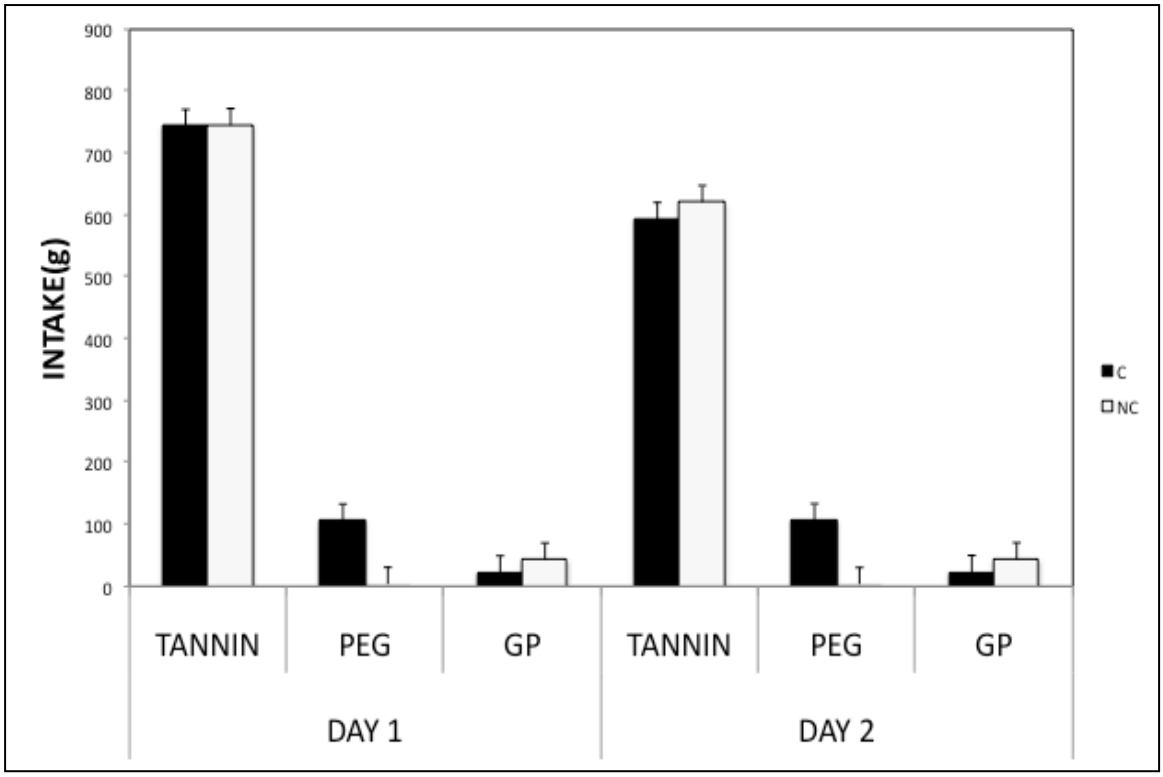


Figure 4-2. Intake of GP and tannin-containing food by ewes during conditioning

Phase 2: Preference for PEG by ewes.

When given a choice between the tannin-containing diet, PEG and GP, ewes previously conditioned to eat PEG (Experienced) had higher intake (106 vs. 23 g; SEM = 26 g; P < 0.05, Table A1) and preference for PEG (71 vs. 19%, SEM=10.28; P < 0.05, Table A2) than naïve ewes (Figures 4-3). Intake of GP did not differ between groups (P = 0.58). Experienced ewes tended to consume more tannin-containing food than naïve ewes during preference tests (744 vs. 607 g; SEM = 77 g; P = 0.23; Figure 4-3).



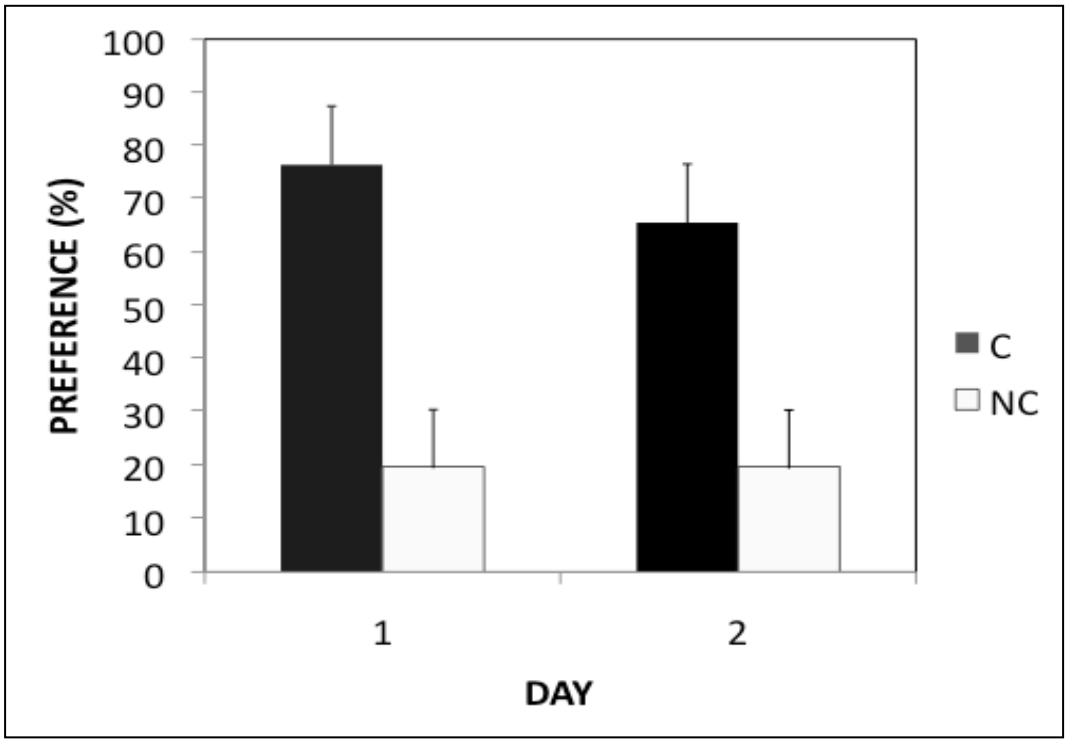


Figure 4-3. Intake of tannin-containing food, PEG and GP (top graph), and preference for PEG by ewes conditioned to eat PEG with tannins (C) and by a naïve group of ewes (NC) (bottom graph).

Phase 3. Transmission of Self-Medicative Behavior

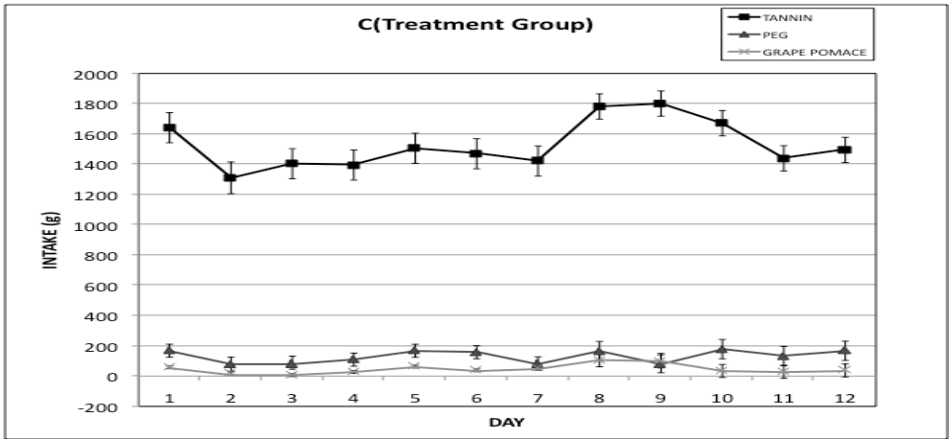
Intake of Test Feeds

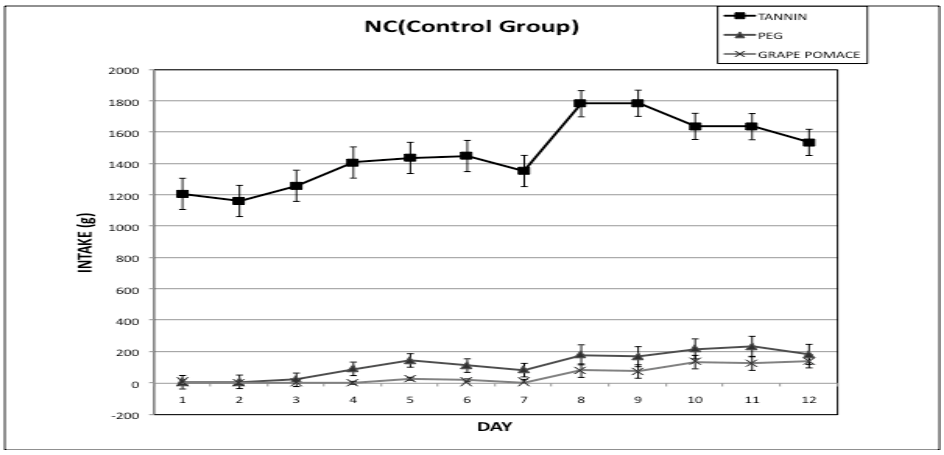
PEG: During the first period (d 1 to 7), ewe-lamb pairs with experienced mothers consumed more PEG than lambs without their mothers (122 vs. 2 g; SEM = 34 g; P = 0.02; Table A3) and tended to consume more PEG than pairs with naïve mothers (122 vs. 68 g; SEM = 34 g; P = 0.27; Table A4). No differences in consumption of PEG were

detected among groups for the second period of the phase (P = 0.62; Figure 4-4; Table A4).

GP: During the first period of the phase (d 1 to 7), ewe-lamb pairs with experienced mothers consumed more GP than pairs with naïve mothers and lambs without their mothers (37 vs. 13 and 4 g, respectively; SEM = 6 g; P < 0.05; Table A5). No differences in consumption of GP were detected among groups for the second period of the phase (P = 0.61; Figures 4-4, Table A6).

Tannin-Containing Diet: Ewe-lamb pairs did not differ in intake of tannin-containing food (P > 0.5), except that for the first day of Phase 1, ewe-lamb pairs with experienced mothers consumed more tannin-containing food than pairs with naïve mothers (P = 0.003; Figure 4-4). Lambs without their mothers ate the least amount of tannin diet in both periods (P < 0.0001; Figure 4-4).





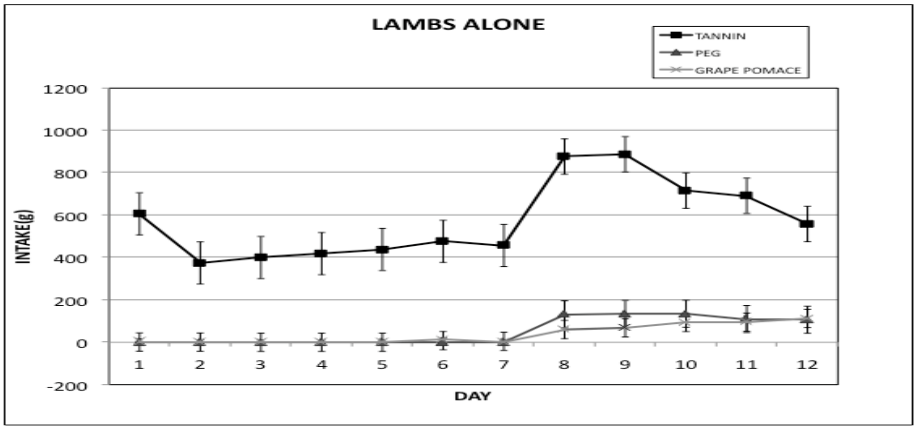


Figure 4-4. Intake of tannin-containing food, medicine (PEG), and GP by two groups of ewe-lamb pairs (C: naïve; NC: treatment) and a group of naïve lambs without mothers (Lambs alone).

Scan sampling

PEG: Experienced and inexperienced ewes did not differ in scans on PEG (6 vs. 3% of ingestive events recorded, respectively; SEM = 3%, Table A9; P = 0.46; Figure 4-5). Likewise, no differences in scans on PEG were detected among groups of lambs (4.4% vs. 3.3% of the ingestive events recorded for lambs with experienced and inexperienced mothers, respectively; SEM = 1.5; P > 0.05; Figure 4-5, Table A9).

GP: No differences among groups of ewes or lambs were detected in scans recorded for GP (P > 0.05, Figure 4-5, Table A11, A12).

Tannin-containing Diet: Naïve and Experienced ewe-lamb pairs did not differ in percent scans consuming the tannin-containing food (Ewes: 94% vs. 88%, SEM = 4%; Lambs: 89 % vs. 86% of ingestive events recorded, SEM = 4%; P > 0.5; Figure 5-5, Table A13, A14).

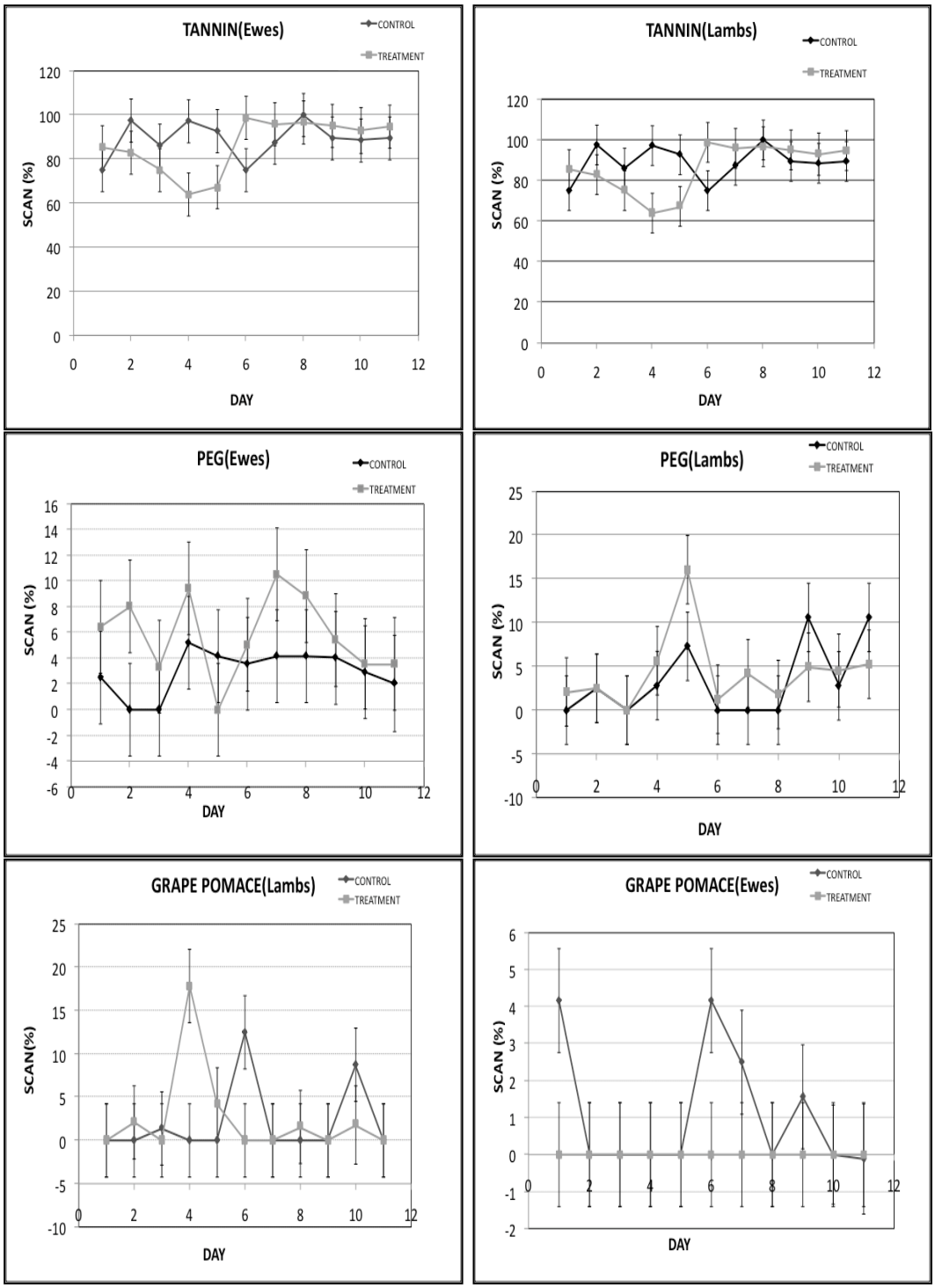


Figure 4-5. Behaviors recorded during scan sampling between ewes and lambs consuming PEG, GP and Tannin when offered the choices.

Phase 4: Preference Tests for lambs

PEG: Lambs exposed with their mothers (experienced and naïve) to PEG and GP while offered a high-tannin food showed a higher preference for PEG than lambs exposed without their mothers (Preference Test 1: 79% and 74% vs. 46%, respectively, SEM = 13%; $P < 0.05$. Preference Test 2: 69% and 72% vs. 42%, respectively; SEM = 11%; $P < 0.0001$) (Figure 4-6). During the first preference test, lambs exposed to PEG with their mothers (experienced and naïve) also consumed more PEG than lambs without their mothers (32 and 29 g vs. 2 g, respectively; SEM = 9 g; $P < 0.05$). In contrast, no differences in intake of PEG were detected among groups during Preference Test 2 (Group x Food interaction; $P = 0.49$; Figure 4-6).

GP: Lambs exposed without their mothers to PEG and GP while offered a high-tannin food tended to show a higher preference for GP than lambs exposed with their mothers (experienced and naïve): Preference Test 1: 54% vs. 20% and 25%, respectively, SEM = 13%; $P = 0.16$; Preference Test 2: 58% vs. 32% and 28%, respectively, SEM= 11%; $P = 0.16$). No differences in intake of GP were observed among groups of lambs ($P > 0.05$), but for Preference Tests 2 the average intake of GP by lambs with experienced and naïve mothers was 28 and 45 g, respectively, whereas intake of GP by lambs without their mothers was 134 g (Figure 4-6).

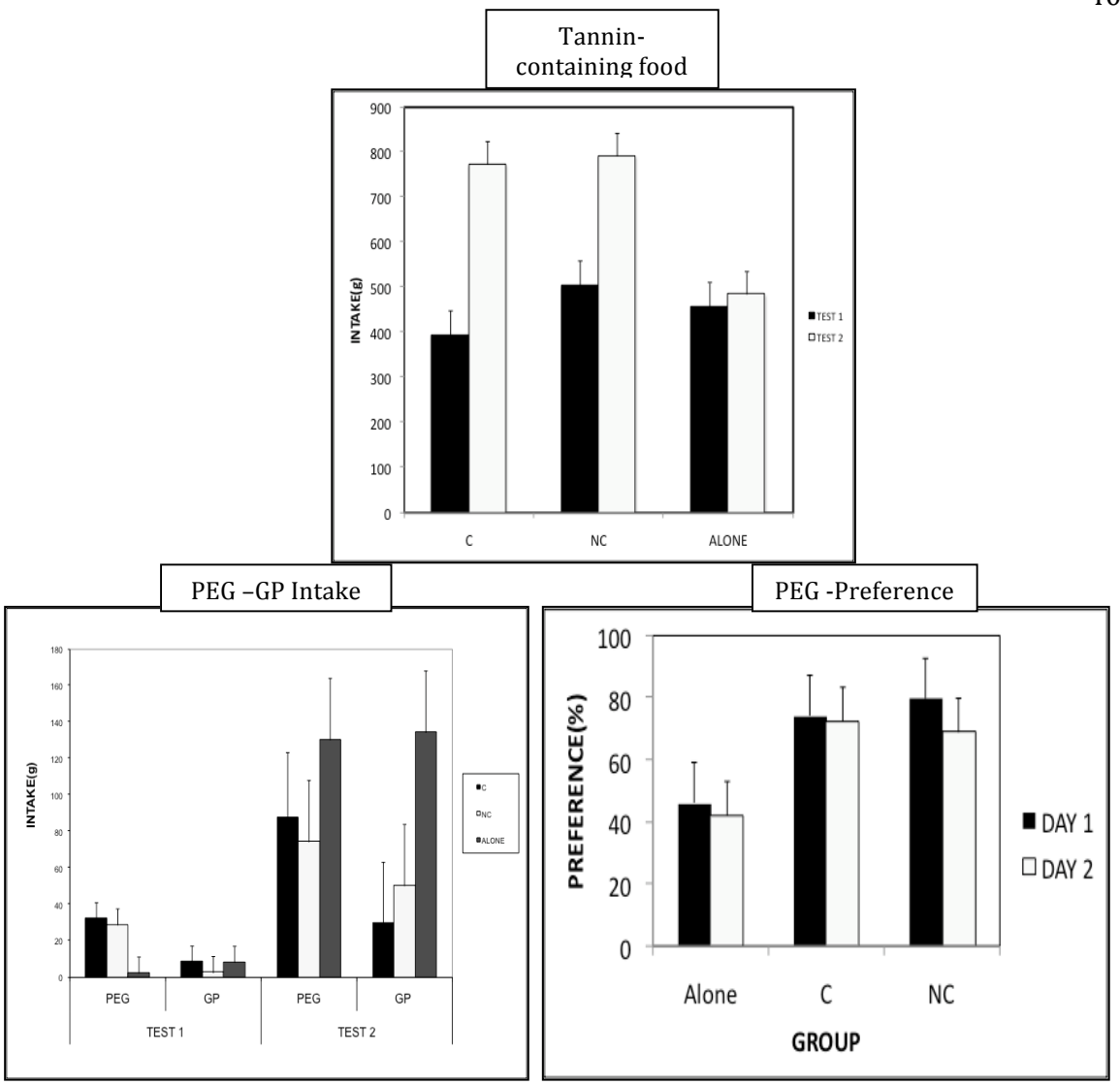


Figure 4-6. Preference for PEG (top graph), intake of PEG and GP (bottom left), and intake of tannin-containing food (bottom right) by lambs with conditioned mothers (C), lambs with unconditioned mothers (NC) and lambs without mothers (ALONE).

Tannin-Containing Food. No differences in intake of tannin-containing food were detected among groups during Preference Test 1 (P = 0.37). However, during Preference Test 2 lambs exposed with their mothers (experienced and naïve) had higher intake of tannin-containing food than lambs exposed without their mothers (773 and 791 g vs. 484 g, respectively, SEM = 51 g; $P < 0.05$; Figure 4-6).

Relationship between intake of PEG by experienced mother and offspring:

For preference test 1, consumption of PEG by experienced mothers was proportional to the consumption of PEG by lambs ($R^2 = 0.33$; P=0.14). In contrast, there was no relationship between consumption of PEG by mothers and offspring during preference test 2 ($R^2 = 0.04$;P=0.64, Figure 4-7).

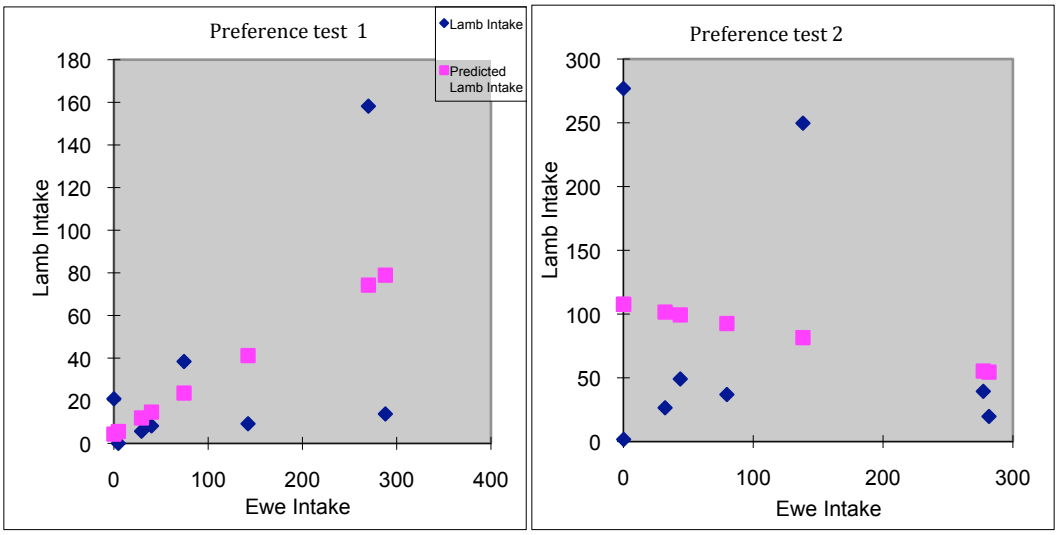


Figure 5-7. Linear regression plot estimating the relationship between consumption of PEG by mothers and by lambs during preference tests 1 and 2.

CHAPTER 5

DISCUSSION

Preference for PEG by ewes

Ewes experienced with the beneficial effects of PEG while consuming tannins preferred PEG over GP. In contrast, naïve ewes ate greater amounts of GP than PEG and their intake of PEG was low. These results are consistent with previous studies showing that lambs fed a high tannin diet discriminate the positive effects of PEG from those provided by a non-medicinal, control diet (Provenza et al. 2000;Villalba and Provenza 2001), and that lambs regulate the amount of PEG that they consume according to the proportion of condensed tannin in their diet (Provenza et al. 2000). Moreover, experienced ewes tended to consume more tannin-containing food than naïve ewes during preference tests, and they ate more tannin-containing food during conditioning with PEG than during conditioning with GP (Figures 4-1 and 4-2). These results suggest that PEG was effective at attenuating the negative post-ingestive effects of tannins, thus 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. 1994). This interaction is irreversible over a wide range of pH, and renders tannins unavailable for the formation of protein-tannin

complexes that adversely affect animal tissues and nutrient absorption (Foley and Moore 2005).

Because naïve ewes were reluctant to eat PEG initially due to its low nutritional value, the ewes were 'enticed' to sample PEG by mixing barley with PEG and gradually decreasing the ratio of barley until the ewes started consuming 100% PEG. The exposure time to PEG and the tannin-containing food was also increased to enhance the acceptance of the food (Pliner 1982, Villalba and Provenza 2001). During conditioning with PEG, intake of tannin-containing food increased across time as the ewes experienced the beneficial effects of PEG (Figure 4-1). Previous studies show that a PEG-condensed tannin ratio of 1:2 totally neutralizes the negative effects of condensed tannins (Silanikove et al 1994). The intake of the tannin-containing food (15% tannin) stabilized at 1000g/d while the intake of PEG stabilized at 78g/d. Hence, the ewes were consuming an average of 150g/d of condensed tannins, which according to previous findings was being completely neutralized by the amount of PEG in the rumen.

During conditioning, the intake of the tannin-containing food and GP by ewes decreased with time, evidently as they began experiencing the negative effects of the high-tannin diet 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 and Singh 1984); they can also condition strong food aversions (Provenza et

al. 1992). Tannins also decrease the permeability of the outer cellular layer of the gut and reduce the passage of nutrients into the body (Kumar and Singh 1984). Tannins bind to the digestive enzymes in the rumen and inhibit digestibility of the food (Kumar and Singh 1984).

Transmission of Self-Medicative Behavior-
Influence of Mother's Experience

Ewe-lamb pairs with experienced mothers tended to consume more PEG and they ate more GP than pairs with naïve mothers. Experienced ewes also had twice the proportion of scans on PEG relative to inexperienced mothers. However, differences in use of PEG between experienced and naïve ewe lamb pairs disappeared during Period 2 of Phase 3 of the study. Likewise, no differences were found in preference for PEG between lambs previously exposed to PEG with experienced or naïve mothers, tannins and GP. This suggests that the presence of mother *per* se was as consequential as experience of the mother for enhancing the ability of naïve offspring to self-medicate with PEG. This might have occurred as a result of enhanced exploratory and novel behavior by the young offspring in the presence of a social model. It was observed that lambs, which were alone, were initially more neophobic with the food choices offered as compared to lambs with their mothers. Mother's experience is important for young lambs to learn which foods to eat (e.g., nutritious) (Mirza and Provenza, 1990). Rat pups also eat the same diet as models do (Galef and Clark 1971a) and are reluctant to eat

foods that adults avoid (Galef and Clark 1971b). Even behaviors without adaptive values such as pith chewing, fur rubbing and leaf swallowing can be passed on from generation to generation (Huffman 1997). Previous studies on leaf swallowing in chimpanzees as a means of physically expelling intestinal parasites appears to originate in the wild from opportunistic feeding behavior where the individuals fed on a variety of plant species. The information is later passed down in the form of a behavioral tradition (Huffman and Hirata 2004). In our study, the presence of mother *per se* may have represented enough visual and olfactory stimuli for lambs - or for the ewe-lamb pair - to learn about the medicinal properties of PEG, compensating the lack of experience by naïve ewes.

Transmission of Self-Medicative Behavior – Influence of Mother per se

Results of preference tests conducted on lambs provide strong evidence that preference for PEG depended on the presence of mother. Lambs exposed to the food choices along with either their experienced or inexperienced mothers showed a much higher preference towards PEG than lambs without their mothers. Hence, the presence of mother markedly impacted lambs to select PEG. Lambs without their mothers exhibited a higher preference for GP, reinforcing the idea that these lambs, as opposed to lambs with their mothers, did not associate the beneficial effect of PEG when receiving a tannin challenge. Moreover, a

higher preference for PEG by lambs exposed with mothers (experienced and naïve) led to greater intakes of the tannin-containing food than lambs exposed without their mothers, supporting the notion that PEG attenuated the aversive effects of tannins.

The close proximity of a lamb to its mother enhances learning by the lamb (Provenza and Balph 1987, 1988, Mirza and Provenza 1990). The transmission of adaptive maternal effects on the feeding behaviors 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 and Fragaszy 1995). The presence of mother encourages the lambs to sample foods and gives the lambs social cues to eat the particular foods mother eats (Mirza and Provenza 1990).

The presence of mother contributed to the emergence of a new behavior (i.e., selection of PEG) within the ewe-lamb pairs. An innovation is likely to arise when an individual or group is faced with a new challenge (i.e., a tannin-containing diet) for which it currently has no workable solution in its existing behavioral repertoire (Huffman and Hirata 2003). Some imitative processes generate new behaviors (Russon and Galdikas1995), and thus the occurrence of ewe-lamb pairs could have enhanced the sampling of PEG relative to lambs exposed alone. The diffusion of behavioral innovation also occurs from younger to older animals (Huffman and Hirata 2003), which also suggests ewes may have

benefited by being exposed to a medicine and a tannin-containing food with their offspring. For instance, while it took 33 d of enticing ewes with grain to achieve a stable consumption of PEG during Phase 1 (Figure 4-1), it only took 4 d for naïve ewe-lamb pairs to consume substantial amounts of PEG which rapidly stabilized over time (Figure 4-4). Thus, it is likely ewes' prior experience with PEG was important but not nearly as significant as the rapid sampling of PEG by naïve ewe-lamb pairs.

Once lambs sampled PEG while consuming a high-tannin food, their individual experience with the beneficial post-ingestive effects of PEG likely reinforced their preference for 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 (Figure 4-6).

On average during preference tests, lambs previously exposed with their mothers ate 68 g (d 1) and 113 g (d 2) of tannin with their diet, and 30 g (d 1) and 75 g (d 2) of PEG during preference tests (Figure 4-6), representing quantities of PEG that closely (d 1) or completely (d 2) neutralized the negative effects of the condensed tannins ingested according to the proposed 1:2 PEG:tannin ratio (Silanikove et al. 1994).

Lambs with experienced mothers with high or low preference towards PEG also tended to consume a higher or lower amount of PEG, respectively, during preference test 1. However, this relationship disappeared in preference test 2. It is likely that intake of PEG by lambs

during preference test 2 was an outcome of both learning through maternal effects and individual experience.

By being in smaller pens, the frequency of interactions between mothers and their offspring were likely enhanced which might have exaggerated the maternal effects that would occur in free-ranging animals. Likewise, ewes and lambs had a limited range of feeds to sample (PEG, tannin-containing diet, and GP) which were presented daily in the pens. However, ruminants develop dietary habits through social learning not only under confined conditions but also on rangelands (Mirza 1994, Key and McIver 1980, Ramus and Tennessen 1992). Nevertheless, it is likely that under range conditions and with more feed alternatives available, mothers' previous experience with the medicinal properties of a supplement would be more consequential than in confinement.

CHAPTER 6

CONCLUSION

My study shows that apart from influencing ingestion of nutritious and toxic foods, mothers also influence the ability of their offspring to self-medicate against diets with high concentrations of secondary compounds. The presence of mother *per se* was as important in the emergence of lambs' self-medicative behavior as mother's experience with the beneficial effects of the medicine. Thus, mother-young interactions may contribute to create new knowledge within a herd, as well as to improve the transmission and maintenance of this knowledge across generations. These results have important implications in animal nutrition, and in the biological control of plants with secondary compounds. Invasive plant species such as *Sericea lespedeza* with high tannin content can be better controlled with social transmission of self-medication with PEG. The inefficiency, delays, and risk of error associated with learning through trial and error based on post-ingestive feedback may provide selective pressure on herbivores to learn through social models (Provenza et al. 1992).

This study is among the first to demonstrate that maternal effects and social learning play an important role in the ability of young animals to associate the medicinal properties of a food item with recovery from malaise. It is widely known that herbivores select their diets through

individual learning and dietary feedback mechanisms. Various sensory cues such as smell, taste and texture of the food further reinforce these dietary choices. In self-medicating herbivores, animals learn to associate recovery through post-ingestive feedback mechanisms. My study demonstrates that apart from individual learned responses, social learning also plays an important role in acquisition of self-medicative behavior by young animals.

My study showed that the presence and previous experience of the mother influenced the ability of lambs to self-medicate. Lambs without mothers were neophobic and did not associate the beneficial effect of consuming PEG with the tannin-containing food compared to lambs with their mothers.

Individual variation also occurs with regard to animal abilities to self-medicate. Lambs with conditioned mothers varied in their intake of tannin and PEG according to the individual preference of the mother ewes to the tannin-PEG association. Lambs with mothers with high preference foe PEG also consumed a higher amount of PEG during the preference tests while lambs with mothers with a low preference towards PEG during conditioning had a low preference for PEG.

I hypothesize that the mechanism through which mothers train their offspring to self-medicate is first initiated with the young lambs following the visual and sensory cues (such as licking mother's mouth)

from the mother and selecting the 'medicine' ingested by the ewe. Lambs then begin to experience the post-ingestive consequences and thus through continual reinforcement learn to distinguish self-medicating substances.

Maternal effects influence offspring behaviors in ways that have survival consequences. Animals learn to self medicate against disease (e.g., endoparasites) primarily through the process of trial and error where they consume small amounts of medicinal plants which contain an array of secondary compounds with anti-parasitic properties) that can be toxic at high doses (Huffman 1997, Lisonbee et al. 2009). Thus, post-ingestive feedback from these compounds calibrates the amount of medicinal plants animals can consume safely. If young animals were trained to self-medicate through social models, this would greatly reduce the risk of consuming toxic compounds. Thus, when medicines are also plant secondary compounds with potential toxic effects (e.g., tannins and terpenes with anti-parasitic properties), the influence of an experienced mother can significantly increase the ability and efficiency of lambs to self medicate. In the natural environment, where young animals cannot afford to rely solely on the trial and error for food selection, transmission of such complex feeding behavior ensures better chances of survival and overall fitness.

My research explored preferences for a medicinal substance (PEG), which is not toxic even at high doses. It would be interesting to conduct further research in the transmission of self-medicative behavior when the medicine is a plant secondary compound with potential toxic effects.

My research also opens interesting avenues for further exploring various mechanisms through which mothers teach their young about their environment through various visual and sensory cues. In mammals in particular, mothers are the most influential "environment" of a young animal, and changes in maternal behavior, brought about by changes in the environment, can significantly impact offspring. Having established that maternal effects have a significant role in the ability of animals to self medicate, it would be interesting to measure the heritability of self-medicative behavior across subsequent generations through cross-fostering methods or twin studies.

An interesting finding of this thesis was that the presence of mother *per se*, without experience to the beneficial effects of PEG, helped naïve lambs use PEG to a similar extent as lambs exposed with experienced mothers. It is possible that efficiency of learning about the medicinal effects of the novel substance PEG was enhanced in the naïve ewe-lamb pair. Many animals respond to environmental stressors by creating a new behavior or using existing behaviors in a novel context

(Kummer and Goodall, 1985). Exploration has been regarded as a precursor to innovative behavior since, combined with learning, it may enable an animal to gather information and develop new behaviors or novel means of exploiting the environment (Kendal et al., 2005). Thus, it is likely that in my study exploratory behavior was enhanced in the ewe-lamb pair relative to the group of lambs without their mothers. Such enhanced exploratory behavior likely promoted increased consumption of PEG by lambs (and ewes), which primed individual learning through experience of the post-ingestive medicinal effects of PEG. As innovative behavior emerged from the ewe-lamb pair, it is possible to assume that not only lambs but also ewes benefited in the process. Naïve ewes with their lambs began consuming significant amounts of PEG even after 4 d of exposure, whereas it took 33 d for individual ewes to stabilize intake of PEG during conditioning. In primates, the prevailing assumption is that young or juvenile individuals are more innovative than adult individuals (Kummer and Goodall 1985). However, recent evidence suggests that exploration and innovation are positively correlated with age, perhaps because innovation frequently builds upon other skills and may require a certain degree of experience (Reader and Laland 2001). Thus, it may be equally likely that ewes or lambs initiated the self-medicative behavior in the naïve group, but certainly the occurrence of the pair was important for the new behavior to occur. It would be interesting to conduct studies that describe the emergence of the new behavior within a naïve ewe-lamb

pair. Additional research is also needed to determine whether prior experience by mothers is more consequential in more complex environments than a pen. For instance, where the sampling capacity of animals, as well as the likelihood of finding a medicine is reduced such as in range conditions -and with a higher number of feed alternatives.

Apart from influencing the offspring's growth and survival in the immediate environment, maternal effects can have long-term implications in changes in gene expression and behavior of species across generations. For instance, maternal exposure to stress enhances the stress response in offspring (Meaney 2009). Some of these responses are mediated through permanent epigenetic changes in gene expression that result from gene methylation or histone acetylation (Fish et al. 2004). Self-medicative behavior in animals might be transmitted across generations through epigenetic change or 'Lamarckian inheritance' of transmission of acquired traits rather than DNA-sequence alleles. Natural selection favors individuals with selective traits, which enable them to better adapt to a changing environment and maintain fitness. Self-medication is a behavioral trait that reduces the physiological health risks of animals from parasites, pathogens and intestinal diseases. Understanding this behavioral trait and its transmission across generations will have important implications in maintaining livestock

health and in understanding populations and communities in an evolutionary scale.

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APPENDICES

Table A1

SAS Output: Intake of PEG by ewes

Least Squares Means								
Effect	Group	Food	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	C			64.5375	18.7359	14	3.44	0.0039
Group	NC			23.5281	18.7359	14	1.26	0.2298
Food		GP		33.0594	18.7359	14	1.76	0.0994
Food		PEG		55.0063	18.7359	14	2.94	0.0108
Group*Food	C	PEG		106.34	26.4966	14	4.01	0.0013
Group*Food	C	GP		22.7312	26.4966	14	0.86	0.4054
Group*Food	NC	GP		43.3875	26.4966	14	1.64	0.1238
Group*Food	NC	PEG		3.6688	26.4966	14	0.14	0.8918
Day			1	43.8781	13.2535	28	3.31	0.0026
Day			2	44.1875	13.2535	28	3.33	0.0024
Group*Day	C		1	64.2500	18.7432	28	3.43	0.0019
Group*Day	C		2	64.8250	18.7432	28	3.46	0.0018
Group*Day	NC		1	23.5063	18.7432	28	1.25	0.2202
Group*Day	NC		2	23.5500	18.7432	28	1.26	0.2193
Food*Day		GP	1	32.8062	18.7432	28	1.75	0.0910
Food*Day		GP	2	33.3125	18.7432	28	1.78	0.0864
Food*Day		PEG	1	54.9500	18.7432	28	2.93	0.0066
Food*Day		PEG	2	55.0625	18.7432	28	2.94	0.0065
Group*Food*Day	C	GP	1	22.4250	26.5070	28	0.85	0.4047
Group*Food*Day	C	GP	2	23.0375	26.5070	28	0.87	0.3922
Group*Food*Day	C	PEG	1	106.07	26.5070	28	4.00	0.0004

Least Squares Means								
Effect	Group	Food	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group*Food*Day	C	PEG	2	106.61	26.5070	28	4.02	0.0004
Group*Food*Day	NC	GP	1	43.1875	26.5070	28	1.63	0.1145
Group*Food*Day	NC	GP	2	43.5875	26.5070	28	1.64	0.1113
Group*Food*Day	NC	PEG	1	3.8250	26.5070	28	0.14	0.8863
Group*Food*Day	NC	PEG	2	3.5125	26.5070	28	0.13	0.8955

Table A2

SAS Output : Preference of PEG by ewes

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	C		70.9955	10.2817	14	6.91	<.0001
Group	NC		19.4406	10.2817	14	1.89	0.0795
Day		1	47.9560	7.7442	14	6.19	<.0001
Day		2	42.4800	7.7442	14	5.49	<.0001
Group*Day	C	1	76.4312	10.9520	14	6.98	<.0001
Group*Day	C	2	65.5597	10.9520	14	5.99	<.0001
Group*Day	NC	1	19.4808	10.9520	14	1.78	0.0970
Group*Day	NC	2	19.4003	10.9520	14	1.77	0.0983

Table A3

SAS Output: Intake of PEG by ewe-lamb pair during day 1-7

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	Alone		2.1321	33.8437	21	0.06	0.9504
Treatment	C		67.5607	33.8437	21	2.00	0.0590
Treatment	T		121.97	33.9395	21	3.59	0.0017
day		1	58.6125	24.8929	124	2.35	0.0201
day		2	29.6030	25.5933	124	1.16	0.2496
day		3	36.9583	24.8929	124	1.48	0.1402
day		4	67.0250	24.8929	124	2.69	0.0081
day		5	104.57	24.8929	124	4.20	<.0001
day		6	92.9083	24.8929	124	3.73	0.0003
day		7	57.5417	24.8929	124	2.31	0.0225
Treatment*day	Alone	1	1.2875	43.1157	124	0.03	0.9762
Treatment*day	Alone	2	0.7625	43.1157	124	0.02	0.9859
Treatment*day	Alone	3	0.01250	43.1157	124	0.00	0.9998
Treatment*day	Alone	4	0.1500	43.1157	124	0.00	0.9972
Treatment*day	Alone	5	0.2875	43.1157	124	0.01	0.9947
Treatment*day	Alone	6	7.9750	43.1157	124	0.18	0.8536
Treatment*day	Alone	7	4.4500	43.1157	124	0.10	0.9180
Treatment*day	C	1	6.6625	43.1157	124	0.15	0.8774
Treatment*day	C	2	9.3875	43.1157	124	0.22	0.8280
Treatment*day	C	3	22.1500	43.1157	124	0.51	0.6084
Treatment*day	C	4	92.1500	43.1157	124	2.14	0.0345

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment*day	C	5	145.71	43.1157	124	3.38	0.0010
Treatment*day	C	6	112.90	43.1157	124	2.62	0.0099
Treatment*day	C	7	83.9625	43.1157	124	1.95	0.0537
Treatment*day	T	1	167.89	43.1157	124	3.89	0.0002
Treatment*day	T	2	78.6590	46.6605	124	1.69	0.0944
Treatment*day	T	3	88.7125	43.1157	124	2.06	0.0417
Treatment*day	T	4	108.77	43.1157	124	2.52	0.0129
Treatment*day	T	5	167.71	43.1157	124	3.89	0.0002
Treatment*day	T	6	157.85	43.1157	124	3.66	0.0004
Treatment*day	T	7	84.2125	43.1157	124	1.95	0.0531
Treatment	Alone		2.1321	33.8437	21	0.06	0.9504
Treatment	C		67.5607	33.8437	21	2.00	0.0590
Treatment	T		121.97	33.9395	21	3.59	0.0017

Table A4

SAS Output: Intake of PEG by ewe-lamb pair during day 8-12

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	Alone		123.56	55.1175	21	2.24	0.0359
Treatment	C		198.23	55.1175	21	3.60	0.0017
Treatment	T		145.96	55.1175	21	2.65	0.0150
day		8	159.67	36.8580	84	4.33	<.0001
day		9	130.07	36.8580	84	3.53	0.0007
day		10	177.60	36.8580	84	4.82	<.0001
day		11	159.39	36.8580	84	4.32	<.0001

Least Squares Means							
Effect	Treatment	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
day		12	152.85	36.8580	84	4.15	<.0001
Treatment*day	Alone	8	132.20	63.8400	84	2.07	0.0414
Treatment*day	Alone	9	134.36	63.8400	84	2.10	0.0383
Treatment*day	Alone	10	134.97	63.8400	84	2.11	0.0375
Treatment*day	Alone	11	109.60	63.8400	84	1.72	0.0897
Treatment*day	Alone	12	106.67	63.8400	84	1.67	0.0984
Treatment*day	C	8	181.50	63.8400	84	2.84	0.0056
Treatment*day	C	9	169.79	63.8400	84	2.66	0.0094
Treatment*day	C	10	219.49	63.8400	84	3.44	0.0009
Treatment*day	C	11	235.80	63.8400	84	3.69	0.0004
Treatment*day	C	12	184.58	63.8400	84	2.89	0.0049
Treatment*day	T	8	165.30	63.8400	84	2.59	0.0113
Treatment*day	T	9	86.0625	63.8400	84	1.35	0.1813
Treatment*day	T	10	178.35	63.8400	84	2.79	0.0065
Treatment*day	T	11	132.76	63.8400	84	2.08	0.0406
Treatment*day	T	12	167.31	63.8400	84	2.62	0.0104
Treatment	Alone		123.56	55.1175	21	2.24	0.0359
Treatment	C		198.23	55.1175	21	3.60	0.0017
Treatment	T		145.96	55.1175	21	2.65	0.0150

Table A5

SAS Output: Intake of GP by ewe-lamb pair during day 1-7

Least Squares Means							
Effect	Treatment	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	Alone		3.6161	5.8669	21	0.62	0.5443
Treatment	C		13.2732	5.8669	21	2.26	0.0344
Treatment	T		37.1849	5.9052	21	6.30	<.0001
day		1	26.2167	5.3297	124	4.92	<.0001
day		2	8.1440	5.5557	124	1.47	0.1452
day		3	5.3083	5.3297	124	1.00	0.3212
day		4	10.5250	5.3297	124	1.97	0.0505
day		5	30.7625	5.3297	124	5.77	<.0001
day		6	23.0542	5.3297	124	4.33	<.0001
day		7	22.1625	5.3297	124	4.16	<.0001
Treatment*day	Alone	1	6.9000	9.2313	124	0.75	0.4562
Treatment*day	Alone	2	2.3000	9.2313	124	0.25	0.8037
Treatment*day	Alone	3	1.3625	9.2313	124	0.15	0.8829
Treatment*day	Alone	4	0.8625	9.2313	124	0.09	0.9257
Treatment*day	Alone	5	0.7125	9.2313	124	0.08	0.9386
Treatment*day	Alone	6	11.9125	9.2313	124	1.29	0.1993
Treatment*day	Alone	7	1.2625	9.2313	124	0.14	0.8914
Treatment*day	C	1	13.9000	9.2313	124	1.51	0.1347
Treatment*day	C	2	4.5375	9.2313	124	0.49	0.6239
Treatment*day	C	3	7.8375	9.2313	124	0.85	0.3975
Treatment*day	C	4	3.1000	9.2313	124	0.34	0.7376
Treatment*day	C	5	28.8000	9.2313	124	3.12	0.0023
Treatment*day	C	6	19.1250	9.2313	124	2.07	0.0404
Treatment*day	C	7	15.6125	9.2313	124	1.69	0.0933
Treatment*day	T	1	57.8500	9.2313	124	6.27	<.0001
Treatment*day	T	2	17.5944	10.3613	124	1.70	0.0920
Treatment*day	T	3	6.7250	9.2313	124	0.73	0.4677
Treatment*day	T	4	27.6125	9.2313	124	2.99	0.0034

Least Squares Means							
Effect	Treatment	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment*day	T	5	62.7750	9.2313	124	6.80	<.0001
Treatment*day	T	6	38.1250	9.2313	124	4.13	<.0001
Treatment*day	T	7	49.6125	9.2313	124	5.37	<.0001
Treatment	Alone		3.6161	5.8669	21	0.62	0.5443
Treatment	C		13.2732	5.8669	21	2.26	0.0344
Treatment	T		37.1849	5.9052	21	6.30	<.0001

Table A6

SAS Output: Intake of GP by ewe-lamb pair during day 8-12

Least Squares Means							
Effect	Treatment	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	Alone		86.0350	35.5098	21	2.42	0.0245
Treatment	C		111.59	35.5098	21	3.14	0.0049
Treatment	T		60.6675	35.5098	21	1.71	0.1023
day		8	82.3625	24.8604	84	3.31	0.0014
day		9	80.3125	24.8604	84	3.23	0.0018
day		10	87.5750	24.8604	84	3.52	0.0007
day		11	83.1083	24.8604	84	3.34	0.0012
day		12	97.1250	24.8604	84	3.91	0.0002
Treatment*day	Alone	8	60.4125	43.0595	84	1.40	0.1643
Treatment*day	Alone	9	68.3500	43.0595	84	1.59	0.1162
Treatment*day	Alone	10	93.6750	43.0595	84	2.18	0.0324
Treatment*day	Alone	11	94.9500	43.0595	84	2.21	0.0302
Treatment*day	Alone	12	112.79	43.0595	84	2.62	0.0105
Treatment*day	C	8	80.7750	43.0595	84	1.88	0.0641

Least Squares Means							
Effect	Treatment	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment*day	C	9	75.2625	43.0595	84	1.75	0.0841
Treatment*day	C	10	135.05	43.0595	84	3.14	0.0024
Treatment*day	C	11	125.65	43.0595	84	2.92	0.0045
Treatment*day	C	12	141.20	43.0595	84	3.28	0.0015
Treatment*day	T	8	105.90	43.0595	84	2.46	0.0160
Treatment*day	T	9	97.3250	43.0595	84	2.26	0.0264
Treatment*day	T	10	34.0000	43.0595	84	0.79	0.4320
Treatment*day	T	11	28.7250	43.0595	84	0.67	0.5065
Treatment*day	T	12	37.3875	43.0595	84	0.87	0.3877
Treatment	Alone		86.0350	35.5098	21	2.42	0.0245
Treatment	C		111.59	35.5098	21	3.14	0.0049
Treatment	T		60.6675	35.5098	21	1.71	0.1023

Table A7

SAS Output: Intake of tannin by ewe-lamb pair during day 1-7

Least Squares Means							
Effect	Treatment	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	Alone		453.21	85.8861	21	5.28	<.0001
Treatment	C		1325.98	85.8861	21	15.44	<.0001
Treatment	T		1449.46	86.0208	21	16.85	<.0001
day		1	1152.18	57.4491	124	20.06	<.0001
day		2	949.31	58.5361	124	16.22	<.0001
day		3	1021.35	57.4491	124	17.78	<.0001
day		4	1073.92	57.4491	124	18.69	<.0001

Least Squares Means							
Effect	Treatment	day	Estimate	Standard Error	DF	t Value	Pr > |t|
day		5	1127.13	57.4491	124	19.62	<.0001
day		6	1132.00	57.4491	124	19.70	<.0001
day		7	1077.62	57.4491	124	18.76	<.0001
Treatment*day	Alone	1	606.65	99.5047	124	6.10	<.0001
Treatment*day	Alone	2	374.68	99.5047	124	3.77	0.0003
Treatment*day	Alone	3	400.36	99.5047	124	4.02	<.0001
Treatment*day	Alone	4	418.78	99.5047	124	4.21	<.0001
Treatment*day	Alone	5	438.08	99.5047	124	4.40	<.0001
Treatment*day	Alone	6	476.73	99.5047	124	4.79	<.0001
Treatment*day	Alone	7	457.23	99.5047	124	4.60	<.0001
Treatment*day	C	1	1208.36	99.5047	124	12.14	<.0001
Treatment*day	C	2	1163.09	99.5047	124	11.69	<.0001
Treatment*day	C	3	1260.04	99.5047	124	12.66	<.0001
Treatment*day	C	4	1408.40	99.5047	124	14.15	<.0001
Treatment*day	C	5	1437.64	99.5047	124	14.45	<.0001
Treatment*day	C	6	1449.96	99.5047	124	14.57	<.0001
Treatment*day	C	7	1354.34	99.5047	124	13.61	<.0001
Treatment*day	T	1	1641.54	99.5047	124	16.50	<.0001
Treatment*day	T	2	1310.18	105.05	124	12.47	<.0001
Treatment*day	T	3	1403.65	99.5047	124	14.11	<.0001
Treatment*day	T	4	1394.58	99.5047	124	14.02	<.0001
Treatment*day	T	5	1505.68	99.5047	124	15.13	<.0001
Treatment*day	T	6	1469.30	99.5047	124	14.77	<.0001
Treatment*day	T	7	1421.30	99.5047	124	14.28	<.0001
Treatment	Alone		453.21	85.8861	21	5.28	<.0001
Treatment	C		1325.98	85.8861	21	15.44	<.0001
Treatment	T		1449.46	86.0208	21	16.85	<.0001

Table A8

SAS Output: Intake of tannin by ewe-lamb pair during day 8-12

Least Squares Means							
Effect	Treatment	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	Alone		746.35	64.3285	21	11.60	<.0001
Treatment	C		1676.47	64.3285	21	26.06	<.0001
Treatment	T		1637.54	64.3285	21	25.46	<.0001
day		8	1480.60	48.2288	84	30.70	<.0001
day		9	1491.79	48.2288	84	30.93	<.0001
day		10	1342.12	48.2288	84	27.83	<.0001
day		11	1255.99	48.2288	84	26.04	<.0001
day		12	1196.78	48.2288	84	24.81	<.0001
Treatment*day	Alone	8	876.81	83.5347	84	10.50	<.0001
Treatment*day	Alone	9	887.84	83.5347	84	10.63	<.0001
Treatment*day	Alone	10	716.36	83.5347	84	8.58	<.0001
Treatment*day	Alone	11	691.81	83.5347	84	8.28	<.0001
Treatment*day	Alone	12	558.94	83.5347	84	6.69	<.0001
Treatment*day	C	8	1783.34	83.5347	84	21.35	<.0001
Treatment*day	C	9	1786.44	83.5347	84	21.39	<.0001
Treatment*day	C	10	1638.81	83.5347	84	19.62	<.0001
Treatment*day	C	11	1636.99	83.5347	84	19.60	<.0001
Treatment*day	C	12	1536.79	83.5347	84	18.40	<.0001
Treatment*day	T	8	1781.66	83.5347	84	21.33	<.0001

Least Squares Means							
Effect	Treatment	day	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment*day	T	9	1801.10	83.5347	84	21.56	<.0001
Treatment*day	T	10	1671.19	83.5347	84	20.01	<.0001
Treatment*day	T	11	1439.16	83.5347	84	17.23	<.0001
Treatment*day	T	12	1494.61	83.5347	84	17.89	<.0001
Treatment	Alone		746.35	64.3285	21	11.60	<.0001
Treatment	C		1676.47	64.3285	21	26.06	<.0001
Treatment	T		1637.54	64.3285	21	25.46	<.0001

Table A9

SAS Output: Scan sampling of ewes for PEG

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	Control		2.9795	2.6758	14	1.11	0.2843
Group	Treatment		5.8384	2.6732	14	2.18	0.0465
Day		1	4.4697	2.5487	140	1.75	0.0817
Day		2	4.0179	2.5487	140	1.58	0.1172
Day		3	1.6741	2.5487	140	0.66	0.5124
Day		4	7.3201	2.5487	140	2.87	0.0047
Day		5	2.0833	2.5487	140	0.82	0.4151
Day		6	4.3110	2.5487	140	1.69	0.0930
Day		7	7.3492	2.5487	140	2.88	0.0046
Day		8	6.5009	2.5487	140	2.55	0.0118
Day		9	4.7222	2.5487	140	1.85	0.0660

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Day		10	3.2442	2.5156	140	1.29	0.1993
Day		11	2.8058	2.5973	140	1.08	0.2819
Group*Day	Control	1	2.5000	3.6044	140	0.69	0.4891
Group*Day	Control	2	-129E-16	3.6044	140	-0.00	1.0000
Group*Day	Control	3	-153E-16	3.6044	140	-0.00	1.0000
Group*Day	Control	4	5.2083	3.6044	140	1.44	0.1507
Group*Day	Control	5	4.1667	3.6044	140	1.16	0.2497
Group*Day	Control	6	3.5714	3.6044	140	0.99	0.3235
Group*Day	Control	7	4.1667	3.6044	140	1.16	0.2497
Group*Day	Control	8	4.1667	3.6044	140	1.16	0.2497
Group*Day	Control	9	4.0278	3.6044	140	1.12	0.2657
Group*Day	Control	10	2.9221	3.6044	140	0.81	0.4189
Group*Day	Control	11	2.0447	3.7407	140	0.55	0.5855
Group*Day	Treatment	1	6.4394	3.6044	140	1.79	0.0762
Group*Day	Treatment	2	8.0357	3.6044	140	2.23	0.0274
Group*Day	Treatment	3	3.3482	3.6044	140	0.93	0.3545
Group*Day	Treatment	4	9.4318	3.6044	140	2.62	0.0099
Group*Day	Treatment	5	-142E-16	3.6044	140	-0.00	1.0000
Group*Day	Treatment	6	5.0505	3.6044	140	1.40	0.1634
Group*Day	Treatment	7	10.5317	3.6044	140	2.92	0.0041
Group*Day	Treatment	8	8.8352	3.6044	140	2.45	0.0155
Group*Day	Treatment	9	5.4167	3.6044	140	1.50	0.1351
Group*Day	Treatment	10	3.5663	3.5102	140	1.02	0.3114
Group*Day	Treatment	11	3.5669	3.6044	140	0.99	0.3241

Table A10

SAS Output: Scan sampling of lambs for PEG

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	Control		3.3266	1.4725	14	2.26	0.0403
Group	Treatment		4.3828	1.4785	14	2.96	0.0102
Day		1	1.0417	2.7684	139	0.38	0.7073
Day		2	2.5000	2.7684	139	0.90	0.3681
Day		3	8.88E-16	2.7684	139	0.00	1.0000
Day		4	4.2336	2.7684	139	1.53	0.1285
Day		5	11.6815	2.7684	139	4.22	<.0001
Day		6	0.6250	2.7684	139	0.23	0.8217
Day		7	2.0833	2.7684	139	0.75	0.4530
Day		8	0.8929	2.7684	139	0.32	0.7475
Day		9	7.7577	2.7684	139	2.80	0.0058
Day		10	3.6542	2.8636	139	1.28	0.2041
Day		11	7.9320	2.7684	139	2.87	0.0048
Group*Day	Control	1	1.33E-15	3.9151	139	0.00	1.0000
Group*Day	Control	2	2.5000	3.9151	139	0.64	0.5242
Group*Day	Control	3	1.78E-15	3.9151	139	0.00	1.0000
Group*Day	Control	4	2.8125	3.9151	139	0.72	0.4737
Group*Day	Control	5	7.2917	3.9151	139	1.86	0.0647
Group*Day	Control	6	-888E-18	3.9151	139	-0.00	1.0000
Group*Day	Control	7	1.11E-15	3.9151	139	0.00	1.0000
Group*Day	Control	8	1.33E-15	3.9151	139	0.00	1.0000
Group*Day	Control	9	10.6047	3.9151	139	2.71	0.0076
Group*Day	Control	10	2.7778	3.9151	139	0.71	0.4792
Group*Day	Control	11	10.6061	3.9151	139	2.71	0.0076
Group*Day	Treatment	1	2.0833	3.9151	139	0.53	0.5955
Group*Day	Treatment	2	2.5000	3.9151	139	0.64	0.5242
Group*Day	Treatment	3	0	3.9151	139	0.00	1.0000

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group*Day	Treatment	4	5.6548	3.9151	139	1.44	0.1509
Group*Day	Treatment	5	16.0714	3.9151	139	4.10	<.0001
Group*Day	Treatment	6	1.2500	3.9151	139	0.32	0.7500
Group*Day	Treatment	7	4.1667	3.9151	139	1.06	0.2891
Group*Day	Treatment	8	1.7857	3.9151	139	0.46	0.6490
Group*Day	Treatment	9	4.9107	3.9151	139	1.25	0.2118
Group*Day	Treatment	10	4.5307	4.1800	139	1.08	0.2803
Group*Day	Treatment	11	5.2579	3.9151	139	1.34	0.1815

Table A11

SAS Output: Scan sampling of ewes for GP

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	Control		1.1162	0.6923	14	1.61	0.1292
Group	Treatment		2.44E-16	0.6897	14	0.00	1.0000
Day		1	2.0833	0.9935	140	2.10	0.0378
Day		2	-386E-19	0.9935	140	-0.00	1.0000
Day		3	1.3E-16	0.9935	140	0.00	1.0000
Day		4	-22E-17	0.9935	140	-0.00	1.0000
Day		5	-173E-18	0.9935	140	-0.00	1.0000
Day		6	2.0833	0.9935	140	2.10	0.0378
Day		7	1.2500	0.9935	140	1.26	0.2104
Day		8	-228E-18	0.9935	140	-0.00	1.0000
Day		9	0.7813	0.9935	140	0.79	0.4330
Day		10	-258E-18	0.9713	140	-0.00	1.0000

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Day		11	-0.05891	1.0246	140	-0.06	0.9542
Group*Day	Control	1	4.1667	1.4051	140	2.97	0.0036
Group*Day	Control	2	-295E-18	1.4051	140	-0.00	1.0000
Group*Day	Control	3	2.07E-16	1.4051	140	0.00	1.0000
Group*Day	Control	4	1.06E-16	1.4051	140	0.00	1.0000
Group*Day	Control	5	1.43E-17	1.4051	140	0.00	1.0000
Group*Day	Control	6	4.1667	1.4051	140	2.97	0.0036
Group*Day	Control	7	2.5000	1.4051	140	1.78	0.0774
Group*Day	Control	8	-18E-17	1.4051	140	-0.00	1.0000
Group*Day	Control	9	1.5625	1.4051	140	1.11	0.2680
Group*Day	Control	10	-175E-18	1.4051	140	-0.00	1.0000
Group*Day	Control	11	-0.1178	1.4917	140	-0.08	0.9372
Group*Day	Treatment	1	5.69E-17	1.4051	140	0.00	1.0000
Group*Day	Treatment	2	2.18E-16	1.4051	140	0.00	1.0000
Group*Day	Treatment	3	5.39E-17	1.4051	140	0.00	1.0000
Group*Day	Treatment	4	-546E-18	1.4051	140	-0.00	1.0000
Group*Day	Treatment	5	-36E-17	1.4051	140	-0.00	1.0000
Group*Day	Treatment	6	-793E-19	1.4051	140	-0.00	1.0000
Group*Day	Treatment	7	1.77E-16	1.4051	140	0.00	1.0000
Group*Day	Treatment	8	-277E-18	1.4051	140	-0.00	1.0000
Group*Day	Treatment	9	-735E-19	1.4051	140	-0.00	1.0000
Group*Day	Treatment	10	-342E-18	1.3415	140	-0.00	1.0000
Group*Day	Treatment	11	3.86E-15	1.4051	140	0.00	1.0000

Table A12

SAS Output: Scan sampling of lambs for GP

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	Control		2.0581	1.2759	14	1.61	0.1290
Group	Treatment		2.4959	1.2841	14	1.94	0.0723
Day		1	-201E-18	2.9922	139	-0.00	1.0000
Day		2	1.0417	2.9922	139	0.35	0.7283
Day		3	0.6944	2.9922	139	0.23	0.8168
Day		4	8.9286	2.9922	139	2.98	0.0034
Day		5	2.0833	2.9922	139	0.70	0.4874
Day		6	6.2500	2.9922	139	2.09	0.0386
Day		7	-423E-18	2.9922	139	-0.00	1.0000
Day		8	0.7813	2.9922	139	0.26	0.7944
Day		9	-81E-17	2.9922	139	-0.00	1.0000
Day		10	5.2679	3.0972	139	1.70	0.0912
Day		11	-192E-17	2.9922	139	-0.00	1.0000
Group*Day	Control	1	2.08E-17	4.2316	139	0.00	1.0000
Group*Day	Control	2	3.21E-17	4.2316	139	0.00	1.0000
Group*Day	Control	3	1.3889	4.2316	139	0.33	0.7432
Group*Day	Control	4	-413E-18	4.2316	139	-0.00	1.0000
Group*Day	Control	5	4.76E-16	4.2316	139	0.00	1.0000
Group*Day	Control	6	12.5000	4.2316	139	2.95	0.0037

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group*Day	Control	7	-18E-17	4.2316	139	-0.00	1.0000
Group*Day	Control	8	6.98E-16	4.2316	139	0.00	1.0000
Group*Day	Control	9	-942E-19	4.2316	139	-0.00	1.0000
Group*Day	Control	10	8.7500	4.2316	139	2.07	0.0405
Group*Day	Control	11	-412E-18	4.2316	139	-0.00	1.0000
Group*Day	Treatment	1	-423E-18	4.2316	139	-0.00	1.0000
Group*Day	Treatment	2	2.0833	4.2316	139	0.49	0.6233
Group*Day	Treatment	3	9.95E-16	4.2316	139	0.00	1.0000
Group*Day	Treatment	4	17.8571	4.2316	139	4.22	<.0001
Group*Day	Treatment	5	4.1667	4.2316	139	0.98	0.3265
Group*Day	Treatment	6	-222E-18	4.2316	139	-0.00	1.0000
Group*Day	Treatment	7	-666E-18	4.2316	139	-0.00	1.0000
Group*Day	Treatment	8	1.5625	4.2316	139	0.37	0.7125
Group*Day	Treatment	9	-153E-17	4.2316	139	-0.00	1.0000
Group*Day	Treatment	10	1.7857	4.5237	139	0.39	0.6936
Group*Day	Treatment	11	-343E-17	4.2316	139	-0.00	1.0000

Table A13

SAS Output: Scan sampling of ewes for tannin

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	Control		93.7022	3.7485	14	25.00	<.0001
Group	Treatment		88.4072	3.7350	14	23.67	<.0001
Day		1	87.1970	5.3350	140	16.34	<.0001
Day		2	89.7321	5.3350	140	16.82	<.0001
Day		3	98.3259	5.3350	140	18.43	<.0001
Day		4	86.4299	5.3350	140	16.20	<.0001
Day		5	85.4167	5.3350	140	16.01	<.0001
Day		6	93.6057	5.3350	140	17.55	<.0001
Day		7	91.4008	5.3350	140	17.13	<.0001
Day		8	87.2491	5.3350	140	16.35	<.0001
Day		9	94.4965	5.3350	140	17.71	<.0001
Day		10	96.3565	5.2164	140	18.47	<.0001
Day		11	91.3914	5.5011	140	16.61	<.0001
Group*Day	Control	1	80.8333	7.5448	140	10.71	<.0001
Group*Day	Control	2	100.00	7.5448	140	13.25	<.0001
Group*Day	Control	3	100.00	7.5448	140	13.25	<.0001
Group*Day	Control	4	94.7917	7.5448	140	12.56	<.0001
Group*Day	Control	5	95.8333	7.5448	140	12.70	<.0001
Group*Day	Control	6	92.2619	7.5448	140	12.23	<.0001
Group*Day	Control	7	93.3333	7.5448	140	12.37	<.0001
Group*Day	Control	8	83.3333	7.5448	140	11.05	<.0001
Group*Day	Control	9	94.4097	7.5448	140	12.51	<.0001
Group*Day	Control	10	97.0779	7.5448	140	12.87	<.0001
Group*Day	Control	11	98.8497	8.0077	140	12.34	<.0001
Group*Day	Treatment	1	93.5606	7.5448	140	12.40	<.0001
Group*Day	Treatment	2	79.4643	7.5448	140	10.53	<.0001
Group*Day	Treatment	3	96.6518	7.5448	140	12.81	<.0001
Group*Day	Treatment	4	78.0682	7.5448	140	10.35	<.0001

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group*Day	Treatment	5	75.0000	7.5448	140	9.94	<.0001
Group*Day	Treatment	6	94.9495	7.5448	140	12.58	<.0001
Group*Day	Treatment	7	89.4683	7.5448	140	11.86	<.0001
Group*Day	Treatment	8	91.1648	7.5448	140	12.08	<.0001
Group*Day	Treatment	9	94.5833	7.5448	140	12.54	<.0001
Group*Day	Treatment	10	95.6350	7.2056	140	13.27	<.0001
Group*Day	Treatment	11	83.9331	7.5448	140	11.12	<.0001

Table A14

SAS Output: Scan sampling of lambs for tannin.

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	Control		88.9335	4.4820	14	19.84	<.0001
Group	Treatment		86.2385	4.4935	14	19.19	<.0001
Day		1	80.2083	6.9110	139	11.61	<.0001
Day		2	90.2083	6.9110	139	13.05	<.0001
Day		3	80.5556	6.9110	139	11.66	<.0001
Day		4	80.5878	6.9110	139	11.66	<.0001
Day		5	79.9851	6.9110	139	11.57	<.0001
Day		6	86.8750	6.9110	139	12.57	<.0001
Day		7	91.6667	6.9110	139	13.26	<.0001
Day		8	98.3259	6.9110	139	14.23	<.0001
Day		9	92.2423	6.9110	139	13.35	<.0001

Least Squares Means							
Effect	Group	Day	Estimate	Standard Error	DF	t Value	Pr > |t|
Day		10	90.7231	7.1346	139	12.72	<.0001
Day		11	92.0680	6.9110	139	13.32	<.0001
Group*Day	Control	1	75.0000	9.7736	139	7.67	<.0001
Group*Day	Control	2	97.5000	9.7736	139	9.98	<.0001
Group*Day	Control	3	86.1111	9.7736	139	8.81	<.0001
Group*Day	Control	4	97.1875	9.7736	139	9.94	<.0001
Group*Day	Control	5	92.7083	9.7736	139	9.49	<.0001
Group*Day	Control	6	75.0000	9.7736	139	7.67	<.0001
Group*Day	Control	7	87.5000	9.7736	139	8.95	<.0001
Group*Day	Control	8	100.00	9.7736	139	10.23	<.0001
Group*Day	Control	9	89.3953	9.7736	139	9.15	<.0001
Group*Day	Control	10	88.4722	9.7736	139	9.05	<.0001
Group*Day	Control	11	89.3939	9.7736	139	9.15	<.0001
Group*Day	Treatment	1	85.4167	9.7736	139	8.74	<.0001
Group*Day	Treatment	2	82.9167	9.7736	139	8.48	<.0001
Group*Day	Treatment	3	75.0000	9.7736	139	7.67	<.0001
Group*Day	Treatment	4	63.9881	9.7736	139	6.55	<.0001
Group*Day	Treatment	5	67.2619	9.7736	139	6.88	<.0001
Group*Day	Treatment	6	98.7500	9.7736	139	10.10	<.0001
Group*Day	Treatment	7	95.8333	9.7736	139	9.81	<.0001
Group*Day	Treatment	8	96.6518	9.7736	139	9.89	<.0001
Group*Day	Treatment	9	95.0893	9.7736	139	9.73	<.0001
Group*Day	Treatment	10	92.9739	10.3966	139	8.94	<.0001
Group*Day	Treatment	11	94.7421	9.7736	139	9.69	<.0001

Table A15

SAS Output: Preference of PEG in lambs during Preference test 1.

Least Squares Means						
Effect	Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	Alone	46.1758	12.7885	21	3.61	0.0016
Group	C	74.1959	12.7885	21	5.80	<.0001
Group	NC	79.6686	12.7885	21	6.23	<.0001

Table A16

SAS Output: Preference of PEG in lambs during Preference test 2.

Least Squares Means						
Effect	Group	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	Alone	42.0260	11.4123	20	3.68	0.0015
Group	C	72.3695	12.2002	20	5.93	<.0001
Group	NC	68.8497	11.4123	20	6.03	<.0001