CHAPTER 1

Introduction

There is currently no known explanation for grass-eating behaviour in the domestic dog (Lindsay, 2001). Dogs are adapted to consuming meat and they have almost no capacity to digest the plant fibre in grass (Case, Carey, Hirakawa, & Daristotle, 2000), but they still eat grass. In addition, there are several grass-related commercial products marketed for dogs as dietary aids, but these claims have not been substantiated. This mysterious contradiction and the unknown explanation for the behaviour provided an opportunity for a series of studies.

This thesis comprises five studies: one observational study and four controlled experiments. Each experiment is presented as an autonomous chapter including all content required for a published article. Therefore, the following introduction contains a broad review of the relevant literature, including phylogeny, feeding ecology of wolves and dogs, and a review of grass-eating behaviour. Information that is more detailed is provided within each applicable chapter, which also comprises relevant information presented in this introduction chapter.

Phylogeny

The domestic dog (*Canis familiaris*) is a member of the order Carnivora, a diverse group of animals with non-homogenous eating behaviours, which includes the almost entirely herbivorous great panda, obligate carnivores such as cats, and omnivorous dogs (Stasiak, 2002; Thorne, 1995). Both wolves (*C. lupus*) and dogs are members of the Family Canidae (the dog family) and genus *Canis* (Nowak, 2003; Wayne & Vilà, 2003). In addition to the wolf and domestic dog, the genus *Canis* also contains the coyote (*C. latrans*), the golden, black-backed, and side-striped jackals *(C. aureus*, *C. mesomelas*, *C. adustus*), and the dingo (*C. lupus dingo* or *C. familiaris* *dingo*; Corbett, 1995; Ewer, 1973; Nowak, 2003). There is some debate whether the dingo descends from the domestic dog, *C. familiaris*, or from the wolf, *C. lupus* (Nowak, 2003). The dog is a descendant of the wolf (Clutton-Brock, 1995; Savolainen, Zhang, Luo, Lundeberg, & Leitner, 2002).

Feeding Ecology

The Wolf

The wolf is adapted for hunting, catching, and consuming prey with its large, long, tapered skull and carnassial teeth (Mech, 1995). Wolves also possess a simple digestive tract with a relatively short hind-gut (Peterson & Ciucci, 2003). *C. lupus* typically hunt in packs and prey on large ungulates such as caribou, elk, moose, and deer, but are known to eat small prey such as birds, lizards, insects, and some vegetation (Ewer, 1973; Mech, 1966, 1995; Murie, 1944). Wild ungulates are the main diet component overall and their presence varies from 54.3% to 84.2% of scats sampled (Gazzola et al., 2005; Meriggi & Lovari, 1996).

The presence of plant material in wolf scats varies depending on season. Long-term studies of Latvian wolves found plant material in 11% of the scats collected from 1997 to 2001 (Andersone & Ozoliņš, 2004) whereas approximately 2.5% of Italian wolf scats contained plant material (Gazzola et al., 2005; Meriggi, Brangi, Matteucci, & Sacchi, 1996). Similarly, 14% of Greek wolf stomachs examined during the autumn and winter contained small quantities of grass (Papageorgiou, Vlachos, Sfougaris, & Tsachalidis, 1994). In North America, grass and sedge (a grass-like plant) occurred as 1.6% of the total number of food items present in wolf scats surveyed from 1939 to 1941 by Murie (1944) in Alaska and in 5.8% of wolf scats collected in 1958 to 1960 by Mech (1966) in Michigan. Thompson (1952) found grass in trace quantities to “sizable wads” in 25% of the timber wolf scats he surveyed in Wisconsin from 1946 to 1948.

Scat collections during the summer, when plants are more plentiful, have produced even higher proportions of plant material (Andersone & Ozoliņš, 2004). Andersone (1998) found plant material in 30% of Latvian wolf scats collected in the summer. These authors believed that plant occurrences in the diet are underestimated because some of the items, particularly berries, are readily digestible. More recently, Stahler, Smith, and Guernsey (2006) found plant material, mainly grass, in 74% of the summer scats sampled in Yellowstone National Park, Wyoming, USA.

Hunting for the wolf takes much energy and effort and may not always be successful. Therefore, the wolf is adapted to go without eating for extended periods (Peterson & Ciucci, 2003). The wolf has a large stomach capacity, and when a hunt is successful, it can gorge on the kill (Mech, 1966, 1995). Wolves have been documented to ingest 20 lb (9.1 kg) of meat in one feeding (Mech, 1995) with the average wolf weighing 72 lb (32.7 kg; Mech, 1966). They may return to the kill later to consume more, or the food may be cached under the snow or in the ground (Murie, 1944).

The Domestic Dog

Similar to the wolf, the domestic dog is adapted to consuming meat with its canine and carnassial teeth and simple digestive system with a short gut (Case, 2005; Case et al., 2000; Peterson & Ciucci, 2003). However, due to human intervention with selective breeding, individual dog breeds vary in their likeness to the head and body structure of the wolf whereas the teeth structure remains relatively similar (A. E. W. Miles & Grigson, 1990). Dogs and wolves have the same dental formula: 3/3 incisors, 1/1 canines, 4/4 premolars, and 2/3 molars, totalling 42 teeth (Bell, 1967; Harvey & Dubielzig, 1985; Mech, 1995).

Domestic dogs have sweet, sour, bitter, and salty taste receptors on their tongues (Overall, 1997) with the most common receptors excited by sugars and various sweet-tasting amino acids (Lindsay, 2000c). Anosmic dogs (those that cannot smell) are able to distinguish meat versus non-meat (cereals), and sweet versus non-sweet items, but not different meats, except pork, which was preferred over lamb (Houpt, Hintz, & Shepherd, 1978). Therefore, dogs use smell to differentiate meats, but use other senses, probably taste and texture, to distinguish meats from non-meats. Taste alone distinguishes sweet from non-sweet items (Houpt & Hintz, 1978).

Initially, dogs will prefer food that smells like meat, but the preference will not be sustained unless the taste is paired with the odour (Houpt et al., 1978). Dogs also prefer canned and semi-moist food to dry food (Kitchell, 1978). Lohse (1974) found that dogs prefer beef to lamb, chicken or horsemeat, canned or cooked meat to raw meat, and fresh ground beef to cubed beef.

If given the opportunity, dogs will graze on food throughout the day, but do not typically eat at night (Mugford, 1977). They also tend to eat rapidly and have the ability to gorge, similar to wolves (Hart, 1985). Some breeds are known to gorge more than other breeds. For example, Mugford (1977) documented a Labrador retriever that consumed 10% of its own body weight in canned food.

Dogs can survive on a vegetarian diet alone (Brown, McIntyre, Redman, & Pluske, 2005; Thorne, 1995), but are more accurately classified as ‘preferentially opportunistic carnivores’ which are adapted to consuming an omnivorous diet

(Lindsay, 2001). Dogs typically digest food of animal origin better than plant origin due to plants containing dietary fibre components such as cellulose and hemicellulose (Case et al., 2000). These components contain beta-bonds, which resist digestion by enzymes in the dog's monogastric digestive system. Therefore, plant dietary fibre is not absorbed in the small intestine and it passes to the large intestine (Case et al., 2000). The large intestine breaks down plant fibre to varying degrees. For instance, pectin is highly fermentable and cellulose is non-fermentable (National Research Council, 2006; Sunvold et al., 1995). However, there is no mechanism for absorption in the non-herbivore large intestine. Therefore, dogs do not receive significant energy from the fermentation of fibre within the large intestine (Case et al., 2000). Instead, fibre fermentation is beneficial to the structure of the large intestine by increasing its weight and mucosal surface area (Reinhart, Moxley, & Clemens, 1994) and stimulating growth of potentially health-enhancing intestinal bacteria (Fahey et al., 1990; Flickinger, Van Loo, & Fahey, 2003).

Grass-Eating Behaviour in Dogs

Wolves and dogs are well-adapted to hunt animals and tear flesh, but they cannot digest the dietary fibre in plants (Case et al., 2000), so it may seem contradictory that dogs have been reported to eat grass (*Gramineae*). Many authors contend that grass eating is a normal behaviour (Hart, 1985; Lindsay, 2001; McKeown, 1996; Overall, 1997) or should at least *not* be considered an abnormal ingestive behaviour (i.e., pica), because the behaviour is so common (Houpt, 2005).

Regardless of its 'normal' classification, grass eating is still considered a problem by owners and was the third most client-reported food problem behind obesity and anorexia (Overall, 1997). Overall does not state in what way grass eating was a problem for owners, but Beaver (1981) speculates that the behaviour is undesirable because it may lead to vomiting. Despite some owners reporting grass eating as a problem, there are several grass-related products marketed toward the pet owner as digestive aids or dietary supplements for their dogs (Organic Pet Grass Kit, ©Wheatgrasskits.com, Springville, UT; Pet Greens® and Pet Grass®, Bell Rock Growers, Inc., San Marcos, CA; Barley Dog, ©Green Foods Corporation, CA).

Although there is documentation of grass-eating observations and there are commercially-available grass products advertised as digestive aids, there is actually no known explanation for grass-eating behaviour as there have been no controlled experiments which investigate it (as noted by Hart, 1985; UC Davis SVMCABP, 2005). Sueda, Hart, and Cliff (2005, 2008) is the first known study which examines plant-eating behaviour in the domestic dog (besides the study presented in Chapter 3: Bjone, Brown, & Price, 2007). There is also a case study that indicates a male miniature poodle ceased its seven-year long plant-eating habit after being fed a high fibre diet (Kang et al., 2007). Kang et al. performed extensive examinations, laboratory tests and eliminated possible medical conditions, allergies, and behavioural problems to explain the subject’s daily plant-eating habit. The authors concluded that the dog’s plant-eating behaviour problem was due to a dietary deficiency. The dog’s diet was changed from an unknown diet to a diet containing 20% dietary fibre and plant eating ceased. While Kang et al. (2007) appear to have addressed a wide variety of medical conditions that might have influenced the dog’s plant-eating behaviour, they did not adequately explain the components of the previous and ‘high fibre’ diets nor did they analyse possible dietary differences which may have accounted for the change in plant eating. Therefore, the validity of the conclusion is questionable.

As there is a paucity of information on grass eating in dogs and Sueda et al. provides an initial understanding of this behaviour, it will be examined in detail. Sueda et al. (2008) comprises two survey-based studies, the first of which was used to determine the prevalence of plant eating in healthy dogs attending an outpatient veterinary service. Dog owners completed a written survey, which included questions regarding the general description, diet, and medical history of the dog as well as information about the consumption of non-food items, such as stones, toys, faeces, and plants. Owners of dogs that consumed plants were asked further questions about the dogs' plant eating habits, including frequency of the dog showing signs of illness before consuming plants or vomiting within 1 hr after plant consumption. Illness was described as depression, loss of appetite, or inactivity as per Hart (1988).

While the dogs were at the clinic, a medical history was obtained and a veterinarian physically examined each dog. Any dog that had a medical condition that could confound the results, such as gastrointestinal disease, diabetes, or administration of medicines that may affect appetite, was eliminated from the study. In addition to the medical condition criterion, dogs were included in the study if the owners reported that they observed the dogs for at least 3 hr per day and the dogs had access to plants for at least 1 hr each day. Of the 78 completed surveys, 47 dogs met the inclusion criteria. 79% (37) of the owners reported that they observed their dogs eating plants at least once or noticed plant material in their dogs' faeces or vomit. Of these plant-eating dogs, 95% primarily ate grass. Some dogs were reported to appear ill before plant consumption (n=4) or regularly vomit afterwards (n=6). Three of these vomiting dogs appeared ill before consumption.

The second study of Sueda et al. (2008) focused on dogs known to eat plants and encompassed the types of plants eaten, frequency of plant eating, incidence of illness before or after plant eating, and influences of age, breed group, gender, gonadal status, and diet. Inclusion criteria required that the owners spent at least 6 hr with their dogs per day and had observed their dogs eating plants on at least 10 occasions. The dogs also needed to be medically acceptable for the study as in Study 1. Due to its online availability, Study 2 reached a larger audience than Study 1 with 3,340 surveys returned of which 1,571 met the inclusion criteria.

Of the plant materials consumed, grass was consumed most frequently (79% of respondents). The remaining 21% of respondents reported that their dogs consumed non-grass plants, such as berries, sticks, or leaves, most frequently. Most dogs (68%) were reported to eat plants weekly or daily and 32% were reported to eat plants monthly or more infrequently. Half of the owners also reported that they had observed their dogs eat plants on more than 100 occasions, 18% reported observing their dogs eat plants on 51-100 occasions, and 32% reported observing their dogs eat plants on 10-15 occasions.

Most owners (92%) reported that their dogs rarely appeared ill before or after eating plants and 78% reported that their dogs rarely vomited after plant consumption. Age of the dog was significantly inversely related to frequency of plant eating: a younger age was associated with an increase in plant eating frequency. There was a significantly increasing tendency for older dogs to primarily eat grass versus non-grass plants. Despite the low overall incidence of signs of illness, older dogs were also reported to have an increasing tendency to show illness before and vomiting after plant consumption. Although causative explanations cannot be determined by these correlational results, it may be that younger dogs sample various plants to determine preferences and have a natural propensity to chew on objects (Lindsay, 2000a) and older dogs become more sensitive to the physical irritation grass may cause (Hart, 1985; McKeown, 1996).

Hound and toy breed groups were more likely to regularly vomit after grass eating than dogs in other major breed groups (mixed, sporting, herding, terrier, working, non-sporting). Those dogs fed a commercial, nutritionally balanced diet were more likely to vomit regularly than dogs fed a raw or homemade diet. Breed group and diet were not significant predictors of plant eating frequency, type of plant eaten, or showing illness before plant consumption.

Sueda et al. (2008) showed that plant eating is a common and widespread behaviour as 79% of the healthy well-cared-for dogs in the first study ate plants and plant eating was prevalent in all major breed groups in Study 2. Most dogs also ate grass daily to weekly. In particular, 98% of respondents indicated that their dogs consumed grass on some occasions, with 79% eating grass most frequently. Data from both studies also showed that most dogs do not show signs of illness before plant ingestion and do not vomit afterwards. The authors contended that because there was a small proportion of dogs that appeared ill before eating plants and that some do vomit after consumption, it is possible that there is a connection between intestinal discomfort and plant eating. Overall, the authors concluded that plant eating is a mostly normal behaviour unassociated with dietary deficiency, illness, or vomiting.

The results of Sueda et al.’s (2008) two surveys provide initial insights into plant eating behaviour in the domestic dog. Surveys such as these provide correlational information, but causative explanations cannot be determined from these data (Gravetter & Wallnau, 2007). Similarly, the case study by Kang et al. (2007) does not prove causation. These surveys are also limited because they are completed by multiple owners who report on their subjective experiences, rather than one objective observer documenting dogs' behaviours in a controlled environment. Therefore, controlled experiments are needed to provide causative explanations for grass-eating behaviour.

Development of Grass-Eating Behaviour in the Domestic Dog

No experimental studies have investigated grass-eating behaviour in dogs, so it is understandable that the development of the behaviour is also unknown. The ontogeny of grass eating is examined in this thesis to further explain grass-eating behaviour.

The domestic dog has retained most of its ancestor's (wolf, *Canis lupus*) eating behaviours (Case, 2005), which possibly include grass and plant eating, because these materials have been found in wolf scats (Andersone & Ozoliņš, 2004; Mech, 1966; Meriggi, Rosa, Brangi, & Matteucci, 1991; Murie, 1944; Stahler et al., 2006; Thompson, 1952) and stomach contents (Papageorgiou et al., 1994). The prevalence of grass eating in wolves and dogs suggests that the behaviour was preserved through domestication of the dog (Sueda et al., 2008). Overall (1997) also contends that plant eating is an innate canine eating behaviour. Additionally, mothers' grass-eating habits may influence the behaviour in their puppies.

Food preferences may become fixed in domestic dogs due to a limited flavour experience as a puppy (Ferrell, 1984; Kuo, 1967). In a study by Kuo (1967), puppies were fed set diets consisting of soybeans, or fruits and vegetables, or a varied diet including fruits, vegetables, meat and animal products, from birth to six months of age. After six months, the puppies were presented with new foods. Puppies that were not fed meat as part of their diet would not eat meat, whereas those puppies that were fed the varied diet ate new and varied foods. Therefore, providing a puppy with a variety of foods will reduce preference fixation (Kuo, 1967; Thorne, 1995).

Kuo (1967) also found that similar food fixations occur in second generation offspring. The puppies from the first fixed diet study were interbred, resulting in 48 second generation offspring. Puppies from mothers of one diet group were exchanged with puppies from another and all puppies ate the diets of the mothers who nursed them. The puppies were tested with new food after six months of age, similar to the first study. They exhibited their nursing mother's diet preferences, which were demonstrated in the first study, and not the diet of their biological parents.

Lindsay (2000a, p. 49) stated that "Kuo (1967) has found that a mother exercises a strong influence on the development of food preferences in her puppies." Although Kuo's studies demonstrated food fixation in puppies, he did not examine the social facilitation effects of the mother. The puppies only had one diet option, the same diet as their nursing mother, from birth to 6 months of age. Therefore, it is logical that the puppies fed the less-varied diets (soybeans or fruits and vegetables) would remain fixed to these familiar foods, similar to the first generation puppies. On the other hand, a puppy sampling new food in the presence of the mother, that it would not eat when alone, may indicate social facilitation by the mother. This, however, was not examined in Kuo (1967).

Social facilitation has been documented in puppies which eat more in the presence of other puppies than individually (James & Gilbert, 1955; Ross & Ross, 1949a). James and Gilbert (1955) fed one litter together for 90 days from weaning and another litter was fed individually. For an additional 40 days, puppies were alternately fed in their respective litter or individually per day. The litter that had been fed in a group for the first 90 days immediately ate more in the social situation than when fed individually. However, in the litter that had been fed individually for the first 90 days, social facilitation took approximately two weeks to become apparent.

Ross and Ross (1949a) fed 10-week-old puppies from two litters individually and with littermates on alternate days. The chow-basenji puppies ate 14% more in the group state than the solitary state and the Irish terrier-dachshund puppies ate 51% more in the group state than the solitary state. The differences between the two litters can be explained by individual puppy performance which ranged from a 3% to 86% increase in food eaten during the group feeding as compared to the individual feeding (Ross & Ross, 1949a).

Social facilitation will occur even to the extent that puppies that have been satiated during individual feeding will eat more if in the presence of other hungry dogs which are actively eating (Ross & Ross, 1949b). James (1960) also examined the onset and development of social facilitation in 10 puppies once they were weaned at 21-days old. Puppies were then fed daily, either individually or in two 5-member groupings from 3- to 8.5-weeks old. The puppies ate more in the social situation than when alone, but the effect of social facilitation only became apparent after an average of 9 and 16 days, respectively, for each group. Social facilitation appeared on the first social feeding for one puppy. Once it appeared, the puppies always ate more in the social situation. Therefore, social facilitation develops early in puppies when they are fed in groups (James, 1960). The puppies were already weaned for these social facilitation studies, and thus, they did not investigate the influence of the mother on her puppies.

Another type of social influence is learning by imitation or observational learning (Lindsay, 2000b). Observational learning has been documented in domestic dog puppies that have observed their trained mothers search for narcotics (Slabbert &

Rasa, 1997) or littermates operate a food cart on a pulley (Adler & Adler, 1977). Adult dogs also learn from observing humans perform a detour (Pongrácz, Miklósi, Kubinyi, Topál, & Csányi, 2003; Pongrácz, Miklósi, Timár-Geng, & Csányi, 2004) or a manipulation task (Kubinyi, Topál, Miklósi, & Csányi, 2003). However, no known studies have documented observational learning to develop food preferences in domestic dogs, whereas it has been documented in other animals, such as moose calves (Edwards, 1976), domestic fowl (Gajdon, Hungerbuhler, & Stauffacher, 2001; Nicol, 2004), and monkeys (Prescott, Buchanan-Smith, & Smith, 2005).

There has also been extensive research into the development of food preferences in sheep, particularly the influence of the mother on those preferences (Black-Rubio, Cibils, & Gould, 2007; Mirza & Provenza, 1990, 1994; Nolte, Provenza, & Balph, 1990; Pfister & Price, 1996; Saint-Dizier, Lévy, & Ferreira, 2007). Saint-Dizier et al. (2007) determined that the development of food preference in lambs was mainly determined by observation of the mother as long as there was access to the food’s olfactory cues. Younger lambs are also influenced more by their mothers’ dietary habits than older lambs (Mirza & Provenza, 1990). However, observational learning to develop food preferences has not been documented in dogs.

Food preferences can be learned through olfaction as documented in adult dogs that acquired food preferences from socializing with recently fed conspecifics (Lupfer-Johnson & Ross, 2007) and in puppies during the peri-natal period (Hepper & Wells, 2006). Hepper and Wells found that pups exposed to aniseed both pre- and post-natally had a significantly higher preference for the aniseed diet as compared to pups exposed just pre- or post-natally. In Lupfer-Johnson and Ross (2007) one dog of a testing pair was fed a diet flavoured with either basil or thyme. Both dogs of the testing pair were then allowed to socialize before the second dog was fed both flavoured foods alone. The second dog preferred the flavoured diet consumed by their partner, suggesting dogs prefer foods smelled on their conspecific's breath (Lupfer-Johnson & Ross, 2007). This study indicates that the first dog to eat facilitated the food preferences of the recipient dog (social facilitation), but the recipient dog did not *copy* the behaviour of a demonstrator dog, as would be the case in observational learning, because the recipient dog did not observe its testing partner eat. The development of grass-eating behaviour in puppies warrants investigation.

Grass-Eating Theories

Grass eating is common, widespread, and normal, and yet there is still no known explanation for the behaviour. References to grass-eating behaviour in dogs within the published literature are typically brief and include the author's speculations on the behaviour. At present, these hypotheses have not been supported as no controlled studies have investigated grass-eating behaviour in the domestic dog (as noted by Hart, 1985; UC Davis SVMCABP, 2005). These unsupported hypotheses will be referred to as "theories," so as not to confuse them with the hypotheses of the current project's individual experiments. This thesis presents these theories as an indication of the current perception of grass-eating behaviour with the understanding that the theories have not been substantiated.

Several theories have emerged from scat surveys or observations of dogs and wolves consuming grass and other vegetation. Three existing theories that have attempted to explain grass-eating behaviour include the carcass paunch theory, self-medication of a worm burden, and gastrointestinal distress self-medication. Each theory is described in more detail in the following sections.

Carcass Paunch Theory

The presence of plant material in wolf scats ranges from 1.6% (Murie, 1944) to 74% (Stahler et al., 2006) of scats and is higher in the summer than winter (Andersone & Ozoliņš, 2004). Mech (1966) maintains that grass is most likely eaten inadvertently, whereas Murie (1944) and Stahler et al. (2006) venture that grass is intentionally eaten by wolves. Similar to Mech, Thompson (1952) suggests that vegetable matter may be inadvertently ingested from the forest floor near a kill or from the paunch of a kill, rather than intentionally eaten. Because wolves ingest all parts of their herbivorous prey, including the digestive tract, partially digested plant material is consumed (Case et al., 2000; Mech, 1995). However, Thompson (1952) is sceptical of this "inadvertent ingestion" theory because needles and twigs were most frequent in scats during times when deer, the primary prey, would not be feeding on these items. Thus, the presence of these items in the wolves' excrement is not likely to have been obtained from the deer's digestive tracts.

The theory of grass ingestion through consumption of carcasses has also been related to grass eating in domestic dogs. Typically, domestic dogs do not consume whole carcasses, but directly feeding on grass may be a compensation for the lack of plant material that would have been consumed through carcass eating (Beaver, 1981; Hart, 1985). Beaver suggests supplementing the diet with fresh vegetables to prevent grass eating. However, Sueda et al. (2008) found that no dogs in the non-plant-eating group of Study 1 had their diets supplemented with vegetables and 27% of plant-eating dogs' diets were supplemented with vegetables or fruit.

Therefore, offering vegetables did not preclude plant eating and plant eating was not induced by the lack of vegetables in the diet, suggesting that the "carcass theory" of grass eating in domestic dogs is not likely. Considering the high prevalence of plant eating reported in dogs (Sueda et al., 2008) and the prevalence of grass in wolf scats (Stahler et al., 2006), it is more likely that grass is eaten for a purpose other than compensation for abundance or lack of vegetable matter present in a diet.

Self-Medication of a Worm Burden

Another proposed theory is that dogs and wolves intentionally eat grass to eject parasites, such as worms (de Baïracli Levy, 1992; Engel, 2002; Hart, 1985; Thorne, 1995). Sueda et al. (2008) suggest that grass eating was preserved through the domestication process for this reason. Murie (1944) observed wolves eating grass during all seasons and some wolf scat contained grass as well as round worms. He also observed a wolf grazing on grass for a few minutes and then vomiting and leaving a watery scat. From his general observations, he hypothesized that grass might be used as an emetic to eject worms via the mouth or as an intestinal scour to dislodge worms from the digestive tract and remove them through excretion.

A similar self-medication behaviour has been documented more extensively in wild chimpanzees (Huffman & Caton, 2001) and bonobos (Dupain et al., 2002). Huffman and Caton (2001) documented chimpanzees deliberately swallowing whole, bristly leaves without chewing and found a significant correlation between this behaviour and the expulsion of nodule worms (*Oesophagostomum stephanostomum*). The leaves swallowed by the chimpanzees were excreted whole and contained no known phytochemical properties, evidenced by chemical analysis and the passing of live, rather than dead, worms (Huffman, 1997). Therefore, it is believed that the physical roughness of the leaves creates a ‘velcro effect’ that scours worms from the digestive tract and expels them through defecation.

Grass may similarly affect worms that dogs harbour. The domestic dog commonly harbours 3 main gastrointestinal nematodes: hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*), roundworm (*Toxocara canis* and *Toxoscaris leonina*), and whipworm (*Trichuris vulpis*; Jacobs et al., 1994). *A. caninum* digest plugs of mucous from the small intestinal mucosa, leaving behind small haemorrhages, which can reduce haematocrit counts in dogs (Roberson & Cornelius, 1980). *T. canis* also reside in the small intestine. Adult *T. vulpis* worms attach to the large intestinal mucosa by burying their heads in the folds, making them difficult to detach (Jubb & Kennedy, 1970). The prepatent period of hookworm, or the period from initial infection to detection in the host’s faeces, is 2-3 weeks, whereas *T. canis* is 4-6 weeks and *T. vulpis* has a prepatent period of 1 to 3 months (Anderson, 1992; Jacobs et al., 1994; Jubb & Kennedy, 1970; Roberson & Cornelius, 1980).

All of these gastrointestinal nematodes reside in the small or large intestine of the dog, so it is possible that the ingestion of grass could scour them, similar to leaf swallowing behaviour in chimpanzees. The theory that dogs eat grass to self-medicate a worm burden is worthy of investigation.

Gastrointestinal Distress Self-Medication

Some researchers suggest that grass may be used to self-medicate some form of gastrointestinal distress (McKeown, 1996; Overall, 1997). Grass may be an emetic (Fox, 1965; Hart, 1985; Houpt, 2005; Thorne, 1995) or a laxative (Hart, 1985;

McKeown, 1996). Acute gastritis usually results from the ingestion of irritating substances (Quinn et al., 1997). Therefore, Fox (1965) and Hart (1985) suggest that grass may irritate the mucosa of the stomach and facilitate vomiting. Similarly, Thorne (1995) postulated that grass ingestion may help expel food that is causing digestive upset. Houpt (2005) suggests that grass may be an emetic because dogs may vomit afterward and the behaviour occurs in dogs with gastritis or other upper gastrointestinal problems. However, it is unclear if self-medication is occurring or just physical irritation caused by grass ingestion (Overall, 1997).

Hart (1985) also offers a different tack and suggests that feather, fur, and bone may have a constipating effect which is remedied by grass consumption, due to its theorized laxative properties (McKeown, 1996). However, no support for these claims was evident within the relevant texts and gastrointestinal distress is a broad term, which could encompass many “digestive upsets,” such as nausea, constipation, or diarrhoea. Self-medication of a mild gastrointestinal distress, specifically large intestinal diarrhoea, is investigated in this thesis.

Research Plan

The current project comprises five studies, including one observational study and four controlled experiments, which explore specific aspects or theories of grass-eating. Sueda et al.’s main survey was available online for 3 weeks in 2004 and its preliminary data were presented at the Waltham International Nutritional Sciences Symposium in September 2005 (Sueda et al., 2005; UC Davis SVMCABP, 2005). An observational study of grass eating in dogs, for the current project, was completed in January 2006. This observational study (Chapter 2) quantified several of the UC

Davis survey questions, such as prevalence of plant eating and vomiting. In addition, dog information, such as age and breed, was collected to compare with the results of Sueda et al. (2008). The observational study was also used to identify grass-eating dogs to be used in the controlled scientific experiments as well as help develop the methodology for these experiments, the first of which is presented in Chapter 3.

Chapter 3's study aimed to provide an initial understanding of grass-eating behaviour by determining the pattern of grass-eating habits during the day as well as the relationship between grass eating and food ingestion. The preference for couch or kikuyu grass was tested in this study. Couch grass (English couch, *Agrospyron canina* aka *Elymus repens*) is considered to be the 'grass of choice' for dogs as its 'dog grass' nickname indicates (Auld & Medd, 2002; de Baïracli Levy, 1992; van Wyk & Wink, 2005). Couch grass is the only grass named in the anecdotal 'grass eating' literature. A local couch grass (*Cynodon dactylon*) which also has the vernacular name "dog grass" (Kapoor, 2001) was used in Chapter 3's study. *C. dactylon* is a fine-leafed, creeping perennial with hairless or lightly haired blades and wiry underground and above ground stems (Muyt, 2001), similar to de Baïracli Levy's couch grass (Auld & Medd, 2002; van Wyk & Wink, 2005). Kikuyu grass (*Pennisetum clandestinum*), a robust, creeping perennial with soft, hairless or lightly haired blades, was also used in the current project (Muyt, 2001).

Chapter 4 investigated the development of grass-eating behaviour and the influence of the nursing mothers' grass-eating habits on five- to seven-week-old puppies, which coincided with the optimum time for socialisation and weaning onto solid foods (Serpell & Jagoe, 1995). Kikuyu grass was presented to half the puppies with their mother present and the remaining half without their respective mother present to determine the effect of puppy age and mother on grass eating.

The theory that dogs eat grass to self-medicate a worm burden was investigated in a controlled laboratory setting (Chapter 5). Dogs that were due for their quarterly de-worming, and were naturally harbouring worms, were presented with kikuyu grass. Their grass-eating behaviours were recorded before half of the dogs were de-wormed and grass-eating behaviours were documented again. Kikuyu's hairless or lightly haired blades are similar to those of couch grass which herbalist Mességué (1991), in his autobiography, briefly conjectured dogs use as a purgative.

Some researchers suggest that grass may be used to self-medicate some form of gastrointestinal distress (McKeown, 1996; Overall, 1997). The study presented in Chapter 6 compared grass-eating behaviours of dogs when producing normal stools and when producing loose, watery stools. Preferences for couch and kikuyu grasses were also investigated.

Grass has also been purported to be an emetic (Fox, 1965; Hart, 1985; Houpt, 2005; Thorne, 1995). This theory was indirectly tested throughout all of the controlled experiments by documenting the number of vomiting events and determining if there was a relationship between vomiting events and time spent grass eating for each study.

The current thesis is formatted so that each of the four controlled experiments is presented as an autonomous chapter. Therefore, Chapters 3 to 6 include relevant information presented within the current *Introduction* in addition to individual method, results, discussion, and references. Chapter 7: *Discussion and Conclusions* presents discussion of the thesis results as a whole.

CHAPTER 2

Grass Eating in Domestic Dogs in a Semi-Naturalistic Home Environment

Introduction

Plant eating in the domestic dog has been investigated in one known article by Sueda, Hart, and Cliff (2008). Sueda et al. (2008) surveyed owners of healthy dogs attending an outpatient clinic about their dogs' feeding habits. Owners were asked questions about their dogs' regular diet and consumption of non-food items, such as stones, toys, faeces, and plants. Owners of dogs that ate plants were asked further questions about the plant-eating habits including frequency of plant eating and frequency of the dog showing signs of illness before consuming plants or vomiting within 1 hr after plant consumption. The authors also performed an online survey, their Study 2, of owners whose dogs were known to eat plants.

Sueda et al. (2008) found that plant eating is a common and widespread behaviour as 79% of the healthy well-cared-for dogs in their first study ate plants. Plant eating was also prevalent in all major breed groups. In particular, 98% of their Study 2 respondents indicated that their dogs consumed grass on some occasions, with 79% eating grass most frequently as compared to other non-grass plants. Data from both Sueda et al. studies also showed that most dogs appear normal before plant ingestion and do not vomit afterwards. However, younger age was associated with an increase in frequency of plant eating and a decrease in appearing ill (depression, loss of appetite, and inactivity as described in Hart, 1988) before or vomiting after plant eating. The authors concluded that plant eating is a normal behaviour that evolved in wild canids and was preserved through the domestication process.

Sueda et al.'s (2008) surveys have provided preliminary information about vegetation-eating behaviour in domestic dogs, but the explanation for the behaviour is still unknown. Conclusions that are more concrete may be drawn from controlled scientific experiments, such as those in the current project. The study presented in the current chapter was used to develop methodology for recording vegetation-eating behaviours, such as prevalence of vegetation eating, type of vegetation eaten, and vomiting, similar to Sueda et al. (2008). The current study's dogs were observed in their home environment to quantify the frequency of these vegetation-eating behaviours and to identify vegetation-eating subjects for the laboratory experiments.

The current study had several aims:

1) Quantify vegetation-eating behaviours from Sueda et al.'s (2008) study
2) Develop methodology for recording vegetation-eating behaviours
3) Identify vegetation-eating subjects for the laboratory experiments.

Methods

Procedure

Thirty privately owned dogs were observed in January 2006 in their semi-naturalistic home environment. They lived in groups in kennels with an adjoining paddock and were fed a varied diet of dry dog food and raw meats and offal. All dogs were given daily access to an adjoining half-acre paddock during feeding and cleaning times. Individual dog information was recorded for each pack member, including gender, breed, and age. The mean age of the dogs was 5.5 yr (standard error = 0.7 yr) and age groups were categorized similar to Sueda et al. (2008): 1- <3 yr, 3-9 yr, or >9 yr. Major breed groups were classified as terrier, hound, and toy.

Previous personal observations indicated that grass-eating behaviour generally occurs quickly on introduction to grass and that the behaviour diminishes within about five minutes after initial access. Therefore, 10-minute observations sessions were chosen to encompass this grass-eating activity. The 10-minute length of time was also manageable to observe several groups within the constraints of the owner's cleaning and feeding schedule. Therefore, dogs (n=30) were observed in groups of 5 dogs for 10-minutes per group as they were released into the paddock around midday. The dogs had access to vegetation only during these times.

Three observation sessions were completed with one session per week. All-occurrences sampling (Altmann, 1974; Martin & Bateson, 1993) was used to document each occurrence of the following behaviours for each dog: number of vegetation-eating events, type of vegetation eaten (grass, clippings, leaves, sticks), vomiting, urination, and defecation (Table 1).

Table 1

Behaviours recorded during observation sessions and their definitions

Behaviour	Definition
Number of vegetation-eating events	A grass-eating event occurred when a dog ingested vegetation until it stopped chewing for two or more seconds or moved to a new position
Type of vegetation eaten	The type of plant eaten during a vegetation eating event was noted as either grass, clippings, leaves, or sticks
Number of vomiting events	A vomiting event occurred when a dog vomited and spasming stopped. A new event was documented if spasming recommenced and the dog vomited again.
Number of urination events	A dog urinated
Number of defecation events	A dog defecated

Instantaneous sampling (Altmann, 1974; Martin & Bateson, 1993) was also used to document which dogs were in view at each minute of the 10-minute sampling period. This sampling method documented whether or not the behaviour (‘in view of observer’) was occurring at each sample point (each minute of the observation session). All efforts were made to observe dogs from a vantage point from which all areas of the paddock could be seen. Nevertheless, at times subjects could have been out of view. Therefore, instantaneous sampling was performed to ensure that each dog’s documented behaviours were representative of the dog and not skewed because the dog was out of the observer’s view.

A second observer was present for a preliminary observation period, before the study formally began, to formalize methodology and the shorthand used to record observations. The shorthand included a one-letter symbol or mark for each behaviour. For instance, a grass-eating event was denoted by an “o” and a “v” symbolized a vomiting event. The second observer also recorded observations in the first observation session to ensure behaviours were being reliably measured by the primary observer (S. Bjone). The University of New England Animal Ethics Committee granted authority (AEC05/198) to conduct this study, in accordance with Section 25 of the Animal Research Act (1985).

Statistical Analysis

The mean number of events per 10-minute observation session were calculated for all behaviours and used for the statistical analyses. Pearson correlation determined the relationship between the vegetation-eating events and urination or defecation. One-way ANOVA examined the relationship between major breed group, gender, or age group and vegetation-eating events.

Normality was assumed for each variable if the statistic for skewness (and kurtosis) divided by the standard error for skewness (and kurtosis) was less than three. Z-scores identified univariate outliers. If z was outside the range of ±3.3, there were no outliers. Hartley’s Fmax tested the homogeneity of variance assumption. If the Fmax value was less than the Fmax critical value in Gravetter and Wallnau (2007), homogeneity of variance was assumed. All assumptions were met. Intra-observer reliability was determined using Pearson correlation with the vegetation eating frequency data from the primary and second observer and was found to be 0.96. All significance levels were set at α=0.05.

Results

One dog was excluded from the study because it was out of view during all scan samples, and therefore, observations were minimal. Dogs were only observed eating grass and not any other vegetation types (grass clippings, leaves, or sticks), even though these items were available in the paddock. Therefore, ‘vegetation-eating events’ will be referred to as ‘grass-eating events’ in the remainder of the current study.

Twenty-seven of the twenty-nine (93%) dogs included in the current study ate grass at some point during the observation sessions. There was an average of 3.8 grass-eating events per dog per 10-minute observation session (Table 2). Table 2 also lists the mean number of grass-eating events per 10-minute observation session for each dog. Only one vomiting event was observed during the three observation sessions; no grass was present in the vomit.

Table **2**

Mean number of grass-eating events per 10-minute observation session for each dog in descending order

Dog	Number of events
Bessie	9.5
Ruby Cool Puppy	8.0
Jewel	7.3
Molly	7.3
TobyJ	7.0
Barney	6.7
Max	6.3
Caramel	6.0
Bonnie	5.7
Pru	5.7
Bindy	5.3
Ginger	4.7
Fugly	4.7
Columbus	4.3
TobyM	3.7
Daisy Mae	2.7
Maddog	2.5
Benjamin	2.0
Sheba	2.0
Jack	2.0
Fluffy	1.5
Jackie	1.3
Peggy Sue	1.3
Sarah	1.0
Little Fluffy	1.0
Snuffy	0.7
Naomi	0.5
Fox	0.0
Rosy	0.0
Mean ± SE	3.8 ± 0.5

There were no significant effects of major breed group (df=2, p=0.61), age group (df=2, p=0.63), or gender (df=1, p=0.83) on the number of grass-eating events (Table 3). There were also no significant correlations between the number of grass-eating events and the number of urination ($r_{(27)}$= -0.05, p=0.80) or defecation ($r_{(27)}$= -0.19, p=0.32) events.

Table **3**

Mean number of grass-eating events and standard error values by major breed group, age group, and gender

Major breed group	N	Mean ± SE
Terrier	14	3.4 ± 0.8
Toy	8	3.9 ± 1.0
Hound	7	4.7 ± 0.9
Age		
1- <3 yr	6	3.0 ± 1.5
3-9 yr	18	3.9 ± 0.6
>9 yr	5	4.6 ± 1.1
Gender		
Male	8	4.0 ±0.9
Female	21	3.7 ± 0.6

Discussion

The main aim of this study was to quantify vegetation-eating behaviour in the dogs' normal environment. Vegetation eating was common: 27 of the 29 dogs ate grass on at least one occasion. Grass was the preferred plant as all vegetation-eating observations were of grass and there were no observations of clippings, leaf, or stick consumption. This high frequency of grass consumption is similar to Sueda et al.

(2008) in which 79% of the healthy, well-cared-for dogs ate plants and of these vegetation-eating dogs, 79% consumed grass most frequently and the remaining 21% consumed non-grass plants most frequently.

We observed no relationship between plant eating and gender or breed. These results should be viewed cautiously because of the limited sample size for males and breed. Despite this, these results are consistent with the findings of Sueda et al. (2008). However, contrary to Sueda et al. (2008), there was no relationship between grass eating and age in the current study. Differences between age groups may have become apparent if a larger sample of dogs was observed with younger dogs under 1 yr as the current sample lacked dogs from Sueda et al.'s (2008) youngest category.

It is likely that the singular vomiting event observed in the current study was due to the subject binge drinking stagnant water only a few minutes prior to the vomiting event and it was not related to the ingestion of grass. The lack of vomiting throughout the current study further supports the theory that grass eating is a mostly normal behaviour (Sueda et al., 2008).

Another purpose of the observational study was to guide the development of the experimental design for the other studies included in this project and to select subjects that eat grass. A shorthand notation developed for the current observational study was an efficient recording method even when simultaneously observing 5 subjects in a large paddock. Therefore, this shorthand should be even more successful in the experimental studies in which there will be smaller groups to observe at one time and these groups will be in a testing pen that is considerably smaller than the paddock. Similarly, the all-occurrences sampling method was adequate for the sufficiently attention-grabbing behaviours (Altmann, 1974) and will be used in the controlled experiments.

Because the dogs were studied in groups of 5 dogs, it is possible that the presence of the other dogs influenced grass-eating behaviour. However, the large size of the paddock (half acre) would have limited crowding and the possible social influences. The possible effect of social influences in a group testing situation will be further addressed in subsequent chapters.

Because no dogs were observed eating leaves or sticks and grass was the most frequently eaten plant in Sueda et al.’s (2008) study, the future studies will concentrate on grass. As there was no effect of major breed group or age, all efforts will be made to select those dogs that exhibited frequent grass eating (as listed in Table 2) as subjects for the remaining studies. In conclusion, grass eating appears to be a normal and common behaviour of domestic dogs. The shorthand and sampling method of the current study will be used in future controlled experiments.

CHAPTER 3

Grass-Eating Patterns in the Domestic Dog, *Canis familiaris*

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Grass eating patterns in the domestic dog, *Canis familiaris*

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Summary

Very little is known about grass eating behaviour in the domestic dog, *Canis familiaris*. This study is the first to investigate grass eating in dogs in a controlled experiment, and attempts to provide an initial understanding of this behaviour by describing the pattern of grass eating during the day and the relationship between grass eating and the ingestion of food. Twelve dogs were presented with both kikuyu and couch grass three times daily for 6 d and grass eating behaviours were observed using an all-occurrences sampling method. The results of this study suggest that grass eating is influenced by satiety and time of day. Dogs spent more time eating grass before ingestion of their kibble meal than after, and the time spent eating grass decreased throughout the day. Grass may be seen as a food source, as the subjects were less likely to eat grass when they were satiated. Couch and kikuyu grasses were equally preferred. We conclude that grass eating is a normal and common behaviour, as all dogs in this study were in good health and readily ate grass. As such, grass-eating should not be seen as a problematic behaviour for most dogs or as indicative of illness.

Keywords: *Canis familiaris*, dog, feeding behaviour, grass eating, satiety

Introduction

It has been suggested that grass eating is a common behaviour in domestic dogs (Houpt, 2005) but there is no known explanation for this behaviour (Lindsay, 2001) and there have been no experimental studies investigating grass eating in this species (Hart, 1985; UC Davis School of Veterinary Medicine Companion Animal Behavior Program, 2005). Dogs have almost no capacity to digest grass (Beaver, 1981). Nonetheless, some researchers contend that grass may influence digestion by acting as an emetic (Fox, 1965; Beaver, 1981; Hart, 1985; de Baïracli Levy, 1992; Thorne, 1995; Lindsay, 2001; Houpt, 2005), a laxative (Hart, 1985), or by providing roughage (McKeown, 1996; Houpt, 2005). There are several products that market grass as a digestive aid or dietary supplement (Organic Pet Grass Kit, ©Wheatgrasskits.com, Springville, UT; Pet Greens® and Pet Grass®, Bell Rock Growers, Inc., San Marcos, CA; Barley Dog®, Green Foods Corporation, CA). However, the claims made for these dietary supplements have not been substantiated (Lindsay, 2001).

While there are no experimental studies, Sueda et al. (2005) performed an owner-completed survey and observed no relationship between plant eating and gender, gonadal status, breed, diet or presence of intestinal parasites. The researchers concluded that plant eating evolved in wild canids and was preserved through the domestication process. While the study of Sueda et al. study provides some preliminary information about grass eating, the scientific value of the study may have been compromised by the subjective nature of its design (information was provided by multiple owners about their individual dog's eating habits). More concrete conclusions may be drawn from controlled scientific experiments.

The current study is the first to scientifically investigate grass eating behaviour in dogs in a controlled experiment. As very little is known about grass eating in dogs, the aim was to provide an initial understanding of the behaviour by determining the pattern of grass eating habits during the day as well as the relationship between grass eating and the ingestion of food.

A preliminary pilot study indicated that dogs eat a few grams of grass per eating episode and prefer to eat grass presented as entire plants growing in pots rather than as cut blades or turf (S.J. Bjone, unpublished data). This preliminary study also illuminated the difficulty of quantifying the amount of grass eaten. As grass blades are light and the pots and soil that contain the grass are heavy in comparison, it was difficult to obtain accurate measurements of the amount of grass eaten. In addition, the dogs often disrupted the grass by urinating or salivating on the grass, digging or tipping the pots. These disruptions further complicated the weighing procedure. Therefore, the method adopted for the current experiment was to measure grass eating behaviours: the amount of time spent eating grass, number of grass interactions, and vomiting events. The current study was devised to explore the following questions:

1. Is grass eating influenced by satiety?
2. Do dogs have a preference for one type of grass over another?
3. Is grass eating influenced by the time of day?

Materials and Methods

Subjects, housing and diet

Twelve mixed-breed dogs which were accustomed to kennel housing, owned by the same owner and known to eat grass (mean age ± standard error (SE) = 6.0 ± 0.3 yr) were housed at the University of New England Dog Research Facility for the duration of the study. The dogs became accustomed to the daily routine and diet during a 7 day habituation period. All dogs were fed a nutritionally complete and balanced diet (Pedigree Advance Adult Chicken®; MasterFoods ANZ, Wodonga, VIC) once daily in amounts calculated to meet maintenance energy requirements and adjusted as necessary to maintain ideal body weight. Fresh water was available *ad libitum*. Dogs were housed in compatible groups according to owner recommendations. Each indoor kennel was outfitted with a trampoline style bed within a secure, centrally-heated facility where dogs slept at night. During the day, dogs were placed in spacious, fully covered, outdoor runs.

Materials

Couch (*Cynodon dactylon*) and kikuyu (*Pennisetum clandestinum*) grasses were used in the study because dogs are known to eat couch grass as its “dog grass” nickname indicates (de Baïracli Levy, 1992), and kikuyu was readily available locally. The grasses were grown in 20 cm pots in a greenhouse.

Ethics

All procedures were undertaken in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 1997). All dogs were privately owned, and written permission was obtained from the owner for the inclusion of the animals in the study. Animals received the highest standard of care throughout the study, in accordance with UNE Animal Ethics Committee guidelines. In addition, a veterinarian assessed each dog to ensure it was healthy and fit to participate in the study. All dogs were returned to the owner at the end of the study.

Procedure

Dogs were observed in compatible groups (*n* = 3) during three 10 minute testing sessions (morning, noon and afternoon at approximately 0900, 1200 and 1500 h) per day for 6 d. Groups were presented with two pots of both kikuyu and couch grass and all occurrences of each behaviour (Table 1) were recorded by the experimenter (SB) from the adjoining kennel (Altmann, 1974). A mini-DV camera also recorded each session for further analysis. The daily feeding time was rotated through the testing session time slots every 2 d. Therefore, the dogs were fed during the morning session for the first two consecutive days, the noon session for next 2 d and the afternoon session for the remaining two testing days. During the sessions in which the dogs were fed, two groups were observed before ingestion of food and two groups were observed after their meal. Group order within testing sessions was determined using a balanced Latin-square design.

Table 1. Behaviours recorded during testing sessions and their definitions. Behaviours marked with an asterisk (*) were recorded for both couch and kikuyu grasses.

Behaviour	Definition
Time spent eating grass*.	A dog chewed and swallowed grass .
Number of grass eating events*.	An event encompassed the dog ingesting grass until it stopped chewing, lifted its head or moved to a new position.
Number of grass interactions*.	Any interaction with the grass which did not entail ingestion or urination.
Number of vomiting events.	A dog vomited.

Statistical Analysis

The total number of grass eating events, grass interactions and vomiting events and the total time spent eating grass for each dog was analysed using a within-subjects repeated measures ANOVA (Tabachnick and Fidell, 2001). A 2 × 2 ANOVA was used to analyse the differences in the behaviours for each grass type before and after the ingestion of food and a 2 × 3 ANOVA was used to analyse the differences in the behaviours for each grass type between the morning, noon and afternoon periods. Repeated measures ANOVA was used to analyse behaviours that did not specifically relate to a grass type.

Significance levels were set at $\alpha = 0.05$ unless otherwise noted. The strength of association was represented by partial eta-squared, pη2 (Tabachnick and Fidell, 2001; Levine and Hullett, 2002). If sphericity could not be assumed for a repeated measures ANOVA, Greenhouse–Geisser values were used and the p-value was labelled with a “G–G” (Tabachnick and Fidell, 2001). There was a severe violation of the homogeneity of variances assumption for the amount of time spent eating grass and a moderate violation for the number of grass eating events for data obtained before and after the ingestion of food. Therefore, more stringent alphas of 0.01 and 0.025, respectively, were chosen rather than interpreting transformed data (Tabachnick and Fidell, 2001).

Results

The results were consistent with a preliminary study of the dogs’ normal grass eating habits in their home kennel environment. All dogs were observed eating grass with a total of 709 grass eating events across all 12 dogs and all 18 testing sessions. Each dog spent an average of 1.1 min (SE = 0.06 min) eating grass during an average of 3.3 grass eating events (SE = 0.2) per 10 min testing session, totalling 3.3 min (SE = 0.2 min) spent eating grass during 9.9 grass eating events (SE = 0.6) per d.

Grass Eating and Ingestion of Food

The dogs spent significantly more time (P = 0.001, $p\eta^2$ = 0.67) eating grass before ingestion of the daily kibble meal than after the meal (Table 2; Figure 1). There was no significant difference between the amount of time spent eating the two grasses (P = 0.74) and no significant grass and time interaction (P = 0.30). A small to medium positive Pearson correlation (Cohen, 1988) was present between the amount of time spent eating grass and the number of hours since the last kibble meal ($r_{(214)}$ = 0.23, P = 0.001).

Similarly, there were significantly more grass eating events before than after the kibble meal (P = 0.001, $p\eta^2$ = 0.63; Table 2, Figure 2). There was no significant difference between the types of grass (P = 0.11) and no significant interaction effect (P = 0.58). There were also no significant differences in the number of interactions before or after the meal (P = 0.953) or for grass type (P = 0.422) and there was no significant interaction effect (P = 0.180).

Although vomiting has been linked with the ingestion of grass, there were only five vomiting events involving three dogs across all 18 testing sessions. All of the vomiting events occurred during testing sessions in which dogs were also fed, and all events were by dogs presented with grass before ingesting the kibble diet. Three of the events occurred in the morning and two occurred in the afternoon.

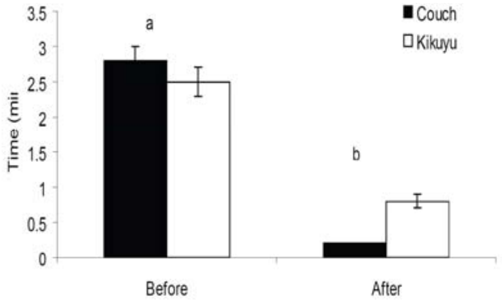

Figure 1. The time spent eating couch and kikuyu grass before and after the ingestion of the kibble meal. Different superscript letters indicate significant differences.

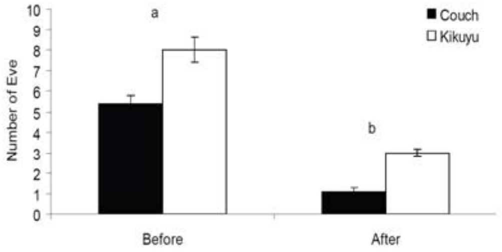

Figure 2. The number of couch and kikuyu eating events before and after the ingestion of the kibble meal. Different superscript letters indicate significant differences.

Table 2. Mean and standard error values for significant statistics. *Grass* represents the total of the couch and kikuyu grasses. Different superscript letters indicate significant differences.

Behaviour	Test statistics	Before meal	After meal				
Time spent eating grass (min)	F(1,11) = 22.71 P = 0.001; pη2=0.67	2.67 ± 0.53^{a}	0.53 ± 0.14^{b}				
Number of grass eating events	F(1,11) = 18.45 P = 0.001; pη2=0.63	6.68 ± 1.42^{a}	2.04 ± 0.57^{b}				
		Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Time spent eating grass (min)	F(5,55) = 2.48; P = 0.042; pη2 = 0.18	4.14 ± 0.23^{a}	3.65 ± 0.25^{ab}	2.32 ± 0.20^{b}	2.46 ± 0.19^{ab}	3.00 ± 0.26^{ab}	4.13 ± 0.28^{a}
		Morning	**Noon**	**Afternoon**			
Time spent eating grass (min)	F(2,22) = 9.17; G–G P = 0.006; pη2 = 0.46	4.45 ± 0.91^{a}	3.26 ± 0.72^{a}	2.15 ± 0.53^{b}			
Number of grass eating events	F(2,22) = 12.84 G–G P = 0.002; pη2 = 0.54	13.73 ± 2.94^{a}	8.92 ± 1.83^{b}	6.92 ± 1.60^{b}			

Daily Pattern of Grass Eating

The dogs spent significantly less time (G–G P = 0.006, $p\eta^2 = 0.46$) eating grass during the afternoon than the morning and noon testing sessions (Table 2, Figure 3), and there was no significant difference between the two grasses (P = 0.49) and no significant grass-by-time interaction (P = 0.30). The amount of time spent eating grass did differ slightly across the six testing days (P = 0.042, $p\eta^2 = 0.18$): the dogs spent more time eating grass on Days 1 and 6, the first and last days of testing, compared to Day 3 (Table **2**). There was no significant difference between grass types (P = 0.49) and no significant day-by-grass interaction (G–G P = 0.56).

The number of grass eating events followed a similar pattern to that for grass eating time; there was a difference between the time of day (G–G P = 0.002, $p\eta^2 = 0.54$), and there was no difference between the grasses (P = 0.40) and no significant interaction effect (G–G P = 0.19). However, there were significantly more grass eating events during the morning testing sessions than during the noon and afternoon sessions (Table 2, Figure 4).

The number of interactions were not significantly different across time of day (P = 0.72) or grass type (P = 0.72) and there was no significant interaction effect (P = 0.81).

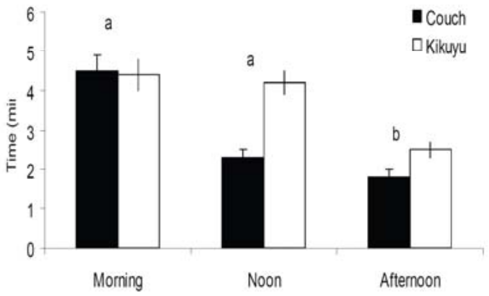

Figure 3. The time spent eating couch and kikuyu grass during the morning, noon and afternoon sessions. Different superscript letters indicate significant differences.

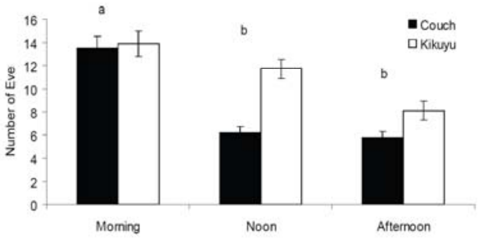

Figure 4. The number of couch and kikuyu eating events during the morning, noon and afternoon sessions. Different superscript letters indicate significant differences.

Discussion

While there are few references to grass eating in domestic dogs in the literature, anecdotal information suggests that couch grass is the grass of choice for dogs (de Baïracli Levy, 1992; Engel, 2002). However, the results of the current study do not support this theory: the dogs did not demonstrate a statistically significant preference for eating either couch or kikuyu grass. This was evident at all levels of the study: around mealtime, throughout the day and across the six testing days.

Many of the theories about grass eating involve digestion (Hart, 1985; de Baïracli Levy, 1992; Overall, 1997; Lindsay, 2001; Engel, 2002). All dogs participating in the current study passed a veterinary health check, and all dogs were dewormed and did not have any known digestive problems. However, in spite of the prevalent digestion theories, all of the subjects ate grass, spending an average of 3.3 min (SE = 0.2 min) eating grass per day. The current study also deflates the theory that dogs eat grass as an emetic as there were only five vomiting events for 709 grass eating events across all 12 dogs and all 18 testing sessions (similar to the results of Sueda et al., 2005).

Grass eating was influenced by time of day. The amount of time spent eating grass decreased throughout the day with less time spent eating grass in the afternoon than morning or noon. It is unlikely that the decrease in grass eating as the day progressed was related to an overall habituation effect as the dogs spent similar amounts of time eating grass at the beginning and end of the trial.

However, satiety may have influenced this grass eating pattern. Possibly, by the afternoon testing session the dogs had their fill of grass and were no longer interested in eating more. As the dogs had already ingested their kibble diet before the afternoon session for 4 of the 6 testing days, they may not have been hungry during these later sessions.

Further support for the effects of satiety on grass eating is evident from results showing that the dogs spent significantly more time eating grass before they ingested their kibble meal than after the meal. Similarly, the correlation between the amounts of time spent eating grass and the number of hours since the last kibble meal also supports the concept that the longer the time sincethe last kibble meal, i.e., the hungrier the dogs are, the more time they will spend eating grass.

The current study endeavoured to provide an initial understanding of grass eating behaviour in dogs by determining the pattern of grass eating during the day and the relationship between grass eating and the ingestion of food. Further studies are currently underway to investigate other aspects of grass eating behaviour in dogs such as its relationship with worm burdens and the development of the behaviour in puppies.

Conclusions

The results of this study suggest that grass eating is influenced by satiety and time of day. As the day progressed, the dogs spent less time eating grass. While the dogs showed no preference for couch or kikuyu grasses, dogs may see grass as a food source and are more likely to eat grass if they are hungry, i.e., before ingesting their regular diet. Grass eating is a normal and common behaviour and should not be seen as a problematic behaviour for most dogs.

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Statement of originality

- All content in this chapter is an original contribution by the candidate except where specified in the statement below.

Statement of contribution by others

- Brown and Price contributed to the concept, design of the study, and preparation of the manuscript.

Signed

Candidate, Samantha Bjone	Date	Principal supervisor, Ian Price	Date
		Co-supervisor, Wendy Brown	Date

CHAPTER 4

The Development of Grass-Eating Behaviour in Puppies and the Effect of the Nursing Mother's Grass-Eating Habits

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The Development of Grass-Eating Behaviour in Puppies and the Effect of the Nursing Mother's Grass-Eating Habits

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Abstract

Very little is known about grass-eating behaviour or its development in the domestic dog, *Canis familiaris*. The current study investigated the development of grass-eating behaviour and the influence of the nursing mother's grass-eating habits on five- to seven-week-old puppies. Puppies were exposed to grass with or without their nursing mother present. All puppies were observed eating grass despite the presence or absence of their mother, suggesting an innate tendency to grass-eating behaviour. Grass eating increased from 5 weeks of age to 6 and 7 weeks of age as the puppies matured. There was no main effect of treatment group: puppies with and without their mothers spent similar amounts of time eating grass, but there was an interactive effect between treatment groups and how frequently mothers ate grass. The puppies with mothers that ate grass more frequently spent more time eating grass than their littermates who were not with their mothers during testing and puppies with infrequent grass-eating mothers. In conclusion, the current study indicates that grass-eating behaviour develops in five- to seven-week-old puppies through innate tendencies and is influenced by the mother's grass-eating habits. The innate nature of grass eating further suggests that grass eating in the domestic dog is a normal behaviour and grass could be provisioned even for very young puppies. The high prevalence of grass eating in both adult dogs and puppies suggests a biological function to the behaviour.

Introduction

Very little is known about grass-eating behaviour or its development in the domestic dog, *Canis familiaris*. Many grass-eating theories exist (Hart, 1985; Houpt, 2005; Lindsay, 2001; McKeown, 1996), but there are only two known published scientific studies on grass-eating behaviour in the domestic dog (Bjone, Brown, & Price, 2007; Sueda et al., 2008). However, although Sueda et al. (2008) and Bjone et al. (2007) found that grass eating is common in the domestic dog, the reasons for this behaviour are still not completely understood. Sueda et al. (2008) found that a large proportion of healthy dogs eat plants (79%) and grass was the preferred plant with 79% of the plant-eating dogs eating grass compared to other non-grass plants. The authors concluded that grass eating is a common behaviour and may be a mechanism to expel internal parasites.

All of the study dogs in Bjone et al. (2007) ate grass. The amount of time they spent eating grass decreased throughout the day, no matter when they were fed their meal. The dogs also spent more time eating grass the longer it had been since their last daily meal. Subsequently, Bjone et al. (2007) suggested that grass may be seen as a food source. By examining the development of grass-eating behaviour in the domestic dog and the possible influence by the nursing mother, we may have a clearer understanding of this behaviour.

Puppies are born deaf and blind but do possess reflexes to assist nursing (Lindsay, 2000a). Nursing starts immediately and peaks at the end of the first week (Houpt, 2005). During the weaning process, puppies progress from suckling their mother’s milk to consuming solid foods and no longer suckling within eight weeks.

Bitches start to wean puppies at three weeks of age. The primary socialisation period occurs from around three to five weeks of age during which puppies exhibit intense exploratory behaviour, including chewing of both animate and inanimate objects (Lindsay, 2000a). Mothers leave the puppies progressively more frequently during these weeks and the puppies begin to wander more (Lindsay, 2000a). Puppies will readily consume semi-solid food around four weeks, and by five to six weeks, they are able to chew and consume dry food (Case, 2005). Puppies no longer need to suckle for nutrition by six weeks of age, but nursing may continue until around seven to eight weeks of age when behavioural weaning is typically completed (Case, 2005). Providing puppies with a variety of foods will reduce food preference fixation (Kuo, 1967; Thorne, 1995).

Development of food preferences may be learned through social influences, or social learning, which includes both social facilitation and observational learning (Lindsay, 2000b). Social facilitation has been documented in puppies that eat more in the presence of other puppies than individually (James & Gilbert, 1955; Ross & Ross, 1949a). This social facilitation will occur even to the extent that after puppies have been satiated during individual feeding, they will eat more if fed again in a group (Ross & Ross, 1949b). James (1960) also found that social facilitation developed early in puppies: from 3 to 8.5 weeks old.

Learning by imitation (observational learning) has been documented in domestic dog puppies that have observed their trained mothers search for narcotics (Slabbert & Rasa, 1997) or littermates operate a food cart on a pulley (Adler & Adler, 1977). Adult dogs also learn from observing humans perform a detour (Pongrácz, Miklósi, Kubinyi, Topál, & Csányi, 2003; Pongrácz, Miklósi, Timár-

Geng, & Csányi, 2004) or a manipulation task (Kubinyi et al., 2003). Dogs can develop food preferences through olfaction as documented in adult dogs that acquired food preferences from socializing with recently fed conspecifics (Lupfer-Johnson & Ross, 2007) and in puppies during the peri-natal period (Hepper & Wells, 2006). Wells and Hepper (2006) proposed that carnivores' dietary choices may be more influenced by postnatal experience, such as observation.

Observational learning to develop food preferences has been documented in other animals, such as moose calves (Edwards, 1976), domestic fowl (Gajdon, Hungerbuhler, & Stauffacher, 2001; Nicol, 2004), and monkeys (Prescott et al., 2005). There has also been extensive research on the development of food preferences in sheep, particularly the influence of the mother on those preferences (Black-Rubio, Cibils, & Gould, 2007; Mirza & Provenza, 1990, 1994; Nolte, Provenza, & Balph, 1990; Pfister & Price, 1996; Saint-Dizier, Lévy, & Ferreira, 2007). Saint-Dizier et al. (2007) determined that the development of food preference in lambs was determined by observation of the mother as long as the lamb also had access to the food's olfactory cues. In addition, younger lambs are influenced more by their mothers' dietary habits than older lambs (Mirza & Provenza, 1990) and lambs' preferences persist longer if exposed to foods with their mothers present (Nolte et al., 1990). However, observational learning to develop food preferences has not been documented in dogs.

The domestic dog has retained most of its ancestor's (wolf, *Canis lupus*) eating behaviours (Case, 2005), which possibly include grass and plant eating, because these materials have been found in wolf scats (Andersone & Ozoliņš, 2004; Mech, 1966; Meriggi, Rosa, Brangi, & Matteucci, 1991; Murie, 1944; Stahler et al., 2006; Thompson, 1952) and stomach contents (Papageorgiou et al., 1994). The occurrence of grass eating in wolves and dogs suggests that the behaviour was preserved through domestication of the dog (Sueda et al., 2008). Overall (1997) also contends that plant eating is an innate canine eating behaviour. Additionally, mothers' grass-eating habits may influence the behaviour in their puppies.

There is minimal information on grass-eating behaviour in domestic dogs and the development of the behaviour is unknown. Therefore, the current study investigated the development of grass eating in five- to seven-week-old puppies, which coincided with the optimum time for socialisation and weaning onto solid foods (Serpell & Jagoe, 1995). We hypothesized that 1) grass eating would increase as the pups were weaned onto solid foods, 2) the behaviour would be innate, and 3) the mother would influence her puppies' grass-eating behaviours (similar to sheep; Saint-Dizier et al., 2007). To test these hypotheses, we presented grass to half the puppies with their mother present and the remaining half without their respective mother present from five to seven weeks of age.

Method

Subjects, Housing, and Diet

Six mixed-breed bitches (age mean ± standard error (SE): 3.9 ± 0.2 yr) and their 4-week-old puppies (n=26) were housed at the University of New England Dog Research facility for the duration of the study. Litter availability and size were the two main criteria for bitch and litter selection. Each litter consisted of either four or five puppies. The mothers and puppies' exposure to grass before arriving at the kennels was unknown. However, the owner reported observing all six bitches eating grass in their home environment at least once. Bitches were individually housed with their respective litters within the centrally heated facility.

Pens were cleaned daily. Dogs were placed in the covered outdoor runs for five to six hours each day and nesting boxes and bedding materials were provided. If weather was inclement, the dogs remained indoors. All dog areas, including kennels, exercise yard, and outdoor runs, were concrete-floored and had no grass available. Grass was only available when provided by the experimenter (S. Bjone) during testing sessions.

The dogs became accustomed to the daily routine and diet during a 7-day habituation period, which coincided with the puppies' fourth week of age. All dogs were fed a nutritionally complete and balanced diet appropriate for growing puppies and lactating mothers (Pedigree Advance Puppy Rehydratable, MasterFoods ANZ, Wodonga, VIC) twice daily at amounts calculated to meet maintenance energy requirements (MER (kcal) = 140 x BW(kg)$^{0.75}$) and adjusted as necessary to maintain ideal body weight. Fresh water was available *ad libitum.*

The puppies' health was maintained according to accepted veterinary practices: puppies were vaccinated (C3+corona) at 6 weeks and de-wormed using Drontal® Allwormer® for small dogs and puppies (active ingredients: Praziquantel 15 mg, Pyrantel 14.9 mg, Febantel 75 mg) at five and seven weeks of age. Mothers were also de-wormed using Drontal® Allwormer® when their respective litters were five weeks old. All medical treatments occurred during non-testing days. Dogs were returned to the owner at eight weeks of age for rehoming.

Materials

Kikuyu grass (*Pennisetum clandestinum*) grown in (28 cm W x 34 cm L x 6 cm H) trays in a UNE greenhouse was used in the study. Kikuyu grass is a robust, creeping perennial with soft, lightly haired or hairless blades (Muyt, 2001). The kikuyu was approximately 40 cm in length with 4-10 mm wide blades. Artificial aquarium plants, corkscrew *Vallisneria*, planted in (28 cm W x 34 cm L x 6 cm H) trays were also used in the study. The *Vallisneria* blades were 30 cm long and 14 mm wide. The artificial grass was included to control for the puppies' natural propensity at this age to chew on objects and to distinguish this behaviour from chewing and eating live grass.

Puppies from the same litter often looked similar. Therefore, each puppy had a specific shaved location on his/her underside and each puppy was also marked with Crayola® washable, non-toxic kid's paints (Binney & Smith; Easton, PA, USA) on the back and head for identification.

Ethics

All procedures were undertaken in accordance with the *Australian code of practice for the care and use of animals for scientific purposes* (National Health and Medical Research Council, 1997). All dogs were privately owned by a local breeder, and written permission was obtained for the inclusion of the animals in the study. Animals received the highest standard of care throughout the study, in accordance with UNE Animal Ethics Committee (AEC06/089) and WALTHAM Foundation guidelines. In addition, a veterinarian assessed each dog to ensure it was healthy to participate in the study.

Procedure

Before testing began, each puppy was randomly allocated to one of two groups per litter, resulting in groups of two to three puppies. One group of each litter was randomly selected to be exposed to grass in the presence of their mother (Group WM; n=13) and the other group was exposed to grass without their mothers present (Group WOM; n=13). Puppies retained their assigned treatment condition throughout the study.

The testing area was an indoor pen (180 cm W x 235 cm L x 210 cm H) that was only used for testing and included a trampoline bed with familiar bedding. The size of the testing pen and grass trays was large enough to allow all subjects in a group individual access to the grass and the surrounding area without crowding. Groups were exposed to one tray each of kikuyu and artificial grass in the testing area, and from outside the testing area, the experimenter (S. Bjone) recorded all occurrences (Altmann, 1974) of each behaviour (Table 4) for each puppy and mother. Whether the WM puppies ate the kikuyu grass before or after their mothers was recorded for the first testing session. A mini-DV camera also recorded each session for further review.

Each testing session was ten minutes in length and occurred during the morning of each testing day (at approximately 08:00-10:00 hr). Therefore, puppies were removed from their respective mothers for no more than 25 minutes per testing day (10 min for each group and time to change groups). At all other times mothers and their respective litters were together. In addition, the puppies were tested in groups to reduce the possible stress caused by separation. Testing was performed on three consecutive days for each of the fifth, sixth, and seventh weeks of age, totalling nine testing days.

Table 4

Behaviours recorded during testing sessions and their definitions. Those behaviours marked with an asterisk (*) were recorded for both kikuyu and artificial grasses.

Behaviour	**Definition**
Time spent eating grass*	The amount of time (in seconds) that a dog spent chewing the artificial grass or chewing and swallowing the kikuyu grass
Number of grass-eating events*	A grass-eating event occurred when a dog chewed (artificial grass) or chewed and ingested (kikuyu) grass until it stopped chewing for two or more seconds or moved to a new position
Number of non-eating grass interactions*	Any interaction with the grass which did not entail eating, such as sniffing or rubbing against grass
Number of vomiting events	A vomiting event occurred when a dog vomited and spasming stopped. A new event was documented if spasming recommenced and the dog vomited again.

Statistical Analysis

The total amount of time spent performing each behaviour and the total number of events (Table 4) were calculated for each subject per week (five, six, and seven weeks of age). Paired t-tests analysed the differences between each type of grass for those behaviours relevant to both grass types: the time spent eating, the number of eating events and the number of non-eating grass interactions.

The effect of puppy group and age on each behaviour was analysed using a mixed design ANOVA in SPSS® (Tabachnick & Fidell, 2007). Normality was assumed if the statistic for skewness (and kurtosis) divided by the standard error for skewness (and kurtosis) was less than three. Z-scores identified univariate outliers. If z was outside the range of ±3.3, there were no outliers. Hartley's Fmax tested the homogeneity of variance assumption. If the Fmax value was less than the Fmax critical value in Gravetter and Wallnau (2007), homogeneity of variance was assumed. Homogeneity of variance-covariance and sphericity were tested using Box's M and Mauchly's check, respectively, as provided in SPSS. All assumptions were met. Significance levels were set at $\alpha=0.05$. The strength of association was represented by partial eta-squared, $p\eta^2$, as provided by SPSS (Levine & Hullett, 2002; Tabachnick & Fidell, 2007). Bonferroni post hoc tests determined the significance of the differences between groups.

Results

Kikuyu versus Artificial Grass

The mothers did not chew the artificial grass, but did interact with it an average of seven times per week. Paired t-tests analysed the differences in the time spent eating, the number of eating events and the number of non-eating grass interactions between each type of grass for the puppies. The puppies spent significantly more time eating the kikuyu grass (mean ± SE:18.52 ± 1.07 min) than chewing on the artificial grass (6.54 ± 0.73 min; $t_{(25)}=10.18$, $p<0.001$). There were also significantly more kikuyu-eating events (53.81 ± 2.87) than artificial grass chewing events (24.42 ± 2.92; $t_{(25)}=9.92$, $p<0.001$). Conversely, there were significantly fewer kikuyu non-eating grass interactions (25.75 ± 1.85) than artificial grass interactions (31.50 ± 1.34; $t_{(25)}=-3.70$, $p=0.001$). The following analyses report on behaviours associated with kikuyu grass only.

Effect of Puppy Age and Mother

All of the puppies were observed eating grass regardless of the presence or absence of their mother. Generally, all puppies ate grass in each of the nine testing sessions, with a few exceptions. Two puppies did not eat grass until Day 3 and Day 4, respectively. One additional puppy did not eat grass on Day 2 and another puppy did not eat grass on Days 5 and 6. Therefore, 24 (Day 1), 23 (Day 2), 25 (Day 3), 26 (Day 4), 25 (Day 5), 25 (Day 6), 26 (Day 7), 26 (Day 8), and 26 (Day 9) puppies out of 26 ate grass for each respective testing day.

Of the puppies that were exposed to grass with their mothers present, 11 of the 13 (85%) ate grass before their mothers did. There were 1,399 grass-eating events and 482 minutes of grass eating recorded across nine testing sessions. Each puppy exhibited 6.0 grass-eating events (SE = 0.06) during an average of 2.1 min (SE = 0.02 min) spent eating grass per 10-minute testing session. There were very few vomiting events, six events, in the current study. One mother accounted for one vomiting event and three WM puppies accounted for the remaining five vomiting events.

Time spent eating grass

The amount of time the mothers spent eating grass did not vary across the three weeks of testing (p=0.41; Figure 1). The owner reported that all six bitches had been observed eating grass at least once while in their home environment, but the total time each mother spent eating grass during the current study was wide-ranging. Three mothers spent less than 2 min each eating grass (Karen, Laura, Legs: 0.0, 0.9, 1.8 min, respectively) whereas the other three mothers spent more than 10 min eating grass (Harriet, Briar, Trouble: 10.4, 11.0, 18.5 min, respectively). Therefore, the mothers were classified as High grass eaters if they spent more than 10 min eating grass or Low grass eaters if they spent less than 2 min eating grass.

A 2x2x3 ANOVA was used to test the differences in the amount of time spent grass eating between treatment group (WM, WOM), mother type (High, Low) and puppy age (five, six, seven weeks of age). There was no age by treatment group interaction (p=0.18), age by mother type interaction (p=0.92), or age by group by mother type interaction (p=0.72). However, there was a significant increase in grass eating from week five to weeks six and seven ($p<0.001$, $p\eta^2=0.34$; Figure 1).

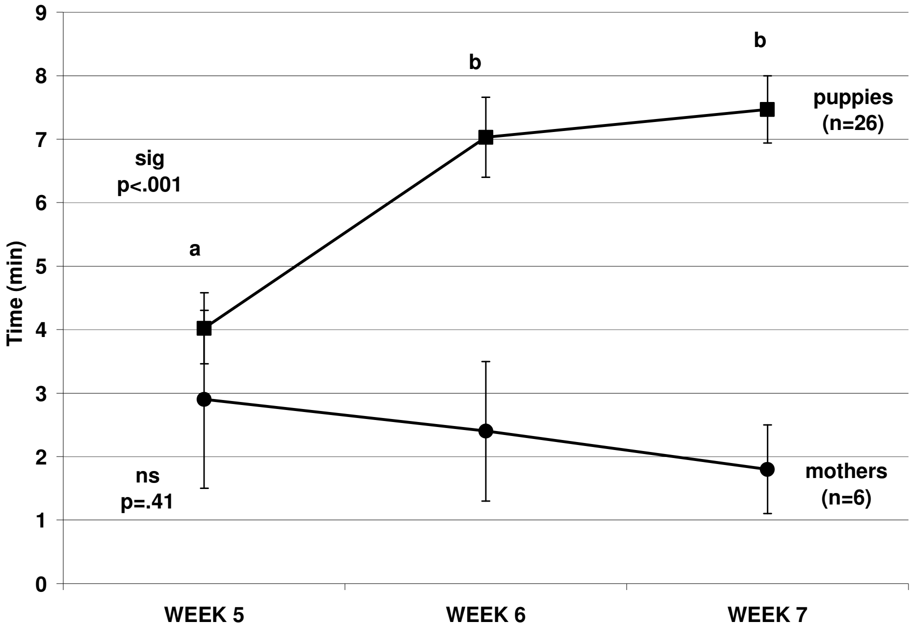

Figure 1. The mean total amount of time spent eating grass per week for the puppies and mothers. Different letters indicate significant differences. 'ns' indicates no significant difference. Error bars indicate ± standard error.

There was no main effect of treatment group (p=0.09) or mother type (p=0.24), but there was a significant group by mother type interaction (p<0.001; $p\eta^2$=0.49; Figure 2). Puppies that were with their high grass-eating mothers spent significantly more time eating grass than their littermates who were not with their mothers (p<0.001). However, there was no significant difference between puppies who were with their low grass-eating mothers and those that were not (p=0.06). In addition, puppies whose low grass-eating mothers were not present spent significantly more time eating grass than those whose high grass-eating mothers were absent (p=0.03). Puppies whose high grass-eating mothers were present spent significantly more time eating grass than puppies whose low grass-eating mothers were present (p=0.001). Table 5 lists the mean and standard error values for the time spent eating grass by group, mother type, and age. Figure 2 shows the interaction between mother type and treatment group.

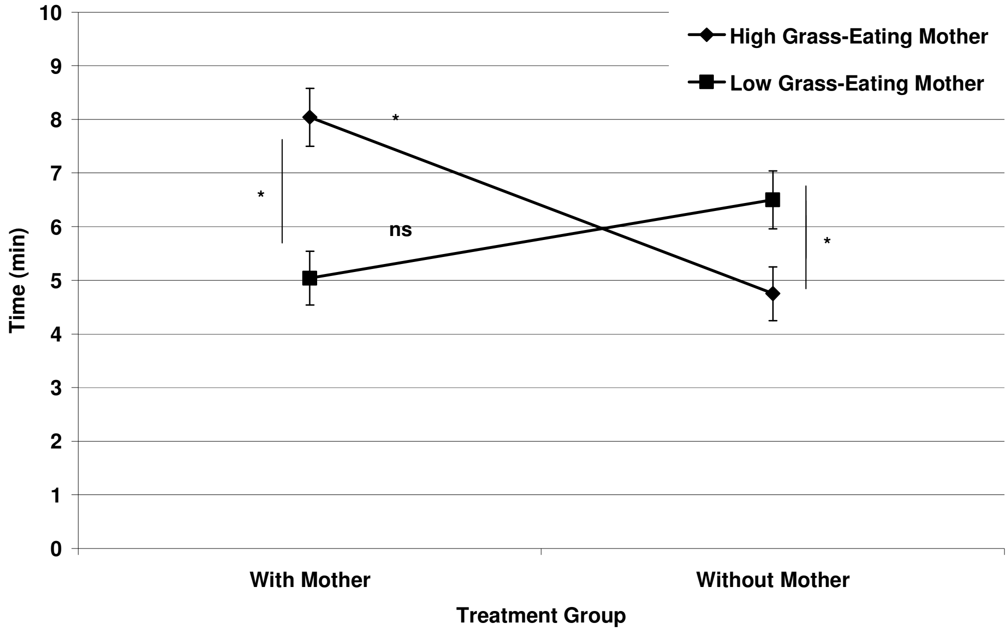

Figure 2. The interaction between the treatment group (with or without mother) and type of grass-eating mother (low or high) for the amount of time spent eating grass. An asterisk (*) indicates a significant difference. 'ns' indicates no significant difference. Error bars indicate ± standard error.

The number of grass-eating events and non-eating grass interactions

The number of grass-eating events of the mothers also did not vary across the three weeks of testing (p=0.68; Figure 3). The number of eating events for the mothers were also similarly divided as the time spent eating grass: three mothers had less than 10 grass-eating events (Karen, Legs, Laura: 0.0, 3.0, 8.0, respectively) whereas the other three mothers had more than 20 grass-eating events (Briar, Trouble, Harriet: 22.0, 25.0, 26.0, respectively). A 2x2x3 ANOVA was also used to analyse the effect treatment group, mother type, and puppy age on the number of grass-eating events and non-eating grass interactions.

Similar to the analysis for time spent eating grass, there was a significant increase in the number of grass-eating events from week five to weeks six and seven ($p<0.001$, $p\eta^2=0.43$; Figure 3). There was no age by treatment group interaction ($p=0.38$), age by mother type interaction ($p=0.41$), or age by group by mother type interaction ($p=0.43$). There was no main effect of treatment group ($p=0.73$) or mother type ($p=0.52$), but there was a significant group by mother type interaction ($p=0.001$; $p\eta^2=0.39$; Figure 4).

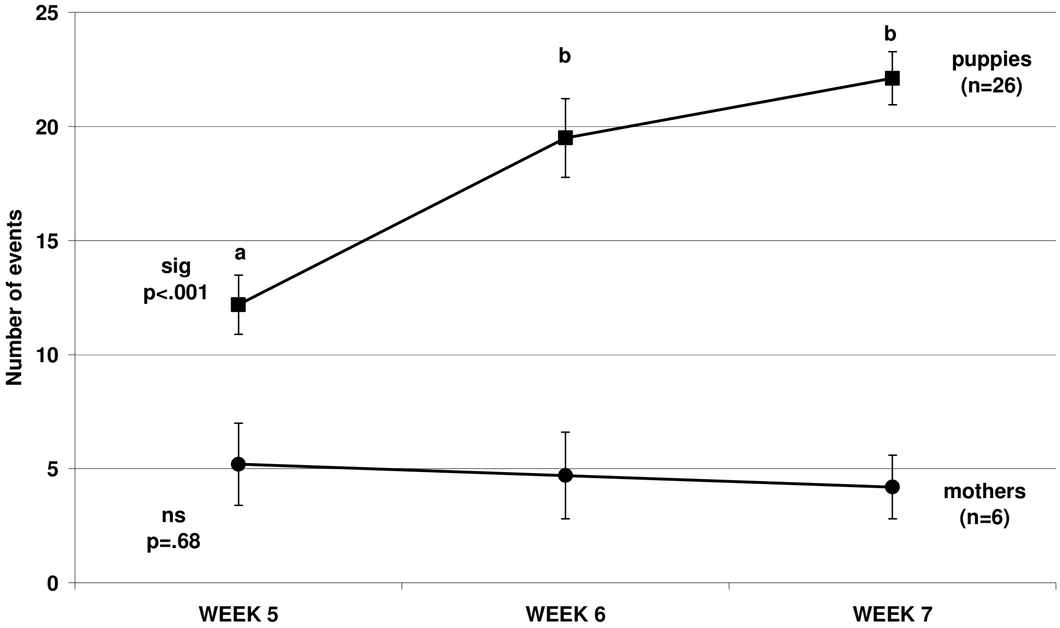

Figure 3. Mean total number of grass-eating events per week. Different letters indicate significant differences. 'ns' indicates no significant difference. Error bars indicate ± standard error.

Puppies that were with their high grass-eating mothers had significantly more grass-eating events than their littermates who were not with their mothers ($p=0.009$).

Puppies that were with their low grass-eating mothers had significantly fewer grass eating events that those without their mothers (p=0.03). In addition, puppies whose low grass eating mothers were not present had significantly more grass eating events than those whose high grass-eating mothers were absent (p=0.005). Puppies whose high grass-eating mothers were present had significantly more grass eating events than puppies whose low grass-eating mothers were present (p=0.04). Table 6 lists the mean and standard error values for the number of grass-eating events by group, mother type, and age. Figure 4 shows the interaction between mother type and treatment group for number of grass-eating events.

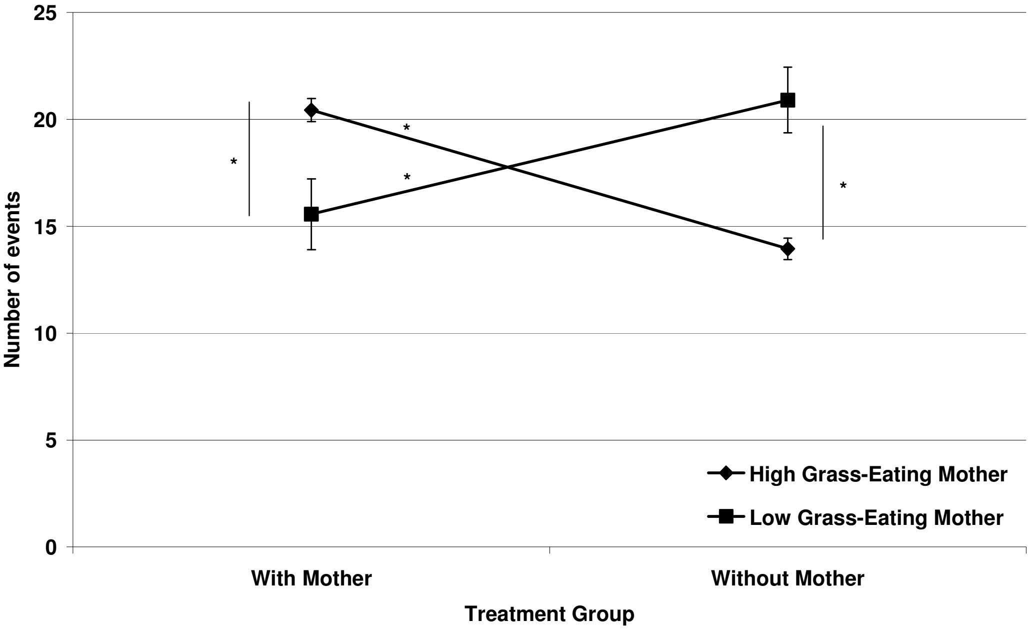

Figure 4. The interaction between the treatment group (with or without mother) and type of grass-eating mother (low or high) for the number of grass-eating events. An asterisk (*) indicates a significant difference. Error bars indicate ± standard error.

The mothers’ number of non-eating grass interactions did not vary across the three weeks (p=0.10). No significant differences were found in the puppies’ number of non-eating grass interactions across the three weeks (p=0.08), treatment groups (p=0.21), and mother type (p=0.19). Similarly, there were no significant interactions: age by treatment group interaction (p=0.78), age by mother type interaction (p=0.57), age by treatment group by mother type interaction (p=0.071), or treatment group by mother type interaction (p=0.08).

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Table 5

Mean and standard error values for the time spent eating grass (min) by treatment group (with or without mother), mother type (low or high grass-eater), and puppy age (5, 6, and 7 weeks of age)

	Week 5			Week 6			Week 7		
	Mother			Mother			Mother		
Treatment group	High mother	Low mother	Total	High mother	Low mother	Total	High mother	Low mother	Total
With mother	6.27±1.33	3.95±0.68	5.2±0.83	9.38±1.03	5.51±1.09	7.59±0.91	8.46±0.66	5.68±0.79	7.18±0.63
Without mother	1.86±0.46	3.69±1.02	2.85±0.62	5.21±1.78	7.55±0.44	6.47±0.88	7.19±1.13	8.25±1.35	7.76±0.87
Total	4.24±0.96	3.81±0.61	4.02±0.56	7.46±1.12	6.61±0.61	7.03±0.63	7.87±0.63	7.06±0.86	7.47±0.53

Table 6

Mean and standard error values for the number of grass-eating events by treatment group (with or without mother), mother type (low or high grass-eater), and puppy age (5, 6, and 7 weeks of age)

	Week 5			Week 6			Week 7		
	Mother			Mother			Mother		
Treatment group	High mother	Low mother	Total	High mother	Low mother	Total	High mother	Low mother	Total
With mother	15.71±1.82	11.67±2.33	13.85±1.51	21.00±1.83	15.83±3.07	18.62±1.81	24.57±3.16	19.17±2.51	22.08±2.12
Without mother	7±1.53	13.57±3.24	10.54±2.04	14.00±4.26	25.86±3.03	20.38±2.98	20.83±1.62	23.29±1.27	22.15±1.03
Total	11.69±1.71	12.69±1.99	12.19±1.29	17.77±2.32	21.23±2.52	19.50±1.72	22.85±1.87	21.38±1.41	22.12±1.16

Discussion

All three hypotheses were supported. The puppies' ages affected their grass-eating habits: as they matured, they spent more time eating grass and had more grass-eating events, supporting our first hypothesis. This is likely due to their natural development and the weaning process. During this time puppies progress from suckling to consuming solid foods by approximately six weeks of age (Case, 2005; Lindsay, 2000a). Puppies also have a natural propensity to explore their environment by chewing items within it (Lindsay, 2000a). However, the puppies in the present study were not consuming the kikuyu (real) grass just to fulfil their developmental need to chew. They had the opportunity to interact with both real kikuyu and artificial aquarium grass, but more time was spent eating the real grass than chewing the artificial grass. The puppies did exhibit more non-eating grass interactions with the artificial grass than the real grass. Thus, the puppies consumed the grass intentionally and not as a way to fulfil their chewing requirements.

The puppies may have learned to eat grass by observing their respective mother eating grass. Observational learning of food preferences has been documented in many species, but not in the domestic dog (Edwards, 1976; Gajdon et al., 2001; Nicol, 2004; Prescott et al., 2005; Saint-Dizier et al., 2007). The current study does not confirm this type of learning for grass eating in puppies. All of the puppies ate grass including puppies that were only exposed to grass in the absence of their mothers, and nearly all of the 'with mother' puppies ate grass before the mother did. This suggests that grass eating is not initially learned through observation, but is innate, supporting our second hypothesis. However, the mothers' exposure to grass before they entered the University dog facility was unknown and this may have confounded this result. It is possible that if the mothers ate grass peri-natally, they may have passed on a grass flavour to the puppies which could have altered their food preferences, similar to Hepper and Wells (2006; Wells & Hepper, 2006). Hepper and Wells found that pups exposed to aniseed both pre- and post-natally had a significantly higher preference for the aniseed diet as compared to pups exposed just pre- or post-natally. Controlling the mothers' environment from pregnancy through experimental procedures will help to eliminate this potential confound.

The mothers' grass-eating habits did influence their puppies' grass-eating behaviours, supporting our final hypothesis. There was an interactive effect between the type of mother, high or low grass eater, and the absence or presence of the mother. The presence of a mother that spent a considerable amount of time (>10 min) eating grass was associated with those puppies spending more time eating grass as compared to their littermates that did not have the mother present and those puppies whose minimal grass-eating mothers (<2 min) were present. However, puppies exposed to grass without their low grass-eating mother present spent more time eating grass than puppies without their high grass-eating mother. Possibly, the high grass-eating mothers uninhibited the grass-eating behaviours of their present puppies, whereas the limited grass eating of the low mothers may have inhibited grass eating in the present puppies. However, it is unclear why puppies without their low grass-eating mothers spent more time eating grass than those without their high grass-eating mothers.

Maternal influence on grass-eating behaviour in puppies could be further investigated by offering two grasses and observing the preferences of mothers and puppies. Studies investigating maternal influence on lambs' food preferences often present two or more grasses (Mirza & Provenza, 1994; Nolte et al., 1990). However, it should be noted that these studies are also aided by sheep's food neophobia which creates strong food preferences, whereas dogs are opportunistic omnivores (Lindsay, 2001).

The effect of littermates on puppies' grass-eating behaviours was not examined in the current study but it could have influenced the behaviour of the puppies. The puppies were intentionally tested in groups throughout the current study to reduce the potential stress induced by separation from littermates. The testing pen and grass trays were large enough so that each puppy could have individual access to the grass or surrounding areas of the pen without crowding or competition and there was more grass available than the puppies could eat. A study that examines the effect of littermates on grass-eating behaviour, would complement the current study by eliminating the mother variable. Methodologies similar to those of James (1960; James & Gilbert, 1955), which fed puppies individually and in groups without their mothers present, and the current study could be utilized for future research.

Some researchers contend that dogs use grass as an emetic (Fox, 1965; Hart, 1985; Houpt, 2005; Lindsay, 2001; Thorne, 1995). However, similar to Bjone et al.'s (2007) study with adult dogs, there were very few vomiting events in the current study with puppies: six events in comparison to 1,399 grass-eating events. This is consistent with Sueda et al.'s study (2008), which found a younger age was significantly associated with a decreased likelihood of vomiting after consuming plants.

The prevalence of grass eating in puppies, both with and without their mothers present, and in adult dogs (Bjone et al., 2007; Sueda et al., 2008) suggests that there may be a biological function for grass eating. The very low incidence of vomiting in the current study and in Bjone et al. (2007) suggests that the ‘emetic theory’ is not probable. However, both of those studies used dogs that were healthy and regularly de-wormed. Sueda et al. (2008) hypothesized that grass eating is a mechanism to expel worms. This theory has been suggested by other authors (Hart, 1985; Thorne, 1995). Gastrointestinal distress has also been suggested as a reason for grass eating (Engel, 2002; Fox, 1965; Overall, 1997). These two hypotheses warrant further investigation.

In conclusion, the current study indicates that grass-eating behaviour develops in five- to seven-week-old puppies through innate tendencies and is influenced by the mother’s grass-eating habits. The innate nature of grass eating further suggests that grass eating in the domestic dog is a normal behaviour and grass could be provisioned even for very young puppies. The high prevalence of grass eating in both adult dogs and puppies suggests a biological function to the behaviour, possibly to self-medicate a worm burden or gastrointestinal distress. Chapters 5 and 6, respectively, of the current project will address each of these theories in adult dogs.

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CHAPTER 5

Naturally Occurring Gastrointestinal Nematode Burden in Domestic Dogs (*Canis familiaris*) and Its Effect on Grass Eating

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Naturally occurring gastrointestinal nematode burden in domestic dogs (*Canis familiaris*) and its effect on grass eating

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Abstract

Grass eating is a common and normal behaviour in domestic dogs, but its function is not known. The current study tested the theory that domestic dogs eat grass to self-medicate a worm burden in a controlled, laboratory setting. Faecal samples were collected from dogs due for their quarterly de-worming and degree of hookworm (*Ancylostoma* and *Uncinaria* spp.), roundworm (*Toxocara canis*), and whipworm (*Trichuris vulpis*) infestation (number of eggs per gram of faeces) was determined. Dogs were presented with kikuyu grass (*Pennisetum clandestinum*) and grass-eating behaviours were observed. Half the dogs were de-wormed (n=9) and grass-eating behaviours were observed again. There was no difference in the amount of time spent eating grass or the number of grass-eating events between the dogs who were harbouring worms and those that were dewormed. The 47 vomiting events were more related to worm-burden status than grass eating. In conclusion, the current study does not substantiate the theory that dogs eat grass to self-medicate a naturally harboured worm burden. It may be beneficial to investigate further this theory using varied grasses and dogs with prolonged worm burden or those that are unaccustomed to periodic worm burden.

Introduction

Numerous explanations have been proposed for grass eating in dogs and wolves (Fox, 1965; Hart, 1985; Houpt, 2005; Murie, 1944; Thorne, 1995). Murie (1944) noted that wolves eat grass during all seasons. He proposed that it might be used as an emetic or an intestinal scour because some wolf scat contained grass as well as roundworms. He also observed a wolf grazing on grass for a few minutes; the wolf then vomited and left a watery scat. However, Murie's observations were made during a general survey of wolf ecology and do not represent systematic experimental findings. Nonetheless, these observations may have founded the theory that vomiting after grass ingestion by domestic dogs may help to eject parasites, such as worms (Hart, 1985; Thorne, 1995). Sueda, Hart, and Cliff (2008) also proposed that grass eating in domestic dogs is preserved through domestication as a possible mechanism to expel intestinal parasites.

A similar self-medication behaviour has been documented more extensively in wild chimpanzees (Huffman & Caton, 2001) and bonobos (Dupain et al., 2002). Huffman and Caton (2001) documented chimpanzees deliberately swallowing whole, bristly leaves without chewing and found a significant correlation between this behaviour and the expulsion of nodule worms (*Oesophagostomum stephanostomum*). The leaves were excreted whole and contained no known phytochemical properties, evidenced by chemical analysis and the passing of live, rather than dead, worms (Huffman, 1997). Therefore, it is believed that the physical roughness of the leaves creates a 'velcro effect' that scours worms.

This velcro effect would also be assisted by the life cycle of *Oesophagostomum*. Infection by *Oesophagostomum* worms is caused by ingestion of infective larvae which are on the vegetation eaten by the chimpanzee (Huffman, 2001). During development, the larvae encapsulate themselves in the small intestinal wall of the host. Adult worms then reside in the cavity of the caecum and large intestine (Anderson, 1992; Huffman, 2001) and presumably would be easier to expel than the larvae which are encapsulated in the intestinal wall.

The domestic dog harbours 3 main gastrointestinal nematodes: hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*), roundworm (*Toxocara canis* and *Toxoscaris leonina*), and whipworm (*Trichuris vulpis*; Jacobs et al., 1994). Hookworm infection is caused by ingestion of food or water contaminated with infective larvae or by their penetration of the skin (Georgi & Georgi, 1992). *A. caninum* can also be passed via the mother's milk to her pups (Roberson & Cornelius, 1980). The larvae settle in the small intestine and haemorrhaging begins within about eight days after infection when the larvae digest plugs of mucous from the small intestinal mucosa (Roberson & Cornelius, 1980). After ingesting several plugs, the adult worms travel to a new site, leaving behind small haemorrhages. One adult *A. caninum* can remove approximately 1.0 ml of blood each day from the host (Jubb & Kennedy, 1970). The prepatent period of hookworm, or the period from initial infection to faecal egg detection, is two to three weeks (Jacobs et al., 1994).

The roundworms, *T. canis* and *T. leonina* infect via oral ingestion (Taylor, 2007). The adults reside in the small intestine (Roberson & Cornelius, 1980), but pathogenic effects are attributed to the larval stages (Jubb & Kennedy, 1970). *T. canis* have liver-lung or somatic migration, including prenatal and transmammary transmission, so its larvae cause lesions along these migratory pathways whereas *T. leonina* are confined to the gut mucosa (Anderson, 1992; Roberson & Cornelius, 1980). The prepatent period of *T. canis* is four to six weeks whereas *T. leonina* is greater than eight weeks (Jacobs et al., 1994).

T. vulpis whipworm is transmitted by ingestion of the eggs often from a contaminated environment (Nitsche, 2007). The adult worm resides in the large intestine, particularly the caecum, and has a prepatent period of one to three months (Anderson, 1992; Jacobs et al., 1994; Jubb & Kennedy, 1970; Roberson & Cornelius, 1980). The adult attaches to the mucosa by burying its head in the folds, making it difficult to detach (Jubb & Kennedy, 1970). Whipworm diagnosis can be difficult because eggs are frequently absent from faeces (Nitsche, 2007). However, dogs can exhibit soft, mucoid, and bloody diarrhoea, which helps to identify this particular worm infection (Roberson & Cornelius, 1980).

All of these gastrointestinal nematodes reside in the small or large intestine of the domestic dog (Georgi & Georgi, 1992; Jubb & Kennedy, 1970; Roberson & Cornelius, 1980), similar to the nodule worms which reside in the large intestine of the chimpanzee (Anderson, 1992; Huffman, 2001). Therefore, the ingestion of grass may scour worms from the intestines of the domestic dog. The current study intended to test the theory that domestic dogs eat grass to self-medicate a worm burden in a controlled, laboratory setting. Grass may act as an emetic to eject worms via the mouth or to purge worms through the digestive tract.

Kikuyu grass (*Pennisetum clandestinum*) was used in the current study. It is a robust, creeping perennial with soft blades (Muyt, 2001). Kikuyu's hairless or lightly haired blades are similar to the blades of couch grass that herbalist Mességué (1991) briefly conjectured dogs use as a purgative. Grass was presented to dogs with and without a naturally occurring worm burden to determine the effect of a worm burden on grass-eating behaviour in domestic dogs. We hypothesized that dogs would spend more time eating grass while harbouring a nematode burden than when worm-free. Advancing the knowledge on this subject would be beneficial to veterinarians, behaviourists, and dog owners and breeders.

Method

Subjects, Housing, and Diet

Eighteen female mixed breed dogs, *C. familiaris*, due for their three-monthly de-worming were selected for the study. All were known to eat grass. Dogs were of a similar age (mean ± standard error (SE): 3.6 ± 0.4 yr) and weight (mean ± SE: 5.2 ± 0.3 kg). All dogs were housed in compatible pairs in kennels at the University of New England, Armidale, NSW, in pens within the centrally heated facility with good ventilation and adjoining outdoor runs. Trampoline-style dog beds were provided in indoor pens. Kennels were provided in outdoor runs for additional shelter.

The dogs became accustomed to the daily routine and diet during a seven-day habituation period before testing began. The dogs were fed Pedigree Meaty-Bites Working Dog® (metabolizable energy=350 kcal/100 g) daily, at maintenance energy requirements (MER), as determined by the formula MER (kcal) = 140 x $BW(kg)^{0.75}$. Any refusals were weighed and recorded. Dogs were weighed weekly and feed amounts were adjusted as necessary to maintain ideal body weight. Water was available *ad libitum*. Dogs were socialized and exercised in compatible groups daily.

Ethics

The UNE Animal Ethics Committee (AEC06/088) granted authority to conduct this study, in accordance with Section 25 of the Animal Research Act (1985). Animals received the highest standard of care throughout the study, in accordance with UNE Animal Ethics Committee guidelines. A qualified veterinarian examined all dogs before experimental procedures began and any animal that was considered unsuitable based on health or temperament was excluded from the study.

Procedure

A worm burden was not artificially induced in the study dogs. Because the study dogs normally lived outside, co-habited with many other dogs (all of which had not been treated with anthelmintics for 3 months), co-habited with various farm animals (cattle, sheep, pigs, geese), and typically ate a varied diet including raw meats and offal, they were likely to be naturally harbouring intestinal nematodes. To confirm this, faecal samples were collected from all dogs selected for the study and the degree of worm infestation (number of eggs per gram of faeces) for each dog was determined by faecal flotation using the McMaster Method (Coles et al., 1992). Faecal flotation is the currently accepted method of determining worm burden, besides necropsy, despite there being a weak correlation between faecal egg count and actual hookworm burden (Kopp, Coleman, McCarthy, & Kotze, 2008; Kopp, Kotze, McCarthy, & Coleman, 2007; Krupp, 1961). Samples were collected over three consecutive days, for a minimum of three samples, because some worm eggs are not shed uniformly across faeces and can even be absent (Nitsche, 2007; Roberson &

Cornelius, 1980; Roth-Johnson, 2007). Hookworm (*A. caninum* and *U. stenocephala*), roundworm (*T. canis*), and whipworm (*T. vulpis*) eggs were counted.

Once each dog's worm burden was determined (during the habituation period), dogs were allocated to compatible testing pairs according to owner recommendation. Each pair of dogs was presented with two 20 cm diameter (19cm deep) pots of kikuyu grass, with grass blade length of approximately 40 cm, daily for 10 minutes over six days (Period 1). Grass pots were randomly selected from 10 available pots for each session. Grass-eating behaviours (Table 7) were observed and videotaped for further review. Dogs were then randomly stratified into two groups (Treatment and Control) of equivalent amounts of time spent eating grass.

The Treatment group (n=9) was treated with Drontal Allwormer® tablets (Bayer Australia Ltd., Pymble, NSW, Australia) while the Control group maintained their worm burdens. Faecal samples were again collected for faecal flotation to confirm the absence of intestinal worms in the Treatment group and degree of worm infestation in the Control group before progressing to Period 2. The testing procedure was then repeated for Period 2 and grass-eating behaviours were recorded daily over six days. The Control dogs were treated with Drontal Allwormer® upon completion of the study and all dogs were confirmed worm-free before returning to their owner.

At the conclusion of each testing period, a single blood sample (4 ml) was collected from each dog as a single indicator of hemopoetic status and analysed for complete blood count using a Cell-Dyn 3500R haematology unit.

Table 7

Behaviours recorded during testing sessions and their definitions

Behaviour	**Definition**
Time spent eating grass	The amount of time (in seconds) that a dog spent chewing and swallowing the kikuyu grass
Number of grass-eating events	A grass-eating event occurred when a dog ingested grass until it stopped chewing for two or more seconds or moved to a new position
Number of non-eating grass interactions	Any interaction with the grass which did not entail eating, such as sniffing or rubbing against grass
Number of vomiting events	A vomiting event occurred when a dog vomited and spasming stopped. A new event was documented if spasming recommenced and the dog vomited again.

Statistical Analysis

The total amount of time spent performing each behaviour and the total number of events for each dog per group and period were analysed using mixed design ANOVA in SPSS® (Tabachnick & Fidell, 2007). Normality was assumed if the statistic for skewness (and kurtosis) divided by the standard error for skewness (and kurtosis) was less than three. Z-scores identified univariate outliers. If z was outside the range of ±3.3, there were no outliers. Hartley's Fmax tested the homogeneity of variance assumption. If the Fmax value was less than the Fmax critical value in Gravetter and Wallnau (2007), homogeneity of variance was assumed. Homogeneity of variance-covariance and sphericity were tested using Box's M and Mauchly's check, respectively, as provided in SPSS. All assumptions were met.

Significance levels were set at $\alpha=0.05$. The strength of association was represented by partial eta-squared, $p\eta^2$, as provided by SPSS (Levine & Hullett, 2002; Tabachnick & Fidell, 2007). Bonferroni post hoc tests determined the significance of the differences between groups. Pearson correlations determined relationships between two variables. Paired sample t-tests determined significant differences in worm burden between periods.

Results

Results of faecal floatation found all dogs had a naturally occurring hookworm (*A. caninum* and *U. stenocephala*) infestation at the commencement of the experiment. Several dogs were also carrying roundworm (*T. canis*) and whipworm (*T. vulpis*). Paired-sample t-tests determined that the Control dogs maintained similar worm burdens throughout both testing periods: there were no significant differences in the hookworm ($p=0.13$), whipworm ($p=0.33$), or roundworm ($p=0.32$) burdens between periods 1 and 2 (Table 8). No worms were detected in faeces of dogs following treatment with the anthelmintic. All haematology values remained within the normal range for dogs during both testing periods (Table 8).

Table 8

Mean ± standard error faecal egg count and haematology values for each group and testing period. Control dogs retained their worm burden throughout the study while Treatment dogs were treated with an anthelmintic after Period 1.

		Faecal egg count			Haematology		
	Group	Hookworm	Whipworm	Roundworm	RBC[a]	HGB[b]	HCT[c]
Period 1	Treatment	1721±565	66±52	123±79	7.3±0.2	17.3±0.5	50.5±1.5
	Control	1821±671	12±12	85±82	6.8±0.3	16.0±0.5	47.4±2.1
Period 2	Treatment	0	0	0	7.0±0.3	15.7±0.7	48.5±2.0
	Control	949±312	57±56	22±22	6.6±0.2	15.7±0.4	46.1±1.4

[a] Normal range for red blood count (RBC): 5.5-8.5x10^{12}cells/l
[b] Normal range for haemoglobin (HGB): 12.0-18.0 g/dl
[c] Normal range for haematocrit (HCT): 37.0-55.0 vol. %
(normal ranges according to Bentinck-Smith, 1980)

There was no significant difference in the amount of time spent eating grass for either period (p=0.16), group (p=0.94), or the interaction term (p=0.99). There was also no significant difference in the number of grass-eating events for period (p=0.55), group (p=0.58), or the interaction term (p=0.71). Each behaviour's mean and standard error values are listed in Table 9.

Table 9

Mean ± standard error values for all behaviours for each period and treatment group. Control dogs retained their worm burden throughout the study while Treatment dogs were treated with an anthelmintic after Period 1.

	Period 1		Period 2	
Behaviour	Treatment	Control	Treatment	Control
Time eating grass (min)	14.6±3.1	14.2±3.5	12.5±3.3	12.1±3.2
Number of grass-eating events	38.4±7.3	31.6±5.3	34.7±8.5	30.7±7.7
Number of non-eating grass interactions	58.0±7.9	45.8±5.6	75.0±11.9	50.9±6.7
Number of vomiting events	2.3±0.9	0.6±0.4	1.0±0.5	1.3±0.5

There were significantly more non-eating grass interactions during Period 2 than Period 1 (p=0.01; $p\eta^2$=0.35), but there was no significant difference between the treatment groups (p=0.12). In addition, there was no interaction between the treatment groups and the period (p=0.14; Figure 5).

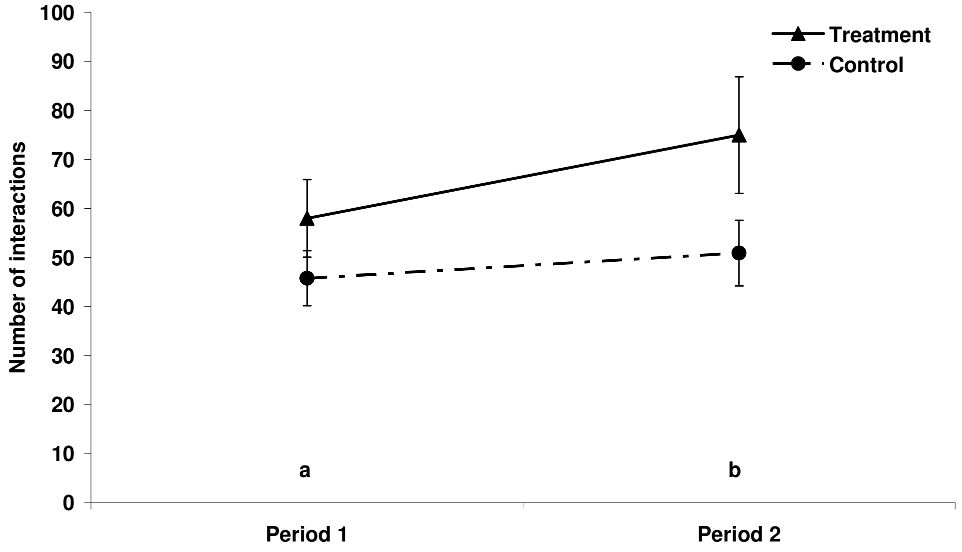

Figure 5. The mean total number of non-eating grass interactions for each group and period. Different letters indicate significant differences. Error bars indicate ± standard error.

There were 47 vomiting events throughout the study. The Treatment dogs had a total of 21 vomiting events while they harboured worms during Period 1 and nine vomiting events during Period 2 after they were dewormed. The Control dogs, which maintained their worm burdens throughout both periods, had a total of five vomiting events during Period 1 and 12 events during Period 2. A mixed design ANOVA indicated that there was no significant difference in the number of vomiting events for either period (p=0.68), treatment group (p=0.21), or the interaction term (p=0.14).

In summary, infestation with gastrointestinal nematodes had no effect on the amount of time spent eating grass or the number of grass-eating events observed in the dogs in this study. However, the dogs exhibited significantly more non-eating interactions with the grass during Period 2 than Period 1.

There were no significant correlations between the amount of time spent eating grass in Period 1 and any of the worm counts during that period: hookworm ($r_{(16)}$= -

0.15, p=0.54), whipworm ($r_{(16)}$=0.30, p=0.23), and roundworm ($r_{(16)}$=0.31, p=0.22). There were also no significant correlations between the number of grass-eating events and the worm counts for Period 1: hookworm ($r_{(16)}$= 0.06, p=0.83), whipworm ($r_{(16)}$=0.40, p=0.10), and roundworm ($r_{(16)}$= -0.05, p=0.86).

Discussion

We hypothesized that dogs infested with gastrointestinal nematodes would spend more time eating grass than dogs that were worm-free. Contrary to our hypothesis, no difference in grass-eating behaviour was observed between dogs that were carrying worms and dogs that were not. Therefore, the data from the current study do not support the theory that dogs eat grass to self-medicate a worm burden.

The number of non-eating grass interactions increased over time, which is dissimilar to the *Grass-Eating Patterns* (Chapter 3) and *Development* studies (Chapter 4), which had no significant differences, and had the same procedure of randomly selecting grass pots for each testing session. However, it is possible that smells from previous dog interactions accumulated on the grass throughout the studies. Dogs use odours as a form of communication (Houpt, 2005) and they possess a renowned sense of smell which is 100 times stronger than that of humans for some odours (Moulton, Ashton, & Eayrs, 1960). Dogs discriminate the smells of family members and even identical twins when tracking (Kalmus, 1955) and they can detect odours of fingerprints left on glass up to six weeks prior (King, Becker, & Markee, 1964). Therefore, it is plausible that the number of non-eating grass interactions, such as sniffing or rubbing on the grass, increased with time in the current study due to odours lingering on the grass.

The self-medication-of-a-worm-burden theory supposes that dogs may vomit to eject parasites from the mouth or they may also try to purge their system of parasites from the anus. Most of the anecdotal evidence in wolves (Murie, 1944), dogs (Engel, 2002), bears, and geese (Huffman, 1997), indicates that grass is passed intact through the digestive tract and is defecated with worms. However, only one live adult worm was seen in the faeces of dogs in the current study, contradictory to chimpanzees' use of leaves to medicate their worm burden and the resultant appearance of live worms around the leaves within faeces (Huffman, 1997; Huffman & Caton, 2001).

Herbalist Maurice Mességué (1991) claims that some dogs use hairy grasses for emetics and couch grass as a purgative, but he did not substantiate this claim. Kikuyu, which was used in the current study, is a lightly haired grass, similar to couch grass. However, chimpanzees utilize "bristly" leaves (Huffman & Caton, 2001), so it is possible that dogs may still utilise "hairier" grasses differently than kikuyu grass to eject worms.

In addition, vomiting incidences in the current study appear to be more related to worm burden than grass eating. Although there was no significant difference in vomiting events for either group or period as analysed by repeated measures ANOVA, trends in the number of vomiting episodes were evident. Those dogs that carried their worms throughout both testing periods vomited more in Period 2 than Period 1, possibly from carrying the worms for an extended time. Conversely, those dogs that were de-wormed before Period 2 vomited less during this period. Therefore, the incidence of vomiting was at least partially related to carrying a worm burden and may not have been altered by the type of grass available.

Dogs from both groups spent similar amounts of time eating grass during each period. In addition, there was a significant positive correlation between the amount of time spent eating grass and the number of vomiting events in Period 1 when all dogs were harbouring worms, but not during Period 2 when half of the dogs were de-wormed. Thus, it is likely that the vomiting events were a symptom of the worm burden and not the ingestion of grass.

The study dogs were active, bright, and alert throughout the study, and exhibited very few external symptoms of their worm burdens. There were no discernible behavioural differences between the Treatment dogs after de-worming and the Control dogs still harbouring worms. Several dogs did occasionally produce mucoid, soft stools outside of testing sessions, with only one dog consistently producing soft stools. Loose, mucoid stools are clinical signs of whipworm and roundworm infestations (Jacobs et al., 1994). This general absence of clinical signs suggests that the degree of worm infestation present in the dogs in this study was not sufficient to compromise animal health. This is further supported by the haematology values, which remained within the normal range throughout the study.

The dogs used in the current study were wormed quarterly and were accustomed to a varied diet including raw meats and offal, potentially containing worms. This likelihood of harbouring worms made these dogs ideal subjects for the current study, but may have also made them hardier than dogs not periodically exposed to parasites. Consequently, although there was no difference between the grass-eating behaviour of the dogs with and without worms in the current study, it is possible that dogs unaccustomed to worm infestation may respond differently. In particular, younger dogs, notably puppies, are less accustomed and more susceptible to the pathogenic effects of worms than adult dogs which can develop resistance (Case, 2005; Stroup, 2007). While seriously infected puppies may provide a better model to test the self-medication theory, puppies were not used in the current study due to ethical concerns and the difficulty of gaining owner consent to use puppies for such a study.

A worm burden that is maintained for a minimum of four to five months would allow the intestinal worms documented in the current study to reach adulthood and their respective prepatent periods. In the current study, the dogs had been de-wormed three months prior and were due for their three-monthly de-worming. Three months following receipt of a broad-spectrum anthelmintic may not have allowed *T. vulpis* to reach the full potential of its one to three month prepatent period (Anderson, 1992; Jacobs et al., 1994; Jubb & Kennedy, 1970; Roberson & Cornelius, 1980). Once the intestinal worm burdens are established, their pathogenic effects may also become more apparent in the haematology values. Therefore, it is recommended that dogs unaccustomed to regular worm burden and dogs who have maintained worm burdens for an extended period of time are studied in future research.

In conclusion, the current study does not substantiate the theory that dogs eat grass to self-medicate a worm burden. It may be beneficial to investigate further this theory using varied grasses and dogs with prolonged worm burdens or those that are not accustomed to periodic worm burden.

Acknowledgements

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CHAPTER 6

Grass-Eating Behaviours in the Domestic Dog, *Canis familiaris*, in Response to a Mild Gastrointestinal Disturbance

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Abstract—193

Grass-eating behaviours in the domestic dog, *Canis familiaris*, in response to a mild gastrointestinal disturbance

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Abstract

The purpose of grass-eating behaviour in the domestic dog remains unknown. The current study investigated the theory that grass could be used to moderate gastrointestinal distress. Grass-eating behaviours were observed in dogs fed a standard diet with and without supplementation of a fructooligosaccharide, which temporarily induced loose, watery stools similar to a mild gastrointestinal disturbance. We hypothesized that dogs would spend more time eating grass when they had diarrhoea compared to when they had normal stools. The current study made several novel findings. Dogs exhibited a strong significant preference for kikuyu (*Pennisetum clandestinum*) compared to couch (*Cynodon dactylon*) grass. Dogs did not use grass as an emetic, as there were only two vomiting events and 374 grass-eating events observed. Dogs spent significantly more time eating grass when fed the standard diet and producing normal stools than when they were fed the supplemented diet and producing loose stools. The results of this study do not support the theory that dogs eat grass in response to a gastrointestinal disturbance. For further clarity, future research should examine the effect of other gastrointestinal disturbances, such as constipation (hard, dry stools) on grass-eating behaviour in the domestic dog.

Introduction

Grass eating is a common behaviour in domestic dogs, *Canis familiaris*, but there is very little research on this behaviour (Lindsay, 2001). Bjone et al. (2007) conducted the first controlled experiment on grass-eating behaviour. In particular, they investigated the pattern of grass eating during the day and the relationship between grass eating and the ingestion of food. The study concluded that grass eating is influenced by satiety and time of day: the subjects were less likely to eat grass when they were satiated and the amount of time spent eating grass decreased throughout the day.

Bjone et al. (2007) suggested that grass may be seen as a food source despite dogs having almost no capacity to digest the plant fibre in grass (Case et al., 2000). Kang et al. (2007) suggest that plant eating is due to a dietary deficiency, particularly fibre. Other researchers suggest that grass may be used to self-medicate some form of gastrointestinal distress (McKeown, 1996; Overall, 1997) as an emetic (Fox, 1965; Hart, 1985; Houpt, 2005; Thorne, 1995) or a laxative (Hart, 1985; McKeown, 1996). However, no scientific research has investigated these claims and gastrointestinal distress is a broad term that could encompass many “digestive upsets,” such as nausea, constipation, or diarrhoea.

The current study investigated the theory that grass could be used to moderate mild gastrointestinal distress by observing grass-eating habits in dogs fed a standard diet with and without BeneoP95® (ORAFTI, Tienen, Belgium), a commercially available fructooligosaccharide (FOS). BeneoP95®, formerly known as RaftiloseP95®, passes undigested through the small intestine and readily ferments in the large intestine. It is added to commercial dog diets as a prebiotic to stimulate growth of potentially health-enhancing intestinal bacteria in the colon (Fahey et al., 1990; Flickinger, Van Loo, & Fahey, 2003). However, in larger doses FOS can degrade faecal consistency and induce loose, watery stools.

The inclusion of 5% FOS had no effect on faecal consistency (Strickling, Harmon, Dawson, & Gross, 2000), but a 6% inclusion of BeneoP95® produced wetter, looser stools (Twomey et al., 2003). At 6% inclusion, the number of unacceptable stools increased but still remained within the ‘ideal’ range. Fahey et al. (1990) documented a similar effect with supplementation of 12.5% sugar beet pulp, a moderately fermentable insoluble fibre, which significantly increased the defecation frequency and water content of stools. In addition, Flickinger et al. (2003) noted that supplementation greater than 20% in adjusted animals or greater than 10% in animals which have not adjusted to the supplementation may cause flatulence and loose stools. Therefore, 10% supplementation of BeneoP95® was used to induce diarrhoea (loose, watery, frequent stools) in the current study to investigate grass-eating behaviours in dogs in response to a mild gastrointestinal disturbance.

The average whole gut transit time in dogs has been documented to be 37 ± 10.4 hr across 13 different dog breeds, ranging from dachshunds to Giant Schnauzers (Hernot, Biourge, Martin, Dumon, & Nguyen, 2005). Therefore, the supplemented diet was fed on two consecutive days during the testing period, followed by a three-day washout period. Number of defecations per day, faecal consistency, and faecal dry matter were documented to verify the effect of the supplementation.

Dogs have been anecdotally documented to self-medicate using couch grass (de Baïracli Levy, 1992; Engel, 2002). Therefore, preferences for couch (*Cynodon dactylon*) or kikuyu (*Pennisetum clandestinum*) grasses were also investigated. Both grasses have been used in previous research (Bjone et al., 2007).

We hypothesized that the dogs would spend more time eating grass when fed the supplemented diet than the standard diet. We also hypothesized that the dogs would prefer couch grass compared to kikuyu grass because couch has been indicated in self-medication.

Method

Subjects, Housing, and Diet

Twelve beagle dogs (3 males; 9 females) of similar age (mean ± standard error (SE) = 3.7 ± 0.5 yr) and weight (mean ± SE = 13.3 ± 0.3 kg) were housed at the University of New England Dog Research Facility for the duration of the study. The dogs were accustomed to the daily routine and diet during a five-day habituation period. All dogs were de-wormed using Drontal Allwormer® (Bayer Australia Ltd., Pymble, NSW, Australia) on the first day of the habituation period. The dogs were fed a nutritionally complete and balanced commercial, meat-based diet consisting of dry (Enduro Plus®; Petco Australia Pty Ltd, Buranda, QLD) and canned food (Pedigree® Advance™ Adult Dog with chicken and rice; MasterFoods ANZ; Wodonga, VIC) once daily between 15:00-17:00 hr. The diet was fed in amounts calculated to meet maintenance energy requirements and adjusted as necessary to maintain ideal body weight. The canned food facilitated easy administration of the supplement during the test periods and only equated to, on average, 32 kcal (SE=0.5 kcal) of energy out of a total of 1,022 kcal (SE=21 kcal) for each dog. Fresh water was available *ad libitum*.

Dogs were individually housed to facilitate collection of faeces and faecal data from individual dogs. Each indoor kennel was outfitted with a trampoline style bed within a secure, centrally heated facility where dogs slept at night. During the day, dogs were placed in spacious, fully covered, outdoor runs. Dogs were socialized and exercised in compatible groups twice daily under supervision.

Materials

Couch (*C. dactylon*) and kikuyu (*P. clandestinum*) grasses grown in 20 cm diameter (19cm deep) pots in a climate-controlled greenhouse were used in the study. Both grasses have hairless or lightly haired blades and they spread through creeping underground and aboveground stems (Muyt, 2001). Kikuyu is a slightly thicker grass with wider (4-10 mm) and longer (5-30 cm) blades and longer underground stems (up to 50 cm) than couch grass (blades 2-4 mm wide, 2-15 cm long; underground stems up to 30 cm) (Muyt, 2001; Spencer, 2005). Both grasses have stems which can grow up to 120 cm tall (Göhl, 1981).

The canned diet (Pedigree® Advance™; ME=125 kcal/100 g) contained 7% crude protein, 8% crude fat, 4% crude fibre, 4% ash, 1% salt as NaCl, 0.36% calcium, 0.29% phosphorus and not more than 78% moisture. The dry diet (Enduro Plus®; ME=403 kcal/100 g) contained 25% crude protein, 14% crude fat, <5% crude fibre, 2.5% calcium, 1.2% phosphorus, 1.14 Omega 6, 0.2 Omega 3, 1% Lioneleic Acid, and <1% NaCl.

BeneoP95® (ORAFTI, Tienen, Belgium) consists of 92% oligofructose (average degree of polymerisation: 2 - 7), 3% H_2O, and a 5% mixture of glucose, fructose, and sucrose. This supplement was added to the diet at an inclusion rate of 10% dietary dry matter to create the supplemented standard diet, which was intended to induce an osmotic-type diarrhoea originating from the large intestine.

Ethics

All procedures was undertaken in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, 1997). All dogs were privately owned, and written permission was obtained from the owners for the inclusion of their animals in the study. Animals received the highest standard of care throughout the study, in accordance with UNE Animal Ethics Committee guidelines (AEC07/144). In addition, a veterinarian examined each dog to ensure it was healthy and fit to participate in the study. All dogs were returned to the owners at the end of the study.

Procedure

The study consisted of one habituation period and replicate baseline and test periods in the following order: Habituation, Baseline 1, Test 1, Washout, Baseline 2, Test 2, and Washout. All dogs were tested on this same schedule (Habituation, Base, Test) for consistency, so that all dogs had the same number of days to adjust to the diet and the kennels before testing began in the baseline period and then the test period. Baseline and Test periods were each two days in length and were followed by three washout days. A ‘day’ began once the dogs were fed their relevant diet in the afternoon (at approximately 16:00 hr) through observation the next morning (at approximately 10:00 hr) until feeding that afternoon. All dogs were fed the standard diet (SD) during the baseline and washout periods and the supplemented standard diet

(SSD) during the test periods. Therefore, the dogs received two SSDs for each test period.

Number of defecations per day, faecal consistency, and faecal dry matter were documented during the baseline and testing periods. The experimenter (S. Bjone) collected this information during two periods each day (07:00-11:00 hr and 15:00-18:00 hr). Faecal consistency was measured using the WALTHAM Faeces Scoring Scale. The WALTHAM Scale uses quarter-grade increments from a chart with photographs corresponding to each half grade. The scoring scale is generally as follows: 1=hard, dry, crumbly; 2=well formed, does not leave a mark; 3=moist, beginning to lose form; 4=most, if not all, of the form is lost, viscous; 5=watery diarrhoea.

Individual stool samples were collected in labelled plastic zip-lock freezer bags and stored at -20°C for later dry matter analysis. At the end of each testing period, individual wet faeces weights were recorded and samples were dried in foil trays at 80°C (for approximately three days) to constant weight and then weighed to determine dry faeces weights for each sample and dog. Faecal dry matter was determined by the formula:

$$\%\ \text{dry matter} = (\text{dry faeces weight} / \text{wet faeces weight}) \times 100$$

Dogs were observed in compatible pairs during one 10-minute testing session (at approximately 10:00 hr) per day of each Test and Baseline period. Pairs were presented with one pot each of kikuyu and couch grass, and from the adjoining kennel, the experimenter (S. Bjone) recorded all occurrences (Altmann, 1974) of each behaviour (Table 10). A mini-DV camera also recorded each session for further review.

Table 10

Behaviours recorded during testing sessions and their definitions. Those behaviours marked with an asterisk (*) were documented for both couch and kikuyu grasses.

Behaviour	Definition
Time spent eating grass*	The amount of time (in seconds) that a dog spent chewing and swallowing the grass
Number of grass-eating events*	A grass-eating event occurred when a dog ingested grass until it stopped chewing for two or more seconds or moved to a new position
Number of non-eating grass interactions*	Any interaction with the grass which did not entail eating, such as sniffing or rubbing against grass
Number of vomiting events	A vomiting event occurred when a dog vomited and spasming stopped. A new event was documented if spasming recommenced and the dog vomited again.

Statistical Analysis

Grass-eating behaviours (total time spent eating grass and total number of grass-eating events, non-eating grass interactions, and vomiting events for each dog) were analysed using a repeated measures ANOVA (Tabachnick & Fidell, 2007). Normality was assumed if the statistic for skewness (and kurtosis) divided by the standard error for skewness (and kurtosis) was less than three. Z-scores identified univariate outliers. If z was outside the range of ±3.3, there were no outliers. Hartley’s Fmax tested the homogeneity of variance assumption. If the Fmax value was less than the Fmax critical value in Gravetter and Wallnau (2007), homogeneity of variance was assumed. Homogeneity of variance-covariance and sphericity were tested using Box's M and Mauchly's check, respectively, as provided in SPSS. All assumptions were met.

Significance levels were set at $\alpha=0.05$. The strength of association was represented by partial eta-squared, $p\eta^2$, as provided by SPSS (Levine & Hullett, 2002; Tabachnick & Fidell, 2007). Bonferroni post hoc tests determined the significant differences between groups. Number of defecations per day, faecal consistency, and faecal dry matter were similarly analysed across both baseline and both test periods to confirm the supplemented diet did induce loose, watery stools.

Results

The study dogs readily ate both the standard and supplemented standard diets and there were no food refusals. Faecal scoring during the first baseline period verified that all dogs were producing 'normal' stools (1.5-2.5) when fed the standard diet. The supplemented standard diet did induce loose, watery faeces as exhibited by significantly greater faecal consistency scores ($p<0.001$, $p\eta^2=0.95$; Figure 6) and significantly reduced faecal dry matter content ($p<0.001$, $p\eta^2=0.84$; Figure 7) during the test periods compared to the baseline periods. The decrease in dry matter and resulting increase in faecal water content during the test period was also exemplified by the significantly higher total faecal output during the test periods than baseline periods ($p<0.001$, $p\eta^2=0.60$; Figure 8). There was no difference in the number of defecations per day across the four periods ($p=0.28$). As seen in Figure 6, dogs produced stools within the ideal range, grade 1.5-2.5, during Baseline 1 and stools returned to the ideal range for Baseline 2.

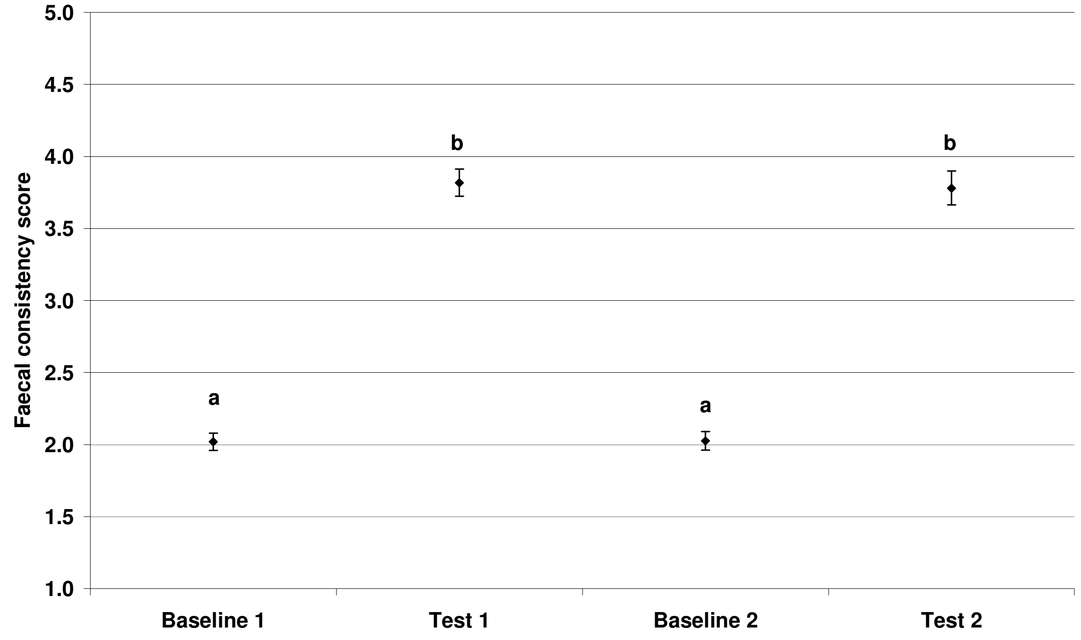

Figure 6. Mean faecal consistency score per period. Faecal consistency was scored on a scale from 1 (hard, dry stool) to 5 (liquid diarrhoea). The ideal faecal consistency range is 1.5 to 2.5. Different letters indicate significant differences. Error bars indicate ± standard error.

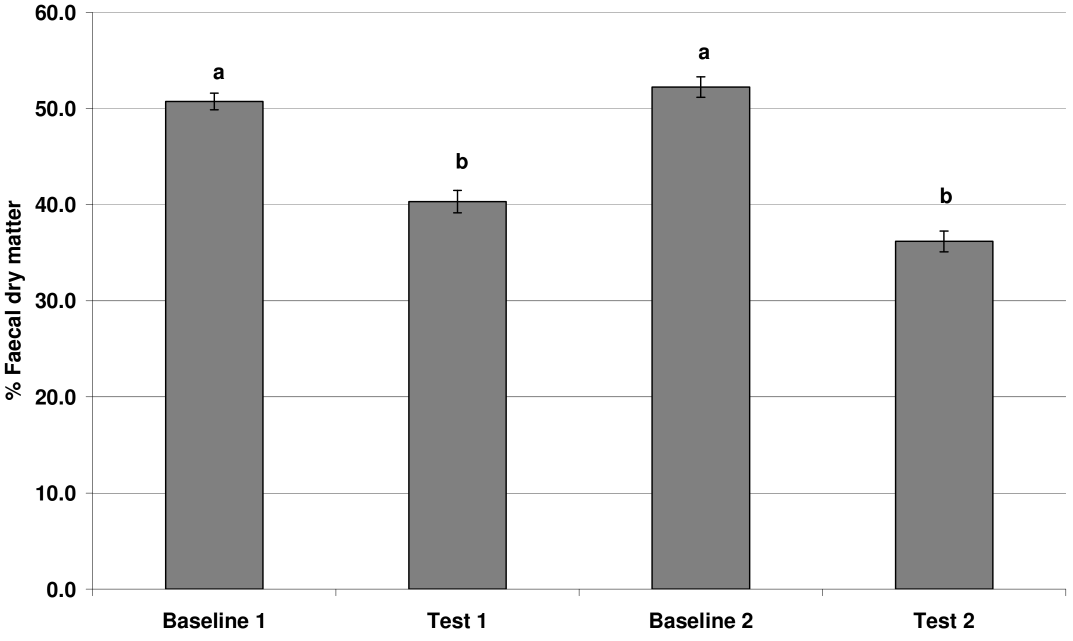

Figure 7. Mean percentage faecal dry matter per period. Different letters indicate significant differences. Error bars indicate ± standard error.

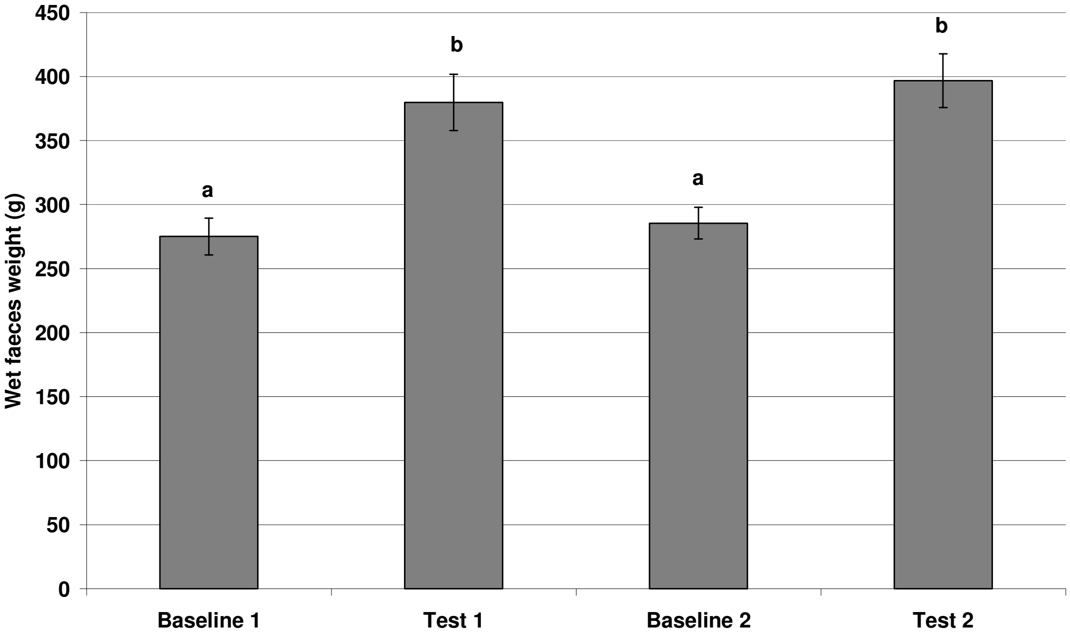

Figure 8. Mean total wet faecal output per period. Different letters indicate significant differences. Error bars indicate ± standard error.

There were 374 grass-eating events observed for all 12 dogs during 80 minutes of observation for each dog. The dogs showed a strong preference for kikuyu compared with couch grass during both the baseline ($t_{(11)}$=3.6, p=0.004) and test periods ($t_{(11)}$=3.0, p=0.01). All but one subject ate kikuyu at some point during the study, totalling 190 minutes spent eating kikuyu, but only six dogs ate couch grass for a total of 13 minutes. Due to the very low number of couch grass episodes and to be statistically conservative, “grass” in the following analyses refers to kikuyu grass.

A repeated measures ANOVA was used to analyse the time spent eating grass for the baseline and test periods. The dogs spent significantly more time eating grass during the baseline period than the test period (p=0.03, $p\eta^2$=0.37; Figure 9). Similarly, there were more grass-eating events during the baseline than the test period (p=0.04,

$p\eta^2$=0.33; Figure 10). However, there was no significant difference in the number of non-eating grass interactions (p=0.78). There were only two vomiting events, occurring in the same subject in one testing session during the first baseline period.

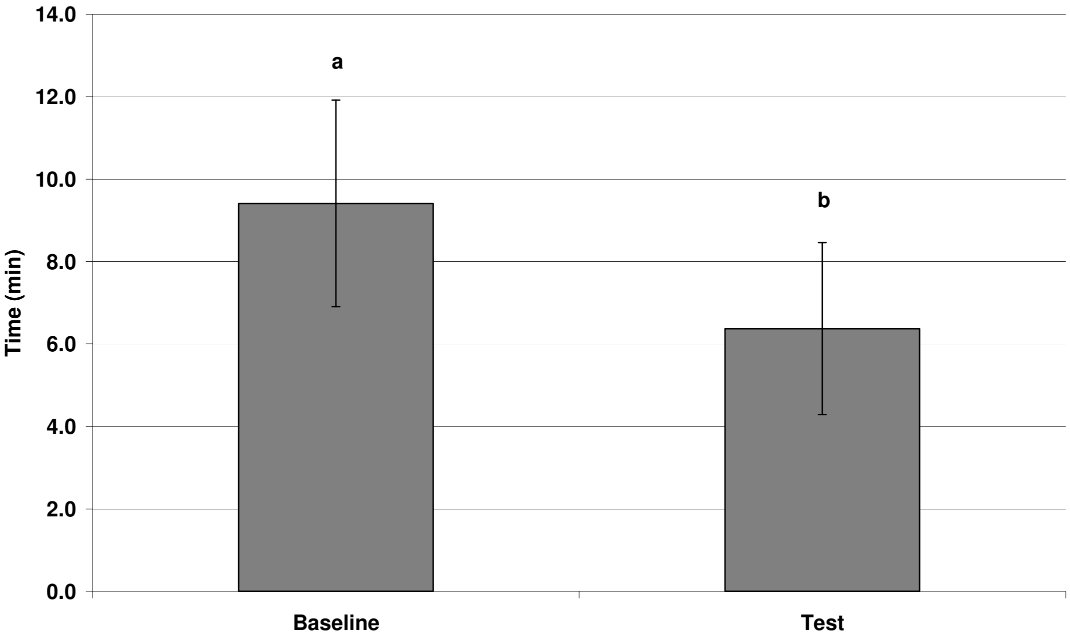

Figure 9. The mean total amount of time spent eating grass for the baseline and test periods. Different letters indicate significant differences. Error bars indicate ± standard error.

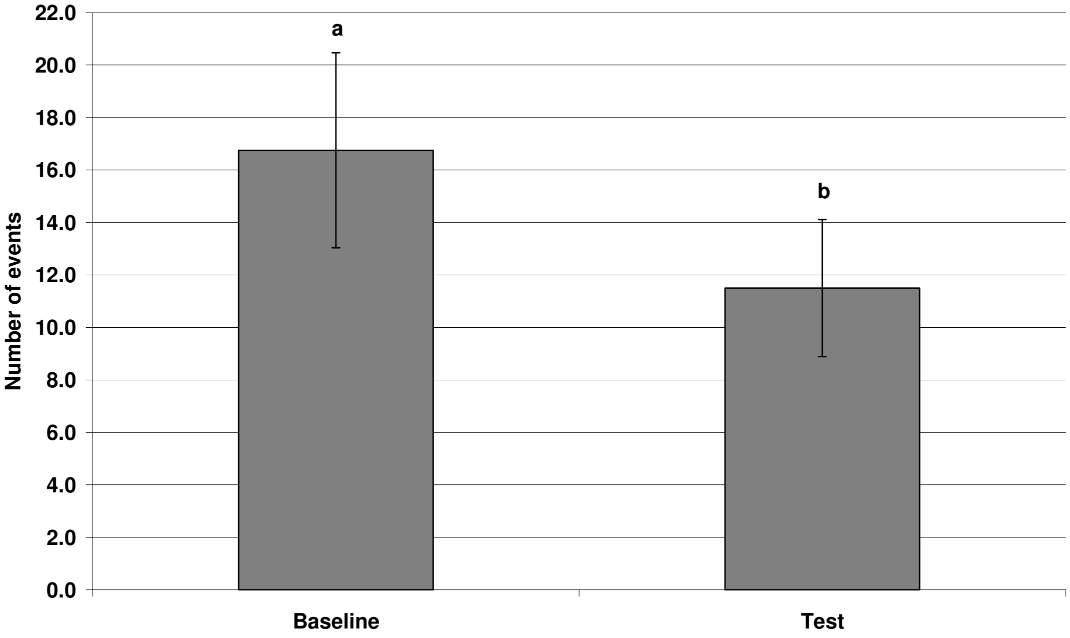

Figure 10. The mean total number of grass-eating events for the baseline and test periods. Different letters indicate significant differences. Error bars indicate ± standard error.

Discussion

Supplementation of the standard diet with 10% BeneoP95® did create looser, wetter stools compared with the dogs' stools when fed the standard diet. Generally, the stools were loosest in the morning before testing and still remained wet through the afternoon feeding. Therefore, observations of grass-eating behaviour coincided with the dogs producing loose, wet stools. However, the frequency of stools did not increase during the test period. Dogs that are given a large, single dose of supplement are potentially more likely to experience flatulence and loose stools compared with multiple, smaller doses (Flickinger et al., 2003). Therefore, it is possible that the large dose in the unadapted subjects used in the current study will have evacuated the bowel of any accessible excrement that normally may not have been ready for excretion. Nonetheless, the faecal consistency scores and faecal dry matter content during the test period confirmed that the dogs had diarrhoea when fed the supplemented standard diet. Therefore, comparisons of grass-eating behaviour in dogs with and without diarrhoea can be made.

The dogs spent less time eating grass and demonstrated fewer grass-eating events when they had diarrhoea compared with when they produced normal stools, not supporting our first hypothesis. Although some researchers suggest that dogs use grass to self-medicate gastrointestinal distress (Hart, 1985; McKeown, 1996; Overall, 1997), the dogs did not eat grass in response to producing loose, watery faeces in the current study. The reduction in grass-eating behaviours when the dogs had loose, water stools may be explained by a reduction in appetite. Chapters 3 (*Grass Patterns*; Bjone et al., 2007), 4 (*Development*), and 5 (*Worm Burden*) have indicated that grass is seen as a food source for dogs. Therefore, it may be that the type of gastrointestinal distress induced in the current study suppressed appetite, which thus reduced grass-eating behaviours.

However, this does not preclude that other forms of gastrointestinal distress may be self-medicated by grass-eating behaviours. We induced osmotic-type diarrhoea, which originated in the large intestine. Diarrhoea can also originate from the small intestine (Strombeck, 1980). Naturally occurring diarrhoea is often of bacterial or viral origin and therefore likely to accompany other clinical symptoms, such as nausea. In addition, grass may be more effective when dogs have constipation (dry, hard, infrequent stools). Hart (1985) and McKeown (1996) have proposed that grass has laxative properties, but there is currently no research on this theory.

Grass contains cellulose, which is indigestible and is not fermented in the large intestine of the dog (Case et al., 2000; National Research Council, 2006; Sunvold et al., 1995). Inclusion of cellulose decreases dry matter digestibility (Burrows, Kronfeld, Banta, & Merritt, 1982; Zentek, 1996) and intestinal transit time (Burrows et al., 1982). Therefore, cellulose may induce a laxative effect in dogs, but it is not known how whole grass, with all of its components, will affect transit time. Further research is needed to determine the putative laxative effects of grass, or its various components.

The dogs ate grass during both the baseline and test periods. Grass eating during the baseline periods, when the dogs were fed the standard diet and had normal stools, further confirms that grass eating is a normal behaviour (similar to Bjone et al., 2007; Sueda, Hart, & Cliff, 2008). Other grass-eating theories were not supported. The dogs had a significant strong preference for kikuyu grass compared to couch grass, despite anecdotal information suggesting couch grass is the self-medication grass of choice for dogs (de Baïracli Levy, 1992; Engel, 2002). Therefore, our second hypothesis was not substantiated. Grass can be more palatable at certain heights or blade lengths (Göhl, 1981). Although couch and kikuyu are approximately the same height, kikuyu has longer grass blades, possibly accounting for the preference for kikuyu in the current study. It is unknown why this kikuyu preference was present in the current study, but not in Bjone et al. (2007; Chapter 3). It may be an individual preference of the dogs used in the current study, which were from different breeders than the dogs used in Chapter 3. Alternatively, although the grass was maintained in a temperature-controlled greenhouse, seasonality may have affected the palatability or nutrient content of the grass (Fulkerson, Slack, Hennessy, & Hough, 1998). The current study was performed in October 2007, the Australian spring, while Bjone et al. (2007) was performed in March-April 2006, the Australian autumn. Seasonality of grass-eating behaviour in dogs would be an area for future investigation.

In addition, grass has also been theorized to be an emetic (Fox, 1965; Houpt, 2005; Thorne, 1995). However, there were only two vomiting events for 374 grass-eating events across all 12 dogs and all eight testing sessions. These data from the current study, in addition to the results of the studies in Chapters 3, 4, and 5, indicate that grass is not used as an emetic.

For further clarity on the effect of grass on gastrointestinal disturbance, future research should examine the effect of constipation (hard, dry stools) on grass-eating behaviour in the domestic dog.

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CHAPTER 7

Discussion and Conclusions

Before the current project, there was no known explanation for grass eating in the domestic dog (as noted by Hart, 1985; UC Davis SVMCABP, 2005). Dogs are adapted to consuming meat and they have almost no capacity to digest the plant fibre in grass (Case et al., 2000), but they still eat grass. This mysterious contradiction and the unknown explanation for the behaviour provided an opportunity for a series of studies.

Developing the Methodology

We intended to investigate grass-eating behaviour in domestic dogs and needed to develop appropriate methodology in the absence of previous experimental studies. Sueda, Hart, and Cliff's (2005, 2008) survey-based study provided initial insights into grass-eating behaviour. However, this study could not determine causative factors of grass-eating behaviour due to its correlational nature. Therefore, we first documented grass-eating behaviours from a single observer's objective viewpoint, rather than multiple owners' subjective experiences.

The *Observational Study* (Chapter 2) determined that it would be most viable to use grass alone, as the dogs did not eat any non-grass plant items that were available to them and most dogs ate grass on at least one occasion. Recording all occurrences of each behaviour and the developed shorthand of this study were sufficient. Consequently, these practices were used in the subsequent controlled experiments. There was no relationship with breed or age, consistent with Sueda et al. (2008) so those dogs that exhibited frequent grass eating were selected as subjects for the laboratory experiments using adult dogs.

Because there were no established sample or effect sizes for grass-eating behaviour in dogs, the current project followed the sample sizes used in worm vaccination trials (six subjects; Hotez et al., 2002; Loukas et al., 2004; Loukas et al., 2005) and nutrition and feeding trials (6 to 12 subjects per group; Brown, Choct, & Pluske, 2005; Brown & McGenity, 2005; Crowell-Davis, Barry, Ballam, & Laflamme, 1995). Data were analysed using repeated measures ANOVA to increase the power of the studies as compared to a standard ANOVA.

Several other methodological issues were discovered when conducting the first controlled experiment presented in Chapter 3. In addition to the problems associated with weighing the amount of grass eaten, which were discussed in Chapter 3, there were other grass difficulties. The dogs selected for Chapter 3's study were known to eat grass, as determined in the *Observational Study* (Chapter 2). However, they did not readily eat the couch grass in the turf or cuttings form that was initially offered. The grass turf and cuttings were both short, approximately 10 cm in length. Consequently, longer couch grass was offered and readily eaten by the Chapter 3 subjects. This couch grass was approximately 40 cm in length and was grown in pots. Kikuyu grass (approximately 40 cm in length) in pots was also added to the study presented in Chapter 3 and it was readily eaten by the study subjects. Subsequent experiments with adult subjects (Chapters 5 and 6) used the longer grasses in pots. The *Development* study (Chapter 4) used long grass as well, but it was presented in trays so that the puppies could reach it.

These adjustments then formed the methodology that was used throughout the controlled experiments of the current project (Chapters 3-6) and would be viable for future research.

General Discussion and Implications for Further Research

Grass eating was very prevalent in the dogs studied in the current project. When the study dogs were healthy, they ate grass. When the dogs were experiencing a naturally occurring worm burden or diarrhoea, they still ate grass. Five- to seven-week-old puppies ate grass as well as adult dogs. Dogs of various breeds ate grass, including beagles, terriers, shih-tzus, kelpies, poodles, and dachshunds. Therefore, grass eating in the domestic dog is a normal and common behaviour. As such, grass eating should not be seen as a problematic behaviour for most dogs or as indicative of illness.

While we might accept that grass eating is a normal and common behaviour, this still does not explain the behaviour in dogs. Many theories exist about grass eating in dogs, and the current project has not supported several of them, including self-medication of a worm burden, grass as an emetic, and the belief that couch grass is the grass of choice for dogs. However, the results of the current project have indicated that grass eating is influenced by satiety, time of day, and the mother's grass-eating behaviours.

Self-Medication of a Worm Burden

Murie's (1944) observations of wolf scats containing grass and roundworms probably founded the theory that dogs eat grass to self-medicate a worm burden. Sueda et al. (2008) postulated that grass eating was preserved through the domestication process for this reason. However, no difference was found in the amount of time spent eating grass or the number of grass-eating events between the dogs who were harbouring worms and those that were dewormed, not corroborating this theory. The general absence of clinical signs of worm burden suggests that the degree of worm infestation present in the dogs in this study was not sufficient to compromise animal health. This may indicate that three months following receipt of a broad-spectrum anthelmintic may not have allowed the worms to reach their full pathogenic capacity. In particular, *Trichuris* whipworm has a lengthy prepatent period and results in clinical signs such as mucoid, loose, bloody stools (Anderson, 1992; Jacobs et al., 1994; Jubb & Kennedy, 1970; Roberson & Cornelius, 1980). Therefore, the conclusion from Chapter 5 may be better phrased: "The current study did not substantiate the theory that dogs eat grass to self-medicate a gastrointestinal nematode burden, which they were naturally harbouring for less than three months." It is also possible that dogs unaccustomed to worm infestation may respond differently. In particular, younger dogs, notably puppies, are less accustomed and more susceptible to the pathogenic effects of worms than adult dogs which can develop resistance (Case, 2005; Stroup, 2007). While seriously infected puppies may provide a better model to test the self-medication theory, puppies were not used in the current study due to ethical concerns and the difficulty of gaining owner consent to use puppies for such a study. It would be beneficial to investigate further this theory using varied grasses and dogs exhibiting clinical signs of worm infestation.

Grass as an Emetic

Several authors have suggested that dogs eat grass to vomit (Engel, 2002; Fox, 1965; Hart, 1985; Houpt, 2005; Lindsay, 2001). This theory was tested indirectly throughout the current project by documenting the number of vomiting events and determining if there was a relationship between vomiting events and the time spent eating grass for each study. Overall, there were very few vomiting events: five events during Bjone, Brown, and Price (2007; Chapter 3), six events in the *Development* study (Chapter 4), 47 vomiting events in the *Worm Burden* study (Chapter 5), and two vomiting events when the dogs had diarrhoea (Chapter 6). Therefore, 60 vomiting events were observed throughout 56 hours of testing, which included 1,444 minutes (24.1 hours) of time spent eating grass and 2,769 grass-eating events.

The higher incidence of vomiting events (47) observed in the *Worm Burden* study seems to indicate that these were at least partially related to the presence of worms rather than anything to do with grass eating, which, if these are removed, leaves very few vomiting events (13). Vomiting can be a clinical sign of some worm infections (Roberson & Cornelius, 1980), but it is not indicative solely of worms or a serious medical condition and can be part of the normal behaviour repertoire in the dog (Houpt, 2005), Therefore, owners should not construe that a dog has worms if it vomits.

The remaining 13 vomiting events may have been due to grass physically irritating the gastric mucosa of the dog (Quinn et al., 1997). Several authors have suggested that grass is eaten for this purpose (Hart, 1985; McKeown, 1996), but it has not been previously substantiated. In the current project, the extremely low frequency of vomiting events does not support the theory that dogs eat grass to vomit. If grass was eaten for a physical irritant attribute, we would expect to see more vomiting events relative to the time spent eating grass and the number of grass-eating events.

Although the current studies do not indicate grass is eaten to vomit, other dogs may eat grass and vomit or intentionally eat grass to vomit, as suggested by Sueda et al. (2008). The concept implies that the dogs experience the vomiting effects of grass ingestion and repeat the behaviour for that purpose. However, experiential learning with an emetic for the domestic dog has not been demonstrated (Rathore, 1984). In Rathore's study, dogs were slow to learn and quick to forget the emetic effects of ingesting lithium chloride-tainted meat. After ingestion, the dogs vomited within a half hour but promptly ate their vomitus, resulting in another vomiting event. A temporary taste aversion lasted for 7.5 hours for all the dogs, but within 24 hours, the dogs had no aversion to the meat. In the current project, the subjects continued to eat grass after 40 of the 60 vomiting events and on three occasions, the dogs also ate the vomitus. Thus far, the evidence is not in favour of dogs eating grass to vomit.

Future research could directly test this theory by inducing nausea, but not to the extent of inducing vomiting, and compare grass-eating behaviours of dogs with and without nausea. In addition, by providing several grasses, including a "hairy" grass, grass preferences may be elucidated and Mességué's (1991) brief conjecture that dogs use hairy grasses as emetics could be tested.

"Dog Grass"

Couch grass was the only grass specifically identified in the anecdotal literature in relation to consumption by dogs (de Baïracli Levy, 1992; Engel, 2002; Mességué, 1991). The botanical name varies for this identified couch grass, depending on the classification system used and the source of information. English couch is known as "dog grass," *Agrospyron canina*, *Agropyrum repens* (de Baïracli Levy, 1992), *Agropyron repens* (Auld & Medd, 2002), *Elymus repens* (van Wyk & Wink, 2005), and some sources refer to it as *Triticum repens* (Barnes, Anderson, &

Phillipson, 2002; British Herbal Medicine Association, 1996). The description of the plants within each of these sources also varies.

Therefore, a couch grass (*Cynodon dactylon*) which also has the vernacular name “dog grass” (Kapoor, 2001), was sourced for the current project. *C. dactylon* is a fine-leafed, creeping perennial with hairless or lightly haired blades (Muyt, 2001). Due to the initial complications with measuring the amount of grass eaten, kikuyu grass (*Pennisetum clandestinum*) was offered in addition to the couch grass to determine any grass preferences.

The grass preferences of the dogs in the current studies do not validate *C. dactylon*’s “dog grass” nickname. The dogs equally preferred *C. dactylon* and *P. clandestinum* for all studies except during the *Gastrointestinal Disturbance* study in which kikuyu was preferred (Chapter 6). The general lack of strong grass preference may be due to the similarities of kikuyu and couch grass. Their blades are hairless or lightly haired, they spread through creeping underground and aboveground stems (Muyt, 2001), and they grow to similar heights (Göhl, 1981). However, kikuyu is a slightly thicker grass with wider and longer blades and longer underground stems (up to 50 cm) than couch grass (Muyt, 2001; Spencer, 2005).

Grass length influenced grass-eating behaviour in Chapter 3 as the dogs did not eat *C. dactylon* grass at 10 cm, but would eat it at 40 cm. Göhl (1981) recommends grazing when *C. dactylon* is at 10-15 cm because it is most palatable for livestock. Possibly, there are optimal grass heights for dogs as well. However, it should be noted that chemical composition of grasses varies widely, depending on maturity, climate, time of day, and soil (Göhl, 1981; van Soest, 1973). Although *C. dactylon* and *P. clandestinum* are approximately the same height, *P. clandestinum* has longer grass blades, possibly accounting for the preference for kikuyu in the *Gastrointestinal Disturbance* study.

Grass as Food

I believe the main purpose dogs eat grass is for food, for five reasons. 1) The domestic dog has retained most of its ancestor's (wolf, *Canis lupus*) eating behaviours (Case, 2005) and the occurrence of grass eating in wolves and dogs suggests the behaviour was sustained through domestication of the wolf into the domestic dog (Papageorgiou et al., 1994; Stahler et al., 2006; Sueda et al., 2008).

2) The presence of an innate tendency for grass eating was supported in the *Development* study in Chapter 4. This study investigated the development of grass-eating behaviour and the influence of the nursing mothers' grass-eating habits on grass eating in five- to seven-week-old puppies by presenting grass to puppies with and without their mother present. All of the puppies ate grass whether in the presence or absence of the mother. Of the puppies that were presented grass with their mothers present, 85% ate grass before their mothers did, indicating an innate tendency to eat grass.

3) Similar to sheep influencing their lambs' food preferences, the puppies' grass-eating behaviours were influenced by their nursing mother in the *Development* study (Black-Rubio et al., 2007; Mirza & Provenza, 1994; Nolte et al., 1990; Saint-Dizier et al., 2007). The *Development* study indicated that the high grass-eating mothers uninhibited the grass-eating behaviours of their present puppies, whereas the limited grass eating of the low mothers may have inhibited grass-eating in the present puppies.

4) In the *Development* study, the amount of time spent eating grass increased from five weeks of age to six and seven weeks of age as the puppies weaned from mother's milk onto solid foods. The puppies were also presented with an artificial grass in addition to the real grass (kikuyu) to control for the puppies' natural propensity to chew on objects and to distinguish this behaviour from chewing and eating live grass. The puppies had more non-eating grass interactions with the artificial grass, but they preferred to spend time eating the kikuyu grass rather than chew on the artificial grass. Thus, the puppies consumed the grass intentionally and not as a way to fulfil their chewing requirements.

5) In Chapter 3 the dogs spent more time eating grass before ingestion of their meal than after and they were more likely to eat grass the longer it had been since their last daily meal, suggesting that grass eating is influenced by satiety (Bjone et al., 2007).

The observed prevalence of grass eating in wolves, adult dogs, *and* weaning puppies suggests a possible biological function to the behaviour. The current project has found that dogs do not eat grass to self-medicate a worm burden or gastrointestinal disturbance or to vomit, but grass eating was affected by satiety.

What dogs could digest from grass is currently unknown. Peterson and Ciucci (2003) and McCay (1949) suggest that grass is a source of vitamins. Papageorgiou, Vlachos, Sfougaris, and Tsachalidis (1994) suggested that dogs obtain Vitamin C from eating grass. Scheer (1996) contends that grass supplements provide enzymes for dogs. However, none of these speculations has been validated and dogs have no dietary requirement for Vitamin C (Case et al., 2000).

The chemical composition of fresh *C. dactylon* has been found to be 10.46% crude protein, 28.17% fibre, 47.81% nitrogen-free extract, 1.80% ether extract, and

11.75% total ash (Kapoor, 2001). However, it should be again noted that chemical composition varies depending on maturity, climate, and soil conditions (Göhl, 1981; van Soest, 1973). Understandably, digestibility of grass has been reported in regard to ruminants, but not non-ruminant animals (Fulkerson et al., 1998; Katiyar & Ranjhan, 1969; N. Miles, de Villiers, & Dugmore, 1995). The nutritional properties of grass and its digestibility in dogs would be an interesting area for future research. Nutritional studies would help determine what, if anything, dogs acquire from eating grass. The results of such research would have implications for the pet industry, in particular pet food companies that currently market diets as "nutritionally complete and balanced."

Grass as a Laxative

The explanation for grass-eating behaviour in dogs may also lie in its indigestible components. In the *Gastrointestinal Disturbance* study (Chapter 6), the dogs ate less grass while experiencing loose watery faeces compared to when they had normal stools. Therefore, grass was not used as a constipator. Instead, grass may have laxative properties.

Dogs are omnivores, but they typically digest food of animal origin better than plant origin due to plants containing dietary fibre components such as cellulose and hemicellulose (Case et al., 2000). Although herbivores can digest these components, plant fibres resist digestion by enzymes in the dog's monogastric digestive system. In particular, cellulose, the most abundant plant polysaccharide, cannot be digested in the small or large intestine (Case et al., 2000; National Research Council, 2006; Sunvold et al., 1995). Cellulose comprises 20 to 50% of the dry matter of most plants (van Soest, 1973). Specifically, *C dactylon* has been documented to consist of 76% plant cell walls, of which 38% is cellulose (van Soest, 1973). Therefore, grass is at least partially indigestible.

Cellulose decreases digestibility of dry matter (Burrows et al., 1982; Zentek, 1996) and intestinal transit time (Burrows et al., 1982) when added to a standard diet. Therefore, this indigestible component of grass may decrease transit time. However, it is unknown how whole grass, with all of its components, will affect transit time. Further research will be needed to determine the chemical composition of grass and its putative laxative effects. For additional clarity on the effect of grass on gastrointestinal disturbance, future research should examine the effect of constipation (hard, dry stools) on grass-eating behaviour in the domestic dog.

Impacts

The results of the current project will be of interest to those who interact with dogs: breeders, owners, veterinarians, behaviourists, and pet food companies. The current project has determined that grass eating is a normal and common behaviour not indicative of illness or vomiting. Therefore, dog owners should not be concerned when their dogs eat grass.

Further investigation of grass constituents may highlight the need for alterations to the current accepted nutrient requirements for dogs. In addition, confirming the laxative properties of grass, through a study on the effect of constipation on grass-eating behaviours, may assist veterinary medicine by providing a natural alternative to laxative medications. Due to the common nature of grass eating in all ages and varied breeds of dogs, it is recommended that dog owners allow their dogs opportunities to eat grass.

Limitations

The current project used dogs from the same colony for all studies except for the *Gastrointestinal Disturbance* study (Chapter 6) which sourced dogs from two local beagle breeders. Sourcing the required number of dogs needed for a study from one or two breeders helped assist the continuity of care as the dogs began a study. Fewer owners also lessened the difficulty of sourcing and gaining permission to use dogs from multiple owners and the compatibility of dogs in the kennels. Sampling from populations different from these few sources may produce results that vary from the current project. However, the results of the current project were in accordance with those reported by Sueda et al. (2008) which sampled from a large population (n=1,571) via the internet. Therefore, the results are believed to be relevant to the general population of domestic dogs.

The limitations of Bjone et al. (2007) were not discussed within the publication and will be discussed here. All dogs were fed in the morning during the habituation period. The dogs were intentionally tested in the same sequence (two days morning, two days noon, and then two days afternoon) to create the rapid change in feeding schedule and to standardize the change in feeding time and the number of hours they had between grass presentations and feedings for all dogs. For future studies, the dogs could be habituated to different feeding times before testing began and the testing schedule could be Latin square randomized. However, possible order and time effects were reduced by randomizing the testing order across and within testing sessions. In addition, the pots of grass were randomly selected from 10 available pots and all pots were hosed down after each session and allowed to dry. Previous grass interactions by other dogs may have an on-going effect on dog behaviour, possibly indicated by an increase in the number of non-eating grass interactions. However, there was no significant difference in non-eating grass interactions across the treatments in all studies, except the *Worm Burden* study (Chapter 5) for those dogs continuing to harbour their worm burden. Therefore, it is believed that the procedure of randomly selecting grass pots for each testing session is acceptable for future studies.

Dogs were tested in groups in all studies. It is possible that dogs within a testing group influenced grass-eating behaviours. Possible group effects were minimized in several ways. 1) Compatible groups were formed by owner recommendation and experimenter observation during the habituation periods. 2) Testing areas in all studies were large enough that the dogs or puppies could have individual access to the grass or surrounding areas of the pen without crowding or competition. 3) There was more grass available than the subjects could eat.

Conclusions

In conclusion, grass eating is a normal and common behaviour in domestic dogs. It should not be considered as indicative of illness. The current project indicates that dogs do not use grass to evoke vomiting or to self-medicate a worm burden or diarrhoea. The dogs also did not substantiate *C. dactylon*'s "dog grass" vernacular name as no preference was shown for it compared with *P. clandestinum*. In addition, longer blades of grass were eaten much more readily than shorter blades of grass.

Future studies should include varied grasses, particularly hairy grasses. Grass eating is affected by satiety as the dogs spend more time eating grass when they were hungry, suggesting that grass is seen as a food source. The methodology in the current project would be applicable to future research, which should include grass nutrient analysis and digestibility studies as well as the effect of constipation on grass eating.

CHAPTER 8

References

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