EFFECTS OF PHOTOPERIOD ON WEIGHT MAINTENANCE IN ADULT NEUTERED MALE CATS

BY

KELLY L. KAPPEN

THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Sciences in the Graduate College of the University of Illinois at Urbana-Champaign, 2012

Urbana, Illinois

Master’s Committee:

Associate Professor Kelly S. Swanson, Advisor, Chair
Professor Emeritus George C. Fahey, Jr.
Assistant Professor Ryan N. Dilger
ABSTRACT

With the continued rise of obesity in humans and companion animals, novel weight management strategies are needed. To date, most strategies have focused on dietary intervention. Strategies aimed at altering physical activity, an important factor in weight maintenance, have been lacking. Due to the drastic decrease in physical activity level noted after gonadectomy, neutered animals are targets for activity-related weight management strategies. Photoperiod is known to cause physiological changes in seasonal mammals, including changes in body weight (BW) and reproductive status. Thus, our objective was to determine the effect of increased photoperiod (longer days) on voluntary physical activity levels, resting metabolic rate (RMR), food intake required to maintain BW, and fasting serum leptin and ghrelin concentrations in adult cats. Eleven healthy, adult, neutered, male domestic shorthair cats were used in a randomized crossover design study. During two 12-wk periods, cats were exposed to either a short day (SD) photoperiod of 8 hr light: 16 hr dark or a long day (LD) photoperiod of 16 hr light: 8 hr dark. Cats were fed a commercial diet to maintain baseline BW. In addition to daily food intake and twice-weekly BW, RMR (via indirect calorimetry), body composition [via dual-energy X-ray absorptiometry (DEXA)], and physical activity (via Actical activity monitors) were measured at wk 0 and 12 of each period, and fasted serum leptin and ghrelin concentrations were measured at wk 0, 6, and 12 of each period. Average hourly physical activity was greater (P=0.008) in LD vs. SD cats (3770 vs. 3129 activity counts/hr), which was primarily due to an increase (P<0.001) in dark period activity (1188 vs. 710 activity counts/hr). This corresponded to a higher (P<0.0001) daily ME intake (mean over 12-wk period: 207 vs. 197 kcal/d), and an increased (P=0.048) RMR in LD cats (9.02 vs. 8.37 kcal/h). Body composition, serum leptin, and serum ghrelin...
were not altered by photoperiod. More research is needed to determine potential mechanisms by which these physiological changes occurred and how they may apply to weight management strategies.
DEDICATION

To my family, friends, and mentors, without whose support this would never have been possible.
ACKNOWLEDGEMENTS

Special thanks are due to my advisor, Dr. Kelly Swanson, for his guidance and instruction over the past two years. I’m very grateful for all of the opportunities for learning and development he has offered me during those years.

I sincerely appreciate the help of everyone in the comparative nutrition lab group, past and present, for their support and their assistance. Their help was essential to the successful completion of this thesis. Particular thanks are due to Ping Deng, Katie Kerr, Alison Beloshapka, Lindsay Garner, Ryan Grant, and Brittany Vester Boler for their help in the planning and execution of this study.

Thank you also to my thesis committee members, Dr. Ryan Dilger and Dr. George Fahey, for their time and assistance with this thesis.

Finally, I owe a huge debt of gratitude to my friends and family, especially my parents, Phil and Diana Kappen, and my brother Michael, for their motivation and encouragement. Without all of you, this never would have been possible.
**TABLE OF CONTENTS**

Chapter 1: Introduction ........................................................................................................ 1

Literature Cited .................................................................................................................. 3

Chapter 2: Literature Review .............................................................................................. 5

Effect of photoperiod on seasonal mammals ...................................................................... 5
Mechanisms of photoperiod response ............................................................................... 13
Feline energy metabolism and obesity ............................................................................. 18
Feline seasonal reproductive response ............................................................................. 19
Feline response to gonadectomy ....................................................................................... 22
Thesis objectives .............................................................................................................. 25
Literature Cited .................................................................................................................. 26

Chapter 3: Effects of Photoperiod on Weight Maintenance in Adult Neutered Male Cats ......................................................................................................................... 31

Abstract ............................................................................................................................ 31
Introduction ........................................................................................................................ 32
Materials and Methods ..................................................................................................... 33
Results ............................................................................................................................... 38
Discussion ......................................................................................................................... 40
Literature Cited .................................................................................................................. 45
CHAPTER 1: INTRODUCTION

Feline obesity is a growing problem in developed nations, and it is estimated that between 25 and 40% of the pet cat population is overweight or obese (Freeman et al., 2006; Lund et al., 2005). Obesity increases risk for numerous health problems, including hepatic lipidosis, dermatological disease, urinary tract disease, and diabetes and, therefore, represents a significant threat to pet health and welfare. While many dietary strategies have been tested to reduce obesity incidence, strategies to alter physical activity have been lacking.

Photoperiod is known to alter many physiologic outcomes in seasonal animals, including food intake, weight gain, and estrus. The response to decreased daylight depends on species, with some gaining weight and others losing weight. Interestingly, the response to shortened day length can usually be predicted by the response to gonadectomy. For example, Siberian hamsters and meadow voles decrease BW and fat mass with gonadectomy (Bartness, 1996; Dark and Zucker, 1984; Wade and Bartness, 1984) or shortened days (Dark and Zucker, 1984; Wade and Bartness, 1984). In contrast, Syrian hamsters, collared lemmings, and prairie voles increase BW and fat mass with gonadectomy (Gower et al., 1994; Kriegsfeld and Nelson, 1996; Morin and Fleming, 1978; Slusser and Wade, 1981) or shortened days (Bartness and Wade, 1984; Gower et al., 1994; Kriegsfeld and Nelson, 1996).

Recent experiments in our laboratory (Belsito et al., 2009; Vester et al., 2009) have observed a significant reduction in physical activity in cats after spaying. While decreased activity was an expected outcome, the differences between the dark and light periods were interesting. While activity counts during light hours were not greatly affected after spaying, physical activity during the dark period decreased dramatically (up to 75%; Belsito et al.,
2009). Given this difference between dark and light periods, we were interested in evaluating the effects of photoperiod on weight management in cats. Because cats are sensitive to photoperiod as it relates to breeding (Dawson, 1941; Michel, 1993), photoperiod also may affect food intake, BW, activity levels, and circulating ghrelin and leptin concentrations; however, this topic has not yet been researched in cats. Cats are known to gain BW and reduce activity in response to gonadectomy (Belsito et al., 2009; Nguyen et al., 2004; Scott et al., 2002; Vester et al., 2009), so if cats respond to photoperiod, it is likely that they will gain BW and reduce activity. The objective of this thesis was to evaluate the effect of photoperiod on weight management in cats, as measured by voluntary activity, resting metabolic rate (RMR), caloric intake necessary to maintain BW, and serum leptin and ghrelin concentrations.
**Literature Cited**


CHAPTER 2: LITERATURE REVIEW

EFFECT OF PHOTOPERIOD ON SEASONAL MAMMALS

Photoperiod (i.e., day length) and ambient temperature are important seasonal cues that lead to physiological changes in seasonal mammals. Under natural conditions, mammals respond to a combination of environmental cues, but in laboratory studies, mammals often respond to photoperiod alone (Heldmaier et al., 1982), indicating that, for these animals, photoperiod is the driving factor for many seasonal physiological changes. Photoperiod exerts a unique effect on various animal species, both in terms of body weight (BW) and in terms of reproductive status. Reduced day length causes weight loss in some species (Bartness et al., 2002; Krol and Speakman, 2007; Wang et al., 2006; Warner et al., 2010; Zhao and Wang, 2005), but it induces weight gain in others (Bartness and Wade, 1984; Kriegsfeld and Nelson, 1996; Nagy et al., 1993; Nieminen et al., 2002).

Photoperiod research techniques

Methodology for photoperiod studies varies widely. Some studies observe physiological changes in outdoor-housed animals that are exposed to natural changes in both photoperiod and ambient temperature. While these studies do not specifically isolate the effect of photoperiod, several studies have found that photoperiod is the primary environmental factor that initiates most physiological changes in animals (Heldmaier et al., 1982; Masuda and Oishi, 1988); therefore, results observed in outdoor-housed animals will be very similar to those observed in indoor-housed animals exposed only to varying photoperiod.

Studies that maintain animals in controlled, indoor environments can be further divided into those that use fixed day lengths [i.e., animals are switched directly from long
day (LD) to short day (SD) or vice versa] and those that use gradually changing photoperiods that imitate a more natural seasonal light cycle. Despite the differing methodologies used to study photoperiod, changes in weight gain are very similar between studies of fixed day length and gradual light cycles (Gorman and Zucker, 1995), which allows for comparison of results from these studies. Due to its simplicity, most studies use fixed day length. The data reported by Gorman and Zucker (1995) validates the use of this methodology as a substitute for more natural photoperiodic cycles.

**Short day-induced weight loss**

Because species that lose weight are more numerous than those that gain weight in response to reduced day length, more research has been done in this area. These species include Siberian or Dzungarian hamsters (*Phodopus sungorus*; Bowers et al., 2005; Hoffmann, 1973; Knopper and Boily, 2000; Wade and Bartness, 1984; Warner et al., 2010), Mongolian gerbils (*Meriones unguiculatus*; Li and Wang, 2005), meadow voles (*Microtus pennsylvanicus*; Dark and Zucker, 1983), root voles (*Microtus oeconomus*; Wang et al., 2006), Brandt’s voles (*Lasiopodomys brandtii*; Li and Wang, 2007; Zhao and Wang, 2005; 2006), bank voles (*Clethrionomys glareolus*; Peacock et al., 2004), and field voles (*Microtus agrestis*; Krol et al., 2005).

The most commonly studied model of SD weight loss is the Siberian hamster. Early studies using indoor-housed animals exposed to natural light demonstrated that Siberian hamsters exhibited cyclical changes in BW and that they were positively correlated with light cycle. One of these studies demonstrated that LD-housed hamsters (16 hr light: 8 hr dark) exhibited greater (P<0.01) weight gain compared to SD-housed hamsters (8 hr light: 16 hr dark) after only 2 wk (Hoffmann, 1973). This difference in weight gain continued to
increase until the end of the trial (37 d), at which time LD-housed hamsters had gained an average of approximately 8 g more than SD-housed hamsters (P<0.01; Hoffmann, 1973). Weight gain did not reach a peak within the 37 d of the trial, so a longer period is likely necessary to see maximum response in Siberian hamsters.

Later studies comparing the effects of photoperiod and ambient temperature on these physiological changes confirmed that the majority of the effects were due to photoperiod (Heldmaier et al., 1982; Masuda and Oishi, 1988). For example, Heldmaier et al. (1982) compared indoor- vs. outdoor-housed hamsters to determine the relative effects of temperature and photoperiod. Outdoor ambient temperatures ranged from 8°C to -12°C, and both indoor and outdoor animals were exposed to naturally changing photoperiod (Frankfurt, Germany), which changed gradually from approximately 16 hr light: 8 hr dark to 8 hr light: 16 hr dark. By comparing the magnitude of change in animals exposed to either decreased temperature, shortened photoperiod, or both, the authors of that study concluded that a majority of the winter physiological changes noted, including decreased BW (photoperiod responsible for 92% of the change), increased basal metabolic rate (BMR; 64% of change), and increased brown adipose tissue (BAT; 100% of change), were due to photoperiod (Heldmaier et al., 1982).

Another study by Wade and Bartness (1984) supported the previous conclusion that photoperiod alone could cause significant weight loss in Siberian hamsters. This study showed that significant weight loss was evident after only 2 wk of SD treatment (P<0.05), but maximum response was not reached until approximately 8-10 wk after SD treatment (Wade and Bartness, 1984). Similar results were observed in another study of Siberian
hamsters in which BW did not start to decline until wk 3 after exposure to a shortened photoperiod and had usually plateaued by wk 16 (Warner et al., 2010).

Wade and Bartness (1984) also observed that SD-induced weight loss in Siberian hamsters was primarily due to changes in fat mass. In that study, fat free dry weight remained constant (P>0.05) in hamsters exposed to SD or melatonin treatment, but fat mass decreased (P<0.01) in intact females, castrated males, and intact males (Wade and Bartness, 1984). No significant difference in fat mass was noted between SD- and LD-housed ovariectomized females because ovariohysterectomy already caused a decrease (P<0.01) in LD fat mass that mimicked SD fat loss. In contrast, castration in males caused small, but insignificant (P>0.05), reductions in all carcass components, indicating that gonadectomy may have a larger and more tissue-specific effect on females of this species than males.

**Short day-induced weight gain**

Species that gain weight in response to reduced day length include Syrian or golden hamsters (*Mesocricetus auratus*; Bartness and Wade, 1984), raccoon dogs (*Nyctereutes procyonoides*; Nieminen et al., 2002), collared lemmings (*Dicrostonyx groenlandicus*; Hunter and Nagy, 2002), and prairie voles (*Microtus ochrogaster*; Kriegsfeld and Nelson, 1996).

Under laboratory conditions with consistent SD photoperiod (8 hr light: 16 hr dark), cold treatment (4°C) caused weight loss after only two wk (28 g loss; 25% of initial BW) in a group of Syrian hamsters (Lyman, 1948). Another group of hamsters under the same conditions in that same study experienced a similar amount of weight loss (29 g loss; 25% of initial BW) during the time necessary to induce hibernation (1-2 mo), indicating that, under these conditions (cold temperature and SD), BW plateaus very quickly (Lyman, 1948).
Another laboratory study, using a control group of Syrian hamsters housed at 20°C and 14 hr light: 10 hr dark, found that SD treatment (2 hr light: 22 hr dark at 20°C) and cold treatment (6°C with 14 hr light: 10 hr dark) both decreased (P<0.05) BW (12.4 and 15.6 g lower than control, respectively), and that these effects were additive (SD and cold treatment led to 29.9 g lower BW than control; P<0.05), indicating that both temperature and photoperiod contribute to changes in BW (Hoffman et al., 1965). Eventually, controlled experiments studying Syrian hamsters that isolated the effects of photoperiod from those of temperature showed that exposure to SD (8 hr light: 16 hr dark) led to a faster (P<0.02) growth rate than LD exposure (16 hr light: 8 hr dark) at both warm (22°C: 32.1 g/wk vs. 25.1 g/wk) and cold temperatures (10°C: 41.5 g/wk vs. 28.3 g/wk; Hoffman et al., 1982).

Similarly, another study showed that SD-maintained Syrian hamsters (10 hr light: 14 hr dark) gained more weight (60% increase in BW from baseline; P<0.001) than LD-maintained hamsters (16 hr light: 8 hr dark; 17% increase in BW from baseline) over a 2 mo period (Campbell et al., 1983). This study only measured initial and final BW, so it is unknown whether or not a 2 mo period is sufficient to elicit a maximum response in Syrian hamsters.

The tissue responsible for weight gain varies by species, but a study of Syrian hamsters indicated that SD-induced weight gain in Syrian hamsters was primarily due to increased (P<0.05) fat mass, while water mass and fat-free dry weight did not change (P>0.05; Bartness and Wade, 1984).

Body weight gain in response to SD also has been reported in prairie voles. One study observed that SD-housed voles (8 hr light: 16 hr dark) had greater growth rates than LD-housed voles (16 hr light: 8 hr dark) in both males (0.710 vs. 0.263 g/d; P<0.001) and females (0.704 vs. 0.328 g/d; P<0.01; Kriegsfeld and Nelson, 1996).
Collared lemmings are very sensitive to photoperiod and also gain weight in response to SD. In a study of lemmings raised under LD conditions (22 hr light: 2 hr dark), decreased light hours (approaching SD conditions) led to increased (P<0.05) BW in both males and females (Nagy et al., 1993). Body weight was increased (P<0.001) above the 22 hr light treatment group (approximately 20-30 g in males and 15-25 g in females) when males were exposed to a light period of 8 to 16 hr, and when females where exposed to a light period of 8 to 14 hr (Nagy et al., 1993). Light periods of 14 or 16 hr still are considered long days, but collared lemmings react to these photoperiods as if they were SD photoperiods. This is likely due to the fact that collared lemmings are native to harsh northern areas where it is especially advantageous for animals to prepare for the cold season as soon as days begin to shorten.

Another study that investigated male collared lemmings reported that the transition from LD (20 hr light: 4 hr dark) to SD (8 hr light: 16 hr dark) led to an average BW increase of approximately 25 g during 80 d of treatment (Nagy, 1993). The greater BW for SD-housed lemmings was not caused by a difference in fat mass, however, but by 9% greater (P<0.001) fat-free dry mass compared to LD-housed lemmings and 7% greater (P<0.001) water content. Another study of collared lemmings reported that while the weight gain (P<0.001) of lemmings transitioned from LD (22 hr light: 2 hr dark) to SD (8 hr light: 16 hr dark) was caused by increases in fat mass (P<0.001), lean tissue mass (P<0.001), and total body bone mineral (TBBM; P<0.05), weight loss (P<0.05) from the transition from SD to LD was caused by a loss of fat mass (P<0.001) and lean tissue mass (P<0.001), but not TBBM (P>0.05; Hunter and Nagy, 2002).
Sex effects on response to photoperiod

In some species, reaction to photoperiod differs due to the sex of the animal. For instance, female Brant’s voles have 10.6% lower (P<0.05) BW when exposed to SD (8 hr light: 16 hr dark) compared to LD (16 hr light: 8 hr dark), but BW of male Brant’s voles was affected only by ambient temperature, not photoperiod (Li and Wang, 2007).

In Siberian hamsters, SD caused both castrated and intact males to first lose (P<0.01) weight, then to reduce (P<0.05) food intake (Wade and Bartness, 1984). In females, however, ovariectomized animals displayed no SD-induced reduction in BW or food intake, which was observed in intact females (BW: P<0.01; intake: P<0.05). The same study demonstrated that gonadectomy in females caused a reduction (P<0.01) in fat mass but not other carcass components. In males, however, all carcass components were numerically reduced (Wade and Bartness, 1984).

Among species that gain weight in response to SD, collared lemmings exhibit a sex effect on seasonal weight gain, with males of this species displaying more sensitivity to photoperiod than females. As mentioned above, in males and females raised under LD conditions (22 hr light: 2 hr dark), males began to gain weight when the light period was reduced to 16 hr or less, while females did not exhibit weight gain until the light period reached 14 hr or less (Nagy et al., 1993). The same study also observed that weight gain of males tended to be slightly greater in males than females (20-30 vs. 15-25 g). Conversely, in Syrian hamsters fed a high-fat diet, females experienced greater weight gain than males (approximately 30 vs. 5 g above LD weight) in response to SD (Bartness and Wade, 1984). This difference was far less dramatic in Syrian hamsters fed a low-fat diet; however, females
still experienced greater weight gain than males (approximately 10 vs. 3 g above LD weight; Bartness and Wade, 1984).

The prairie vole is another species that gains more weight when exposed to SD than LD, and this effect was enhanced by gonadectomy in males, but not in females (Kriegsfeld and Nelson, 1996). Among SD-housed males in that study, castrated males experienced faster (P<0.01) rates of weight gain than intact males, but ovariectomy had no effect on the rate of weight gain in females (P>0.05). It is evident from these studies that the effect of sex varies greatly between species, so we cannot be certain how other seasonal animals, such as the domestic cat, may be affected by this.

**Photoperiod link to gonadectomy in seasonal mammals**

An interesting pattern is observed among seasonal mammals that experience BW changes in response to photoperiod. As noted by Bartness et al. (2002), the BW response to shortened day length can usually be predicted by their response to gonadectomy. For example, species such as the Siberian hamster that lose weight in response to SD also lose weight in response to gonadectomy (Wade and Bartness, 1984). Likewise, species such as the Syrian hamster, which gains weight in response to SD, also gain weight in response to gonadectomy (Bartness and Wade, 1984; Hoffmann, 1978). Seasons of increased sexual activity are sometimes associated with increased BW, but in other cases are associated with decreased BW. Although both Syrian and Siberian hamsters are LD breeders (Hoffmann, 1978; Tamarkin et al., 1976), Siberian hamsters have an increase in BW during the breeding season while Syrian hamsters have a decrease in BW. Because most small mammals are LD breeders (Amoroso and Marshall, 1960), this may explain why a correlation is observed between response to gonadectomy and response to SD. Gonadectomy in LD breeders may
simulate SD conditions, so LD breeders that gain weight in response to SD will also gain weight in response to gonadectomy. In contrast, LD breeders that lose weight in response to SD will also lose weight in response to gonadectomy.

**MECHANISMS OF PHOTOPERIOD RESPONSE**

Many mechanisms have been proposed for the photoperiod-induced weight gain/loss that occurs in seasonal mammals. Evidence to support these mechanisms is not always observed in all species, however, indicating that different mechanisms may be responsible for photoperiod response in different species. The mechanisms with greatest evidence have been outlined below.

**Activity and energy**

*Voluntary physical activity*

One potential mechanism contributing to seasonal weight gain or weight loss is a decrease or increase in voluntary physical activity levels, respectively. A study of Siberian hamsters indicated that exposure to SD (8 hr light: 16 hr dark), caused a temporary increase (P<0.05) in physical activity compared to LD (16 hr light: 8 hr dark) treatment (Warner et al., 2010). After 4 wk of exposure to SD, male hamsters exhibited increased (P<0.05) physical activity, but by wk 8, activity had returned to basal levels (Baseline: ~45 successive beam breaks; wk 4: ~95 beam breaks; wk 8: ~40 beam breaks). In this case, increased activity was temporary, but BW continued to decline through the entire SD period (P<0.001).

Conversely, a study of female Syrian hamsters found that SD (8 hr light: 16 hr dark) treatment caused lower (P<0.05) activity (measured as the number of 6 min periods per day that were at least half filled with activity) than LD (16 hr light: 8 hr dark) treatment (~25 vs. ~48 6 min periods/d; Widmaier and Campbell, 1980). Additionally, when these hamsters
were ovariectomized, physical activity decreased (P<0.001) in LD hamsters (decrease of ~18
6 min periods/d), whereas in SD-housed ovariectomized hamsters, activity remained constant
(Widmaier and Campbell, 1980).

*Energy intake*

Because changes in physical activity alone cannot fully explain changes in BW, others have hypothesized that altered feeding habits also contribute. Siberian hamsters that were transitioned to SD (8 hr light: 16 hr dark) from LD (16 hr light: 8 hr dark) had a decreased (P<0.01) energy intake (4.8 kJ/d) and BW (1.5 g) immediately, but there was a 2 wk delay before the authors noted a decrease in energy expenditure (Knopper and Boily, 2000). They concluded that the decrease in energy intake was sufficient to account for the decrease in BW. In that study, because the reduced body mass preceded the decreased energy expenditure, the authors hypothesized that the reduction in BW led to reduced basal energy requirements (Knopper and Boily, 2000). This would explain why the decline in BW was unaffected when energy expenditure also began to decline; however, physical activity was not monitored and also may have contributed to the reduction in energy expenditure.

Contrary to the results of this study, however, other authors observed that decreased energy intake in male Siberian hamsters did not occur until after BW had already begun to decrease (Wade and Bartness, 1984). In that study, both BW (P<0.01) and feed intake (P<0.05) declined in response to SD (8 hr light: 16 hr dark), but significant declines in BW were observed by wk 2 (P<0.05), whereas energy intake did not begin to decline until wk 4. The fact that BW decreased independently of feed intake indicates that there may still be other contributing factors to these seasonal BW changes.
Energy requirement

Another possible factor that could contribute to seasonal weight gain is a change in the BMR/energy requirement of the animal. Unlike those studies mentioned above, some studies have demonstrated that Syrian and Siberian hamsters lose weight despite maintaining the same food intake, indicating that the energy requirement of these animals had increased (Bartness and Wade, 1985). Another study showed that BMR (corrected for BW) of Siberian hamsters increased (gradual cycle with maximum of approximately 1.90 ml O\textsubscript{2} consumption/g BW/hr in January and minimum of approximately 1.45 ml/g/hr in July) in response to decreasing day length (Frankfurt, Germany; natural light), but that thermal insulation, as measured by fur depth, increased (P<0.001; summer: 7.84±0.18 mm, winter: 9.58±0.15 mm) and BW decreased (gradual cycle with maximum of approximately 40 g in September and minimum of 25 g in February) so that total energy requirements were actually lower during SD (Heldmaier and Steinlechner, 1981).

One contributing factor to the energy requirement of an animal is non-shivering thermogenesis. In species that experience SD-induced weight loss, BAT mitochondrial protein and/or uncoupling protein-1 (UCP-1) concentrations often increase, increasing non-shivering thermogenesis, even when animals are housed at a constant temperature (Wang et al., 2006; Zhao and Wang, 2005). This response occurs even when environmental temperatures are held constant (i.e., in conditions when no practical need for improved SD thermogenesis exists), suggesting that this response is largely due to photoperiod.
Hormones

Melatonin

Melatonin is a hormone found ubiquitously in both plant and animal species. In vertebrates, synthesis occurs primarily in the pineal gland, and secretion is synchronized with the light/dark cycle. Peak blood concentrations occur during dark periods, while light causes a sharp decline to relatively low baseline concentrations (Leyva et al., 1984). Since investigations of seasonal weight change first began, melatonin has been suggested as a key signaling hormone contributing to such change (Hoffmann, 1973). In Siberian hamsters, implantation of a melatonin/beeswax pellet in hamsters exposed to LD (16 hr light: 8 hr dark) negated LD-induced weight gain, causing hamsters to maintain similar growth to that of the SD-housed (8 hr light: 16 hr dark) hamsters (Hoffmann, 1973). Untreated LD-housed hamsters and sham implanted LD-housed hamsters both experienced higher (P<0.01 and P<0.05, respectively) weight gain than the SD-housed and melatonin-implanted LD-housed hamsters (Hoffmann, 1973).

As mentioned above, most melatonin synthesis occurs in the pineal gland, so it might be expected that removal of the pineal gland would prevent any melatonin-induced physiological changes. Actual results, however, are contradictory. In Syrian hamsters, pinealectomy prevents SD-housed animals (1 hr light: 23 hr dark) from undergoing seasonal reproductive inactivity (Hoffman and Reiter, 1965), but not from experiencing SD (8 hr light: 16 hr dark)-induced weight gain (Bartness and Wade, 1984). This lack of effect of pinealectomy on weight gain seems to be in conflict with the fact that this same group of animals (Bartness and Wade, 1984), when exposed to LD (16 hr light: 8 hr dark), experienced weight gain (P<0.05) in response to melatonin supplementation (high fat diet:}
approximately 40 g above LD untreated control; control diet: approximately 15 g above control). This would seem to indicate that although melatonin causes weight gain during LD, its removal does not prevent weight gain during SD, suggesting that other mechanisms are involved. Because the researchers in that study did not measure serum melatonin concentrations, one possibility is that pinealectomy did not actually prevent melatonin secretion in response to SD. Alternatively, it is also possible that there are alternative mechanisms that compensate for the removal of melatonin as a signaling hormone.

Just as melatonin supplementation can mimic SD conditions, melatonin antagonists can also mimic LD conditions in some mammals. Dormice usually gain weight in response to reduced day length or melatonin treatment, but treatment with a melatonin antagonist prevented this seasonal weight gain response (Le Gouic et al., 1996). The authors of that study observed increased BW in SD-housed mice (6 hr light: 18 hr dark; 45±7% BW increase) compared to LD-housed mice (16 hr light: 8 hr dark; no BW change noted). When mice housed under natural photoperiod (gradually decreasing from approximately 16 hr light: 8 hr dark to 11hr light: 13hr dark) were administered a melatonin agonist, weight gain occurred 2 wk earlier than natural photoperiod controls, whereas natural photoperiod mice that were administered a melatonin antagonist exhibited no SD increase in BW.

*Leptin resistance*

Circulating leptin concentrations are usually negatively correlated with food intake, with high concentrations of leptin suppressing appetite and limiting weight gain. In certain seasonal mammals, however, animals exhibit feeding behavior that is inconsistent with serum leptin concentrations. For example, Li and Wang (2007) studied Brandt’s voles and Mongolian gerbils and observed that SD-housed voles and gerbils (8 hr light: 16 hr dark) had
27.4% and 48.1% lower (P<0.01) serum leptin concentrations, respectively, than LD-housed voles and gerbils (16 hr light: 8 hr dark). This decline in serum leptin, however, was not accompanied by increased food intake, suggesting that leptin resistance may contribute to SD-induced loss of BW in these species.

Siberian hamsters demonstrate signs of leptin resistance during LD (16 hr light: 8 hr dim red of <1 lux) but not SD (8 hr light: 16 hr dim red) treatment (Rousseau et al., 2002). Leptin treatment reduced BW during SD (P<0.05; ~4 g below baseline), but failed to change BW during LD, indicating a reduced sensitivity to leptin during seasons of normal weight gain. Similar results have been observed in field voles, with reduced (P<0.001) BW observed in SD-housed leptin-infused voles compared to saline-infused voles, but no difference noted between LD-housed leptin-infused voles and their saline-infused controls (Krol and Speakman, 2007).

**Feline energy metabolism and obesity**

Feline obesity is a growing problem in developed nations and it is estimated that between 25 and 40% of the pet cat population is overweight or obese (Freeman et al., 2006; Lund et al., 2005). Obesity increases risk for numerous other health problems, such as hepatic lipidosis, dermatological disease, urinary tract disease, and diabetes and, therefore, represents a significant threat to pet health and welfare.

Currently, the main focus of obesity-reduction strategies has been on diet, while strategies focused on altering physical activity have been relatively lacking. Gonadectomy is one of the major risk factors for developing obesity, and recent experiments have demonstrated significant reduction in physical activity after spaying (Belsito et al., 2009; Vester et al., 2009). While activity during light hours was not greatly affected 12 wk after
spaying, physical activity during the dark period decreased (P<0.001) up to 75% (Belsito et al., 2009). Activity during the light period decreased to amounts similar to that of the dark period between wk 12 and 24 (Belsito et al., 2009). Considering the dramatic decline in activity level after gonadectomy, weight maintenance strategies aimed at modifying voluntary activity could be very useful in reducing incidence of obesity. Given how photoperiod affects activity, food intake, BW, and metabolism of seasonal mammals, similar responses may be expected in cats.

**Feline Seasonal Reproductive Response**

Most research regarding the effects of photoperiod in cats has focused on changes in their reproductive physiology. It has long been known that cats are seasonal breeders, but more recently, researchers demonstrated that domestic cats respond to photoperiod even when other environmental cues are held constant (Hurni, 1981). Domestic queens are seasonally polyestrous, with a period of anestrous that usually extends from September or October to January or February in the Northern Hemisphere (Concannon, 1991). A period of diminished sexual activity that mirrored the period of anestrous in females has also been observed in male cats although this occurred only after reducing sensory feedback by surgically removing a section of the dorsal nerve of the penis (Aronson and Cooper, 1966). Only under these specific circumstances, however, is there evidence of seasonal behavioral changes in sexual activity among male domestic cats.

Among other felid species, the Pallas’ cat demonstrates particularly drastic seasonal reproductive cycles, with the females experiencing an extended period of anestrous from May through December (animals housed outdoors in various zoos throughout the USA; Brown et al., 2002). Males of this species have improved seminal traits (e.g., semen volume,
sperm concentration and motility, and % normal sperm) during the months of January through April (animals housed indoors at North Carolina State University with artificial simulation of natural light cycle), which matches the breeding season noted in females in other studies (Mellen, 1998; Newell-Fugate et al., 2007).

Unlike observations of wild felids, domestic cats maintained under laboratory conditions, with unchanging photoperiod (12 hr light: 12 hr dark), show a lack of cyclicity in the number of estrous cycles per month (Wildt et al., 1978). During seasons when wild felids experience anestrus, laboratory-housed queens still exhibit shortened (P<0.05) estrous periods (1 to 2.5 d shorter in June, September, October, and November than in March, April, May, August, or December), but they do not experience extended periods of anestrus that have been observed in cats exposed to the naturally changing photoperiod typical of temperate latitudes (Concannon, 1991; Wildt et al., 1978). Similar results were observed in a survey of laboratory animal breeders who housed cats under natural conditions. The results of that survey demonstrated that latitude had a strong impact on the breeding season of the animals (Hurni, 1981). Cats at the equator (12 hr light: 12 hr dark) produced litters year-round, but at higher latitudes, the breeding season steadily shortened until it reached approximately 6 mo among populations in the polar circle.

Although behavioral signs of estrus are not always an accurate indication of mature follicle activity (Wildt et al., 1978), studies that measure hormonal changes in Pallas’ cats show results similar to those observed in behavioral studies. Both male and female cats experience hormonal changes in response to photoperiod. Two small-scale studies demonstrated that male Pallas’ cats have increased fecal testosterone concentrations during the breeding season. Brown et al. (2002) observed an average increase in fecal testosterone
concentrations in three male Pallas’ cats (one housed outdoors at the Conservation and Research Center at the National Zoo and two housed indoors with exposure to natural light at the Cincinatti Zoo) during the breeding season (November-April) compared to the non-breeding season (352.3±30.3 vs. 82.1±3.3 ng/g). Newell-Fugate et al. (2007) also observed increased fecal testosterone during the breeding season (mid-January to mid-March) compared to the non-breeding season in three individually-housed males (Individual #1: 1113±368 vs. 576±311 ng/g; #2: 2204±891 vs. 876±472 ng/g; and #3: 1948±985 vs. 625±320 ng/g) and one male that was paired with a female during the breeding season (4264±2644 vs. 852±353 ng/g). These cats were all housed in an indoor facility at North Carolina State University with artificial lighting simulating natural photoperiod. Males also had increased serum testosterone concentrations [February: 5.3±0.9 vs. December: 3.3±0.8, April: 3.7±1.8, and June: 3.6±0.4 ng/ml (Newell-Fugate et al., 2007); breeding season: 1.27±0.41 vs. non-breeding season: 0.33±0.29 ng/ml (Swanson et al., 1996)], seminal volume [February: 103.5±45.5 vs. December: 51.7±31.5, April: 164±61.5, and June: 76.8±15.8 µl (Newell-Fugate et al., 2007); breeding season: 210±10 vs. non-breeding season: 190±10 µl (Swanson et al., 1996)] and seminal viability (as determined by measures of motility and percentage of structurally normal sperm) during the breeding season (Newell-Fugate et al., 2007; Swanson et al., 1996). Female Pallas’ cats also had increased fecal estrogen concentrations during the breeding season (January-April) compared to the non-breeding season (128.4±18.9 vs. 15.2±8.5 ng/g) in six females (four housed indoors and two housed outdoors) in various zoos and research centers in the United States (Brown et al., 2002).
Several observations also have been made of seasonal increases in BW immediately prior to the breeding season in Pallas’ cats. Swanson et al. (1996) studied a single male Pallas’ cat, but did not observe significant difference in BW between the defined breeding and non-breeding seasons (5.47±0.34 vs. 4.68±0.25 kg, P>0.05); however, BW exhibited clear seasonal cyclicity. Newell-Fugate et al. (2007) evaluated four male Pallas’ cats and reported peak BW during breeding season (February: 5.2±0.2 vs. December: 4.8±0.1, April: 4.4±0.6, and June: 4.0±0.4 kg). Although these studies have been performed with a limited number of animals, these results are consistent with the changes reported in other seasonal mammals. Further investigation is needed to determine if these seasonal changes in BW are also present in domestic cats.

To date, only one study has investigated photoperiod effect on seasonal BW changes in domestic cats. Bermingham et al. (2012) observed mostly outdoor-housed (n=17, young and old) and some indoor-housed (n=8, young) domestic cats. Those cats exhibited seasonal fluctuations in BW (P<0.001), with all three groups demonstrating increased BW during winter months. That study also observed an interaction of season and age in regards to energy expenditure, where both indoor- and outdoor-housed young cats exhibited increased (P=0.05) energy expenditure during summer months, but old cats experienced no change.

**Feline response to gonadectomy**

Energy requirements for gonadectomized animals are significantly lower than those for intact animals, likely due to reduced BMR and/or physical activity. Root et al. (1996) reported that neutered male cats had 28% lower heat production, and spayed females had 33% lower heat production than intact animals. Body weight and activity were not reported for that study, so it is unknown whether gonadectomized animals also gained weight as a
result of reduced BMR, or if physical activity had any impact on BMR. Another study reported similar results in a group of female cats in which energy intake necessary to maintain BW was 24-30% lower for ovariec
tomized females, despite observing no reduction in physical activity (Flynn et al., 1996). Unfortunately, measurement of physical activity in that study was conducted by human observers for limited periods of time (out-of-cage: 5 min/wk, in-cage: 10 min/wk), so it is possible that the presence of the observer influenced activity, and/or there was not a long enough period to measure such changes.

Although Flynn et al. (1996) did not observe differences in activity level between intact and spayed cats, other studies using more objective measures of physical activity have demonstrated dramatic decreases in voluntary physical activity after spaying (Belsito et al., 2009; Vester et al., 2009). Belsito et al. (2009) observed that, during the first 12 wk after spaying, activity during the dark period decreased (P<0.001) dramatically (~75% decrease), but light activity did not change. During the next 12 wk after spaying (wk 12-24), light activity decreased (P<0.05), leading to a total decrease in activity from baseline of 52% [P<0.001; 30.0 vs. 14.3 activity counts per epoch (15 sec.)]. Similarly, Vester et al. (2009) observed decreased physical activity (P<0.001) 24 wk after ovariectomy in both cats fed a high-protein (HP) diet [26.9 vs. 11.7 activity counts per epoch (15 sec.)] and those fed a moderate-protein (MP) diet (34.4 vs. 15.8 activity counts per epoch). This reduction in activity was caused by a reduction in both light (HP: 24.9 vs. 13.1 activity counts per epoch; MP: 31.1 vs. 16.1 activity counts per epoch) and dark activity (HP: 30.7 vs. 8.8 activity counts per epoch; MP: 41.1 vs. 14.9 activity counts per epoch). The increase (P=0.02) in the light:dark activity ratio (0.8 vs. 2.1), however, indicated that dark activity decreased more than light activity.
In addition to observations of domestic cats, gonadectomy also has been observed to cause weight gain in feral cat populations. In a study of 14 feral male cats, BW was reported to increase by 40% 1 yr after neutering, with an average increase in fat pad area of 420% (Scott et al., 2002).

Increased weight gain in gonadectomized cats is exacerbated by *ad libitum* feeding of a high fat-diet (Nguyen et al., 2004). As expected, gonadectomized cats in that study gained more (P<0.05) weight that intact cats (53.80±5.79% vs. 27.11±5.79% over baseline), but weight gain was numerically less pronounced with *ad libitum* feeding of a low- (14.4 MJ/kg) vs. high-fat (18.4 MJ/kg) diet in both males and females. Castrated males gained more fat mass (P<0.05) when fed a high-fat diet than when fed a low-fat diet (292.23±47.85% increase in body fat vs. 108.06±44.83% increase in body fat). This response to gonadectomy mirrors that of photoperiodic changes in other seasonal mammals. Syrian hamsters gained more weight during SD when fed a high-fat diet (2 parts basal diet + 1 part vegetable shortening; 5.3 kcal/g) than when they were fed a lower calorie diet (basal diet; 3.4 kcal/g) (Bartness and Wade, 1984). Although photoperiod-induced weight gain occurs in hamsters fed a low-fat diet, the results are far less dramatic than those fed a high-fat diet (Bartness and Wade, 1984).

Although physical activity and BMR are affected, the post-neuter weight gain is also likely due to a decreased ability to self-regulate food intake. Researchers have observed that ovariectomized cats refuse less food than intact animals, indicating a diminished ability to self-regulate caloric intake (Flynn et al., 1996). When cats are fed *ad libitum*, food intake increases significantly after ovariohysterectomy, despite increasing blood leptin concentrations (Vester et al., 2009). This lack of intake regulation, combined with a high-fat
diet, will most likely lead to increased energy intake and could be one cause for the greater weight gain observed when gonadectomized cats are fed such diets.

Several mechanisms have been proposed for the weight gain observed in gonadectomized cats. While some researchers have noted lower heat production in gonadectomized animals, despite similar levels of activity, others have noted a decrease in voluntary physical activity. It is also probable that gonadectomized animals are less able to self-regulate their caloric intake, especially when fed highly energy-dense diets. A combination of all three of these factors, and likely other factors as well, lead to lower energy requirements for gonadectomized cats and an increased risk for obesity.

**Thesis Objectives**

The prevalence of feline obesity is increasing, and with it comes a wide array of associated diseases. There has been much emphasis on dietary intervention, as well as some effort to modify behavior, but the potential effects of photoperiod on weight management in the cat have not been studied. Therefore, the objectives of this thesis were to determine the effects of photoperiod on cats as regards: 1) voluntary activity levels, resting metabolic rate (RMR), body composition, and caloric intake necessary to maintain BW, and 2) serum concentrations of the appetite control hormones, leptin and ghrelin. We can use the pattern established in small seasonal animals to hypothesize that because cats gain weight in response to gonadectomy, they also will gain weight in response to shortened day length.
LITERATURE CITED


Mellen, J. 1998. Pallas’ cat (Otocolobus manul) international studbook. Disney’s Animal Kingdom, Buena Vista, FL.


CHAPTER 3: EFFECTS OF PHOTOPERIOD ON WEIGHT MAINTENANCE IN

ADULT NEUTERED MALE CATS

ABSTRACT

With the continued rise of obesity in humans and companion animals, novel weight management strategies are needed. To date, most strategies have focused on dietary intervention. Strategies aimed at altering physical activity, an important factor in weight maintenance, have been lacking. Due to the drastic decrease in physical activity level noted after gonadectomy, neutered animals are targets for activity-related weight management strategies. Photoperiod is known to cause physiological changes in seasonal mammals, including changes in body weight (BW) and reproductive status. Thus, our objective was to determine the effect of increased photoperiod (longer days) on voluntary physical activity levels, resting metabolic rate (RMR), food intake required to maintain BW, and fasting serum leptin and ghrelin concentrations in adult cats. Eleven healthy, adult, neutered, male domestic shorthair cats were used in a randomized crossover design study. During two 12-wk periods, cats were exposed to either a short day (SD) photoperiod of 8 hr light: 16 hr dark or a long day (LD) photoperiod of 16 hr light: 8 hr dark. Cats were fed a commercial diet to maintain baseline BW. In addition to daily food intake and twice-weekly BW, RMR (via indirect calorimetry), body composition [via dual-energy X-ray absorptiometry (DEXA)], and physical activity (via Actical activity monitors) were measured at wk 0 and 12 of each period. Fasting serum leptin and ghrelin concentrations were measured at wk 0, 6, and 12 of each period. Average hourly physical activity was greater (P=0.008) in LD vs. SD cats (3770 vs. 3129 activity counts/hr), which was primarily due to an increase (P<0.001) in dark period activity (1188 vs. 710 activity counts/hr). This corresponded to a higher (P<0.0001) daily
ME intake (mean over 12-wk period: 207 vs. 197 kcal/d), and an increased (P=0.048) RMR in LD cats (9.02 vs. 8.37 kcal/h). Body composition, serum leptin, and serum ghrelin were not altered by photoperiod. More research is needed to determine potential mechanisms by which these physiological changes occurred and how they may apply to weight management strategies.

**INTRODUCTION**

Feline obesity is a growing problem in developed nations and it is estimated that between 25% and 40% of the pet cat population is overweight or obese (Freeman et al., 2006; Lund et al., 2005). Obesity increases risk for numerous other health problems, including hepatic lipidosis, dermatological disease, urinary tract disease, and diabetes, and therefore represents a significant threat to pet health and welfare. While many dietary strategies have been tested to reduce obesity incidence, strategies to alter physical activity have been lacking.

Photoperiod is known to alter many physiologic outcomes in seasonal animals, including food intake, weight gain, and estrus. The response to decreased daylight depends on species, with some gaining weight and others losing weight. Interestingly, the response to shortened day length can usually be predicted by the response to gonadectomy. For example, Siberian hamsters and meadow voles decrease BW and fat mass with gonadectomy (Bartness, 1996; Dark and Zucker, 1984; Wade and Bartness, 1984) or shortened days (Dark and Zucker, 1984; Wade and Bartness, 1984). In contrast, Syrian hamsters, collared lemmings, and prairie voles have increased BW and fat mass with gonadectomy (Gower et al., 1994; Kriegsfeld and Nelson, 1996; Morin and Fleming, 1978; Slusser and Wade, 1981)
or shortened days (Bartness and Wade, 1984; Gower et al., 1994; Kriegsfeld and Nelson, 1996).

Recent experiments with cats in our laboratory (Belsito et al., 2009; Vester et al., 2009) have observed significant reduction in physical activity after spaying. While decreased activity was an expected outcome, the differences between the dark and light periods were interesting. Moreover, while activity counts during light hours were not greatly affected after spaying, physical activity during the dark period decreased dramatically (up to 75%; Belsito et al., 2009). Given this difference between dark and light periods, we were interested in evaluating the effects of photoperiod on weight management in cats.

Because cats are sensitive to photoperiod as it relates to breeding (Dawson, 1941; Michel, 1993), photoperiod also may affect food intake, BW, activity levels, and circulating ghrelin and leptin concentrations; however, this topic has not yet been researched in cats. Cats are known to gain BW and reduce activity in response to gonadectomy (Belsito et al., 2009; Nguyen et al., 2004; Scott et al., 2002; Vester et al., 2009), so if cats respond to photoperiod, it is likely that they will gain BW and reduce activity. The objective of this thesis was to evaluate the effect of photoperiod on weight management in cats as measured by voluntary activity, resting metabolic rate (RMR), body composition, caloric intake necessary to maintain BW, and serum leptin and ghrelin concentrations.

**MATERIALS AND METHODS**

**Animals**

Eleven healthy adult (4 yr old) neutered male domestic shorthair cats (existing colony at the University of Illinois) of healthy body condition (5/9 body condition score) were used. Cats were group housed for most of each day (22 hr) but were isolated in individual stainless
steel cages (0.61 × 0.61 × 0.61 m) for 1 hr twice daily so food could be offered and individual intake measured. One group of cats (n=6) was housed in a room measuring 12 m² and a second group of cats (n=5) was housed in a room measuring 26 m². Cats had access to various toys and scratching posts for behavioral enrichment. All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee before animal experimentation.

Diet and treatments

Cats were fed a dry commercial diet (Whiskas® Meaty Selections®, Mars, Inc., Mattoon, IL) to maintain BW (within 5% of baseline BW) throughout the experiment. One half of daily food intake was offered at 9 am and 4 pm each day. Uneaten food was weighed daily so food intake could be calculated. Water was available ad libitum throughout the experiment.

Four weeks prior to the experiment (wk -4), cats were acclimated to the diet and daily feeding schedule, and were exposed to 12 hr light: 12 hr dark. The experiment was performed using a crossover design composed of two 12-wk periods. Cats were randomly assigned to one of two groups, and with groups being randomly assigned to room. Cats remained in the same rooms throughout the study. For the first 12-wk period, Group 1 was exposed to 16 hr light: 8 hr dark (LD), while Group 2 was exposed to 8 hr light: 16 hr dark (SD). For the second 12-wk period, Group 1 was exposed to a SD photoperiod and Group 2 was exposed to a LD photoperiod. Daily food intake and twice-weekly BW were measured throughout the study.
Chemical analyses

Diet samples were ground through a 2 mm screen with dry ice using a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) in preparation for chemical analyses. Diets were analyzed for dry matter (DM) and organic matter (OM) according to the Association of Official Analytical Chemists (AOAC, 2006; methods 934.01, 942.05). Crude protein (CP) was determined according to the Association of Official Analytical Chemists (AOAC, 2006; method 992.15) using a Leco Nitrogen/Protein Determinator (model FP-2000, Leco Corp., St. Joseph, MI). Fat concentrations were determined by acid hydrolysis (AACC, 1983) followed by diethyl ether extraction (Budde, 1952). Total dietary fiber was determined according to Prosky et al. (1992). Gross energy was determined by use of a bomb calorimeter (model 1261, Parr Instrument Co., Moline, IL). Diet composition is reported in Table 3.1.

Blood sampling

Overnight-fasted blood samples were collected via jugular or femoral venipuncture during wk 0, 6, and 12 of each period. Blood was immediately transferred to serum separator Vacutainer® tubes, and tubes were centrifuged for 10 min at 2350 rpm at 4°C. After centrifugation, serum was collected and stored at -80°C until analyzed. Serum chemistry profile and non-esterified fatty acids (NEFA) were analyzed by the University of Illinois Clinical Diagnostic Laboratory using a Hitachi 911 Clinical Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Serum leptin concentrations were determined using a multi-species leptin radio-labeled immunoassay (RIA; Millipore, St. Charles, MO) previously validated for use in the cat (Backus et al., 2000). Serum ghrelin concentrations were determined using an enzyme immunoassay kit (Ghrelin Canine EIA Kit, Phoenix...
Pharmaceuticals, Inc., Burlingame, CA) following a 10-fold dilution of serum (validated for use in our laboratory using parallel determination from increasing dilutions of serum of at least four cats).

*Body composition*

Body composition (lean mass, fat mass, and bone mineral mass) was measured during wk 0 and 12 of each period by dual-energy X-ray absorptiometry (DEXA), which has been previously validated in cats (Backus et al., 2000; Speakman et al., 2001). Cats were placed in ventral recumbency and body composition was analyzed using a Hologic model QDR-4500 Fan Beam X-ray Bone Densitometer and software (Hologic Inc., Waltham, MA).

*Indirect calorimetry*

Indirect calorimetry measurements took place during wk 0 and 12 of each period in calorimetry chambers measuring 0.52 x 0.52 x 0.41 m, with airflow of 5L/min. A silica drying column was used to equilibrate humidity.

Respiratory exchange ratio (RER), heat, and flow of air through the chambers were measured using an open-circuit Oxymax System (Software, Version 5.0, Columbus Instruments, Columbus, OH). System settle time for each chamber was 60 sec and system measure time for each chamber was 30 sec. Calibration of the calorimetry chambers was performed daily using standard gas mixtures containing known concentrations of CO₂, O₂, and N₂ against known calibration gas standards. Room temperature was maintained at 25 ± 1 °C.
The following calculations were used:

\[
RER = \frac{L \, CO_2 \, produced}{L \, O_2 \, consumed}
\]

Heat production (kcal) = \(3.82 \times L \, O_2 \, consumed + 1.15 \times L \, CO_2 \, produced\)

\[
Heat/\text{metabolic body size} = \frac{\text{heat production (kcal)}}{BW^{0.75} (kg)}
\]

Cats were fasted overnight before indirect calorimetry and acclimated to the calorimetry chambers for at least 4 hr, after which time measurements were taken for 2 hr. Data from these 2 hr were extrapolated to obtain 24 hr daily energy expenditure values. Calorimetry system conditions and calculations used were performed according to Hoenig et al. (2007).

Physical activity level

Voluntary physical activity was measured using Actical activity monitors (Mini Mitter, Bend, OR) prior to wk 0 and 12 of each period. Activity collars were worn around the neck for seven consecutive days, previously validated for use in research cats (Lascelles et al., 2008). Collars contained an accelerometer, which monitored both the intensity and duration of movements and converted this information into an electrical current of varying magnitude. After 7 d of activity measurements, collars were removed and data were analyzed, compiled, and converted into arbitrary numbers referred to as ‘activity counts’ by Actical software. Activity counts were summed and recorded at intervals of 15 sec. Activity data are represented as activity counts per hr.

Statistical analysis

Data were analyzed by the Mixed Models procedure (SAS Institute, Cary, NC). Light:dark activity ratios, and serum glucose and potassium concentration data were
transformed to obtain normality and homogeneity of variance (using log transformations for activity and glucose, and squared transformation for potassium). Statistical analysis and differences were determined utilizing the transformed data; however, observed means are presented in the tables, figures and text. Food intake, and serum concentrations of ghrelin, leptin, cholesterol, NEFA, and triglycerides, were analyzed using repeated measures analysis. The fixed effects of photoperiod, time, and treatment x time were tested. Cat (nested within room) and period were considered random effects. For the remaining variables, the fixed effect of photoperiod was tested. Cat (nested within room) and period were considered random effects. Least squares means were separated using LSD with a Tukey adjustment. A $P < 0.05$ was considered statistically significant and a $P < 0.10$ was considered to be a trend.

Reported pooled SEM values were determined according to the Mixed Models procedure of SAS.

**RESULTS**

*Food intake*

Short day-housed cats required lower ($P < 0.0001$) energy intake to maintain BW than LD-housed cats (197.49 vs. 206.88 kcal/d; data not shown). On a weekly basis, the difference in food intake became significant at wk 7 and remained significant until wk 10 (Figure 3.1).

*Blood hormones and metabolites*

No differences were noted in serum leptin ($P = 0.696$) or ghrelin ($P = 0.692$) concentrations between SD and LD groups (Table 3.2). Using combined averages of wk 6 and wk 12 data, serum cholesterol tended to be higher and NEFA tended to be lower in SD-
housed cats (P=0.085 and P=0.097, respectively). Albumin was higher (P=0.038) in LD than SD cats, and creatinine tended to be higher (P=0.090) in LD cats (Table 3.3).

**Body composition**

No significant differences were noted between SD and LD cats as it pertains to body composition (fat: P=0.451, lean: P=0.579, mineral: P=0.237) as measured by DEXA (Table 3.4).

**Indirect calorimetry**

Resting metabolic rate was lower (P=0.048) in SD-housed cats compared to LD-housed cats (8.371 vs. 9.025 kcal/hr), resulting in lower daily energy expenditure (200.9 vs. 216.6 kcal/d). The RER tended to be lower (P=0.064) in LD-housed vs. SD-housed cats (0.765 vs. 0.773; Table 3.5).

**Physical activity level**

Total voluntary activity was lower (P=0.008) in SD-housed vs. LD-housed cats (3129 vs. 3770 activity counts/hr; Table 3.6). Using averages of all light and dark hours, light activity remained similar (P=0.192) between treatments, but average hourly dark activity was greater (P<0.001) during SD treatment than LD treatment (1852 vs. 991 activity counts/hr). This led to a higher (P<0.001) light:dark ratio during LD treatment than SD treatment (11.75 vs. 1.61; Table 3.6). Comparing only the 8 hr that were light during both treatments (9:00 am-5:00 pm) and the 8 hr that were dark during both treatments (9:00 pm-5:00 am), however, the results differ. With average hourly light and dark activity counts from these hours, average light activity still was similar (P=0.528) between treatments, but average hourly dark activity was lower (P<0.001) in SD- than LD-treated cats (710 vs. 1188 activity counts/hr; Table 3.6). This resulted in a lower (P=0.001) light:dark activity ratio during LD treatment.
than SD treatment (5.98 vs. 9.11). The 8 hr average values more closely express the patterns seen in the daily activity profile, which indicates additional activity peaks during the dark period for LD-housed cats (Figure 3.2).

**DISCUSSION**

As discussed above, feline obesity is becoming increasingly prevalent, especially in developed countries. Dietary strategies are important in treating obesity, but alternative treatments, such as those designed to increase activity or basal energy requirement, can also be valuable. The objectives of this study were to determine the effect of photoperiod on weight management parameters, including feed intake necessary to maintain BW, voluntary activity, RMR, body composition, and blood concentrations of appetite control hormones.

In order to make a crossover design possible, cats were fed to maintain BW throughout the study to prevent excessive weight gain between treatment periods. Because cats were not fed *ad libitum*, it was not possible to determine if photoperiod induced a change in voluntary food intake, but SD-housed cats required reduced energy intake to maintain BW. Food intake was different between SD- and LD-housed cats between wk 7 through 9 and tended to be different in wk 6 and 10, but did not remain significant through the end of the study. It is unclear if this was because the variance was too large to determine significance, or if the photoperiod effect on food intake was only transitory. A longer trial is necessary to determine how long a photoperiod-induced change in energy requirement can be sustained.

Resting metabolic rate was higher in LD-housed cats, which is likely responsible, in part, for the increased energy intake necessary for the cats to maintain BW. If the daily increase in energy expenditure due to higher RMR is extrapolated to an annual value, it means a difference of up to 5730 kcal/yr between LD and SD cats. This is equivalent to a
potential weight loss of approximately 0.74 kg or 1.6 lb/yr, based on the assumption that 1 lb of body fat is equivalent to 3500 kcal (Wishnofsky, 1958). Although there are many factors that could affect the annual energy requirement of cats, this illustrates that relatively small changes in daily energy expenditure can have large long-term effects. The Respiratory Exchange Ratio is used to indicate which macronutrient is being oxidized as the primary source of energy, with higher values indicating a higher rate of carbohydrate oxidation and lower values indicating a higher rate of fat oxidation. Although there was a trend indicating a lower RER in LD-housed cats, this difference is insufficient to have any biological significance.

The increase in voluntary physical activity in LD-housed cats was also likely responsible, in part, for the increased energy intake necessary for the cats to maintain BW. This combined with the higher basal energy expenditure suggests that, if cats had been allowed to feed *ad libitum*, cats exposed to LD would have gained less weight than cats exposed to SD.

In previous feline activity studies, during which photoperiod remained constant, total or average light and dark activity have been considered, as well as the light:dark activity ratio. In our study, however, due to the changing photoperiod, total light and total dark activities could not be compared between SD and LD treatments, and hourly averages also can be misleading since they do not account for differing levels of activity throughout the day. If we use these hourly averages, they indicate that light activity remains similar between treatments, but average hourly dark activity is greater during SD treatment than LD treatment, which decreases the light:dark ratio for SD-housed cats. If we look at the daily activity profile, however, we see that this is misleading because the LD-treated cats rarely
have lower activity than SD-treated cats at any time point, and they appear to have additional activity peaks during the dark period, which are not present in SD-housed cats. The average hourly light and dark activity values are misleading, because they compare 16 hr of dark during SD treatment to only 8 hr of dark during LD treatment. The 16 hr of dark during SD treatment include a large portion of one of the two activity peaks that center around feeding times for the cats. As a result, in order to avoid this unequal comparison of light and dark hours, it may be more informative to compare only the 8 hr that were light during both treatments (9:00 am-5:00 pm) and the 8 hr that were dark during both treatments (9:00 pm-5:00 am). Using average hourly light and dark activity counts from only these hours, we observe that average light activity still remains similar between treatments, but average hourly dark activity is actually lower in SD- than LD-treated cats. This results in a decrease in the light:dark activity ratio for LD-treated cats. These 8 hr average values more accurately reflect the trends seen in the cats’ daily activity profile, and indicate that total activity is increased primarily by an increase in nocturnal activity.

Because all cats were fed to maintain BW and were fed the same diet, it may not be surprising that most of the serum hormones and metabolites were similar (P>0.05) for both treatment groups. The difference noted in serum albumin, and the trends observed in serum cholesterol, NEFA, and creatinine were still within the normal range for cats and not large enough to be of biological significance. Indeed, a study of Syrian hamsters observed that, contrary to our results, plasma cholesterol was actually lower (P<0.01) in SD-housed hamsters (10 hr light: 14 hr dark; ~100 mg/dl) compared to LD-housed hamsters (14 hr light: 10 hr dark; ~175 mg/dl), but similar to our study, they observed no differences in plasma triglycerides or glucose (Vaughan et al., 1982). Because none of the trends noted resulted in
serum metabolite concentrations outside of the normal range, we conclude that there were no biologically relevant differences in any serum hormones or metabolites between treatment groups.

It is possible that, if cats had been allowed to feed *ad libitum*, differences in leptin and ghrelin may have been observed, with cats potentially showing the same seasonal leptin resistance as has been reported in other species (Krol and Speakman, 2007; Li and Wang, 2007; Rousseau et al., 2002). Melatonin is also an important hormone that may play a role in photoperiodic regulation of physiological changes. Melatonin was not measured in this study because we were unable to collect all blood samples within a short enough period of time. Due to the large cyclical variation in melatonin concentrations throughout the day (Leyva et al., 1984), comparisons cannot be made between samples that were not collected at the same time.

Body composition also remained constant between treatment groups, which is again likely due to the fact that cats were fed to maintain BW. If future studies are designed to allow cats to feed *ad libitum*, it may be possible to determine how photoperiod affects weight gain or loss and if specific tissue components respond differently. Cats gaining weight after gonadectomy have increased body fat percentage with little changes in lean and bone mass (Belsito et al., 2009; Vester et al., 2009), so it is likely that if cats gain weight during SD, this same response would result.

Future research should continue to investigate the effect of photoperiod and also temperature on seasonal changes in cats. Studies using *ad libitum* feeding could help determine how photoperiod might affect weight gain (e.g., changes in voluntary energy intake vs. changes in energy requirement), and if photoperiod affects body composition.
Research with female cats also is necessary to determine if photoperiod affects them differently than males. The effect of sex on response to photoperiod varies greatly between species (Bartness and Wade, 1984; Kriegsfeld and Nelson, 1996; Li and Wang, 2007; Nagy et al., 1993; Wade and Bartness, 1984), so it is uncertain if/how female cats would react differently than males. Given that female domestic cats react to photoperiod with a very strong reproductive response (Concannon, 1991; Hurni, 1981), whereas there is almost no evidence for a male reproductive response, it is possible that females would exhibit a more dramatic weight response to photoperiod. Melatonin and melatonin agonists have also been noted as important mechanisms regulating photoperiod-induced changes in BW (Hoffmann, 1973; Le Gouic et al., 1996), so future research also should investigate the potential of melatonin agonists to simulate LD increases in energy requirements in cats.

As our understanding of the effect of photoperiod on energy requirements in domestic cats improves, this could be used to develop season-specific feeding recommendations. Depending on the length of time that photoperiod-induced weight changes can be sustained, this could also lead to novel weight management strategies to reduce obesity. Although the results observed in domestic cats do not appear to be as dramatic as those reported in other seasonal mammals, as our understanding of photoperiod effect on cats grows, there is still potential for this to be used in the development of behavioral and/or hormonal treatments for obesity.
LITERATURE CITED


### TABLES AND FIGURES

Table 3.1. Chemical composition of diet fed to cats

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration in diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (DM), %</td>
<td>93.0</td>
</tr>
<tr>
<td>Organic matter (OM)</td>
<td>92.6</td>
</tr>
<tr>
<td>Crude protein (CP)</td>
<td>36.6</td>
</tr>
<tr>
<td>Acid hydrolyzed fat</td>
<td>14.7</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>7.1</td>
</tr>
<tr>
<td>Nitrogen-free extract</td>
<td>41.6</td>
</tr>
<tr>
<td>Gross energy, kcal/g DM</td>
<td>5.13</td>
</tr>
<tr>
<td>Calculated ME(^1), kcal/g DM</td>
<td>3.70</td>
</tr>
</tbody>
</table>

\(^1\) ME = metabolizable energy; calculated using modified Atwater factors (8.5 kcal ME/g fat, 3.5 kcal/g CP, and 3.5 kcal/g nitrogen-free extract)
Table 3.2. Serum hormone and lipid concentrations of short day (SD)- and long day (LD)-housed cats after 6 and 12 wk of treatment

<table>
<thead>
<tr>
<th>Serum component</th>
<th>SD</th>
<th>LD</th>
<th>Pooled SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Photoperiod Week</td>
<td>Photoperiod × Week</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td></td>
<td></td>
<td>0.30</td>
<td>0.696</td>
</tr>
<tr>
<td>6 wk</td>
<td>2.79</td>
<td>2.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 wk</td>
<td>2.89</td>
<td>3.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ghrelin (ng/mL)</td>
<td>5.37</td>
<td>5.26</td>
<td>0.64</td>
<td>0.692</td>
</tr>
<tr>
<td>6 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 wk</td>
<td>5.71</td>
<td>5.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>145.64</td>
<td>149.36</td>
<td>7.68</td>
<td>0.085</td>
</tr>
<tr>
<td>6 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 wk</td>
<td>164.64</td>
<td>151.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA(^1) (mEq/L)</td>
<td>0.20</td>
<td>0.18</td>
<td>0.03</td>
<td>0.097</td>
</tr>
<tr>
<td>6 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 wk</td>
<td>0.18</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglyceride (mg/dL)</td>
<td>31.01</td>
<td>34.09</td>
<td>2.89</td>
<td>0.238</td>
</tr>
<tr>
<td>6 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 wk</td>
<td>36.82</td>
<td>38.72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) NEFA = non-esterified fatty acids
Table 3.3. Serum metabolite concentrations of short day (SD)- and long day (LD)-housed cats after 12 wk of treatment

<table>
<thead>
<tr>
<th>Serum component</th>
<th>SD</th>
<th>LD</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.51</td>
<td>1.63</td>
<td>0.09</td>
<td>0.090</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>23.35</td>
<td>24.01</td>
<td>2.05</td>
<td>0.384</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.24</td>
<td>7.30</td>
<td>0.15</td>
<td>0.493</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.41</td>
<td>3.52</td>
<td>0.04</td>
<td>0.038</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.26</td>
<td>9.40</td>
<td>0.10</td>
<td>0.168</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.83</td>
<td>3.76</td>
<td>0.17</td>
<td>0.626</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>148.55</td>
<td>149.91</td>
<td>0.61</td>
<td>0.105</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>3.97</td>
<td>4.10</td>
<td>0.13</td>
<td>0.194</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>116.82</td>
<td>117.73</td>
<td>0.48</td>
<td>0.157</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>94.88</td>
<td>82.48</td>
<td>11.31</td>
<td>0.881</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>9.88</td>
<td>13.36</td>
<td>2.48</td>
<td>0.124</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>50.17</td>
<td>45.38</td>
<td>6.94</td>
<td>0.453</td>
</tr>
<tr>
<td>γ-glutamyltransferase (U/L)</td>
<td>0.28</td>
<td>0.17</td>
<td>0.17</td>
<td>0.566</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.18</td>
<td>0.18</td>
<td>0.03</td>
<td>0.993</td>
</tr>
<tr>
<td>CO₂ (mmol/L)</td>
<td>16.25</td>
<td>16.39</td>
<td>1.34</td>
<td>0.678</td>
</tr>
</tbody>
</table>
Table 3.4. Body composition of cats as determined by dual-energy X-ray absorptiometry analysis after 12 wk of short day (SD)- or long day (LD)-treatment

<table>
<thead>
<tr>
<th>Tissue</th>
<th>SD</th>
<th>LD</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (g)</td>
<td>688</td>
<td>706</td>
<td>71</td>
<td>0.451</td>
</tr>
<tr>
<td>Lean (g)</td>
<td>3531</td>
<td>3509</td>
<td>71</td>
<td>0.579</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>105</td>
<td>102</td>
<td>6</td>
<td>0.237</td>
</tr>
<tr>
<td>Total (g)</td>
<td>4322</td>
<td>4318</td>
<td>117</td>
<td>0.921</td>
</tr>
</tbody>
</table>
Table 3.5. Resting metabolic rate (RMR), respiratory exchange ratio (RER), and estimated daily energy expenditure of short day (SD)- and long day (LD)-housed cats as determined by indirect calorimetry

<table>
<thead>
<tr>
<th>Item</th>
<th>SD</th>
<th>LD</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMR (kcal/hr)</td>
<td>8.37</td>
<td>9.02</td>
<td>0.854</td>
<td>0.048</td>
</tr>
<tr>
<td>RER</td>
<td>0.773</td>
<td>0.765</td>
<td>0.008</td>
<td>0.064</td>
</tr>
<tr>
<td>Daily energy expenditure (kcal)</td>
<td>200.90</td>
<td>216.59</td>
<td>20.490</td>
<td>0.048</td>
</tr>
</tbody>
</table>
Table 3.6. Total hourly activity, light hourly activity (average of all light hours), dark hourly activity (average of all dark hours), 8-hr light activity (average hourly activity from 9:00 am-5:00 pm), and 8-hr dark activity (average hourly activity from 9:00 pm-5:00 am) of short day (SD)- and long day (LD)-housed cats

<table>
<thead>
<tr>
<th>Item</th>
<th>Activity counts/hr</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>LD</td>
<td></td>
</tr>
<tr>
<td>Total hourly average</td>
<td>3128.73</td>
<td>3770.02</td>
<td>465.85</td>
</tr>
<tr>
<td>Light hourly average</td>
<td>5693.89</td>
<td>5182.44</td>
<td>865.75</td>
</tr>
<tr>
<td>Dark hourly average</td>
<td>1851.85</td>
<td>990.51</td>
<td>215.69</td>
</tr>
<tr>
<td>Light:dark ratio</td>
<td>3.15</td>
<td>5.93</td>
<td>0.44</td>
</tr>
<tr>
<td>8-hr light average</td>
<td>5788.63</td>
<td>6131.48</td>
<td>1216.31</td>
</tr>
<tr>
<td>8-hr dark average</td>
<td>710.44</td>
<td>1188.28</td>
<td>321.92</td>
</tr>
<tr>
<td>8-hr light:dark ratio</td>
<td>9.11</td>
<td>5.98</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Figure 3.1. Weekly averages of daily food intake (± Pooled SEM) of cats. (*) indicates significance (P<0.05) and (#) indicates a trend (P<0.10) between treatments.
Figure 3.2. Daily activity profile of short day (SD)- and long day (LD)-housed cats. Dark periods for SD and LD treatments are represented by solid lines located at the top of the graph. Feeding times are highlighted by the dark bars located at the bottom of the graph.