

# Pancosma Comparative Gut Physiology Symposium: All About Appetite Regulation: Effects of diet and gonadal steroids on appetite regulation and food intake of companion animals<sup>1</sup>

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**ABSTRACT:** The prominent incidence of overweight and obese pet animals not only results in higher morbidity and mortality, but also poses a threat for the quality of life, longevity, and well-being of dogs and cats. To date, strategies to prevent BW gain or to induce weight loss have had modest success in the pet population. In part, due to the complexity and the multifactorial nature of this disease, which involves pet–human interaction, environmental and dietary factors, and an intertwined metabolic process that still is not fully understood. As such, research methods to investigate the role of physiological hormones and dietary management on mechanisms related to the control of feelings of satiety and hunger in pet animals is warranted. Increasing interest exists in exploring gut chemosensing mechanisms, the crosstalk between metabolic-active tissues, and the interface between the gut microbiota and the nervous system (gut–brain axis). The noninvasive nature of research conducted in companion animals focuses on systemic approaches to develop environmental, nutritional, or therapeutic interventions that can be translated from research settings to pet-owned households. Because the

majority of the pet population is spayed or neutered, it is important to determine the effect that sex hormones might have on appetite regulation and fasting metabolic rate of these animals. In general, studies have revealed that gonadectomy may establish a new “set point” characterized by increased food intake and BW, accompanied by physiological and behavioral changes. Some studies have also shown associations between gonadectomy and alterations in appetite-related hormones (e.g., ghrelin, leptin, adiponectin, glucagon-like peptide-1). Manipulation of macronutrients in diets of dogs and cats have also been investigated as a mean to improve satiety. Most of the research in this area has focused on high-protein diets, predominantly, for cats, and the use of dietary fiber sources of contrasting fermentability and viscosity profiles. Dietary fibers may affect the production of fermentative end products and gut microbiome, digestive and absorptive processes, appetite-related hormones, and promote “gut fill” and satiety. More recent studies have reported profound effects of dietary manipulation on the phylogeny and functional capacity of gut microbial communities of dogs and cats.

**Key words:** cat, dog, gonadectomy, hormone regulation, obesity, satiety

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## INTRODUCTION

Appetite regulation involves the integration of nutrition, gut physiology, nutrient metabolism, and environment and behavior factors. The desired outcomes of appetite regulation may vary depending on animal species. For example, in livestock species, stimulation of appetite and

food intake can be advantageous to maintain or improve reproductive performance, maximize growth and feed efficiency, and support animal health during challenging physiological stages (i.e., weaning, postpartum, etc.). In contrast, in companion animals that are more similar to humans, interest in regulation of appetite has focused mainly on aspects pertaining to health status and quality of life, and ability to mitigate chronic diseases and morbidity associated with BW gain and obesity.

A newer area in the field of appetite regulation is related to gut chemosensing. In addition to facilitating digestion and absorption of nutrients, the gastrointestinal tract also is in the forefront of handling, interacting, and sensing a variety of heterogeneous compounds in an external milieu (i.e., digesta). The presence of receptors, mostly enteroendocrine cells, on apical membranes of the gastrointestinal tract serves as the primary sensors of ingested nutrients, toxins, internal secretions, microorganisms, and physicochemical alterations. The signaling of these receptors elicits the secretion of gut peptides that may exert endocrine, paracrine, and/or neuroendocrine functions (Furness et al., 2013; Psichas et al., 2015). These gut peptides, in turn, modulate a series of physiological responses including appetite and satiety, gastric emptying, gut motility, epithelial morphology and barrier function, release of digestive enzymes, and immune responses (Furness et al., 2013). In the past few years, associations among gut hormones, microbiota, food intake, and obesity have just started to be recognized as being important in companion animal nutrition (Lubbs et al., 2010; Park et al., 2015; Li et al., 2017; Schauf et al., 2018). However, the noninvasive nature of research with companion animals prohibits tissue-specific and in-depth mechanistic approaches *in vivo* in contrast to research conducted in laboratory animals and livestock. Consequently, most of the recent scientific investigations have focused on systemic responses and applied sciences that can be easily translated from a research setting to a pet-owned household. In addition, research conducted directly on client-owned pets has become popular. This review offers an up-to-date synopsis of the current knowledge of the influence that sex hormones and dietary strategies might have on appetite regulation and food intake in companion animals. Most of this work has been carried out through the lenses of managing or preventing pet obesity.

## PET OBESITY PANDEMIC

Similar to humans, obesity is a pandemic health issue in the pet population. Obesity is defined as an excessive body fat accumulation that may impair health and is a result of energy imbalance between caloric intake and expenditure. Since 1975, obesity in humans has almost tripled worldwide, with over 1.9 billion adults being overweight and with a third of those being considered obese. Higher mortality is observed in overweight subjects vs. underweight subjects in most countries around the globe (WHO, 2017). In addition, an epidemiological study reported that life expectancy may be reduced by 6 to 7 yr in obese subjects (Peeters et al., 2003). Unfortunately, the same is true for the pet population. In 2016, it was estimated that 54% of dogs and 59% of cats were overweight or obese, which translates to approximately 42 million dogs and 51 million cats affected by this disorder (APOP, 2017). In that same survey, less than 1% of the canine and feline population were considered severely malnourished in the United States. A lifelong study of 48 Labrador Retrievers demonstrated that food-restricted (by 25%) dogs lived about 1.8 yr longer than dogs with *ad libitum* access to feed and had delayed onset of clinical signs of chronic diseases (Kealy et al., 2002).

Dogs and cats are considered overweight or obese when their BW exceeds 15% or 30%, respectively, of their ideal BW (Burkholder and Toll, 2000). Body condition scoring systems are qualitative methods to assess body composition. They are widely utilized by veterinarians, researchers, and pet owners, primarily because they are noninvasive, easily performed, and have virtually no cost. The etiology of companion animal obesity shares many similarities with human obesity. Domestication and anthropomorphism of pet animals have drastically changed the environment and social behavior of these animals, predisposing them to obesity. Nowadays, most pets are kept indoors without significant changes in ambient temperature, with an abundance of food, and with minimal reproductive purpose, all of which contribute to lower energy requirements. Additional risk factors include breed, age, sex, and dietary management (Zoran, 2010). Obese pets also are more predisposed to suffer from endocrine, orthopedic, cardiorespiratory, reproductive, urogenital, and neoplastic disorders (German, 2006).

Most common therapeutic options to manage or prevent obesity involve dietary intervention (mainly by energy restriction) and stimulation of

voluntary physical activity. However, these options have a poor success rate. Thus, a deeper understanding of the interface among nutritional status, endocrine signaling, and energy and glucose homeostasis would assist in the development of targeted therapeutic strategies to improve the quality of life and well-being of pet animals.

### ROLE OF SEX HORMONE ON APPETITE REGULATION

Gonadectomy is one of the risk factors that predisposes dogs and cats to gain weight over time (Crane, 1991), contributing to the pet obesity pandemic. Although the mechanisms involved are poorly understood, it is known that gonadal hormones have pluripotent physiological effects that are independent of their primary reproductive function in the hypothalamic–pituitary–gonadal axis (Pfaff et al., 2002) and that hormonal alterations may subdue appetite regulation (Wara and Datz, 2013). In rodents, gonadectomy affects appetite and food intake. Early work in this field demonstrated that sex hormones affect eating differently in female and male rats, which have been noted as increased meal size and decreased meal frequency, respectively (Blaustein and Wade, 1976; Chai et al., 1999). Androgens (e.g., testosterone) are anabolic hormones, resulting in increased food intake and lean tissue mass, whereas estrogens are catabolic, decreasing food intake and BW (Mystkowski and Schwartz, 2000). Physiological responses due to hormonal withdraw might be a species-specific response, and results observed in laboratory animals and/or humans may differ from domestic dogs and cats. In companion animals, there is an increasing literature on the modulation of appetite via sex hormones. A few studies have examined the impact of ovariectomy or neutering on food intake, BW and composition, voluntary physical activity, and hormones involved in appetite regulation. However, most of this work has been carried out on kittens and adult cats (Flynn et al., 1996; Root et al., 1996; Feetman et al., 1997; Harper et al., 2001; Hoenig et al., 2002; Kanchuk et al., 2002, 2003; Martin et al., 2006; Belsito et al., 2009; Alexander et al., 2011; Wei et al., 2014), with very few studies published in puppies and female dogs (Haupt et al., 1979; Le Roux, 1983; Salmeri et al., 1991). The author of this review speculates that this greater interest in the association of gonadectomy and weight gain in cats is due to these animals' greater predisposition to develop type 2 diabetes mellitus in comparison with dogs, in addition to

other comorbidities commonly observed with BW gain (i.e., osteoarthritis, lower urinary tract disease, hepatic lipidosis, and cardiovascular disease; Wara and Datz, 2013), as well as practical limitations in increasing cats' voluntary physical activity, as a mean to maintain energy balance.

Recently, no canine studies have been published investigating the influence of sex hormones on appetite regulation. One of the first studies to verify the effect of sex and reproductive status on food intake and BW in adult dogs was published in 1979. Daily caloric intake varied in female dogs based on their reproductive status: being lowest during estrus (149 kcal/kg of BW), intermediate during metestrus (159 kcal/kg of BW), and greatest during anestros (175 kcal/kg of BW; Haupt et al., 1979). In that same study, food intake (20%) and BW (4-fold) increased after 10 d after ovariectomy when compared with the sham-operated group. A similar study in adult female dogs evaluated the effect of ovariectomy ( $n = 15$ ) and autotransplantation ( $n = 15$ ) of the ovaries to the portal vein drainage area and compared them with a sham-control group ( $n = 15$ ). Body weight did not differ among groups after 55-wk postsurgery. However, reduced ( $P < 0.05$ ) 17- $\beta$  estradiol and tri-iodothyronine uptake and free thyroxine index were observed in ovariectomized when compared with sham- or autotransplanted bitches (Le Roux, 1983). In a 15-mo study in puppies, age of gonadectomy was tested on outcomes related to skeletal growth, weight gain, food intake, body fat, secondary sex characteristics, and behavioral development (Salmeri et al., 1991). Those authors tested 3 treatment groups: 1) neutering at 7 wk ( $n = 14$ ); 2) neutering at 7 mo ( $n = 8$ ); and 3) sexually intact (control;  $n = 10$ ). Early neutering (7 wk) resulted in the slowest ( $P < 0.05$ ) growth plate closure in contrast to the other treatments. However, neutering did not affect food intake, BW gain, and subcutaneous fat depot. Likewise, only minor behavioral changes were observed among treatments. General activity and excitability were rated higher for both neutered groups vs. the sexually intact animals (Salmeri et al., 1991).

Early work conducted by Flynn et al. (1996), Root et al. (1996), and Feetman et al. (1997) started examining the relationship between sex hormones, food intake, and BW gain in domestic cats using indirect calorimetry. Root et al. (1996) showed that sexually intact male and female cats had a greater ( $P < 0.05$ ) heat coefficient of 28% and 33%, respectively, when compared with sex-matched gonadectomized cats. Feetman et al. (1997) also reported a



reduced fasting metabolic rate in male and female cats after gonadectomy. Body weight and fat mass were greater in neutered and spayed cats in contrast with their intact counterparts (Feetman et al., 1997). Similarly, impaired ability to self-regulate food intake was observed in gonadectomized cats (Flynn et al., 1996; Feetman et al., 1997). However, no differences were observed in physical activity (Flynn et al., 1996) or indices of glucose homeostasis and thyroid hormone concentrations (Feetman et al., 1997) in castrated cats vs. intact cats.

Harper et al. (2001) evaluated the implications of ovariectomy on the onset of obesity and dietary management of domestic cats. Forty-nine cats were given ad libitum access to fed, and 11 cats were meal fed to maintain their preneutered BW. In the ad libitum group, 13 cats were fed dry pet foods, and 36 cats canned foods, whereas all cats in the meal-fed group were fed dry pet foods. After 12-mo postsurgery, cats fed ad libitum had a 31% increase in BW, predominantly driven by an increase in fat mass (40%) as assessed via dual-energy X-ray absorptiometry. No effects of diet were observed, but a negative correlation between BW gain and age of neutering was noted. Data stratification by age showed that cats older than 8 yr gained less weight as a percentage of their initial BW when compared with younger cats (i.e., 4 to 8 yr, and <4 yr of age).

A study of normal and lipoprotein lipase (LPL)-deficient male cats revealed no effects of LPL deficiency on BW gain associated with gonadectomy cats vs. intact cats (Kanchuk et al., 2003). In support of previous literature, food intake was increased by 12% and fat body mass was increased by 100% after 36-wk postneutering. These outcomes were independent of genotype. Mean daily energy expenditures and plasma concentrations of glucose and triacylglycerides did not differ in neutered cats vs. intact cats. Plasma leptin concentration was significantly greater in gonadectomized cats (6.0 pg/L) than in intact cats (2.7 pg/L) at 36 wk. Similarly, insulin also was greater ( $P < 0.05$ ) in neutered cats, increasing incrementally (285 to 292 pmol/L) from 2 to 36 wk in comparison with intact cats (202 to 210 pmol/L). Overall, it was concluded that BW gain was driven by increased food intake and not by a lower fasting metabolic rate in these cats. Insensitivity to the insulin and leptin signaling may further contribute to BW gain and that seems to be independent of removal of gonadal hormone inhibition of adipose LPL activity (Kanchuk et al., 2003). Findings from a more recent study using 9 intact specific-pathogen-free adult cats supported that initial postneutering weight gain (up to 6

mo) was a result of increased food intake, without negatively affecting energy expenditure (Wei et al., 2014). Serum concentrations of leptin (6.3 ng/mL) and ghrelin (855 pg/mL) increased ( $P < 0.05$ ) 6-mo postneutering vs. preneutering (3.9 ng/mL and 727.3 pg/mL, respectively), whereas serum concentrations of adiponectin decreased (2.6  $\mu$ g/mL postneutering vs. 7.8  $\mu$ g/mL preneutering). Increased serum concentration of ghrelin could have positively influenced food intake and consequent BW gain following neutering. The authors suggested that long-term dietary management to control hyperphagia is necessary in neutered male cats (Wei et al., 2014). Backus et al. (2006) investigated the role of cholecystokinin (CCK) on the regulation of food intake of gonadectomized cats. Sixteen adult male cats were evaluated 8 wk prior and 57 wk after neutering ( $n = 8$  intact group;  $n = 8$  neutered group). Normalized area under the curve (AUC) of plasma CCK concentration for meal size was 34% greater ( $P < 0.05$ ) in neutered cats vs. intact cats. No changes in food intake were observed based on plasma circulating CCK concentrations. As such, these results did not support previous findings in which plasma CCK concentration was augmented by ingestion of macronutrients (i.e., proteins, fats, and carbohydrates; Backus et al., 1995). Because CCK is an anorexigenic hormone, those authors hypothesized that plasma concentrations of CCK would be inversely related to food intake.

Belsito et al. (2009) assessed the effects of ovariectomy and food intake on body composition and voluntary physical activity in cats that were food restricted and had ad libitum access to fed. After a 4-wk baseline period, healthy female young adult cats ( $3.52 \pm 0.72$  kg BW) were spayed. Following this procedure, cats were fed to maintain BW for an additional 12 wk. A decrease of approximately 30% in food intake was required to avoid BW gain during this period. Previous research also demonstrated that neutered cats require 24% to 30% fewer calories to maintain BW (Flynn et al., 1996). After spaying, total voluntary physical activity decreased (52% lower at 24 wk vs. baseline; Belsito et al., 2009), which differs from previous literature in dogs (Flynn et al., 1996). However, in the latter study, physical activity was determined only by observing the cats for short periods of time (~5 min) and during only light hours of the day, whereas the former study used omnidirectional sensors capable of determining intensity and duration of movements and providing continuous monitoring of voluntary physical activity for extended periods of time. In that same study, severe increase in

body fat mass was observed in cats given ad libitum access to fed from 12 (~3.5 kg) to 24 wk (~4.7 kg; [Belsito et al., 2009](#)). Similar increases in BW and fat mass have been observed in other studies evaluating cats that had undergone ovariohysterectomy ([Harper et al., 2001](#)).

Post hoc analysis was used to explore the effects of neutering on food intake, BW, and body composition in female kittens ([Alexander et al., 2011](#)). Twelve pairs of 11-wk-old female kittens were chosen from litters born from 12 breeding queens that had been given ad libitum access to a commercial cat food throughout gestation, lactation, and weaning periods. Each of the 12 paired kittens was randomized to 2 groups, either intact or spay. Kittens assigned to the spay group were spayed at 19 wk of age. Data collection started when kittens were 11 wk old and continued to 52 wk of age. Food intake and BW were increased ( $P < 0.05$ ) in spayed kittens vs. intact kittens, with maximal difference in food intake of 17% at 10-wk postspaying. At 52 wk, BCS was also greater ( $P < 0.05$ ) in spayed kittens (6.1) vs. intact kittens (5.2). In that same study, it was observed the ratio of actual food intake and theoretical ME for the intact kittens declined by 7% and 21% of their theoretical ME at 26 and 52 wk of age, respectively ([Alexander et al., 2011](#)). These values were in agreement with previous literature presenting predicted energy requirement equations for adult cats ([Bermingham et al., 2010](#)). Those authors concluded that the current [NRC \(2006\)](#) ME requirements for growing kittens were not reflective of the ME requirements of their cat colony ([Alexander et al., 2011](#)).

Most recent published research ([Allaway et al., 2017](#)) investigated the impact of the time of spaying on total energy requirements in female kittens. These kittens were spayed at either early age (19 wk old;  $n = 7$ ) or conventional age (31 wk old;  $n = 7$ ), and data were collected from kittens between 15 and 52 wk of age. The early-age spayed kittens gradually increased BW until 38 wk of age. Conventional-age kittens had lower energy intake during 24 to 32 wk; however, BW and food intake sharply increased after spaying at 31 wk of age. Food intake restriction was necessary for almost all kittens in this study to maintain ideal BW. Differently than observed in adult cats ([Belsito et al., 2009](#)), voluntary physical activity of the female kittens did not differ between spaying groups, both showing a 25% decrease on daily activity from 19 to 52 wk of age. Overall, it appears that conventional-age spaying might be less desirable due to more rapid increase in food intake and BW after gonadectomy. It was

hypothesized that estrogen regulation and its subsequent loss following spaying could be the driver for hyperphagia and overweight ([Allaway et al., 2017](#)).

## EFFECT OF DIET ON APPETITE REGULATION

Consumption of foods that stimulate satiety for longer periods can be beneficial for BW management in humans and companion animals. Among the macronutrient categories, proteins have been on the front stage as having the most satiating effects, followed by carbohydrates and lipids ([Blundell and MacDiarmid, 1997](#); [Paddon-Jones et al., 2008](#)). Potential mechanisms underlying the enhanced feelings of satiety associated with ingestion of dietary proteins are related to induced thermogenesis, hormonal regulation, slow passage rate, and sensorial experience during food consumption ([Halton and Hu, 2004](#); [Veldhorst et al., 2008](#); [Masic and Yeomans, 2013](#)). Although control of food intake and energy expenditure seems to be possible in humans and rodents, little is known on this topic in pet animals. A few studies have examined the effect of high vs. moderate protein concentrations in diets of adult cats and kittens ([Vester et al., 2009a,b](#); [Vester et al., 2012](#)).

[Vester et al. \(2009b\)](#) investigated the effects of ovariohysterectomy on BW and composition, blood metabolite concentrations, and voluntary physical activity level of adult female cats ( $n = 8$ ) fed either a high-protein diet [53% CP and 10% nitrogen-free extract (NFE)] or a moderate-protein diet (34% CP and 34% NFE). All cats were spayed at baseline (week 0) and had ad libitum access to feed for additional 24 wk. Food intake and body fat mass increased following gonadectomy, independent of diet. In agreement with previous findings ([Belsito et al., 2009](#)), a lower voluntary physical activity was observed in cats after 24 wk of spaying, but no effects of diet were noted ([Vester et al., 2009b](#)). Plasma leptin concentrations were positively correlated with fat mass ( $r = 0.80$ ;  $P < 0.05$ ). A significant diet  $\times$  week interaction was observed for fasted glucose concentrations from weeks 0 to 24, suggesting decreased insulin sensitivity with BW gain ([Vester et al., 2009b](#)).

The same group of researchers also evaluated in utero and postnatal feeding of a high-protein (53% CP and 10% NFE) or a moderate-protein (34% CP and 34% NFE) diets on blood metabolites and voluntary physical activity in kittens for 9 mo ([Vester et al., 2009a](#)). High-protein diet-fed kittens had increased fat mass (17% body fat)

when compared with kittens fed the moderate-protein diet (12% body fat). It was suggested that the higher fat mass at 2 mo of age was related to a potential greater milk intake or smaller litter size of the high-protein-fed group. However, at 8 mo of age, differences in fat mass and BW between the 2 groups were no longer detectable. In addition, kittens fed the high-protein diet tended to have higher nocturnal voluntary physical activity (43.1 vs. 26.1 activity counts/epoch), but lower light:dark activity ratio (0.56 vs. 0.87 light:dark activity counts/epoch) at 6 mo of age when compared with kittens fed the moderate-protein diet. At 6 mo of age, serum leptin (4.7 ng/mL) and glucose (4.6 mmol/L) concentrations were greater ( $P < 0.05$ ) in kittens fed the moderate-protein diet in comparison with kittens fed the high-protein diet; 2.8 ng/mL and 4.1 mmol/L, respectively (Vester et al., 2009a). The effects of diet and age on serum metabolites, ghrelin, and leptin concentrations were assessed in weanling kittens (Vester et al., 2012). Sixteen kittens fed moderate protein and 9 kittens fed high protein as described in previous research (Vester et al., 2009a,b) were evaluated at 4, 8, 12, and 16 wk of age. In moderate-protein-fed kittens, serum cholesterol concentrations were greater ( $P < 0.05$ ) at 4 wk of age, and serum ghrelin also tended ( $P < 0.10$ ) to increase in this dietary group (Vester et al., 2012).

Only a few canine studies evaluated different nutrients on satiety regulation. The literature in dogs has focused on manipulation of macronutrient profiles in diet matrices (i.e., protein, fat, fiber concentrations) or using single dose of a sole ingredient. Lubbs et al. (2010) investigated changes in hormone concentrations after a bolus of either 50 g of protein (canned chicken breast), 50 g of carbohydrate (maltodextrin), 25 g of fat (lard), or 200 mL of water (control) using 8 adult dogs in a replicated  $4 \times 4$  Latin square design. Although no difference in postprandial ghrelin concentrations was observed among treatments, the glucagon-like peptide-1 (GLP-1) concentrations tended to be elevated in dogs fed protein (8.1 pmol/L) vs. fat (4.4 pmol/L). It was also noted in this work that longitudinal satiety hormone responses (24 h) were highly variable throughout the day and should be considered in future research (Lubbs et al., 2010).

The same group of researchers determined the effect of different animal protein sources on satiety in adult dogs (Vester Boler et al., 2012). Ten female hounds were used in a replicated  $5 \times 5$  Latin square design and were fed 100 g of one of the protein-meals in each experimental period (i.e., beef, chicken, pork, salmon, and Pollock fillet). Blood

samples were collected at 0-, 5-, 15-, 30-, 60-, 90-, and 120-min postprandial to determine blood metabolites, peptides and AA concentrations, and 24-h food intake. Baseline plasma glucose, GLP-1, and serum total ghrelin concentrations did not differ based on protein sources tested. Pork, salmon, and chicken pre-meals had reduced ( $P < 0.05$ ) incremental GLP-1 concentration in contrast with beef. Postprandial glucose concentration was lowest ( $P < 0.05$ ) in dogs fed chicken and Pollock, while postprandial insulin concentration was greatest in dogs fed salmon. In addition, baseline and(or) incremental variations of AA were affected by protein source; however, no effect on food intake was noted, thus the authors' hypothesis that fish protein would be most satiating was not supported (Vester Boler et al., 2012). A similar scientific approach of Vester Boler et al. (2012) was used to investigate the impact of high-carbohydrate or high-fat diets on food intake and satiety-related hormones in dogs (Schauf et al., 2018). Twelve beagles were fed either a high-carbohydrate (44% starch; 10% fat) or a high-fat diet (27% starch; 21% fat) using a crossover design (7 wk/period). At the end of each experimental period, blood samples were collected at 0-, 30-, 60-, 120-, 180-, and 360-min postfeeding. Food intake and BW did not differ ( $P > 0.05$ ) between diets. However, a greater ( $P < 0.05$ ) basal and AUC for GLP-1 concentrations were detected in dogs fed the high-carbohydrate diet (Schauf et al., 2018).

Regulation of appetite by variation in feeding frequency and manipulation of dietary macronutrient were investigated in domestic adult cats (Deng et al., 2013b). Three animal trials were conducted using 12 male cats in a crossover design. In each trial, cats had a 15-d adaptation to the dietary treatment, and on day 16, a baseline and postprandial blood samples were collected. During the first trial, cats were fed twice or 4 times daily a control diet, and at the end of each experimental period, a blood sample was collected prior to the first meal of the day and every 2 h for a 24-h period. Cats fed twice daily had greater variation in glucose and insulin concentrations and higher concentration of these metabolites vs. cats fed 4 times daily. In contrast, cats fed 4 meals/d had lower incremental leptin, but higher incremental ghrelin vs. cats fed 2 meals/d. These findings indicate that increasing feeding frequency may be effective to control appetite regulation in adult cats. During trial 2, acute response to a single macronutrient dose (i.e., 20 g of maltodextrin dissolved in 20 mL of water, 9 g of lard, 27 g of canned chicken, or 20 mL of water) was tested. No difference in food intake was observed



among dietary treatments. However, as expected, the carbohydrate dose (maltodextrin solution) resulted in greater ( $P < 0.05$ ) incremental glucose and insulin concentration for 6- and 4-h postprandial, respectively. Large variations were observed with incremental ghrelin and leptin concentrations. The latter was below baseline concentration among all treatments, but cats fed the carbohydrate dose tended ( $P < 0.10$ ) to have increased postprandial leptin concentration in contrast to protein-fed cats. During trial 3, fasting and postprandial blood glucose and hormone concentration were determined in response to feeding a control diet (formulated to have an even distribution of macronutrients; 33% of ME coming from carbohydrate, fat, and protein) or diets containing high-carbohydrate, high-protein, or high-fat concentrations (designed to have 50% of ME from each macronutrient, respectively). Food intakes were similar among dietary treatments, and a few hormonal alterations were observed. The control diet resulted in greater incremental glucose concentration during the first 6-h postprandial in contrast to the high-protein and high-fat diets, whereas the high-carbohydrate diet had a greater incremental glucose concentration 12-h postprandial when compared with the high-protein and high-fat diets (Deng et al., 2013b). The authors suggested that the lack of hormonal response to the dietary treatments could occur due to the fact that cats used in this study were healthy and lean. Thus, they suggested that future research should use similar strategies, but in obese or diabetic cats.

The effects of dietary fiber on voluntary feed intake and satiety-related hormones were tested by Bosch et al. (2009). Diets containing 8.5% cellulose (nonfermentable;  $n = 8$ ) or 8.5% beet pulp plus 2% inulin (fermentable;  $n = 8$ ) were fed to adult dogs for 7 wk. Dogs fed the fermentable-fiber diet tended ( $P < 0.10$ ) to have lower voluntary food intake vs. dogs fed the nonfermentable diet. As expected, fecal fermentative end-product concentrations were increased ( $P < 0.05$ ) in dogs fed the fermentable diet [short-chain fatty acids (SCFA): 0.54; acetate: 0.32; and propionate: 0.14 mmol/g DM] in comparison with the nonfermentable fiber (SCFA: 0.26; acetate: 0.14; and propionate: 0.06 mmol/g DM). Neither diet in this experiment affected the plasma metabolites analyzed (i.e., glucose, insulin, peptide YY, GLP-1, or ghrelin), suggesting the lower food intake of the fermentable fiber diet was not directly regulated through the hormones measured in this study. The effects of dietary fiber fermentability and viscosity on second-meal effect (i.e., the ability of an ingredient to affect a subsequent meal)

were investigated in adult healthy dogs (Deng et al., 2013a). Two experiments were conducted using a replicated  $3 \times 3$  Latin square design ( $n = 6$  per treatment), dogs were adapted to the dietary treatments for 6 d, and on 7 d, blood samples were collected before and after an oral glucose tolerance test (25-g maltodextrin) administered 4 h after the morning meal to verify the effect of dietary fiber on glucose, insulin, and GLP-1 determination. In the first experiment, dogs were assigned to 1 of 3 treatments: low fiber (1% cellulose), low-fermentable fiber (5% cellulose + 3% soybean hulls), and high-fermentable fiber (5% pectin + 3% short-chain fructooligosaccharides). Dogs fed the high-fermentable fiber treatments had lower glucose incremental  $AUC_{0-180\text{min}}$  in contrast with the other 2 treatments. However, incremental GLP-1 and insulin did not differ among treatments. In experiment 2, dogs were fed either a low-viscosity diet (5% cellulose + 3% soybean hulls), a moderate-viscosity diet (2% cellulose, 2% soybean hulls, 2% pectin, and 2% psyllium), or a high-viscosity diet (4% pectin and 4% psyllium). Baseline GLP-1 concentration was greater ( $P < 0.05$ ) in dogs fed the high-viscosity diet (16.6 pmol/L) vs. low- or moderate-viscosity treatments (7.3 and 8.3 pmol/L, respectively). No differences in incremental glucose, GLP-1, and insulin were noted among treatments. Altogether, these results suggest that the control of appetite regulation and food intake in pet animals is complex and involves the orchestration of several different physiological systems, and it is not subject to strict homeostasis and hormonal control.

More recently, several diseases have been associated with microbial dysbiosis in humans, including obesity, diabetes, and gastrointestinal disorders (Turnbaugh et al., 2006; Dicksved et al., 2008; Larsen et al., 2010; Ley, 2010). Crosstalk between gut microbiota and the nervous system (gut-brain axis) has been shown to influence appetite regulation, feeding behavior, and gut motility (Schwartz, 2000; Diaz et al., 2011; Tehrani et al., 2012). For the last 5 yr, advances in companion animal research have started investigating associations among the gastrointestinal microbiota and host health. Some of these efforts have focused on manipulation of dietary macronutrient composition of dogs and cats (Hang et al., 2012; Bermingham et al., 2013a,b; Hooda et al., 2013; Deusch et al., 2014; Li et al., 2017) and the relationship between obesity, appetite hormones, and gut microbiota (Park et al., 2015).

The domestic dog and cat belong to the order carnivore; however, the domestic cat has evolved as a strict carnivore consuming a protein-fat-rich

animal-based diet. Physiologically, the cat has higher minimum protein and fat requirements when compared with dogs, humans, and rodents and is fit to a lower-glucose and higher-protein metabolism (MacDonald et al., 1984). High-protein diets are appealing to some pet owners as an evolutionary feeding strategy and perceived as a healthier or, at least, more natural dietary approach. However, interactions among dietary macronutrient composition, gut microbiota, nutrient utilization, feeding behavior or preferences, and consequently host health are just now beginning to be explored in pet animals. Hooda et al. (2013) evaluated the effect of dietary protein:carbohydrate ratio on fecal microbiota of kittens. Fourteen male kittens ( $n = 7$  per treatment) were weaned at 8 wk from breeding queens fed either a high-protein or a moderate-protein diet (same diets as in Vester et al., 2012) and continued to be fed the same diet during the duration of the experimental period (8 wk). Fresh fecal samples were collected at 8, 12, and 16 wk and were analyzed using 16S rRNA gene-based 454-pyrosequencing. Little effect of age was observed in the fecal microbiota. A distinct dual hierarchical clustering based on the 50 most abundant bacterial genera exist between kittens fed the high-protein or moderate-protein diets. Principal component analysis (PCA) of UniFrac distance also revealed distinct clustering between the 2 dietary groups. Fusobacteria was almost absent in feces of kittens fed the moderate-protein diet, but was the second most prevalent phylum in fecal samples of high-protein-fed kittens. Feeding a high-protein diet also resulted in greater ( $P < 0.05$ ) abundance of fecal *Collinsella*, *Faecalibacterium*, *Eubacterium*, *Anaerotruncus*, *Ruminococcus*, *Fusobacterium*, *Blautia*, and *Clostridium*, in contrast to greater ( $P < 0.05$ ) abundance of *Megasphaera*, *Bifidobacterium*, and *Mitsuokella* from feces of moderate-protein-fed kittens. Principal component analysis of fecal bacterial families with blood hormones and metabolites revealed a positive association between *Lactobacillus* and blood leptin concentration and between Bifidobacteriaceae with blood ghrelin (positive) and triglyceride concentrations (negative). These findings may have implications to host health and metabolism, as leptin and ghrelin are important hormones involved in appetite control and satiety.

Metagenomic analysis using Illumina shotgun sequencing of the fecal samples from Hooda et al. (2013) resulted in differences of the functional capacity of the gut microbiome of kittens fed high-protein diets vs. moderate-protein diets (Deusch et al., 2014). Fecal microbiome of high-protein-fed kittens

had greater ( $P < 0.05$ ) species diversity and richness than moderate-protein-fed kittens. Functional capacity of the microbiome resulted in over 2,000 enzyme functions and 190 pathways that differed based on diet. Kittens fed the moderate-protein diet had greater abundance of genes related to amino acid biosynthesis and metabolism, oxidative phosphorylation, and glycolytic, tricarboxylic acid cycle, and pentose phosphate pathways (Deusch et al., 2014). A recent study examined the dietary protein:carbohydrate ratio in lean and obese adult dogs of 2 breeds [i.e., Labrador Retrievers ( $n = 32$ ) and Beagles ( $n = 32$ ); Li et al., 2017]. After 4 wk of being fed a commercial diet, dogs of each breed and phenotype were assigned to either a high-protein diet or a low-protein diet. Fecal DNA was extracted and analyzed using 16S rRNA gene-based Illumina Mi-seq. Principal coordinate analysis of weighted UniFrac distance showed diet effect on fecal microbial community clustering of dogs fed high-protein diets vs. low-protein diets, with a stronger dietary influence on obese dogs. No effect of breed was observed in this study. In addition, differential taxa abundance using linear discriminant analysis effect size analysis were noted between the 2 dietary groups. Bacterial taxa from Bacteroidetes and Fusobacteria phyla were more abundant in low-protein-fed dogs, whereas bacterial taxa from Firmicutes were most predominantly in high-protein-fed dogs. The authors also suggested that a lower Bacteroidetes:Firmicutes ratio and the greater abundance of genes related to flavone and flavonol biosynthesis pathway may contribute to weight management in dogs (Li et al., 2017). Another study has examined the gut microbiota of lean and diet-induced obese Beagles with peripheral concentrations of leptin, adiponectin, serotonin, and lipid profile. Fourteen dogs were fed a commercial diet to main ideal BW ( $n = 7$ ) or ad libitum ( $n = 7$ ) for 6 mo (Park et al., 2015). A fresh fecal sample was collected at the end of the experimental period and used for microbial community analysis using 16S rRNA gene. After 6 mo of ad libitum feeding, obese dogs had increased ( $P < 0.05$ ) BW, BCS, and leptin and triglyceride concentrations in comparison with lean dogs. However, serotonin and adiponectin concentrations were decreased ( $P < 0.05$ ) in obese dogs. A distinct clustering was observed between lean and obese dogs using PCA. Shannon diversity index was also greater in lean dogs. Abundance of Proteobacteria phylum in fecal samples of obese and lean dogs differed; obese dogs had greater abundance of *Psychrobacter* (36%) and *Pseudomonas* (31%) genera, whereas lean dogs



had greater abundance of unclassified bacteria (38%), *Sutterella* (27%), and *Achromobacter* (15%). Proteobacteria is a major phylum of gram-negative bacteria that have been associated with increased levels of intestinal lipopolysaccharides, which may be a potential mechanism of low-grade inflammation in obesity.

### SUMMARY AND CONCLUSION

Overall, most of the scientific evidence on appetite regulation of companion animals has been garnered from studies examining the effect of gonadal steroids (or lack of), and dietary manipulation of macronutrients and their ratios on physiological responses of dogs and cats. These studies have only started laying the foundation from which to work and, thus far, have yielded conflicting results, specially related to the effect of sex hormones and dietary interventions on appetite-related hormones and blood metabolites. Furthermore, because most of the current studies have been carried out on healthy dogs and cats, it would be important that additional studies would be conducted to test similar interventions on diseased populations (e.g., obese or diabetic pets). Future research should also explore signaling metabolites and physiological pathways that can be targeted to control appetite and prevent BW gain. The use of novel scientific approaches and high-throughput technology will be crucial to further our knowledge on associations and cause-and-effect relationships among gut and sex hormones, gut microbiota, food intake, and obesity, without using invasive techniques. Finally, a deeper understanding of metabolic and physiological processes related to appetite regulation and obesity in pets may also be beneficial to humans, as the etiology of this disease is similar between them.

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