

Macronutrient intake of dogs, self-selecting diets varying in composition offered ad libitum

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Summary

The diet of the domestic dog has changed significantly from that of its wolf ancestor, with to date only two studies having examined macronutrient self-selection in dogs. Whilst the first focused solely on protein intake, determining an intake of 30% metabolisable energy (ME), the second investigated dietary protein, fat and carbohydrate (PFC), indicating an intake ratio of 30:63:7% by energy. This study's aim was to further elucidate macronutrient intake by providing greater macronutrient range, energy content, and to investigate over a longer duration than previous studies. Fifteen adult dogs were given access to three wet diets providing 500% of daily ME, twice daily over 10 days. The diets were nutritionally complete and formulated using the same four ingredients in different proportions to supply high levels of protein (58% ME), fat (86% ME) or carbohydrate (54% ME). Overall fat and carbohydrate consumption significantly declined from 6,382 to 917 kcals per day ($p < 0.001$) and 553 to 214 kcals day⁻¹ ($p < .01$) respectively. Protein intake, however, remained constant over the study and ranged from 4,786 to 4,156 kcals day⁻¹. Such results impacted on percentage total energy intake, with fat decreasing from 68% to 52% ($p < .001$) and protein increasing from 29% to 44% ($p < .01$). Our findings suggest that dogs still possess a "feast or famine" mentality, wherein energy dense fat is prioritised over protein initially. With continued feeding over 10 days, a transition to a more balanced energy contribution from both macronutrients is evident. The study also shows that given the option, dogs do not select carbohydrate to be a significant portion of the diet. The health implications of such dietary selection are of interest.

KEYWORDS

dietary composition, dog, macronutrient, self-selection

1 | INTRODUCTION

Whilst archaeological records cannot determine whether domestic dogs originated from a single wolf population or arose from multiple populations at different times (Frantz et al., 2016; Vilà et al., 1997), dogs are the only large carnivore to have been domesticated, most likely over a wide geographic area (vonHoldt et al., 2010). By inheriting

such wolf ancestry, the domestic dog is classified as a carnivore, with teeth adapted for grasping and tearing; however, they also possess omnivorous traits (Serpell, 1995). The dog has a requirement for both protein and fat (Association of American Feed Control Officials, 2016; National Research Council, 2006), but not for carbohydrate, despite recent findings that show that domestic dogs may have evolutionary adaptations for improved carbohydrate digestion (Axelsson et al., 2013).

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The macronutrient composition of modern dog foods can vary significantly depending on the format fed. This is largely due to the manufacturing processes required to produce the food. For example, kibbled diets typically contain 16%–38% protein, 6%–18% fat and 40%–60% carbohydrate (dry matter basis). However, wet/raw diets typically contain no or low levels (<10%) of carbohydrate combined with higher levels of protein and fat (45% and 50% respectively). From a dietary perspective commercial dry dog food is by far the most popular feeding option, being fed to over 88% of dogs (New Zealand Companion Animal Council 2016). Indeed whilst any impact on health from feeding a differing dietary format (dry or wet) and macronutrient composition has yet to be determined, obesity and its many associated conditions such as diabetes, cardiorespiratory disease and urinary disorders are an increasing health risk for companion animals (German, 2006). Indeed in the past 10 years, approximately 30%–40% of pet dogs are classified as being overweight, whilst an additional 5%–20% termed obese (Witzel et al., 2014). Although the establishment of a targeted macronutrient intake will not in itself highlight any impact on health, it may serve as a starting point for future research, whereby a specific dietary macronutrient composition could be assessed in reference to the impact on markers of health.

The ability of animals to select a macronutrient ratio that optimises fitness costs (such as lifespan and rate of reproduction) has to date been proven in a range of species (Lee et al., 2008; Simpson, Sibly, Lee, Behmer, & Raubenheimer, 2004). Moreover, establishing the macronutrient profile “targeted” by dogs could highlight the potential difference between what they want to consume, and what most commercial diets are providing. Further questions may also be addressed as to whether a dog would under-eat some nutrients, and over-eat others in an attempt to reach an intuitive predetermined macronutrient profile when provided with an inappropriate dietary composition.

From a nutritional standpoint, whilst feeding biologically appropriate diets to pet dogs has not currently been shown to provide any health benefits, raw meat diets have been demonstrated to be highly digestible, resulting in low faecal volume and desirable faecal quality (Beloshapka, Duclos, Vester Boler, & Swanson, 2012). In addition, it is clearly apparent that domesticated dogs are currently eating diets that differ substantially from what their ancestors consumed. Highlighting this, Bosch, Hagen-Plantinga, and Hendriks (2015) found that the dietary composition of wild wolves showed the selected protein–fat–carbohydrate profile was 54:45:1 by energy.

To date, only two studies have examined dietary macronutrient selection in the dog. Whilst the first study appeared to demonstrate a preference for protein over carbohydrates (consuming 30% protein by energy), the impact of fat was not fully determined (Romsos & Ferguson, 1983). A more recent study did, however, allow for all three macronutrients to be self-selected by dogs of differing breeds, suggesting an overall protein/fat/carbohydrate ratio (P:F:C) of approximately 30:63:7% by energy when fed complete and balanced wet-based diets (Hewson-Hughes et al., 2012). However, the restriction of daily total food intake in certain experimental stages (e.g., 100% of MER for the first six 3-day cycles of the learning phase) may have limited the extent by which the animals could fully select the provided diets. In addition,

the structuring of different feeding phases and diet composition selected may potentially have influenced the dogs feeding patterns.

Existing literature suggests that when dogs are provided with the ability to self-select a macronutrient ratio, they will consume 30% of their maintenance energy requirements from protein. However, a number of commercial wet diets contain in excess of this value, with reports from dog owners that an increase in palatability is linked with this factor. Therefore, the hypothesis of the study was that dogs would select a diet consisting of more than 30% of total energy from protein. The aim of this study was, therefore, to establish the self-selective macronutrient intake of dogs by providing them with a range of diets, each specifically higher in energy sourced from protein and fat over a longer duration. This consequently will enable the intuitive macronutrient capabilities of the domestic dog to be examined in a deeper manner than has previously been conducted. Subsequently our findings will either reinforce or challenge those of the previously conducted studies, with the potential to highlight that a dog may still possess a similar macronutrient intake to that of their wild ancestors.

2 | MATERIALS AND METHODS

2.1 | Ethics

Ethical approval was gained from the Massey University Animal Ethics Committee (MUAEC 15/75), before commencing the experiment. The dogs were housed at Massey University Canine Nutrition Unit (Palmerston North, New Zealand), in accordance with the Animal Welfare (Companion Dogs) Code of Welfare (2007).

2.2 | Animals

Fifteen Harrier hound dogs (five male and 10 female) were used throughout the study, comprising of four neutered and one entire male and three neutered and seven entire females. The dogs were all deemed healthy based on a physical examination. The mean age of the dogs used in the study was 7.68 years \pm 0.73 SEM. The dogs were housed in pairs in 10 m x 10 m (100 m²) outdoor pens or in groups of 4 in grass paddocks measuring 700 m² for 8 hr a day. Overnight the dogs were housed indoors in pairs with water and bedding provided.

2.3 | Diets

A high protein (HP), high fat (HF) and high carbohydrate (HC) diet (Table 1) was formulated to meet AAFCO Dog Food Nutrient Profiles for adult maintenance (Association of American Feed Control Officials, 2015). All diets consisted of the same four ingredients at different inclusion levels, namely maize, lamb loin fat, green tripe and venison mechanically deboned meat (MDM) (Table S1). The levels of protein, fat, ash, moisture and NFE (nitrogen free extract) were analysed for each diet (Table 1).

A 5-day period was used to adapt the dogs onto the test diets, consisting of a 20% day on day increase of an equal mixture of the HF, HP and HC diets, whilst concurrently decreasing their existing commercial dry diet (protein–fat–carbohydrate profile 21:23:56 by energy)

TABLE 1 Macronutrient profiles of high protein (PFC 57.6:41.7:0.7%), high fat (PFC 12.8:86.7:0.5%) or high carbohydrate (PFC 17.8:27.7:54.5%) diets offered at 500% maintenance energy requirements to adult dogs ($n = 15$) for 10 days

Nutrient DM (g/100 g)	HF	HC	HP
Moisture (as fed)	41.2	26.2	73.0
Protein	23.9	19.3	71.2
Fat (ether extract)	66.4	12.4	21.2
Ash	7.5	4.8	5.5
Carbohydrate	0.9	59.3	0.9
Crude fibre	1.3	4.2	1.2
ME (Kcal kg ⁻¹) ^a	6,512	3,805	4,325

DM, Dry Matter; HP, High Protein; HF, High fat; HC, High Carbohydrate; ME, Metabolisable energy.

^aCalculated from modified Atwater factors (National Research Council, 2006)

by 20%. Therefore, by the last day of the adaption period, the dogs were being fed solely an equal combination of the experimental diets, at which point they were deemed to have been fully transitioned.

The 10-day experimental phase of the study then started, consisting of the dogs being offered 250% of their daily ME requirement of each diet, twice a day (8 a.m. and 2 p.m.; 1,500% ME, total per day for all three diets). All three diets were offered simultaneously.

2.4 | Experimental protocol

The dogs were weighed at the start (day 1), middle (day 5) and end (day 10) of the experimental period (Table 2). In order to assess self-selected macronutrient consumption, three large plastic bowls each containing 250% of the daily energy requirement of the HF, HC and HP diets were provided to each dog (twice daily, at 8 a.m. and 2 p.m.) for 10 days (Figure 1). The position of each bowl was interchanged at each feeding time to prevent positional bias. A number of feeding dynamics were also observed both directly by an observer during each feeding period and afterwards via the use of a video recording camera (Sony Handycam HDR-SR11E/SR12E) to verify results. These observations involved which diets were approached first, which diets consisted of any consumption first and which diets were completely avoided. Dogs were offered the diets until satiated status was achieved. This was defined as the point whereby the animal lost interest in any of the diets.

TABLE 2 Mean bodyweight of dogs ($n = 15$) offered high protein (PFC 57.6:41.7:0.7%), high fat (PFC 12.8:86.7:0.5%) or high carbohydrate (PFC 17.8:27.7:54.5%) diets at 500% maintenance energy requirements for 10 days

	Day 1	Day 5	Day 10	<i>p</i> -value
Mean	25.9 ^c	27.0 ^b	27.5 ^a	<.001
SEM	0.72	0.77	0.77	

SEM, Standard error of mean.

The superscripts are significantly different from one another ($p < .05$).

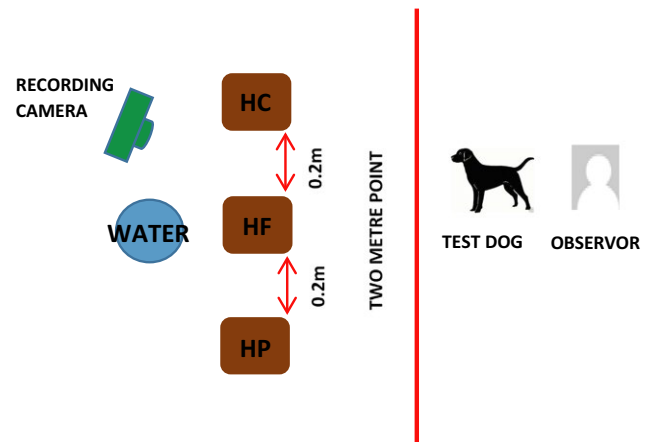


FIGURE 1 Experimental design involving dogs offered high protein HP (PFC 57:42:1% by energy), high fat HF (PFC 13:86:1% by energy) or high carbohydrate HC (PFC 18:28:54% by energy) diets for 10 days [Colour figure can be viewed at wileyonlinelibrary.com]

2.5 | Calculations

Protein, fat and carbohydrate energy intakes were determined by applying modified Atwater factors (protein/carb 3.5 Kcal g⁻¹ fat 8.5 Kcal g⁻¹) (National Research Council, 2006). As these data were known for each specific diet, total energy consumption was calculated by subtracting the total of each diet provided to each dog from that remaining after each dietary exposure. Additionally macronutrient ratio was determined as the overall percentage energy contribution that each macronutrient made to each diet. Therefore, by adding the different quantities of each diet consumed and the respective protein, fat and carbohydrate energy contribution, the total energy from each macronutrient could be established.

2.6 | Statistical analysis

Separate analyses were conducted for each of the response variables (i.e., protein, fat, carbohydrate and protein:fat ratio) against measurement day, using a random coefficients regression model which allowed for separate slopes and intercepts to be fitted for each dog. As the experiment involved dogs of both sexes (5 male and 10 female), the factors "sex" and "reproductive" were assessed separately, but no significant differences were found, so these factors were not included in the model. Modelling was undertaken using R software (R Core Team, 2016). All data were reported as intercept and slope with associated standard error (SE).

Fisher's exact test was used to compare the proportions of first approached and first consumed for each of the diets (HP, HF and HC). The test was performed with the statistical software package MINITAB® 16 (2010).

Binary logistic regression analysis was used to test the effect of diet on diet avoidance, with diet avoidance as the binary response variable (avoided vs. not avoided) and the diet (HP, HF and HC) as predictor.

Bodyweight was analysed with a repeated measurements linear mixed model (REML) with the factor measurement day (levels 1,

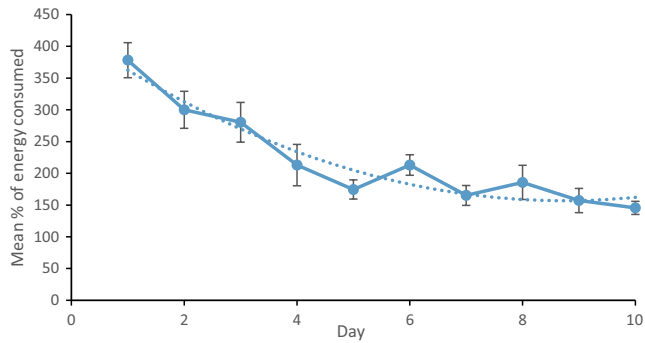


FIGURE 2 Over the 10-day period, the percentage of energy consumed by the dogs ($n = 15$) reduced ($p < .001$) according to the quadratic equation: $\%ME = 419.1 (\pm 31.8 SE) - 60 (\pm 8.78 SE) \times \text{Day} + 3.43 (\pm 0.78 SE)$ when offered high protein (P:F:C 57:42:1% by energy), high fat (P:F:C 13:86:1% by energy) or high carbohydrate (P:F:C 18:28:54% by energy) diets for 10 days [Colour figure can be viewed at wileyonlinelibrary.com]

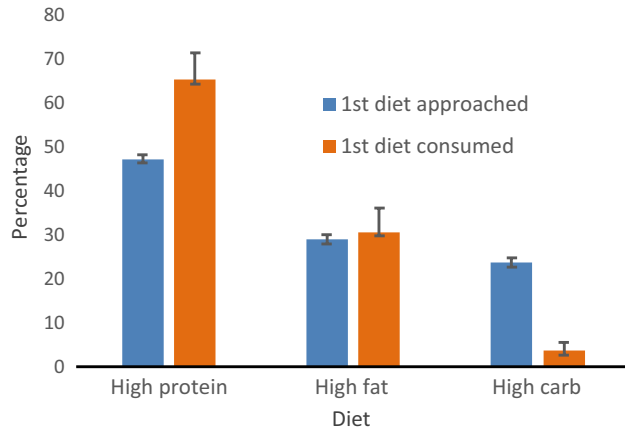


FIGURE 3 Percentage of high protein (P:F:C 57:42:1% by energy), high fat (P:F:C 13:86:1% by energy) or high carbohydrate (P:F:C 18:28:54% by energy) experimental diets which were approached first by adult dogs ($n = 15$) for 10 days were $47 \pm 3.7\%$, $29 \pm 3.5\%$ and $24 \pm 3.0\%$. Those involving any level of first consumption were $64.3 \pm 6.0\%$, $29.7 \pm 5.5\%$ and $3.7 \pm 1.9\%$. For both the high protein and high carbohydrate diets, there were significant differences between the percentage first approached and first consumed ($p < .001$) [Colour figure can be viewed at wileyonlinelibrary.com]

5 and 10). Analysis was conducted using GenStat 18th edition (VSN International, 2016). Results are presented as means and associated standard error of the mean (SEM).

3 | RESULTS

3.1 | Bodyweight

Bodyweight increased significantly ($p < .001$) over the 10-day study (Table 2). At the start of the study, the mean bodyweight of the dogs was $25.9 \text{ kg} \pm 0.72 \text{ SEM}$ which increased to $27.5 \text{ kg} \pm 0.77 \text{ SEM}$ on day 10.

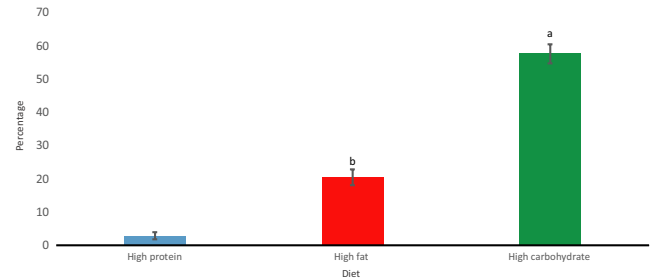


FIGURE 4 Percentage of high protein (PFC 57:42:1%), high fat (PFC 13:86:1%) or high carbohydrate (PFC 18:28:54%) experimental diets completely avoided by dogs ($n = 15$) over 10 days. The percentage of high carbohydrate diets which were completely avoided was significantly different to the percentage of high fat diets, which in turn was significantly different to the high protein diet ($p < .001$) [Colour figure can be viewed at wileyonlinelibrary.com]

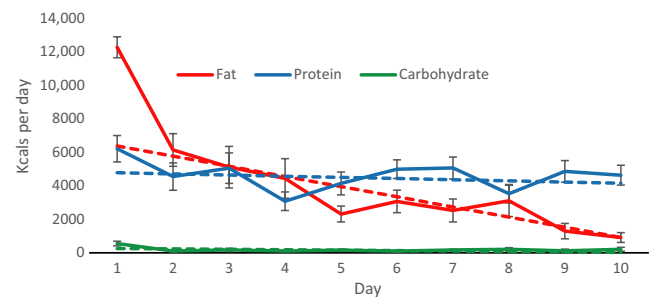


FIGURE 5 Mean macronutrient daily consumption (kcal day^{-1}) for adult dogs ($n = 15$) offered high protein (P:F:C 57:42:1% by energy), high fat (P:F:C 13:86:1% by energy) or high carbohydrate (P:F:C 18:28:54% by energy) diets for 10 days. The consumption of carbohydrate ($\text{kcal} = 284.09 (\pm 64.12 SE) - 26.04 (\pm 8.33 SE) \times \text{Day}$), and fat ($\text{kcal} = 6,989.38 (\pm 1,197.65 SE) - 607.24 (\pm 124.10 SE) \times \text{Day}$) declined over the study ($p < .01$ and $p < .001$ respectively). Consumption of protein remained constant ($\text{kcal} \text{ day}^{-1} = 4,856.21 (\pm 921.20 SE) - 70.00 (\pm 96.95 SE) \times \text{Day}$) [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Energy intake

Over the course of the study, the dogs reduced ($p < .001$; Table 3) their percentage of energy consumed from 363 to 162 per cent of energy intake according to the quadratic equation: $\%ME = 419.1 (\pm 31.8 SEM) - 60 (\pm 8.78 SEM) \times \text{Day} + 3.43 (\pm 0.78 SEM) \times \text{Day}^2$ (Figure 2).

3.3 | Feeding dynamics

Throughout the duration of the experiment, the percentage of dogs which first approached and first consumed a diet was determined (Figure 3). For HP, the percentage of dogs which approached the diet first was $47\% (\pm 3.7 SEM)$ and first consuming it $64\% (\pm 6.0 SEM)$ ($p < .001$), for carbohydrate $24\% (\pm 3.0 SEM)$ first approached the diet, with $4\% (\pm 1.9 SEM)$ first consuming it ($p < .001$). The high fat diet displayed no significant differences between those first approached $29\% (\pm 3.5 SEM)$ and first consumption $30\% (\pm 5.5 SEM)$

TABLE 3 Linear and quadratic responses to analysis of total energy consumed, grams of macronutrients consumed, specific overall macronutrient energy intake and ratios in dogs ($n = 15$) offered high protein (PFC 57.6:41.7:0.7%), high fat (PFC 12.8:86.7:0.5%) or high carbohydrate (PFC 17.8:27.7:54.5%) diets at 500% maintenance energy requirements for 10 days

Response	Model	α	SE	β_1	SEM	β_2	SE
Total Energy Consumed (unit)	Linear	373.10***	40.42	-23.97***	3.28	-	-
	Quadratic	419.10***	31.8	-60.00***	8.78	3.43***	0.78
Protein intake (% of overall ME)	Linear	27.77***	3.17	1.60**	0.36	-	-
Fat intake (% of overall ME)	Linear	69.95***	3.14	-1.81***	0.37	-	-
Carbohydrate intake (% of overall ME)	Linear	2.28***	0.62	0.21	0.27	-	-
Protein (kcal day ⁻¹)	Linear	4,856.21***	921.20	-70.00	96.95	-	-
Fat (kcal day ⁻¹)	Linear	6,989.38***	1,197.65	-607.24***	124.10	-	-
Carbohydrate (kcal day ⁻¹)	Linear	284.09***	64.12	-26.04**	8.33	-	-
Protein:Fat Ratio	Linear	0.40***	0.07	0.05***	0.01	-	-

α , Intercept; SE, Standard error; β_1 , Coefficient of Linear term; β_2 , Coefficient of Quadratic term.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

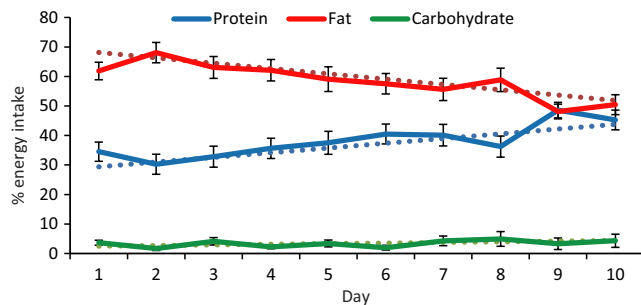


FIGURE 6 Mean self-selected macronutrient total energy intake (solid line) and linear fitted response (dotted line) of adult dogs ($n = 15$) (27.77 ± 3.17 protein % $+1.60 \pm 0.36 \times$ Day), (69.95 ± 3.14 fat % $-1.81 \pm 0.37 \times$ Day), (2.28 ± 0.62 carbohydrate % $+0.21 \pm 0.27 \times$ Day), offered high protein (PFC 57:42:1%), high fat (PFC 13:86:1%) or high carbohydrate (PFC 18:28:54%) diets for 10 days. Protein (% energy intake) increased ($p < .01$) and fat decreased ($p < .001$) [Colour figure can be viewed at wileyonlinelibrary.com]

($p = .720$). Significant differences ($p < .001$) were observed between the percentage of each diet completely avoided, with 58% (± 2.9 SE) of the carbohydrate diet being completely avoided, 20% (± 2.3 SE) of the fat diet and 3% (± 1.0 SE) of the protein diet (Figure 4). No changes in this behaviour were observed over the duration of the study ($p = .206$).

3.4 | Kcal per day of each macronutrient consumed

Over the course of the study, the daily consumption of carbohydrate reduced ($p < .01$; Figure 5) from 554 on day 1 to 214 kcal day⁻¹ on day 10 (kcal = 284.09 ± 64.12 SE) -26.04 ± 8.33 SE \times Day (Table 3). The kcal per day of fat consumed also reduced ($p < .001$; Figure 5) from 6,382 on day 1 to 917 kcal day⁻¹ on day 10 (kcal = $6,989.38 \pm 1,197.65$ SE) -607.24 ± 124.10 SE \times Day (Table 3). Consumption

of protein remained constant over the study ranging from 4786 on day 1 to 4,156 kcal day⁻¹ on day 10 (kcal day⁻¹ = $4,856.21 \pm 921.20$ SEM) -70.00 ± 96.95 SEM \times Day (Table 3).

3.5 | Macronutrient consumption and ratio

Protein intake (as a proportion of total ME) increased ($p < .01$; Figure 6) from 29.4% ME on day 1 to 44% ME (ME% = 27.77 ± 3.17 SE) $+1.60 \pm 0.36$ SE \times Day; Table 3) by day 10. Fat intake decreased ($p < .001$; Figure 6) from 68% ME on day 1 to 52% ME (ME% = 69.95 ± 3.14 SEM) -1.81 ± 0.37 SE \times Day (Table 3) by day 10. No significant difference in carbohydrate intake was observed (Figure 6) over the study (2.5% ME on day 1 and 4.4% ME by day 10: ME% = 2.28 ± 0.62 SE) $+0.21 \pm 0.27$ SE \times Day; Table 3).

The P:F ratio reflects these differences, increasing significantly ($p < .001$) from day 1 to 10 of the study (P:F = 0.40 ± 0.07 SE) $+0.05 \pm 0.01$ SE \times Day; Table 3). A P:F:C ratio of 34:62:4% was selected by the dogs on day 1, which gradually changed to 45:51:4% by day 10 (see Figure 6, raw data solid lines), driven by this increase ($p < .01$) in protein intake (ME day⁻¹) and decrease ($p < .001$) in fat intake (ME day⁻¹).

Using the fitted regression line of Protein% = $27.8 + 1.6$ Day, Protein % intake on Day 5 was calculated to be 35.8%, increasing to 43.8% by day 10 (Table 3, Figure 6).

4 | DISCUSSION

Our study shows that when dogs are allowed to self-select from diets varying in macronutrient composition, they will consume at least 30% of their energy from protein, thus in agreement with our hypothesis. Moreover, whilst mean protein intake over the course of the study was 37%, the energy consumption altered over the duration of the study, with an increase from 29% total energy on day 1 to 44% by day 10. This increase in protein intake was associated with

a decrease in fat consumption over the experiment, with the dogs consuming 68% on day 1 and 52% by day 10. Thus, the protein:fat ratio increased from 0.45 on day 1 to 0.90 by day 10. Although both protein and fat intake altered significantly during the study, carbohydrate consumption remained steady at 3% throughout the study. These changes in macronutrient selection by the dogs are illustrated in Figures 7a,b. A nutrition triangle was utilised, to represent a multidimensional assessment of dietary composition information (Raubenheimer, 2011). Collectively, these changes in macronutrient intake resulted in energy consumption decreasing from of 363% on day 1 to 162% on day 10.

Based on raw data energy intake, the dogs selected an average macronutrient P:F:C ratio of 38:59:3% (by energy) during the study. It must be noted, however, that the P:F:C ratio on day 1 (35:62:3 by energy) was different to that consumed on day 10 (45:51:4% by energy).

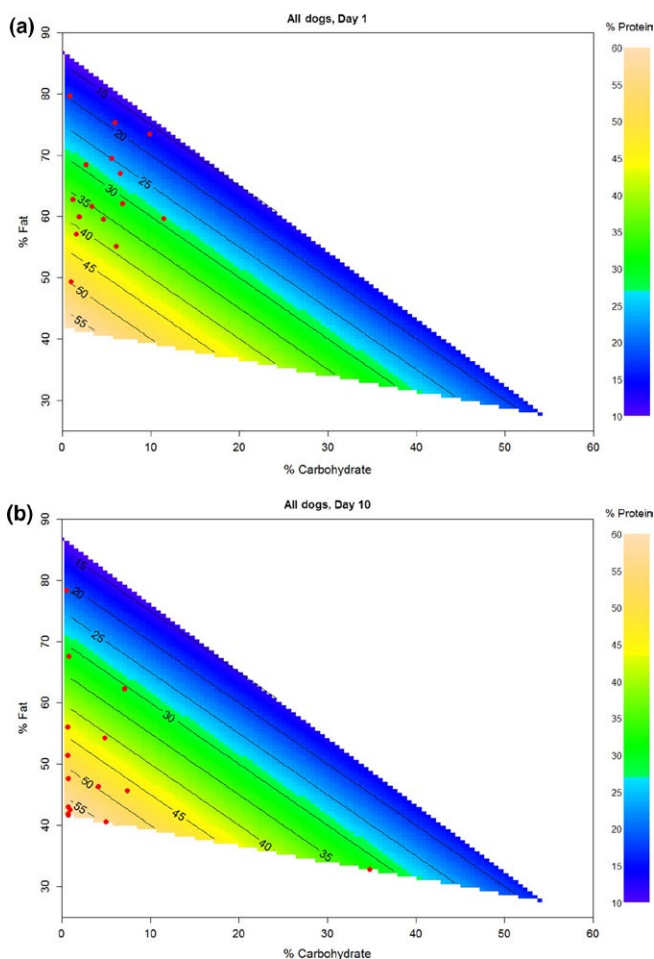


FIGURE 7 (a, b) Macronutrient total energy intake of individual adult dogs ($n = 15$) offered high protein (P:F:C 57:42:1% by energy), high fat (P:F:C 13:86:1% by energy) or high carbohydrate (P:F:C 18:28:54% by energy) diets. The y axis of both a and b represents fat intake, with the x axis signifying the carbohydrate intake (by energy). The graphs depict partial contour plots with lines representing protein intake (also colour coded with the legend showing the range of colours). Percentage of macronutrient total energy intake values for all dogs are symbolised by red dots [Colour figure can be viewed at wileyonlinelibrary.com]

This difference was driven by the decrease in fat energy consumption ($6,382-917 \text{ kcal day}^{-1}$) rather than any drop in protein intake ($4,786$ to $4,156 \text{ kcal day}^{-1}$) over the duration of the study. Such a reduction in energy consumption is likely as a result of increasing body fat in the dogs as the experiment progressed, with plasma leptin levels likely to be rising (Ishioka et al., 2002). As leptin serves as a signalling pathway between adipose tissue and the central nervous system, the consequence of this may be a reduction in energy intake (Akers & Denbow, 2008).

The initial targeting of fat dense food sources has also been demonstrated in the predatory beetle *Agonum dorsale* (Carabidae) (Raubenheimer, Mayntz, Simpson, & Tøft, 2007). Within the study, the beetles were assessed in regard to their nutrient intake over 10 days, with the first 2 days involving targeting a diet rich in fat after which protein intake increased. Although this study involved determining macronutrient intake after emergence from hibernation, and thus differed from our investigation, we did observe the same macronutrient pattern. Our dogs also targeted a high fat diet initially, with energy contribution from protein increasing thereafter, which may indicate an evolutionary past, whereby limited prey availability would predispose dogs to initially select fat sources. Although the dogs used in our study were maintained at a healthy body condition score, variations in how a score relates to body fat content can occur (Ishioka et al., 2005). Further studies in dogs investigating the association between body composition, macronutrient selection, total energy intake and factors such as leptin involved in influencing food intake would help better understand both the macronutrient and energy intake of dogs.

When comparing the average P:F:C ratio of 38:59:3% selected by the dogs in our study to that determined by Hewson-Hughes et al. (2012) of 30:63:7 (% by energy), several key factors could explain the differences, namely the length of the study period, the calculation of the P:F:C and the experimental structure.

In Hewson-Hughes et al. (2012), the experienced phase was 7 days in duration, whereas in the present study, it was 10 days. In the current study, when macronutrient selection was examined across the study period, it was apparent that major differences in the P:F:C selected occurred during the latter stages of the study, averaging 47:49:4 (% by energy) on days 9 and 10. Thus, the shorter timeframe in Hewson-Hughes et al. (2012) may have resulted in missing this apparent key macronutrient transitional period. It is likely that providing an average macronutrient ratio across the whole of the experimental period may fail to interpret the true nutritional movement the dogs made over relatively short testing periods (7–10 days). For example, the established average macronutrient ratio observed by Hewson-Hughes et al. (2012) over a 7-day period (30:63:7% by energy) was similar to the raw data over the initial 7 days of our study (36:61:3% by energy). However, only when average macronutrient values are teased apart for each day and examined in detail, do these key timeframes become obvious. The macronutrient selection by the dogs within our study varied significantly over the 10-day period, with a decrease in fat intake (68% vs. 52% by energy) and increase in protein (29% vs. 44% by energy) observed. It remains to be determined if the macronutrient selection by the dogs had stabilised after 10 days, or whether protein intake would continue to increase.

Secondly the experimental structure of the Hewson-Hughes et al. (2012) study involved three distinct phases, of differing duration and feeding patterns. These consisted of naïve self-selection (having access to all three meal options simultaneously for 7 days), learning (eight, 3-day periods, whereby the dogs were restricted to a specific diet (HC, HF, HP) for a day of each period) and experienced (the same as the naïve phase). Thus, the potential exists whereby within the self-selective phases, the feeding period ended just as the dogs were starting to regulate their macronutrient intake. In between these phases, the learning stage may also have confused dogs already starting to target a macronutrient intake, that is by confining each to a specific diet for 24 hours and repeating the process eight times. Therefore, the combination of a shorter study period, and the inclusion of a learning phase, limiting the dogs to specific diets in Hewson-Hughes et al. (2012) may have affected the dog's ability to target the macronutrient intake we observed.

Romsos and Ferguson (1983) also addressed macronutrient selection in the domestic dog; however, their primary aim was to understand the regulation of protein intake. In a 4-week study, two different sets of diets were offered to the dogs, differing not just in protein content, but also in fat and carbohydrate. Whilst the results showed the animals selected 30% of their metabolisable energy from protein, limitations in regard to nutrient movement, primarily due to the carbohydrate content varying from 20% to 42% ME within the test diets, could potentially have masked the true macronutrient ratio the dogs wished to select.

The self-selected macronutrient profile has also been reported for the domestic cat (*Felis catus*) using an approach similar to that applied to the domestic dog. Hewson-Hughes et al. (2011), established that macronutrient energy profile (P:F:C) was 52:36:12 (% by energy). In addition the study also suggests that cats have a carbohydrate ceiling of 300 kJ day⁻¹, which constrains them to deficits in protein and fat (relative to the determined intake target) when restricted to high carbohydrate diets (Hewson-Hughes et al., 2011). As with the dog study, a lack of reporting relating to macronutrient intake over the duration of the project was apparent. However, using another member of the felid family the mink (*Mustela vison*), it was demonstrated that within the first 24 hr of being allowed to self-select a P:F:C (with carbohydrate fixed at 15%) from a number of complementary foods, the mink selected a diet consisting of (P:F) of 35:50 (% by energy) (Mayntz et al., 2009). This ratio was observed throughout the 11-day study, with additionally when confined to diets that did not allow the desired protein:fat ratio to be achieved, the closest possible to that previously established being targeted.

In the current study, it is evident that over the 10-day experimental period, the dogs made a dietary "switch," reducing fat and increasing protein intake on an energy basis. To better understand the dietary switch, the feeding dynamics of the diets were explored.

When the overall percentage of dogs which first approached and first consumed a given diet was determined (Figure 3), it was clear that the HF diet displayed similar values of 29% and 31%, thus indicating most of the dogs which approached the diet first, consumed some of it. However, when the HP diet was examined, 47% of dogs approached it first, with 64% then consuming some of it first. This difference can be explained by results from the HC diet, which 24%

of dogs approached first; however, only 4% then consumed any. The majority of the dogs which approached the HC diet decided to move away and consume at least some of the HP diet instead. Throughout the study, the percentage of times that each diet was approached and consumed remained consistent. This highlighted that the initial decision to consume a specific diet at the start of the investigation was maintained during the study. In addition, the data also show that the HC diet was much most likely to remain untasted (58%), than the HF and HP diets (20% and 3% respectively) (Figure 4). Collectively, these feeding dynamics may indicate that there was an olfactory difference between the diets. As with the percentage of diets first approached and consumed, the proportion of each diet completely avoided were similar over the duration of the study. This would indicate the preference of dogs to target or avoid specific diets from day 1 of the investigation, remained consistent over the subsequent 9 days.

Indeed whilst we did not attempt to ensure palatability of our diets were consistent (e.g., with the use of a palatant), the same key ingredients were used in all the diets, just in different proportions. Interestingly research conducted by Salaun, Le Pailh, Roberti, Niceron, and Blanchard (2016) found that whilst the application of a palatability enhancer increased food intake in domestic cats, they were still capable of macronutrient regulation when offered pairs of differing diets. Moreover, a recent study has also indicated that the domestic cat is able to detect and maintain a macronutrient preference, despite changes in flavour (Hewson-Hughes, Colyer, Simpson, & Raubenheimer, 2016), with cats still preferring a diet containing a protein:fat ratio of 70:30 (by energy), even when the diet was flavoured with (apparently) negative flavours.

In the current study, lamb green tripe was used as the ingredient to manipulate the dietary protein content. Whilst the dogs migrated over the course of the study to a macronutrient ratio with a greater energy contribution from protein, it may be argued that this indicated a preference for green tripe, rather than a desire for protein *per se*. A similar argument could also be raised regarding the carbohydrate source used throughout the experiment (maize). Evidently carbohydrates played a minimal role in regard to selected dietary composition in the dogs; however, it is possible that this specific carbohydrate source was disliked more so than others that are also typically used in dog foods (e.g., rice or barley). Future studies could address these questions, where dogs are offered diets of similar macronutrient ratios, using different protein, fat or carbohydrate sources. Similarly, moisture content was not consistent between diets in the current study, with the HC diet having less moisture than the HP diet. At present, it is unknown if this had any impact on the resulting macronutrient profile, but studies have indicated in cats that energy intake and food consumption reduce as the level of water in a diet increases (Wei, Fascetti, Villaverde, Wong, & Ramsey, 2011).

In conclusion, the study clearly demonstrated that over a ten-day experiment, the test dogs selected a diet dominated by consumption of energy derived primarily from fat and protein, with carbohydrate playing a minimal role in contributing to overall energy intake. However, only after the completion of much deeper investigations into the selective capabilities and mechanisms influencing these dietary

decisions, will we truly have a grasp on what it is undoubtedly a fascinating and highly complex area of study.

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