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Influence of high-protein and high-carbohydrate diets on serum lipid and fructosamine concentrations in healthy cats

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Abstract

Objectives: The aim of this study was to determine whether high-protein and high-carbohydrate diets exert differential effects on serum cholesterol, triglyceride and fructosamine concentrations in healthy cats.

Methods: A randomised, crossover diet trial was performed in thirty-five healthy shelter cats. Before enrolment in the study, cats were fed a commercial maintenance diet. Following baseline health assessments, cats were randomised into groups receiving either the high-protein or high-carbohydrate diet for four weeks. The cats were then fed a washout diet for four weeks before being transitioned to whichever of the two studied diets they had not yet received. Fasting serum cholesterol, triglyceride and fructosamine concentrations were determined at the end of each four-week diet period.

Results: Cats on the high-carbohydrate diet had significantly lower serum cholesterol ($P<0.001$) concentrations than cats fed the baseline diet. Cats on the high-protein diet had significantly higher serum cholesterol ($P<0.001$) and triglyceride ($P<0.001$) concentrations, yet lower fructosamine ($P<0.001$) concentrations compared to baseline measurements. In contrast, overweight cats (body condition score (BCS) >5) had lower cholesterol ($P=0.007$) and triglyceride ($P=0.032$) concentrations on the high-protein diet than cats within other BCS groups.

Conclusions and relevance: Diets higher in protein and lower in carbohydrates appear beneficial for short-term glucose control in healthy cats. Indeed, the high-protein diet was associated with significantly elevated cholesterol and triglyceride concentrations amongst healthy cats even though the increase was significantly less pronounced in cats with a BCS>5. This finding suggests that overweight cats process high-protein diets, cholesterol and triglycerides differently than leaner cats.

1.Introduction

The cat is a true carnivore with various nutritional peculiarities adapted to a diet high in protein and low in carbohydrates [1]. While older research postulated that high-carbohydrate diets increased the risk for obesity in cats [2], more recent literature; however, presents contrasting findings [3]. Obesity in cats is associated with an increased risk for the development of diabetes mellitus (DM) [2]. Dietary therapy for diabetic cats should focus on reducing obesity, increasing muscle mass, decreasing postprandial hyperglycaemia and controlling blood glucose fluctuations by minimising the need for ß-cells to produce insulin [4] . Current evidence suggests that a high-protein, low-carbohydrate diet – relative to a high-carbohydrate diet – can benefit cats with DM [5-8]. Furthermore, clinical signs, blood glucose measurements and fructosamine concentrations can be used to monitor glycaemic control and response to therapy [9-15]. While researchers agree that high-protein diets can help treat DM and obesity in cats [5-8, 16], there is limited literature as to whether this type of diet would be advantageous to the healthy cat.

There are conflicting reports about how carbohydrates and fats influence the glycaemic response in healthy cats. High-fat diets are associated with diminished glucose clearance and ß cell function [17] in contrast to high-carbohydrate diets [18, 19] . There is contradicting evidence regarding the influence of fibre on glycaemic control in cats. While some have demonstrated better glycaemic control [20], others have failed to replicate these findings [6]. Most of the research that has assessed how diet composition influences the feline glycaemic response has focused on DM [5, 6, 8, 20], with only a few studies also including healthy cats [17, 18, 21, 22]. Furthermore, these investigations differ widely in study design, feeding protocols, population size as well as diet composition, which makes comparisons between studies difficult.

Several studies have reported that a high-protein and low-carbohydrate diet does not significantly affect serum triglyceride concentrations in cats [19, 23, 24]. Comparatively, cats fed diets high in fat had significant increases in triglyceride concentrations [17, 25, 26]. Importantly, high-fibre foods lower triglyceride concentrations in diabetic cats [20] but increase cholesterol concentrations in healthy cats [27]. Previous studies evaluating the effects of high-fat diets on cholesterol concentrations have been contradictory. Some have reported that high-fat diets do not contribute to hypercholesterolaemia [25, 28, 29] in comparison to others in which high fat diets do contribute to hypercholesterolaemia in healthy cats [17]. Moreover, diabetic cats with increased serum cholesterol concentrations are 65% less likely to achieve diabetic remission than cats with normal serum cholesterol concentrations [30]. This suggests that hypercholesterolaemia plays a primary role in the progression of diabetes in cats, possibly preventing the recovery of ß-cell function [30]. This theory is supported by several studies in mice that have shown that elevated cholesterol concentrations can impair ß-cell function [31, 32]. Current literature has also reported increased cholesterol concentrations in lean, overweight and diabetic cats on the traditional high-protein diet prescribed to diabetic cats [23].

The aim of this study was to determine the effect of three (high-protein, high-carbohydrate and washout) diets on serum cholesterol, triglyceride and fructosamine concentrations in lean, normal and overweight non-diabetic cats.

2. Materials and methods

2.1. Experimental design

The study was a randomised, crossover clinical trial and all procedures involving animals were approved by the Animal Ethics Committee of the University of Pretoria (V079-18).

2.2. Animals

Forty cats were recruited from an animal shelter, with thirty-five cats completing the study. Three animals were excluded due to early renal insufficiency while two further animals were excluded due to behaviour-related issues. To assist in proper identification, all of the cats were microchipped (Backhome, Virbac RSA., Centurion, South Africa). The inclusion criteria were:

- Age over one year
- Not affected with renal, liver disease; DM; hyperthyroidism
- Feline immunodeficiency and leukaemia virus (FIV and FeLV) negative
- Not receiving medical treatment
- Acceptance of restraint, venipuncture, and all diets
- No history of chronic vomiting or diarrhoea
- Known birthdate and complete vaccination record.

2.3. Feeding Protocol

Prior to enrolment, participating cats were fed a commercial maintenance diet (Table 1). Following baseline health assessments, cats were randomised into groups that were fed a high-protein or high-carbohydrate diet for four weeks. After these four weeks, cats were fed a washout diet for four further weeks. Thereafter, they were transitioned to the cross-over diet (Table 1 and Figure 1). Each cat was transitioned between diets over seven days. Cats were fed *ad libitum,* receiving 750 grams of each dry diet and 468 grams of the high-protein wet diet per room per day. Body condition score (BCS) was determined based on the nine-point BCS chart [33]. Clinical examinations, BCS, weight and environmental temperature (non-contact infrared thermometer, Electromann SA, Pretoria, South Africa) measurements, were conducted on a weekly basis.

2.4. Laboratory Tests and Health Assessment

Cats were determined as healthy based on history, physical examination, and laboratory tests. Blood samples were collected from all forty cats prior to the start of the study. All cats were fasted for 12 hours before blood collection. Blood was collected from the jugular vein by needle venipuncture and placed into one serum and one EDTA tube. Serum cholesterol, triglyceride, alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), blood urea nitrogen (BUN), creatinine, glucose, albumin, globulin and total serum protein (TSP) levels were measured using the Cobas Integra 400 plus analyser (Roche Diagnostics, Risch-Rotkreuz, Switzerland). The ADVIA 2120 Hematology System (Siemens Healthineers, Erlangen, Germany) was used to obtain all complete blood counts (CBCs), while total thyroxine (TT4) concentrations were measured with the Immulite 2000 immunoassay system (Siemens Healthineers). Fructosamine concentration assessments were performed using a colorimetric method on the Cobas Integra 400 plus analyser. A SNAP Combo plus (Idexx Laboratories), enzyme-linked immunosorbent assay for the simultaneous detection of FeLV antigen and antibodies for FIV was performed. All biochemistry and complete blood counts were performed at the Clinical Pathology Laboratory at the Faculty of Veterinary Science, University of Pretoria while fructosamine concentrations were measured at a commercial laboratory (Idexx Laboratories, Johannesburg, South Africa). Blood samples were collected from the cats after the four-week feeding period with the high-protein, high-carbohydrate and washout diets. Serum and plasma were centrifuged, separated and refrigerated overnight (within 1 hour of collection). Thereafter, serum and plasma were aliquoted and frozen at 80^0C within 24 hours of collection. At the end of the study, all collected specimens were analysed in a single batch.

2.6. Statistical Analyses

Data were assessed for normality of distribution by plotting histograms, calculating descriptive statistics and performing the Anderson-Darling test (MINITAB Statistical Software, Release 13.32, Minitab Inc, State College, Pennsylvania, USA). Right-skewed data were transformed using the natural logarithm. Categorical data were described using proportions and 95% confidence intervals (CI) while quantitative data were described using medians and interquartile ranges (IQR). Quantitative data were further evaluated by creating boxplots using the ggplot2 package (Wickham, 2009) within R (R Development Core Team, 2017). Categorical data were compared between cats based on the first diet assignment groups using chi-square tests (Epi Info, version 6.04, CDC, Atlanta, GA). Quantitative data were compared between initial diet assignment groups using independent t-tests on the raw or natural logarithm transformed data. Mann-Whitney U tests were used when the normality assumption was violated. Mixed-effects linear models were created to determine the effect of diet and BCS on serum cholesterol, triglyceride and fructosamine concentrations. Cat was included as a random effect in all models and the correlation among repeated measures was modelled using a first-order autoregressive (AR1) covariance structure. Evaluated fixed effects included diet, ordinal BCS groupings, sex, breed, age, experimental room, room temperature, and pairwise interactions between BCS and diet. Complete models were fit and a backwards stepwise process was employed to remove predictors with the largest P values until all remaining variables had significant slope parameters. Unless otherwise stated, SPSS (IBM SPSS Statistics Version 25, International Business Machines Corp., Armonk, NY, USA) was used for all statistical analyses. Significance was set at $p < 0.05$.

3. Results

3.1. Baseline Data

There were no significant differences in the baseline data between the two initial diet groups (Table 2 and 3).

3.2. Body Weight

Having been fed a high-protein diet (P=0.001), being male (P<0.001) and having a BCS>5 (P=0.002) were significant predictors of heavier body weights (Table 4).

3.3. Cholesterol

Median cholesterol concentrations were highest on the high-protein diet (Figure 2). Cholesterol concentrations were above the reference range in 10% (15/145) of the samples, of which 87% (13/15) represented the high-protein diet and 13% (2/15) the washout diet. The cholesterol concentrations of the remainder of the samples, 90% (130/145), were either within or just below the reference range. Cats on the high-carbohydrate diet had significantly lower (P<0.001) cholesterol concentrations than cats on either the high-protein (P<0.001) or washout diets (P<0.001; Table 5). Moreover, cats with a BCS >5 and that were fed a high-protein diet had significantly lower (P=0.007) cholesterol concentrations than cats from other BCS groups.

3.4. Triglycerides

Median triglyceride concentrations were lowest on the washout diet (Figure 3). None of the triglyceride concentrations were above the reference range. Cats that had been fed the washout diet had significantly lower ($P=0.009$) concentrations of triglycerides, whereas cats fed the high-protein diet had significantly higher ($P<0.001$; Table 6) concentrations. Cats with a BCS >5 and fed a high-protein diet had significantly ($P=0.03$) lower triglyceride concentrations than those from other BCS groups.

3.5. Fructosamine

Median fructosamine concentrations were highest in cats fed the high-carbohydrate diet (Figure 4). Only 6% (9/143) of the fructosamine samples were below the reference range; of these, four represented cats on the high-protein diet, four on the washout diet, and one on the baseline diet. The remaining 94% (134/143) of samples were within the reference range. Cats on either the high-protein or washout diets had significantly lower ($P<0.001$) fructosamine concentrations than other cats (Table 7).

4. Discussion

This study showed that cats on a high-carbohydrate diet had significantly lower serum cholesterol concentrations than cats on the maintenance diet. Cats on a high-protein diet had significantly higher serum cholesterol and triglyceride concentrations, yet lower fructosamine concentrations compared to baseline measurements. In contrast, overweight cats (BCS>5) had lower cholesterol and triglyceride concentrations on a high-protein diet than cats representing other BCS groups.

Neutered male cats are at an increased risk of obesity compared to intact males, and are thus at greater risk for developing DM [22, 34, 35]. In the current study, neutered male cats were significantly heavier than female cats, which is in agreement with previous findings [36, 37]. It has been postulated that high-carbohydrate diets increase the risk for obesity in cats [2]; however, there are few epidemiological studies available that either support or refute this claim [35]. In the current study, cats on a high-carbohydrate diet were not heavier than cats on other diets. This supports prior reports that cats limit their total energy intake when consuming a high-carbohydrate diet [18, 38]. Dietary protein is an important component of weight loss diets [39], as high-protein diets have been shown to promote fat loss in cats [16]. However, offering overweight cats an *ad libitum* high-protein diet increases food intake – perhaps due to increased palatability – without any noticeable changes in body weight or composition [24]. Our *ad libitum* experiment demonstrated that cats fed a high-protein diet were heavier than cats on other diets.

In DM cats, hypercholesterolaemia reduces the chance of remission by almost 65%. Although hypercholesterolaemia can contribute to the pathogenesis of DM in cats [30], its effects on healthy cats are still debateable. Prior research has reported that diabetic, lean and overweight cats fed a high-protein diet had higher serum cholesterol concentrations [23]. In this current study, cats that were fed a high-protein diet had significantly higher cholesterol concentrations compared to other cats. Cats fed the high-protein diet had elevated median cholesterol concentrations among all three BCS groups. Interestingly, overweight cats on the high-protein diet did not have a large increase in serum cholesterol concentrations. The mechanism through which ingested protein is coupled to upregulated cholesterol production requires further study.

Studies have indicated that insoluble fibre is positively associated with cholesterol concentrations in overweight cats. It has been speculated that fibre can interfere with the absorption of specific fat components that could subsequently alter which lipoproteins are synthesised in the liver [27]. It should be noted that the high-protein diet of the current study had considerably higher crude fibre content than the high-carbohydrate diet. The high-protein diet had nearly double the amount of fat that was in the high-carbohydrate diet. It has been reported that a high-fat diet does not contribute to hypercholesterolaemia [25, 28, 29]. Nevertheless, prior studies of cats fed a high-fat diet reported higher cholesterol concentrations compared to cats fed a high-carbohydrate diet [17]. Cats in the current study fed the high-carbohydrate diet, which has low fibre, protein and fat content, had lower cholesterol concentrations while cats fed the high-protein diet, which has high fibre, protein and fat content, had increased cholesterol concentrations. Interactions between different dietary components might exert an additive role in the presented results; for this reason, lipoprotein fractions should be assessed in future studies.

There are conflicting reports of the influence of diet on triglyceride concentrations in cats. Several studies reported that a high-protein and low-carbohydrate diet did not significantly affect triglyceride concentrations in cats [19, 23, 24]. In contrast, cats fed a high-fat diet had a significant increase in triglyceride concentrations [17, 25, 26]. Nevertheless, the current study revealed that a high-protein diet increased triglyceride concentrations among healthy cats. The discrepancies between our findings and previous reports might be due to the differences in study design. Overweight cats generally have higher triglyceride concentrations than healthy cats [40]. In this current study, lean and normal cats fed a high-protein diet and overweight cats fed a high-carbohydrate diet had the highest median triglyceride concentrations. In this current study, cholesterol and triglyceride concentrations in overweight cats decreased when fed a high-protein diet.

Diabetic cats can benefit from high-protein and low-carbohydrate diets due to higher diabetic remission rates [6], lower fructosamine concentrations [8], and improved glycaemic control [5, 8]. However, most reports concerning how diet composition affects feline glycaemic response have focused on diabetic cats [5-8, 20], with only a few studies involving healthy cats [17, 18, 21, 22]. In this current study, cats fed the high-carbohydrate diet had the highest median fructosamine concentrations. There are conflicting reports about how carbohydrates and fats influence the glycaemic response in healthy cats. Cats fed high-fat diets had diminished glucose clearance and ß-cell function relative to cats fed a high-carbohydrate diet [17]. These findings contrasted with reports that high-carbohydrate diets cause higher insulin [26] and post-prandial glucose concentrations[18, 19] compared to healthy cats fed high-fat and high-protein diets. In this current study, a complex link between diet and fructosamine concentrations among healthy cats was demonstrated, as elevated carbohydrate content increased fructosamine concentrations, and increased fat content decreased fructosamine concentrations in healthy cats, which partly agrees with recent findings.

In this current study, cats on both high-protein and washout diets had significantly lower fructosamine concentrations compared to cats on the other diets. These findings agree with the finding that healthy cats fed high-protein diets with either low or moderate levels of starch had significantly decreased glucose and fructosamine concentrations in comparison to moderate-protein and high-starch diets [41]. There is conflicting evidence regarding the influence of fibre on the glycaemic control in cats. Glycaemic control among DM cats improved when fibre intake was increased to moderate levels [20] in contrast to other studies that showed the opposite [6]. Cats continue with post-prandial gluconeogenesis from protein, which might explain why fibre is potentially less effective in this species [42, 43]. Even though the exact mechanisms underlying these findings remain unknown, dietary fibre seems to affect the nutrient transit rate in the gut, which, subsequently, reduces glucose absorption along with post-prandial glycaemia and enhances glycaemic control [20, 44]. This current study suggests that diets high in protein, fibre, and fat, but low in carbohydrates, could contribute to decreased glucose concentrations in healthy cats.

This study had several limitations. The included cats were not fed according to their individual nutritional requirements, but *ad libitum* to simulate the situation in private, multi-cat households. *Ad libitum* feeding is regarded as a risk factor for obesity, and the feeding strategy in this study might have inadvertently predisposed the participating cats to gain weight [45]. The amount of food that each cat ingested was not recorded and thus some cats might have preferred one type of food to another, which could have introduced bias. Additionally, a hierarchical structure will occur with group housing of cats with dominant animals eating more and submissive animals eating less. While this might have introduced bias, an adaptation period of 2 months to identify these cats was performed to reduce this limitation. Two such cats were identified and excluded. Finally, wet food was only used for the high-protein diet and this could have influenced presented findings; however, the dietary contents of the wet and dry high-protein diets had very similar protein, carbohydrate and fat composition. Additionally, both types of feed(wet and dry) of this specific diet would traditionally be given to a cat in the clinical setting, and we attempted to replicate the decision a clinician would face in private practice.

In conclusion, diets with high protein, but low carbohydrate content, might be beneficial for short-term glucose control in healthy cats. The reduction in cholesterol and triglyceride concentrations among overweight cats on a high-protein diet, relative to lean and normal cats on the same diet, is a novel result that warrants further investigation. The finding that a high-protein diet significantly increased cholesterol and triglyceride concentrations and a high-carbohydrate diet significantly decreased cholesterol concentrations in healthy cats relative to other diets also warrants further investigation.

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Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

This work involved the use of experimental animals and the study therefore had ethical approval from an established committee as stated in the manuscript.

Informed consent

Informed consent (either verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (either experimental or non-experimental animals) for the procedure(s) undertaken (either prospective or retrospective studies). No animals or humans are identifiable within this publication, and therefore additional informed consent for publication was not required.

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Table 1. Comparison of the diets(reported on a per kilocalorie basis) used in 35 healthy cats enrolled in a cross-over study investigating the effects of diet on serum lipid and hormone profiles.

Type of diet	**Manufacturer**	**Protein (**percentage of kcal in the diet)	**Fat (**percentage of kcal in the diet)	**Carbohydrate** (percentage of kcal in the diet)	**Crude Fibre (**grams per 100 kcal ME)
Maintenance	Whiskas Beef, Lamb and Rabbit flavour	29.9%	28.5%	41.7%	0.9g

	with meaty nuggets				
High-protein	Hill's M/D dry food	42.8%	40.6%	16.6%	0.9g
High-protein	Hill's M/D wet food	43.5%	42.3%	14.1%	1.7g
High-carbohydrate	Hill's Science plan, Feline mature adult 7+ sterilized cat	32.5%	22.4%	45.2%	0.3g
Washout	Hill's Science Plan, Feline Mature Adult 7+ Hairball Control)	30.7%	41.5%	27.9%	2.1g

ME = metabolisable energy.

Table 2. Comparison of baseline demographics and serum chemistry parameters in 40 healthy cats randomised into groups receiving either a high-carbohydrate (HC; n=20) or high-protein (HP; n=20) diet first during a cross-over study investigating the effects of diet on serum lipid and hormone profiles.

	HC diet first		**HP diet first**		
Variable	**n/d**	**PE* (Interval†)**	**n/d**	**PE* (Interval†**	**P value‡**

				)	
Categorical data					
Female sex	15/20	0.75 (0.53, 0.90)	15/20	0.75 (0.53, 0.90)	1.0‡
DSH	17/20	0.85 (0.64, 0.96)	12/20	0.60 (0.38, 0.79)	0.07‡
Quantitative data					
Age (yr)	20/20	4 (2,7)	20/20	4 (3, 6)	0.84§
Albumin (g/L)	20/20	33.1 (32.3, 34.4)	20/20	34.0 (31.2, 37.7)	0.80¶
ALP (U/L)	20/20	35.5 (26.8, 44.8)	20/20	31.5 (24.3, 45.5)	0.87¶
ALT (U/L)	20/20	43. 7 (35.2, 54.2)	20/20	40.3 (33.8, 49.4)	0.31¶
BCS (/9)	20/20	5 (4, 6)	20/20	5 (4, 6)	1.0¶
BUN (mmol/L)	20/20	7.1 (5.9, 9.0)	20/20	7.8 (6.2, 9.8)	0.34¶
Cholesterol (mmol/L)	20/20	2.46 (1.98, 3.02)	20/20	2.46 (1.99, 2.76)	0.80#
Creatinine (umol/L)	20/20	112 (104, 121)	20/20	118 (104, 131)	0.39¶
Fructosamine (mmol/L)	20/20	247 (227, 270)	20/20	239 (214, 275)	0.44#
GGT (U/L)	20/20	0 (0, 1)	20/20	0 (0, 0)	0.12§
Globulin (g/L)	20/20	39.7 (34.4, 43.4)	20/20	36.8 (33.8, 41.7)	0.30¶
Glucose (mmol/L)	20/20	4.2 (3.8, 4.6)	20/20	4.1 (3.7, 4.6)	0.86§
Triglycerides (mmol/L)	20/20	0.39 (0.31, 0.52)	20/20	0.33 (0.27, 0.38)	0.17#
TSP (g/L)	20/20	73.5 (69.9, 77.1)	20/20	70.2 (67.5, 74.6)	0.36¶
TT4 (nmol/L)	20/20	22.1 (20.1, 26.4)	20/20	23.6 (21.1, 29.4)	0.93#
Weight (kg)	20/20	3.7 (3.6, 4.3)	20/20	3.9 (3.5, 4.2)	0.99#

n/d = numerator/denominator; ALP = alkaline phosphatase; ALT = alanine transaminase; BCS = body condition score; BUN = blood urea nitrogen; CI = confidence interval; DSH = domestic short hair breed; GGT = gamma-glutamyl transferase; TSP = total serum protein; TT4 = Total thyroxine.

*PE = point estimate, corresponding to the proportion for categorical variables and the median for quantitative data

†Interval is the 95% confidence interval for categorical data and the interquartile range for quantitative data

‡Based on chi-square tests

§Based on Mann-Whitney U tests

¶Based on independent t-tests on untransformed data

#Based on independent t-test on natural log-transformed data

Table 3. Comparison of complete blood count results in 40 healthy cats randomised into groups receiving either a high-carbohydrate (HC; n=20) or high-protein (HP; n=20) diet first during a cross-over study investigating the effects of diet on serum lipid and hormone profiles.

	HC diet first		**HP diet first**		
Variable	**n/d**	**Median (IQR)**	**n/d**	**Median (IQR)**	**P value**
Band neutrophils (×10^9/L)	16/20	0 (0, 0.05)	17/20	0 (0, 0)	0.81*
Basophils (×10^9/L)	16/20	0 (0, 0)	17/20	0 (0, 0)	0.76*
Eosinophils (×10^9/L)	16/20	0.44 (0.26, 0.88)	17/20	0.54 (0.42, 0.76)	0.91†
Hematocrit (L/L)	16/20	0.35 (0.31, 0.38)	17/20	0.37 (0.32, 0.40)	0.59‡
Hemoglobin (g/L)	16/20	125 (121, 139)	17/20	140 (113, 147)	0.60‡
Lymphocytes (×10^9/L)	16/20	3.61 (3.10, 5.07)	17/20	3.39 (2.76, 5.41)	0.68*
MCHC (g/dL)	16/20	36.8 (35.5, 38.7)	17/20	36.5 (35.6, 37.5)	0.73‡

MCH (pg)	16/20	15.7 (14.4, 16.2)	17/20	14.1 (13.4, 15.8)	0.17†
MCV (fL)	16/20	41.6 (38.8, 44.2)	17/20	39.2 (37.3, 42.3)	0.24‡
Monocytes ($\times10^9$/L)	16/20	0.29 (0.16, 0.60)	17/20	0.26 (0.11, 0.39)	0.38†
Neutrophils ($\times10^9$/L)	16/20	5.97 (4.29, 9.25)	17/20	6.25 (5.51, 7.95)	0.71†
Platelets ($\times10^9$/L)	16/20	204 (151, 316)	17/20	316 (189, 523)	0.10†
RCC ($\times10^{12}$/L)	16/20	8.49 (7.52, 9.81)	17/20	8.81 (7.61, 10.60)	0.38*
RDW %	16/20	14.5 (14.0, 15.2)	17/20	14.7 (14.4, 15.1)	0.83‡
WCC ($\times10^9$/L)	16/20	11.9 (8.2, 13.6)	17/20	10.6 (9.0, 14.0)	0.86†

n/d = numerator / denominator; IQR = interquartile range; MCH= mean corpuscular haemoglobin; MCHC= mean corpuscular haemoglobin concentration; MCV= mean corpuscular volume; RCC= red cell count; RDW= red cell distribution width; WCC= white cell count.

*Based on Mann-Whitney U tests

†Based on independent t-test on natural log-transformed data

‡Based on independent t-tests on untransformed data

Table 4. Multivariable associations between body weight*, diet, and body condition score (BCS) in 35 healthy cats enrolled in a cross-over study investigating the effects of diet on serum lipid and hormone profiles.

Variable	**Level**	**Estimate (95% CI)**	**t statistic**	**P value**
Diet	HC	0.005 (-0.016, 0.026)	0.477	0.63
	HP	0.036 (0.015, 0.058)	3.421	0.001

	Washout	0.025 (-0.001, 0.050)	1.920	0.05
	Baseline	Reference		
Sex	Male	0.187 (0.103, 0.270)	4.531	<0.001
	Female	Reference		
BCS < 5	Yes	-0.071 (-0.104, -0.038)	-4.250	<0.001
	No	Reference		
BCS > 5	Yes	0.047 (0.017, 0.078)	3.100	0.002
	No	Reference		
Room temperature	1 C increase	-0.009 (-0.015, -0.003)	-2.812	0.006

CI = confidence interval; HC = high carbohydrate; HP = high protein.

*Data were natural log-transformed prior to statistical analysis

Table 5. Multivariable associations between serum cholesterol*, diet, and body condition score (BCS) in 35 healthy cats enrolled in a cross-over study investigating the effects of diet on serum lipid and hormone profiles.

Variable	Level	Estimate (95% CI)	t statistic	P value
Diet	HC	-0.122 (-0.186, -0.059)	20.628	<0.001
	HP	0.479 (0.408, 0.551)	-3.814	<0.001
	Washout	0.166 (0.099, 0.233)	13.323	<0.001
	Baseline	**Reference**		
BCS > 5	Yes	0.001 (-0.085, 0.088)	0.031	0.97
	No	**Reference**		
BCS > 5 and HP diet	Yes	-0.157 (-0.269, -0.044)	-2.766	0.007
	No	**Reference**		

CI = confidence interval; HC = high carbohydrate; HP = high protein.

*Data were natural log-transformed prior to statistical analysis

Table 6. Multivariable associations between serum triglycerides*, diet, and body condition score (BCS) in 35 healthy cats enrolled in a cross-over study investigating the effects of diet on serum lipid and hormone profiles.

Variable	Level	Estimate (95% CI)	t statistic	P value

Diet	HC	0.068 (-0.029, 0.164)	1.386	0.16
	HP	0.223 (0.114, 0.332)	4.045	<0.001
	Washout	-0.134 (-0.233, -0.034)	-2.669	0.009
	Baseline	**Reference**		
BCS > 5	Yes	-0.009 (-0.131, 0.112)	-0.152	0.87
	No	**Reference**		
BCS > 5 and HP diet	Yes	-0.192 (-0.366, -0.017)	-2.180	0.03
	No	**Reference**		

CI = confidence interval; HC = high carbohydrate; HP = high protein.

*Data were natural log-transformed prior to statistical analysis

Table 7. Associations between serum fructosamine* and diet in 35 healthy cats enrolled in a cross-over study investigating the effects of diet on serum lipid and hormone profiles.

Variable	**Level**	**Estimate (95% CI)**	**t statistic**	**P value**
Diet	HC	-0.037 (-0.077, 0.002)	-1.878	0.06
	HP	-0.110 (-0.149, -0.070)	-5.514	<0.001
	Washout	-0.133 (-0.169, -0.097)	-7.338	<0.001
	Baseline	**Reference**		

CI = confidence interval; HC = high carbohydrate; HP = high protein.

*Data were natural log-transformed prior to statistical analysis. No multivariable model fit the data.

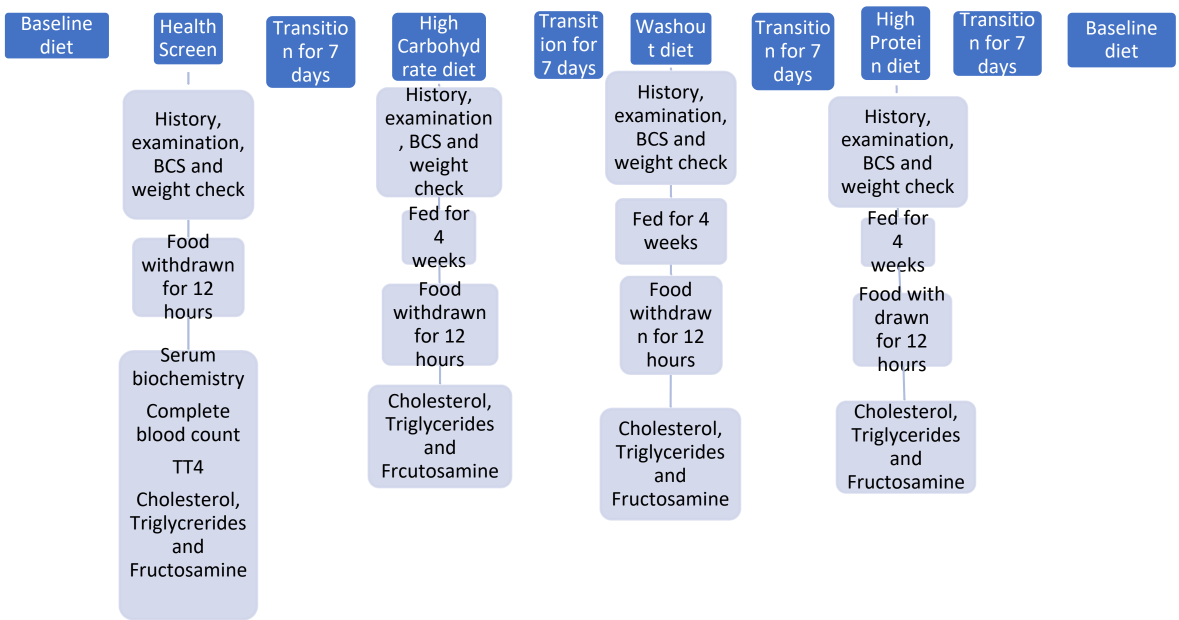

Figure 1: Flow chart highlighting the research process in 40 healthy cats randomised into groups receiving either a high-carbohydrate or high-protein diet first during a cross-over study investigating the effects of diet on serum lipid and hormone profiles

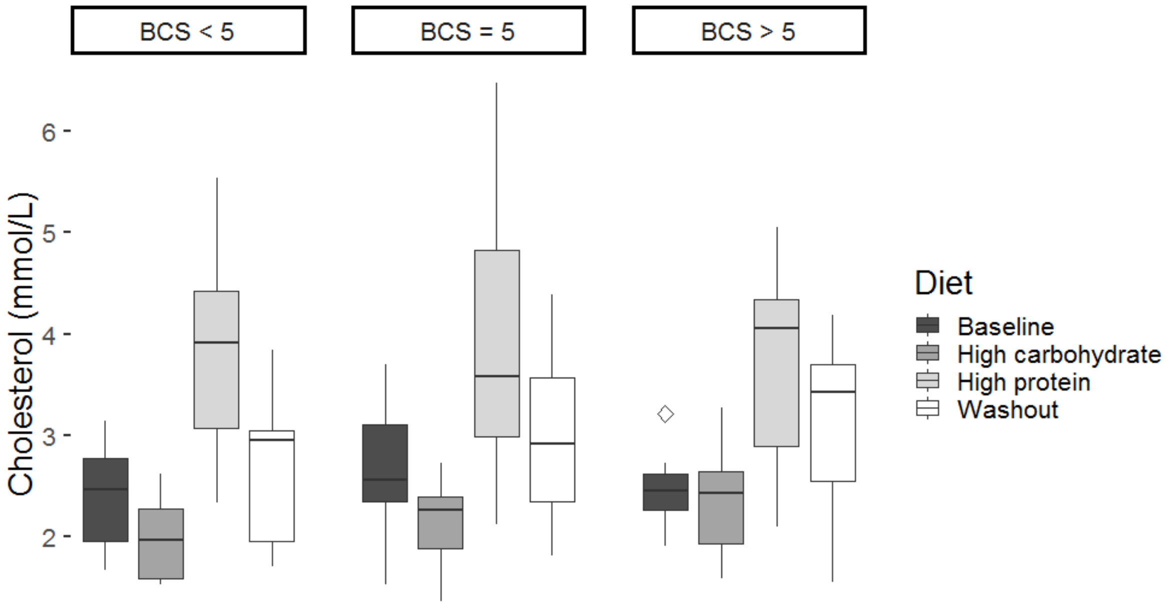

Figure 2. Illustration of serum cholesterol values for 35 healthy cats – separated according to body condition score (BCS) - in a cross-over study investigating the effects of diet on serum lipid and hormone profiles.

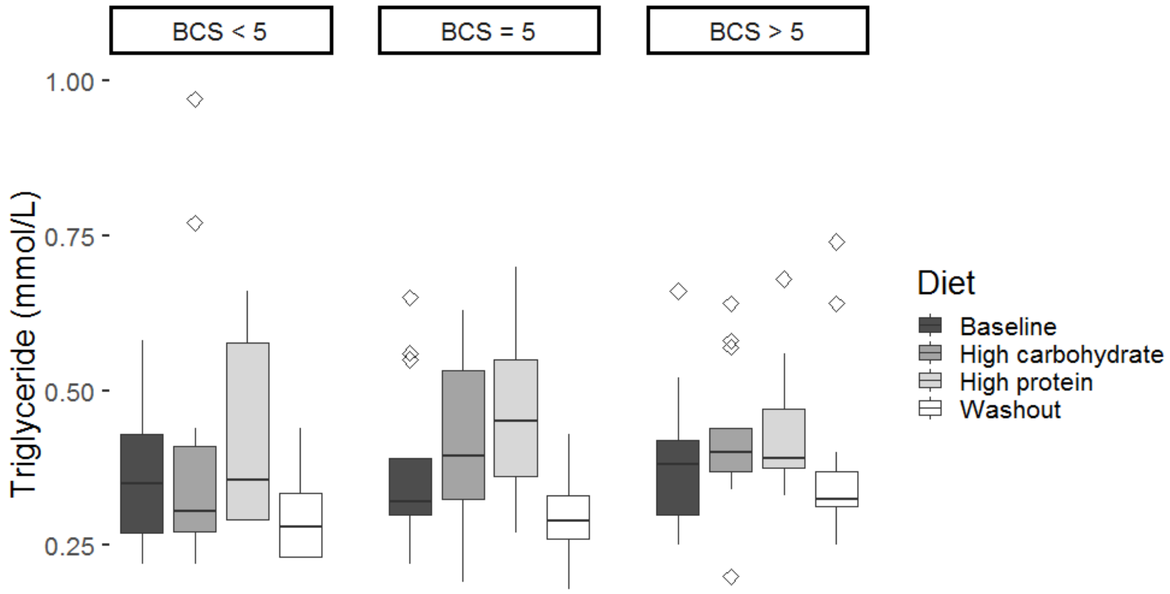

Figure 3. Illustration of serum triglyceride values for 35 healthy cats – separated according to body condition score (BCS) - in a cross-over study investigating the effects of diet on serum lipid and hormone profiles.

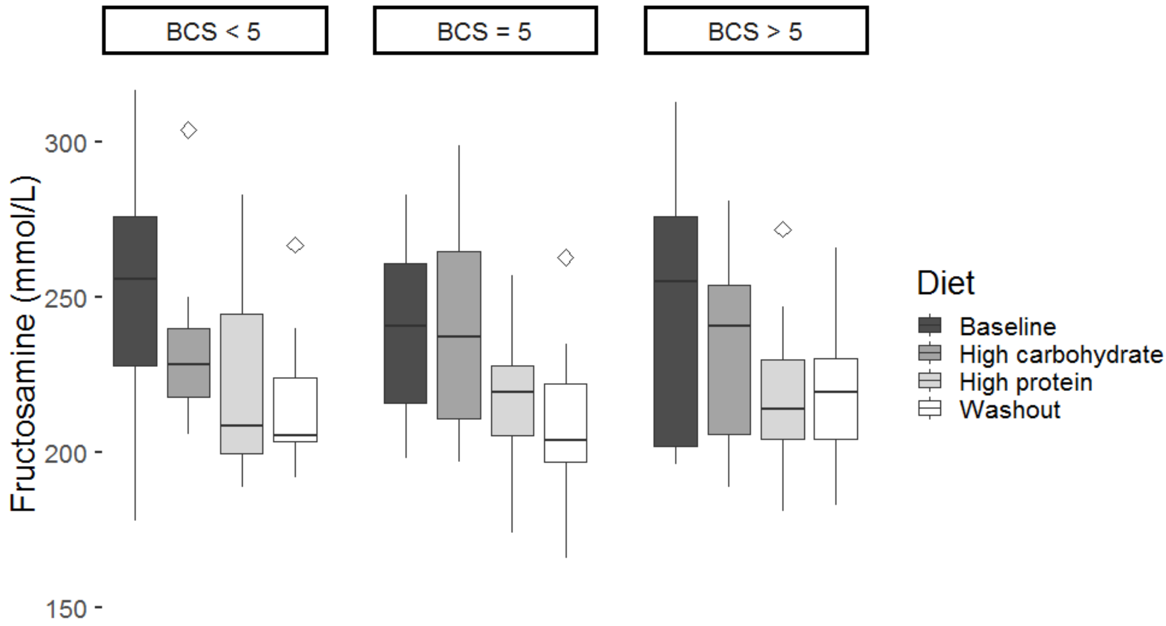

Figure 4. Illustration of serum fructosamine values for 35 healthy cats – separated by body condition score - in a cross-over study investigating the effects of diet on serum lipid and hormone profiles.