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

by

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Submitted in partial fulfilment of the requirements for the degree of

MMedVet (Med)

in the Faculty of Veterinary Science

at the

University of Pretoria

SUPERVISOR: Prof Johan Schoeman

DATE 19 July 2021

UNIVERSITY OF PRETORIA

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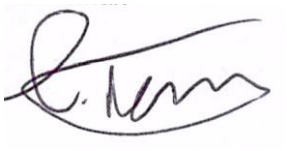
Signature of student: ....  ……..

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PROJECT TITLE	**Influence of diet on the lipid and hormone profile in a population of healthy cats.**
PROJECT NUMBER	**REC072-18**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Chad Berman**

DISSERTATION/THESIS SUBMITTED FOR	**MMedVet**

SUPERVISOR	**Johan Schoeman**

APPROVED	Date **12 October 2018**
CHAIRMAN: UP Research Ethics Committee	Signature

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Animal Ethics Committee

PROJECT TITLE	**Influence of diet on the lipid and hormone profile in a population of healthy cats**
PROJECT NUMBER	**V079-18**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. CF Berman**

STUDENT NUMBER (where applicable)	**U_28087543**
DISSERTATION/THESIS SUBMITTED FOR	**MMedVet**

ANIMAL SPECIES/SAMPLES	**Domestic cats**
NUMBER OF ANIMALS	**40**
Approval period to use animals for research/testing purposes	**September 2018 –September 2019**
SUPERVISOR	**Prof. J Schoeman**

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 8 October 2018
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S4285-15

Acknowledgements

I would like to extend my gratitude to the following people and organisations for their assistance during the course of the study. Firstly, I acknowledge Prof Geoffrey Fosgate for all his help and advice given throughout the study, and Prof E Zini for all of the relevant guidance. I am grateful to the staff of the Animal Anti-Cruelty League for allowing me to use their facilities and animals throughout the duration of the study. In particular, Cindy Price for assisting me with all the logistics of the study. I would like to thank the nurses of Bryanston Veterinary Hospital that helped me with all the blood collections. I am grateful for my wife, especially for her devotion and support during this period of our life. I acknowledge the South African Veterinary Foundation and the Health and Welfare Sector Education and Training Authority for funding the study. I would also like to extend my gratitude to Hills, who sponsored all the food used in this study, along with Virbac, who provided all of the microchips. The work covered in this mini-dissertation was presented as a poster at the online ECVIM congress in September 2020. Lastly, I would like to extend a massive thank you to my supervisor Prof J P Schoeman and my co-supervisor Dr R G Lobetti for their never-ending guidance and support during the study.

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Summary

Influence of diet on the lipid and hormone profiles of cats

Berman, CF. University of Pretoria, 2021

Diabetic cats can benefit from a high-protein and low-carbohydrate diet in terms of higher diabetic remission rates, lower fructosamine concentrations and improved glycaemic control. Hypercholesterolaemia in cats with diabetes mellitus has been associated with lower remission rates. A recent publication demonstrated that a high-protein and low-carbohydrate diet resulted in significantly elevated serum cholesterol concentrations in lean, overweight and diabetic cats. The concept that a traditional high-protein and low-carbohydrate diet causes increased cholesterol concentrations in healthy cats is relatively new and requires further investigation.

This mini-dissertation focused on clarifying whether high-protein and high-carbohydrate diets exert differential effects on serum cholesterol, triglyceride, and fructosamine concentrations in healthy cats.

This mini-dissertation describes the results of a randomised, crossover diet trial that was performed in thirty-five healthy shelter cats. Prior to enrolment, cats were fed a commercial baseline 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 diet they had not yet been subjected to. Fasting serum cholesterol, triglyceride and fructosamine concentrations were determined at the end of each four-week diet period. Weekly body condition score (BCS), body weight measurements and environmental temperatures were evaluated throughout the study.

Cats on the high-protein diet had significantly higher serum cholesterol and triglyceride, yet significantly lower serum fructosamine concentrations than cats on the baseline diet ($P<0.001$). Among the cats on the high-protein diet, the observed increases in cholesterol and triglycerides were significantly more pronounced in cats with BCS < 5. In contrast, cats on the high-carbohydrate diet had significantly lower serum cholesterol concentrations ($P<0.001$) relative to cats on the baseline diet.

The empirical evidence base currently suggests that diets with high protein but low carbohydrate contents may 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 cats on the same diet, suggests that overweight cats process cholesterol and triglycerides differently from lean cats. These findings from healthy cats, that a high-protein diet significantly increased cholesterol and triglyceride concentrations while a high-carbohydrate diet significantly decreased cholesterol concentrations relative to baseline diets, warrants further investigation.

Key terms:

Key words: High-protein, high-carbohydrate, lipid profile, fructosamine, cholesterol

List of abbreviations

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AR1	first-order autoregressive covariance
BCS	body condition score
BUN	blood urea nitrogen
CBC	complete blood count
CI	confidence interval
DLH	domestic long hair
DM	diabetes mellitus
DMH	domestic medium hair
DSH	domestic short hair
FeLV	feline leukaemia virus
FFA	free fatty acid
FIV	feline immunodeficiency virus
GGT	gamma-glutamyl transferase
HC	high-carbohydrate
HDL	high-density lipoprotein
HP	high-protein
IQR	interquartile range
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
ME	metabolisable energy
RCC	red cell count
RDW	red cell distribution width
TSP	total serum protein
TT4	total thyroxine concentration
WCC	white cell count

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Chapter 1: Introduction

A nutritional characteristic of cats is that they are adapted to diets high in protein and low in carbohydrates (Morris, 2002). Obesity is becoming an increasingly common nutritional disorder that predisposes cats to insulin resistance and diabetes mellitus (DM) (Clark and Hoenig, 2016). Current nutritional recommendations suggest that a high-protein, low-carbohydrate diet – relative to a high-carbohydrate diet – can benefit cats with DM (Mazzaferro et al., 2003, Bennett et al., 2006, Frank et al., 2001, Hall et al., 2009). While most researchers agree that high-protein diets can help both treat DM and obesity in cats (Mazzaferro et al., 2003, Bennett et al., 2006, Frank et al., 2001, Hall et al., 2009, Laflamme and Hannah, 2005), there is still limited evidence regarding whether this type of diet would also be advantageous to healthy cats.

Diabetic cats with increased serum cholesterol concentrations are 65% less likely to achieve diabetic remission than cats with normal serum cholesterol concentrations. This suggests that hypercholesterolaemia plays a primary role in the progression of diabetes in cats, eventually preventing the recovery of ß-cell function (Zini et al., 2010). This theory is supported by several studies in rodents which have shown that elevated cholesterol concentrations can impair ß-cell function (Hao et al., 2007, Ishikawa et al., 2008). Recent literature has also reported increased cholesterol concentrations in lean, obese and diabetic cats on the traditional high-protein diet prescribed to diabetic cats (Zapata et al., 2017).

The concept that a traditional high-protein and low-carbohydrate diet increases cholesterol concentrations among healthy cats has been investigated during recent years, but requires further investigation for eventual verification. This mini-dissertation focusses on several questions relating to the influence of dietary constituents on serum lipid and fructosamine concentrations in healthy cats; more specifically, will high-protein and high-carbohydrate diets show differential response on serum cholesterol, triglyceride and fructosamine concentrations?, and will a cat's serum lipid and hormone profile be correlated with any of the study variables and/or diets?

In summary, if one of the diets has a significant influence on the hormone or lipid profile it will broaden our understanding of dietary selection in the domestic cat and possibly elucidate dietary strategies for reducing the risk of DM in this species.

Chapter 2: Literature review

2.1 Nutrition of the healthy cat

As obligate carnivores, cats require higher protein levels in their diet compared with other mammals. In cats, the minimum requirement for protein is 16% of metabolisable energy (ME) (Council, 2006). The aims of dietary therapy in healthy cats should be directed towards the prevention of obesity in young cats and promoting weight loss in older obese cats (Zoran and Rand, 2013). Dietary protein is important in weight loss diets (Laflamme, 2012), as high-protein diets have been shown to promote fat loss in healthy cats (Laflamme and Hannah, 2005). Among overweight cats, increasing dietary protein from 35 to 45% of metabolizable energy resulted in more than 10% fat loss (Laflamme and Hannah, 2005). This may be attributable to the thermogenic effect of dietary protein (Hoenig et al., 2007a), i.e., increased protein turnover and synthesis (Laflamme, 2012). For example, Vasconcellos et al. (2009) reported that cats fed a high-protein diet achieved the same rate of weight loss as cats fed traditional diets despite exhibiting almost 10% greater calorie intake (Vasconcellos et al., 2009). In obese cats under a weight loss regime, the consumption of a high-protein, low-fat diet significantly reduced markers of oxidative stress (inflammatory cytokines, c-reactive protein and interleukin-6) when compared to levels in cats fed a low protein diet (Tanner et al., 2006). However, providing excess energy through *ad libitum* feeding of neutered and inactive cats should be discouraged (Lund et al., 2006, Rand and Marshall, 2005, Nguyen et al., 2004). Furthermore, the *ad libitum* consumption of a high-protein diet does not effectively promote weight loss in obese cats (Wei et al., 2011), yet calorie restriction coupled with a high-protein diet may support weight loss through the maintenance of lean body mass(des Courtis et al., 2015). Therefore, feeding strategies should focus on including enough protein to prevent muscle loss while reducing food energy content and adjusting energy intake to promote weight loss (Zoran and Rand, 2013). Cats can efficiently digest carbohydrates (Thiess et al., 2004), with a high-carbohydrate diet previously postulated to be an indirect risk factor for the development of DM (Hoenig et al., 2007a). This type of diet has been suggested to result in a higher postprandial insulin response, which can potentially lead to overstimulation of the pancreatic ß-cells and, following ß-cell exhaustion, to DM (Miller and Colagiuri, 1994). However, in healthy cats, insulin production will not be impaired by the consumption of a large amount of carbohydrates, and thus, the increased demand for insulin production can be met (Slingerland et al., 2009). Recent literature

has not shown the consumption of carbohydrates to exert any adverse effects on young and mature cats (Backus et al., 2010). Interestingly, it has been reported that cats limit their total energy intake when consuming a high-carbohydrate diet (Farrow et al., 2013), with some studies suggesting that they gain more weight on a high-protein diet than a high-carbohydrate diet (Coradini et al., 2011). This finding, i.e., a high-protein diet is linked with higher energy consumption than a high-carbohydrate diet, may highlight why there is no clear evidence of how dietary composition influences a cat's predisposition to DM. For example, the long-term consumption of a high-protein and high-fat diet may lead to obesity and, as such, counters the initial beneficial effects of lower glucose concentrations associated with these diets (Farrow et al., 2013). These findings do not directly support the theory that a high-carbohydrate diet promotes insulin resistance and the development of feline DM (Hoenig et al., 2007b, Slingerland et al., 2009). Thus, it would seem that obesity, rather than dietary composition, leads to reduced insulin sensitivity and insulin resistance (Hoenig et al., 2007b).

There are conflicting reports about how carbohydrates and fats influence the glycaemic response in healthy cats. When compared to high-carbohydrate diets, high-fat diets have been shown to result in diminished glucose clearance and ß cell function(Thiess et al., 2004, Farrow et al., 2013, Mimura et al., 2013). There is conflicting evidence about how fibre content influences glycaemic control in cats. More specifically, glycaemic control was shown to improve when fibre intake among DM cats was increased to moderate levels (Nelson et al., 2000), while other studies reported the opposite (Bennett et al., 2006). In cats, post-prandial gluconeogenesis is usually driven by absorbed amino acids, which may explain why fibre is not as effective at mediating glycaemic control.

Most of the research that has assessed how diet composition influences the feline glycaemic response has focussed on diabetic cats (Nelson et al., 2000, Bennett et al., 2006, Mazzaferro et al., 2003, Hall et al., 2009), with only a few studies also including healthy cats (Mori et al., 2009, Hoenig et al., 2007b, Farrow et al., 2013, Thiess et al., 2004). Furthermore, these investigations differ widely in study design, feeding protocol, population size as well as diet composition, which makes comparisons between studies difficult.

2.2 Nutrition of the diabetic cat

The cat is a true carnivore and, as such, is adapted to a diet high in protein and low in carbohydrates (Morris, 2002). While older research postulated that high-carbohydrate diets increase the risk for obesity in cats (Scarlett et al., 1994), more recent literature has provided contrasting findings (Cave et al., 2012). Obesity in cats is associated with an increased risk for developing diabetes mellitus (DM) (Scarlett et al., 1994). 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 (Zoran and Rand, 2013). Normalizing glucose concentrations will allow the ß-cells to recover from glucose toxicity (Zoran and Rand, 2013). The aim of nutritional therapy should always be achieving diabetic remission, and the current evidence suggests that a high-protein, low-carbohydrate diet can benefit cats with DM when compared to a high-carbohydrate diet (Mazzaferro et al., 2003, Bennett et al., 2006, Frank et al., 2001, Hall et al., 2009). In addition, the current recommendations state that diabetic cats should receive longer-acting insulin preparations, injected twice daily, for optimal diabetic control (Sparkes et al., 2015). Furthermore, clinical signs, blood glucose measurements and fructosamine concentrations can be used to monitor glycaemic control and response to therapy (Crenshaw et al., 1996, Link and Rand, 2008, Plier et al., 1998, Kaneko et al., 1992, Reusch et al., 1993, Lutz et al., 1995, Martin and Rand, 2007). While most researchers agree that high-protein diets can help both treat DM and obesity in cats, (Mazzaferro et al., 2003, Bennett et al., 2006, Frank et al., 2001, Hall et al., 2009, Laflamme and Hannah, 2005), there is still limited evidence as to whether this type of diet would be advantageous to healthy cats.

Cats with DM require high-quality protein in their diet. A high-protein diet may benefit diabetic cats in terms of reduced demand for insulin (Slingerland et al., 2009). It has recently been reported that diabetic animals can lose amino acids in the urine because of abnormal hormonal signals and glomerulonephropathies (Verbrugghe and Hesta, 2017). Inadequate insulin concentrations can lead to a loss of lean body mass, which can be explained by poor cellular amino acid uptake and increased protein metabolism to drive hepatic gluconeogenesis (Kirk, 2006). Thus, a diet which includes limited carbohydrates needs to be supplemented with high levels of proteins to maintain adequate hepatic gluconeogenesis and glucose production (Kirk, 2006).

Dietary carbohydrates have been postulated to increase the risk of DM in cats (Buffington, 2008, Rand et al., 2004). This is based on the theory that cats have a limited ability to process large glucose loads (Verbrugghe and Hesta, 2017). It is thought that the excess consumption of carbohydrates places a large demand on pancreatic ß-cells for increased insulin secretion. This high demand for insulin secretion can lead to ß-cell failure, especially in situations of insulin resistance (Porte, 1991, Miller and Colagiuri, 1994). When the amount of dietary carbohydrates is reduced, blood glucose can be maintained solely by hepatic gluconeogenesis (Kirk, 2006). The shift in substrate use, from carbohydrates to fats and protein, has been called the "metabolic shift". In other words, it has been suggested that diets that are low in carbohydrates but high in protein and fat result in a shift from glucose oxidation to fat metabolism (Atkins, 2004, Kirk, 2006).

2.3 Influence of diet on lipids

Lipids are large, heterogeneous, fatty and related compounds that are insoluble in water (Ganong, 2016). Lipoproteins, are essential components of cell membranes and are important in the stabilization and transport of lipids in plasma(Ganong, 2016). Lipoproteins are classified by size, density, electrophoresis, and apoprotein content(Ganong, 2016). The four classes of lipoproteins are chylomicrons, very-low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs)(Ganong, 2016). Triglycerides (glycerol and fatty acids) are transported as chylomicrons and VLDLs. Cholesterol is transported as LDLs and HDLs(Ganong, 2016). The serum HDL cholesterol concentrations are higher in cats than in many other species and the cat is often referred to as the "HDL animal" because it possesses higher serum HDL cholesterol concentrations than serum LDL cholesterol concentrations(Ginzinger et al., 1999).

In terms of DM, cats share certain similarities with humans. For instance, as in humans, obesity in cats is associated with substantial changes in lipid metabolism, i.e., significant increases in serum cholesterol and triglyceride concentrations (Szabo et al., 2000, Hoenig et al., 2003). However, unlike diabetic humans, obese cats seems to be protected from atherosclerosis; more specifically, obese cats show higher levels of high-density lipoproteins (HDLs) than lean cats (Hoenig et al., 2003).

Several studies have found that a high-protein and low-carbohydrate diet does not significantly affect serum triglyceride concentrations in cats (Zapata et al., 2017, Wei et al., 2011, Mimura et al., 2013). Importantly, in the Zapata et al. (2017) study, cats that were fed the control diet for the same duration of the high-protein diet were not included in the study, which may have biased the results. Comparatively, diets high in fat have been reported to significantly increase triglyceride concentrations (Trevizan et al., 2010, Thiess et al., 2004, Keller et al., 2017). Moreover, Nelson et al. (2000) found high-fibre foods to lower triglyceride concentrations in diabetic cats.

Previous studies of whether high-fat diets influence cholesterol concentrations in cats have produced contradictory results. For example, some researchers have reported that high-fat diets do not contribute to hypercholesterolaemia in cats (Butterwick et al., 2012, Jordan et al., 2008, Trevizan et al., 2010), while other groups have provided evidence for how high-fat diets contribute to hypercholesterolaemia in healthy cats (Thiess et al., 2004). Additionally, high-fibre diets increase cholesterol concentrations in healthy cats (Fischer et al., 2012). This is relevant because diabetic cats with increased serum cholesterol concentrations are 65% less likely to achieve diabetic remission than cats with normal serum cholesterol concentrations (Zini et al., 2010). Hence, hypercholesterolaemia has been theorised to play a primary role in the progression of diabetes in cats, possibly through preventing the recovery of ß-cell function (Zini et al., 2010). This theory is supported by several studies in mice, with the results showing that elevated cholesterol concentrations can impair ß-cell function (Hao et al., 2007, Ishikawa et al., 2008). Additionally, mice with hypercholesterolaemia showed an over 50% decrease in the number of pancreatic islets (Ishikawa et al., 2008). Moreover, increased cholesterol concentrations have been reported in lean, overweight and diabetic cats on the traditional high-protein diet prescribed to diabetic cats (Zapata et al., 2017).

Chapter 3: Methodology

3.1 Objective of the research covered in this mini-dissertation.

To compare the effects of either a high-protein or a high carbohydrate diet on serum cholesterol, triglyceride, and fructosamine concentrations in healthy cats.

3.2 Hypotheses

Null hypothesis: Populations of cats fed either a high-protein or high-carbohydrate diet will not differ in terms of serum cholesterol, triglyceride or fructosamine concentrations.

Alternative hypothesis: Cats fed a high-protein diet will have higher serum cholesterol and triglyceride concentrations than cats fed a high-carbohydrate diet. Cats fed a high-protein diet will have lower serum fructosamine concentrations than cats fed a high-carbohydrate diet.

3.3 Benefits

a) The results may elucidate whether a high-protein or high-carbohydrate diet cause changes in lipid and/or fructosamine concentrations in healthy cats.
b) This mini-dissertation serves as a partial fulfilment of the MMedVet (Med) degree of the main author, whose name appears on the title page of this dissertation.

3.4 Design of the research covered in this mini-dissertation

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) and the Faculty Research Ethics committee (REC072-18). Cats were selected from an animal shelter. The study area comprised two separate rooms (10 by 10 metres) with 10 cats in each room, with the remaining cats entering the study area after the first groups had finished the study. Upon completion of the study, all of the cats returned to their original cages

at the shelter. Environmental temperature changes were monitored(using a non-contact infrared thermometer, Electromann SA, Pretoria, South Africa) during the study. Before the study was started, all of the cats were transitioned over a period of two months to assess which cats would tolerate the entire process. Furthermore, cats were transitioned from one diet to the other over a period of seven days to reduce the stress of dietary changes. All of the cats had a local anaesthetic cream applied to their necks, 10-15 minutes before venipuncture to reduce the stress and pain of blood collection.

3.5 Population of cats used in the research

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

- Age over one year
- Not affected by renal disease (normal BUN, creatinine concentrations), liver disease (normal ALT, ALP, GGT concentrations), DM (normal blood glucose concentrations), or hyperthyroidism (normal TT4 concentrations)
- Feline immunodeficiency and leukaemia virus (FIV and FeLV) negative
- No concurrent medical treatment
- Acceptance of restraint, venipuncture, and all diets
- No history of chronic vomiting and/or diarrhoea
- Known birthdate and complete vaccination record.

Furthermore, BCS was determined according to the nine-point BCS chart (Laflamme, 1997). The cats that started on the high-carbohydrate diet had the following breed distribution: 17 domestic shorthairs (DSH); two domestic longhairs (DLH); and one Siamese cross. In comparison, the cats that started on the high-protein diet consisted of 12 DSH, four DLH, one domestic medium hair (DMH), one Siamese cross and two Persian crosses (Tables 3.1 and 3.2).

Table 3.4. Comparison of baseline demographics and serum chemistry parameters in 40 healthy cats randomised into groups based on their initial diet allocation, i.e. receiving either a high-carbohydrate (HC; n=20) or high-protein (HP; n=20) diet first.

Variable	HC diet first		HP diet first		P value‡
	n/d	PE* (Interval†)	n/d	PE* (Interval†)	
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/L0	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.

*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-tests on natural log-transformed data

Table 3.5. Comparison of baseline 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.

	HC diet first		**HP diet first**		
Variable	**n/d**	**Median (IQR)**	**n/d**	**Median (IQR)**	**P value**
Band neutrophils ($\times 10^9$/L)	16/20	0 (0, 0.05)	17/20	0 (0, 0)	0.81*
Basophils ($\times 10^9$/L)	16/20	0 (0, 0)	17/20	0 (0, 0)	0.76*
Eosinophils ($\times 10^9$/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 ($\times 10^9$/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 ($\times 10^9$/L)	16/20	0.29 (0.16, 0.60)	17/20	0.26 (0.11, 0.39)	0.38†
Neutrophils ($\times 10^9$/L)	16/20	5.97 (4.29, 9.25)	17/20	6.25 (5.51, 7.95)	0.71†
Platelets ($\times 10^9$/L)	16/20	204 (151, 316)	17/20	316 (189, 523)	0.10†
RCC ($\times 10^{12}$/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 ($\times 10^9$/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.

*Based on Mann-Whitney U tests

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

‡Based on independent t-tests on untransformed data

3.6 Feeding protocol

Prior to enrolment, the participating cats were fed a commercial maintenance diet (Table 3). Following baseline health assessments, cats were randomised into groups and started on either a high-protein or high-carbohydrate diet for four weeks. All cats received the dry and wet high-protein diet and then the dry high-carbohydrate diet. After the four weeks, cats were fed a washout diet for an additional four weeks. Thereafter, they were transitioned to the cross-over diet (i.e., cats that had started with the high-carbohydrate diet were switched to the high-protein diet, and vice versa; Table 3.3 and Figure 3.1). Each cat was transitioned between diets over seven days. Following the completion of the study, the cats were transitioned back to their original diet. All of the cats in the study were fed *ad libitum*, receiving measured 750 grams each of the dry food and 468 grams of the high-protein wet food per room per day. Clinical examinations, BCS, weight and environmental temperature (non-contact infrared thermometer, Electromann SA, Pretoria, South Africa) measurements, were conducted on a weekly basis. All BCS and weights measured throughout the study were included within the statistical models. The temperature of each room was measured(around 10-11 am each week) with the device when the animals were weighed. Four separate readings were taken from each corner of the room and the mean was then recorded.

Table 3.6. Comparison of the diets used in this study (reported on a per kilocalorie basis).

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 with meaty nuggets	29.86%	28.48%	41.65%	0.89g
High-protein	Hill's M/D dry food	42.84%	40.62%	16.59%	0.9g
High-protein	Hill's M/D wet food	43.5%	42.28%	14.12%	1.7g
High-carbohydrate	Hill's Science plan, Feline mature adult 7+ sterilised cat	32.48%	22.38%	45.18%	0.3g
Washout	Hill's Science Plan, Feline Mature Adult 7+ Hairball Control)	30.70%	41.45%	27.88%	2.1g

BCS = body condition score; CBC=complete blood count; TT4=total thyroxine.

Figure 3.1. Flow chart of the research process

3.7 Health Assessment and Laboratory Tests

Cats were determined to be 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 (3ml syringe and a 14-gauge needle) 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 for the complete blood counts (CBCs). Total thyroxine (TT4) concentrations were measured with the Immulite 2000 immunoassay system (Siemens Healthineers). Fructosamine concentration was determined using a colorimetric method on the Cobas Integra

400 plus analyser. A SNAP Combo plus (Idexx Laboratories, Johannesburg, South Africa) enzyme-linked immunosorbent assay was used for the simultaneous detection of FeLV antigen and FIV antibodies. All of the biochemical assays, complete blood counts, and TT4 were run at the Clinical Pathology Laboratory at the Faculty of Veterinary Science, University of Pretoria, whereas fructosamine concentrations were measured at a commercial laboratory (Idexx Laboratories). Blood samples were collected from the cats after the four-week high-protein, high-carbohydrate and washout diets. Blood samples were centrifuged and serum separated and refrigerated (at 4°C) overnight (within 1 hour of collection). Thereafter, serum were aliquoted and frozen at -80^{0}C within 24 hours of collection. At the end of the study, all of the assays (excluding the CBC's) were performed as a single batch.

3.8 Data management

All laboratory data was entered into a spreadsheet program (Microsoft Excel®, Microsoft Corporation, Redmond, WA, USA).

3.9 Statistical analyses

The sample size was estimated using the main study variable of triglyceride concentrations with a single sample t-test, which was chosen because of the randomised crossover design. Based on previously reported results, it was assumed that obese cats would have a mean difference of 1.74 (+/- 2.19 mmol/L SD) for triglycerides when on a high-protein versus a high-carbohydrate diet (Mimura et al., 2013). The desired power was set as 90% with a two-sided significance level of 5%. The calculated sample size was 19 obese cats, which was increased by 1 to account for potential animal exclusion. As independent comparisons for lean cats were desired, the overall sample size was doubled to 40 cats in the absence of specific data. It was further assumed that the source population would include relatively equal numbers of obese and lean cats. The 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). Differences in the quantitative data for baseline diet assignment groups were evaluated for significance using independent t-tests on the raw or natural logarithm transformed data. Mann-Whitney U tests were used when the normality assumption were violated. The correlation between quantitative variables was estimated using Spearman's rho. 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 were significant based on the significance of slope parameters. A similar mixed-effects model was fit to evaluate the effect of diet and other predictors on body weight. A variance components analysis was performed to partition the variance in each of the three serological outcomes based on diet and cat demographics. 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 $p < 0.05$.

Chapter 4: Results

4.1. Baseline Data

There were no significant differences in the baseline data between the two initial diet groups (Tables 3.1 and 3.2).

4.2. 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 found to be significantly associated with heavier body weights (Table 4.1).

Table 4.8. Multivariable associations between body weight*, diet, and body condition score (BCS) in 35 healthy cats.

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.

BCS<5: n=13 on baseline diet, n=12 on HC diet, n=10 on HP diet, n=10 on washout diet.

BCS=5: n=9 on baseline diet, n=13 on HC diet, n=11 on HP diet, n=14 on washout diet.

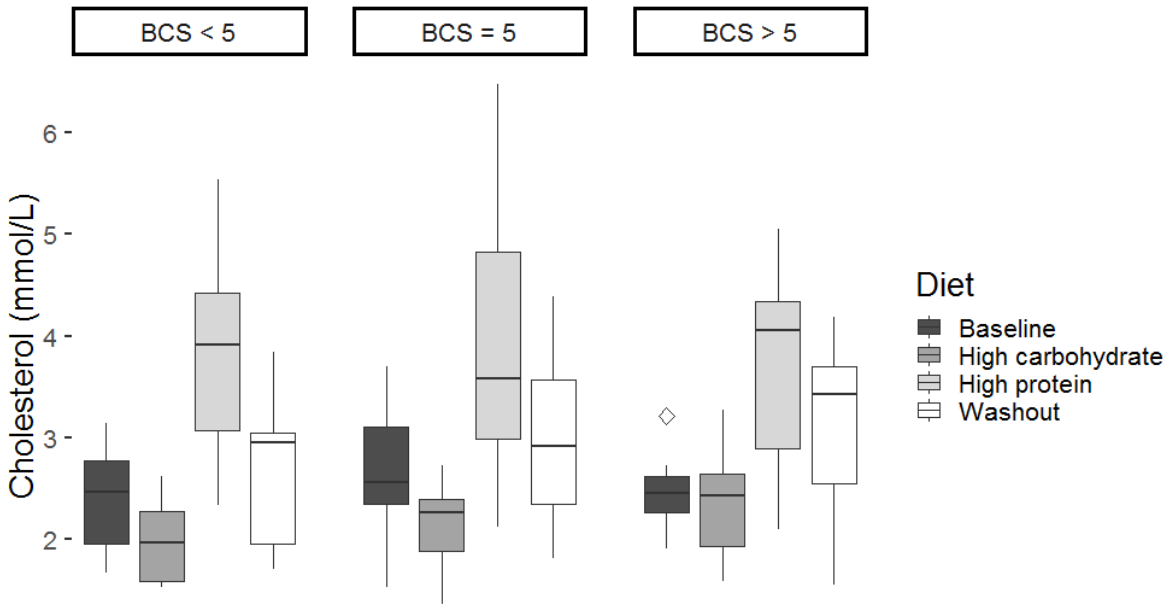
BCS>5: n=13 on baseline diet, n=10 HC diet, n=14 on HP diet, n=11 on washout diet.

*Data were natural log-transformed prior to statistical analysis

4.3. Cholesterol

The high-protein diet resulted in the highest median cholesterol concentrations among the tested cats (Figure 4.1). Cholesterol concentrations were above the reference range (1.8-4.1mmol/L) 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 remaining 90% of the samples (130/145) were either within or just below the reference range. Cats on the high-carbohydrate diet showed significantly lower (P<0.001) cholesterol concentrations than cats on either the high-protein (P<0.001) or washout diet (P<0.001; Table 4.2). Moreover, cats with a BCS >5 that were fed the high-protein diet showed significantly lower (P=0.007) cholesterol concentrations than cats(on the high-protein diet) from other BCS groups. Additionally, 39% of the variability observed in the cholesterol concentrations was due to the diet, 34% of the variability stemmed from variation between individuals, 13% was due to sex, and the remaining 14% of the variability could not be attributed to the evaluated factors (i.e., residual errors). BCS did not explain any of the variability in cholesterol concentrations.

Figure 4.3. Illustration of serum cholesterol values for 35 healthy cats – separated according to body condition score (BCS).



BCS<5: n=13 on baseline diet, n=12 on HC diet, n=10 on HP diet, n=10 on washout diet.

BCS=5: n=9 on baseline diet, n=13 on HC diet, n=11 on HP diet, n=14 on washout diet.

BCS>5: n=13 on baseline diet, n=10 HC diet, n=14 on HP diet, n=11 on washout diet.

Table 4.9. Multivariable associations between serum cholesterol*, diet, and body condition score (BCS) in 35 healthy cats.

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.

BCS<5: n=13 on baseline diet, n=12 on HC diet, n=10 on HP diet, n=10 on washout diet.

BCS=5: n=9 on baseline diet, n=13 on HC diet, n=11 on HP diet, n=14 on washout diet.

BCS>5: n=13 on baseline diet, n=10 HC diet, n=14 on HP diet, n=11 on washout diet.

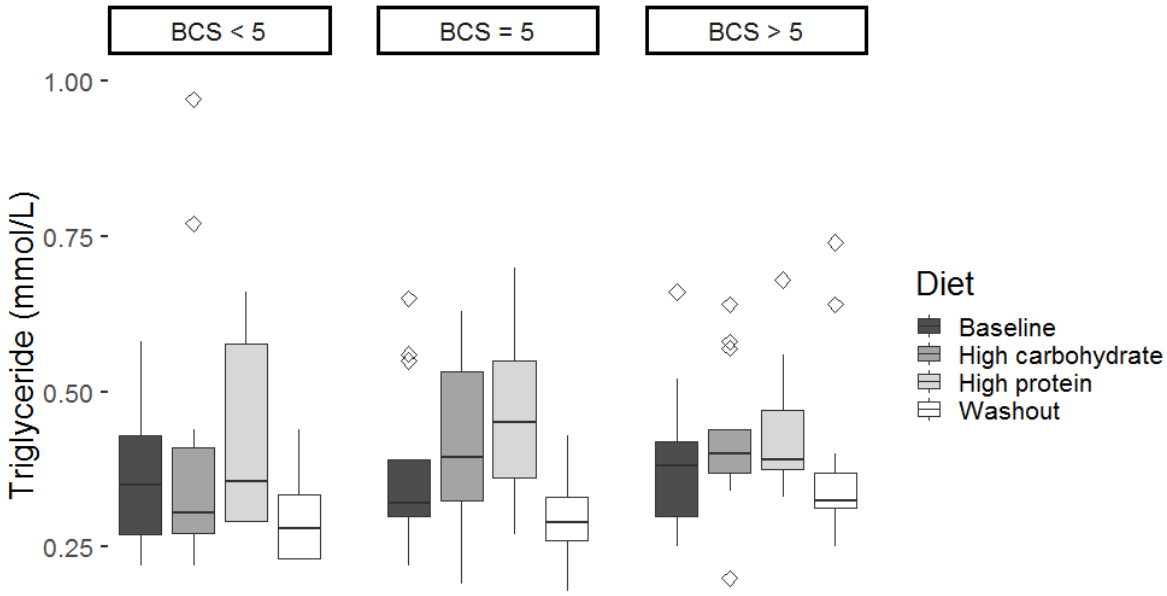
*Data were natural log-transformed prior to statistical analysis

4.4. Triglycerides

The washout diet resulted in the lowest median triglyceride concentrations(P=0.009) in the tested cats (Figure 4.2), whereas cats fed the high-protein diet showed significantly higher (P<0.001; Table 4.3) triglyceride

concentrations than other cats. None of the samples demonstrated triglyceride concentrations above the reference range(0.6-1.4mmol/L). Cats with a BCS >5 that were fed the high-protein diet showed significantly (P=0.03) lower triglyceride concentrations than those from other BCS groups fed the HP diet. Furthermore, diet explained 13% of the variability observed for triglyceride concentrations, while 48% of this variability was explained by variation between individuals. Only 2% of the observed variation was explained by sex, with the remaining 37% classified as residual errors.

Figure 4.4. Illustration of serum triglyceride values for 35 healthy cats – separated according to body condition score (BCS).



BCS<5: n=13 on baseline diet, n=12 on HC diet, n=10 on HP diet, n=10 on washout diet.

BCS=5: n=9 on baseline diet, n=13 on HC diet, n=11 on HP diet, n=14 on washout diet.

BCS>5: n=13 on baseline diet, n=10 HC diet, n=14 on HP diet, n=11 on washout diet.

Table 4.10. Multivariable associations between serum triglycerides*, diet, and body condition score (BCS) in 35 healthy cats.

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.

BCS<5: n=13 on baseline diet, n=12 on HC diet, n=10 on HP diet, n=10 on washout diet.

BCS=5: n=9 on baseline diet, n=13 on HC diet, n=11 on HP diet, n=14 on washout diet.

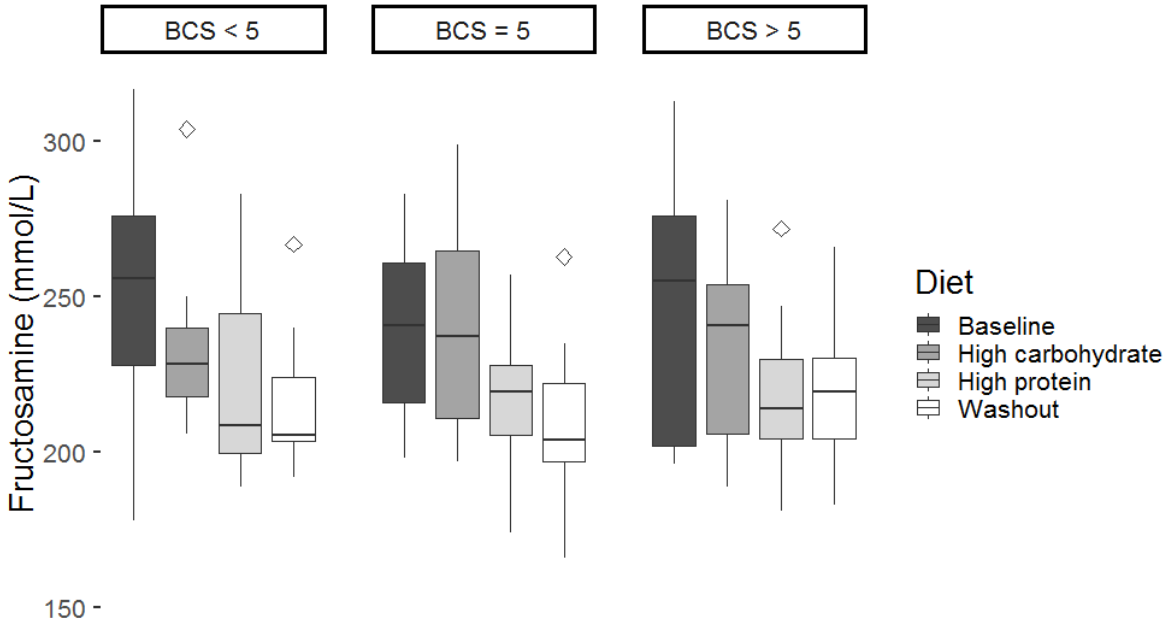
BCS>5: n=13 on baseline diet, n=10 HC diet, n=14 on HP diet, n=11 on washout diet.

*Data were natural log-transformed prior to statistical analysis

4.5. Fructosamine

The high-carbohydrate diet resulted in the highest median fructosamine concentrations in the tested cats(229umol/L in cats with a BCS<5, 238umol/L in cats with BCS=5 and 241umol/L in cats with a BCS>5) (Figure 4.3). Only 6% (9/143) of the fructosamine samples were below the reference range(230-350umol/L); of these, four represented the high-protein diet, four represented the washout diet, and one represented the baseline diet. The remaining 94% (134/143) of the samples were within the reference range. Cats on either the high-protein or washout diet showed significantly lower (P<0.001) fructosamine concentrations than other cats (Table 4.4). Additionally, BCS was not correlated with fructosamine concentrations(Table 4.5). Furthermore, 16% of the variability observed for fructosamine concentrations was a result of diet, while variation between individuals accounted for 47% of the variability. The observed variability in fructosamine concentrations could not be attributed to sex, with the remaining variability (37%) classified as residual errors.

Figure 4.5. Illustration of serum fructosamine values for 35 healthy cats – separated by body condition score.



BCS<5: n=13 on baseline diet, n=12 on HC diet, n=10 on HP diet, n=10 on washout diet.

BCS=5: n=9 on baseline diet, n=13 on HC diet, n=11 on HP diet, n=14 on washout diet.

BCS>5: n=13 on baseline diet, n=10 HC diet, n=14 on HP diet, n=11 on washout diet.

Table 4.11. Associations between serum fructosamine* and diet in 35 healthy cats.

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.

BCS<5: n=13 on baseline diet, n=12 on HC diet, n=10 on HP diet, n=10 on washout diet.

BCS=5: n=9 on baseline diet, n=13 on HC diet, n=11 on HP diet, n=14 on washout diet.

BCS>5: n=13 on baseline diet, n=10 HC diet, n=14 on HP diet, n=11 on washout diet.

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

4.6. Comparison between variables

Overall, weak to moderate correlations were found between the various variables. Cholesterol and triglycerides concentrations were positively correlated overall (ρ=0.362; P<0.001; Table 4.5), whereas cholesterol and fructosamine concentrations were negatively correlated (ρ=-0.185; P=0.02) in all cats. Room temperature was negatively (ρ=-0.301;P<0.001) and positively (ρ=0.2; P=0.01) correlated with cholesterol and fructosamine concentrations, respectively (Table 4.5). Fructosamine was negatively correlated with body weight in cats with a BCS>5 (ρ=-0.342; P=0.01;Table 4.6). Triglyceride concentrations were positively correlated (ρ=0.221; P=0.007) with body weight in all cats.

Table 4.12. Correlations between serum cholesterol, fructosamine, and triglyceride values and quantitative data for 35 healthy cats.

Variable 1	**Variable 2**	**N**	**Spearman's rho (ρ)**	**P value**
Cholesterol	Fructosamine	143	-0.185	0.02
	Triglyceride	145	0.362	<0.001
	Age	145	0.148	0.07
	Weight	145	0.066	0.42
	Body condition score	145	0.154	0.06
	Room temperature	145	-0.301	<0.001
Fructosamine	Triglyceride	143	0.090	0.28
	Age	143	0.134	0.11
	Weight	143	-0.043	0.60
	Body condition score	143	-0.036	0.67

	Room temperature	143	0.200	0.01
Triglyceride	Age	145	0.140	0.09
	Weight	145	0.221	0.007
	Body condition score	145	0.254	0.002
	Room temperature	145	-0.054	0.51

Table 4.13. Correlations between age, weight, serum cholesterol, fructosamine, and triglyceride values stratified by body condition score (BCS) in 35 healthy cats.

Cat BCS	**Variable 1**	**Variable 2**	**n**	**Spearman's rho**	**P value**
BCS < 5	Cholesterol	Fructosamine	45	-0.281	0.06
		Triglyceride	45	0.408	0.005
		Age	45	-0.115	0.45
		Weight	45	-0.125	0.41
	Fructosamine	Triglyceride	45	0.064	0.67
		Age	45	0.047	0.76
		Weight	45	0.014	0.92
	Triglyceride	Age	45	-0.243	0.10
		Weight	45	0.117	0.44
BCS = 5	Cholesterol	Fructosamine	50	0.025	0.86
		Triglyceride	50	0.363	0.009
		Age	50	0.259	0.06

		Weight	50	-0.075	0.60
	Fructosamine	Triglyceride	50	0.281	0.04
		Age	50	0.203	0.15
		Weight	50	0.223	0.11
	Triglyceride	Age	50	0.300	0.03
		Weight	50	-0.057	0.69
BCS > 5	Cholesterol	Fructosamine	48	-0.282	0.05
		Triglyceride	50	0.248	0.08
		Age	50	0.182	0.20
		Weight	50	0.112	0.43
	Fructosamine	Triglyceride	48	-0.073	0.62
		Age	48	0.161	0.27
		Weight	48	-0.342	0.01
	Triglyceride	Age	50	0.194	0.17
		Weight	50	0.480	<0.001

4.7. Comparison between diets

Overall, weak to moderate correlations were observed between the tested variables. In all cats, dietary protein was positively correlated with cholesterol (ρ=0.295; P<0.001) and triglyceride (ρ=0.226; P=0.006) concentrations, but negatively correlated with fructosamine concentrations (ρ=-0.202; P=0.01; Table 4.7). Moreover, dietary fat content was positively correlated with cholesterol levels (ρ=0.449; P<0.001) and negatively correlated with fructosamine (ρ=-0.315; P=0.001) and triglyceride concentrations (ρ=-0.166;

P=0.04). Analyses that included all of the studied cats showed carbohydrate concentrations to be negatively correlated with cholesterol levels (ρ=-0.592; P<0.001) and positively correlated with fructosamine concentrations (ρ=0.281; P=0.001). Crude fibre content was positively correlated with cholesterol concentrations (ρ=0.449; P<0.001).

Table 4.14. Correlation between different diet components and serum cholesterol, fructosamine, and triglyceride values, stratified by cat body condition score (BCS), in 35 healthy cats.

		Cholesterol	**Fructosamine**	**Triglyceride**
Cat BCS	**Diet component**	**Spearman's rho (P value)**	**Spearman's rho (P value)**	**Spearman's rho (P value)**
All cats	Protein (% kcal)	0.295 (<0.001)	-0.202 (0.016)	0.226 (0.006)
	Fat (% kcal)	0.449 (<0.001)	-0.315 (0.001)	-0.166 (0.046)
	Carbohydrate (% kcal)	-0.592 (<0.001)	0.281 (0.001)	-0.032 (0.703)
	Crude fibre (g per 100 kcal ME)	0.449 (<0.001)	-0.315 (<0.001)	-0.166 (0.046)
BCS < 5	Protein (% kcal)	0.286 (0.057)	-0.266 (0.078)	0.151 (0.323)
	Fat (% kcal)	0.491 (0.001)	-0.312 (0.037)	-0.084 (0.594)
	Carbohydrate (% kcal)	-0.651 (<0.001)	0.286 (0.057)	-0.092 (0.547)
	Crude fibre (g per 100 kcal ME)	0.491 (0.001)	-0.312 (0.037)	-0.084 (0.584)
BCS = 5	Protein (% kcal)	0.299 (0.035)	-0.211 (0.142)	0.282 (0.048)
	Fat (% kcal)	0.453 (0.001)	-0.376 (0.007)	-0.234 (0.102)

	Carbohydrate (% kcal)	-0.600 (<0.001)	0.307 (0.030)	-0.039 (0.790)
	Crude fibre (g per 100 kcal ME)	0.453 (0.001)	-0.376 (0.007)	-0.234 (0.102)
BCS > 5	Protein (% kcal)	0.292 (0.040)	-0.146 (0.321)	0.273 (0.055)
	Fat (% kcal)	0.414 (0.003)	-0.265 (0.068)	-0.266 (0.061)
	Carbohydrate (% kcal)	-0.535 (<0.001)	0.258 (0.077)	0.069 (0.632)
	Crude fibre (g per 100 kcal ME)	0.414 (0.003)	-0.265 (0.068)	-0.266 (0.061)

ME = metabolisable energy.

Chapter 5: Discussion

5.1 General discussion

The results presented in this study showed that cats on a high-carbohydrate diet had significantly lower serum cholesterol concentrations than cats on a maintenance diet, while a high-protein diet was found to significantly increase serum cholesterol and triglyceride concentrations, but decrease fructosamine concentrations, relative to baseline measurements. In contrast, overweight cats (BCS>5) on a high-protein diet had lower cholesterol and triglyceride concentrations than cats representing other BCS groups.

It has been shown that neutered male cats are at an increased risk of obesity relative to intact males, and therefore at greater risk for developing DM (Hoenig et al., 2007a, Hoenig et al., 2007b, Lund et al., 2005). In this current study, comprising both neutered male and female cats, the neutered males were significantly heavier than neutered female cats, which could result from sexual dimorphism. It has been suggested that the availability of energy-dense, highly palatable dry foods at least partly explains the recent increase in feline obesity; however, there are few epidemiological studies available that either support or refute this claim (Lund et al., 2005). In this 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 (Farrow et al., 2013, Hewson-Hughes et al., 2011). Dietary protein is an important component of weight loss diets (Laflamme, 2012), because high-protein diets have been shown to promote fat loss in cats (Laflamme and Hannah, 2005). However, offering overweight cats an *ad libitum* high-protein diet has been shown to increase food intake – perhaps due to increased palatability – without any noticeable changes in body weight or composition (Wei et al., 2011). This study, which used *ad libitum* feeding showed that cats fed a high-protein diet gained weight more in comparison to cats on the other diets.

In DM cats, hypercholesterolaemia has been postulated to reduce the chance of remission by almost 65%. However, causality between hypercholesterolaemia and diabetic remission has not been conclusively demonstrated (Zini et al., 2010), Although hypercholesterolaemia may contribute to the pathogenesis of DM in cats (Zini et al., 2010), its effects on healthy cats are still debateable. The concept that a traditional high-protein and low-carbohydrate diet can increase cholesterol concentrations in healthy cats is a relatively new

idea. Prior research has shown that diabetic, lean and overweight cats fed a high-protein diet show heightened cholesterol concentrations (Zapata et al., 2017), which is similar to the results of this study. However, the Zapata et al. (2017) study, cats that were fed the control diet for the same duration of the high-protein diet were not included in the study. In addition, diet explained the largest share of the variability observed in cholesterol concentrations. The high-protein diet resulted in elevated median cholesterol concentrations among all three BCS groups, even though cholesterol concentrations exceeded the reference range in only 10% of samples. Interestingly, overweight cats on the high-protein diet did not show a large increase in cholesterol concentrations. Hence, the mechanism through which ingested protein is coupled to upregulated cholesterol production requires further investigation.

Dietary fibre and fat were both positively correlated with cholesterol concentrations among all of the studied cats. It should be noted that both the washout and high-protein diets had considerably higher crude insoluble fibre content than the high-carbohydrate diet. Previous research has indicated that insoluble fibre is positively associated with cholesterol concentrations in overweight cats (Fischer et al., 2012). It has been speculated that fibre may interfere with the absorption of specific fat components that could alter which lipoproteins are synthesised in the liver (Fischer et al., 2012). The high-protein and washout diets had similar fat content - nearly double the amount that was in the high-carbohydrate diet. It has been reported that a high-fat diet does not contribute to hypercholesterolaemia (Butterwick et al., 2012, Jordan et al., 2008, Trevizan et al., 2010). Nevertheless, it has previously been reported that cats fed a high-fat diet show higher cholesterol concentrations than cats fed a high-carbohydrate diet (Thiess et al., 2004). These discrepancies may be explained by differences in study design, sample size, feeding regimen, and actual fat content. This current study showed that a high-carbohydrate diet with a low fibre, protein and fat content, was negatively correlated with cholesterol concentrations. As interactions between different dietary components may have exerted an additive role in these finding, future studies are required to specifically address lipoprotein fractions.

There are conflicting reports of how diet influences triglyceride concentration in cats. Several studies have shown that a high-protein and low-carbohydrate diet did not significantly affect triglyceride concentrations

(Zapata et al., 2017, Wei et al., 2011, Mimura et al., 2013); whereas others have shown a high-fat diet significantly increased triglyceride concentrations (Trevizan et al., 2010, Thiess et al., 2004, Keller et al., 2017). This current study showed that a high-protein diet increased triglyceride concentrations in healthy cats. The discrepancies between these findings and previous reports may be ascribed to differences in study design. Furthermore, overweight cats generally have higher triglyceride concentrations than healthy cats (Hoenig et al., 2003). This study also showed that overweight cats fed a high-carbohydrate diet showed the highest median triglyceride concentrations. While none of the samples showed triglyceride concentrations exceeded the reference range, the washout diet resulted in significantly lower triglyceride concentrations than the other diets. Additional findings in this current study was a negative relationship between crude fibre content and triglyceride concentrations, which is in agreement with previous results that high-fibre foods lower triglyceride concentrations in diabetic cats (Nelson et al., 2000); and a high-protein diet resulted in decreased triglyceride concentrations in overweight cats.

Diabetic cats can benefit from high-protein and low-carbohydrate diets in terms of higher diabetic remission rates (Bennett et al., 2006), lower fructosamine concentrations (Hall et al., 2009), and improved glycaemic control (Hall et al., 2009, Mazzaferro et al., 2003). The majority of studies on the effect of diet composition on feline glycaemic response have focussed on diabetic cats (Nelson et al., 2000, Bennett et al., 2006, Mazzaferro et al., 2003, Hall et al., 2009, Frank et al., 2001), with only a few looking at the response in healthy cats (Mori et al., 2009, Hoenig et al., 2007b, Farrow et al., 2013, Thiess et al., 2004). This current study showed that, although 94% of fructosamine results were within reference range, cats on the high-carbohydrate diet tended to have the highest median fructosamine concentrations. Additionally, the fat and carbohydrate contents of a diet were negatively and positively correlated, respectively, with fructosamine concentrations among all cats. In the literature, there are conflicting reports on the effect of carbohydrates and fats on the glycaemic response in healthy cats. One study showed that cats on a high-fat diet had a diminished glucose clearance and ß-cell function relative to cats on a high-carbohydrate diet (Thiess et al., 2004). There is also evidence that high-carbohydrate diets result in higher insulin (Keller et al., 2017) and post-prandial glucose concentrations (Farrow et al., 2013, Mimura et al., 2013) than high-fat and high-protein diets in healthy cats. There seems to be a complex link between diet and fructosamine concentrations among healthy cats; more specifically, the

research covered in this mini-dissertation revealed that carbohydrate and fat contents were positively and negatively, respectively, linked with fructosamine concentrations in healthy cats.

This current study showed that cats on either high-protein or a washout diet had significantly lower fructosamine concentrations than the cats on other diets. Protein and crude fibre contents were also negatively correlated with fructosamine concentrations. These findings support the finding that healthy cats fed high-protein diets with either low or moderate levels of starch had showed significantly lower glucose and fructosamine concentrations than cats on moderate-protein and high-starch diets (Musco et al., 2017). There is conflicting evidence regarding the influence of fibre on glycaemic control in cats with some studies showing that increasing fibre intake to moderate levels improves glycaemic control among DM cats (Nelson et al., 2000); whereas opposing results are reported in other studies (Bennett et al., 2006). Cats can rely on ingested protein to drive post-prandial gluconeogenesis, which may explain why fibre has a potentially smaller effect on glycaemic control than protein (Eisert, 2011, Colagiuri and Brand Miller, 2002). Even though the exact mechanisms underlying these findings remain unknown, it is speculated that dietary fibre affects the nutrient transit rate in the gut, which, subsequently, reduces glucose absorption along with post-prandial glycaemia and enhances glycaemic control (Nelson et al., 2000, Costacou and Mayer-Davis, 2003). Results from this current study are suggestive that diets high in protein, fibre, and fat, but low in carbohydrates, may contribute to decreased glucose concentrations in healthy cats.

This study showed that cholesterol and triglyceride concentrations were positively correlated in lean and normal cats, but not in overweight cats, which is agreement with the findings of Hoenig et al. (2003), who theorised that this may be a positive adaptation in obese cats (Hoenig et al., 2003). It has also previously been suggested that cholesterol concentrations are linked to environmental temperature changes, as cholesterol may alter the cell membrane composition in response to hyperthermic temperatures (Cress and Gerner, 1980). According to this theory, intracellular cholesterol concentrations increase, while circulating cholesterol levels decrease, as temperature increases (Halonen et al., 2011). This study showed that room temperature was negatively and positively correlated with cholesterol and fructosamine concentrations, respectively. In addition, fructosamine concentrations were negatively correlated with body weight among overweight cats, which corroborates that overweight cats can maintain euglycaemia (despite peripheral insulin resistance) by

producing high levels of insulin (Hoenig et al., 2011, Hoenig et al., 2013). The finding that triglyceride concentrations were positively correlated with weight and BCS in overweight cats in this study seems to agree with previous research which demonstrated that overweight cats have elevated triglyceride concentrations(Jordan et al., 2008).

5.2 Limitations of this study

This study had several limitations. 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 (Russell et al., 2000), and thus the feeding strategy in this study may have inadvertently predisposed the participating cats to gain weight. Moreover, commercial available diets were used which allowed for less precise control over individual nutritional components such as fat content and fatty acid composition. The amount of food that each cat ingested was not recorded; thus, some cats may have preferred one type of food to another, which could have introduced bias. Furthermore, total cholesterol rather than the individual cholesterol fractions were measured. This may have biased the results as a particular cholesterol fraction may have been elevated from the given diets. Additionally, a hierarchical structure will naturally occur when cats are housed in groups, with dominant animals potentially eating more and submissive animals eating less. While this may have introduced bias, a two-month adaptation period – during which various characteristics of the studied cats were identified - was performed to reduce this limitation. More specifically, two cats that may have been problematic in a group setting were identified and excluded. Additionally, these cats had been living with each other for months and should thus have had established social structures. Finally, only wet food was used for the high-protein diet. Nevertheless, the wet and dry high-protein diets had very similar protein, carbohydrate and fat contents. Additionally, both types of food would traditionally be given to a diabetic cat, and we tried to replicate the decision a clinician would face in private practice.

5.3 Future implications

Future experimental studies should focus on assessing how the different classes of lipoproteins are affected by different diets. This type of research could yield insight that will help researchers better understand whether

overweight cats have a protective adaptation against atherosclerosis (Hoenig et al., 2003) and if certain lipoproteins are more influenced by diet than others. Furthermore, this mini-dissertation has presented strong evidence that high-protein diets have beneficial effects for short-term glucose control, yet long-term studies are required to determine whether these benefits are maintained over longer periods of time. Moreover, future studies should include temperature-controlled environments to elucidate how temperature influences lipid concentrations in cats.

The results presented in this mini-dissertation have also opened new avenues for future research that could potentially broaden our understanding of the optimal diet for healthy cats. The hypotheses that high-protein diets contribute to hypercholesterolaemia, hypertriglyceridaemia and lowered fructosamine concentrations deserve further attention. The next logical step would be performing long-term dietary trials in healthy cats to gather further information that can be used to identify the best way to feed the domestic cat.

Chapter 6: Conclusion

This study demonstrated that diets with high protein, fat and fibre, but low carbohydrate contents may be beneficial for short-term glucose control in healthy cats. This finding may be explained by the thermogenic effect of dietary protein (Hoenig et al., 2007b), the influence of dietary fibre on glycaemic control (Nelson et al., 2000, Costacou and Mayer-Davis, 2003), and the metabolic shift from glucose oxidation to lipolysis associated with replacing the carbohydrates in a diet with protein and fat (Kirk, 2006).

While factors such as individual variation and environmental temperature may have influenced the results, 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 concept that warrants further investigation. This finding suggests that overweight cats process high-protein diets, cholesterol and triglycerides differently from lean cats and this may represent a protective adaptation against atherosclerosis (Hoenig et al., 2003).

The finding that a high-protein diet significantly increased cholesterol and triglyceride concentrations, while a high-carbohydrate diet significantly decreased cholesterol concentrations, in healthy cats relative to cats on other diets also warrants further exploration.

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