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**Changing the metabolism of dogs (*Canis familiaris*)  
and cats (*Felis catus*) at rest and during exercise by  
manipulation of dietary macronutrients**

**Shay Rebekah Hill**

**2010**

**Changing the metabolism of dogs (*Canis familiaris*)  
and cats (*Felis catus*) at rest and during exercise by  
manipulation of dietary macronutrients**

**A thesis presented in partial fulfillment of the requirements for  
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**Shay Rebekah Hill**

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## Abstract

Worldwide, dogs are used for a vast range of activities, however, in New Zealand the most valuable and common are those that play a key role in the daily management of farms. These dogs are invaluable for farmers and therefore providing them with optimum care and nutrition is of paramount importance. However, despite their importance, there is very little information regarding the nutrition of these 'intermediate' working dogs and others such as hunt and agility dogs, although a significant amount of research exists focussing on sled dogs and greyhounds; the marathon runners and sprinters of the canine world. Today many farmers in New Zealand feed home kill meat to their dogs, often supplemented with commercial dry biscuits. The major issues associated with these diets are that home kill can be deficient in many micronutrients; while commercial dog biscuits contain high carbohydrate levels and large quantities of both of these feeds need to be consumed for the dog to meet its nutrient requirements. The ideal diet for working dogs should be highly digestible, palatable and energy dense as their stomach capacity is limited and they are often too tired to eat a large amount after exercise. Ultimately the goals of nutrition for working dogs should be to maintain health and immune function, minimise injury and optimise performance by providing a sustained energy source during their long periods of exercise.

The aims of this thesis were to evaluate the effects of dietary macronutrients on animal metabolism during exercise and at rest in cats and dogs. This was achieved by determining apparent digestibility, post-prandial glycaemic and insulinaemic responses, large intestinal carbohydrate fermentation, weight maintenance, exercise performance, immunity and fuel utilisation during exercise in working dogs (with exercise or at rest) and/or cats (at rest). Diets with macronutrient profiles giving better glycaemic control and increased satiety may be beneficial for the cat especially because the incidence of feline obesity, the most common form of malnutrition seen in cats in the western world, and diabetes mellitus are increasing worldwide. It was hypothesised that a diet high in protein and low in carbohydrate would be beneficial for dogs and cats at maintenance and working dogs; being closer to their natural diets (carnivorous) than many of the commercial diets available today (Zoran 2002; Rand and Marshall 2005; Bradshaw 2006; Kirk 2006; Backus *et al.* 2007) and perhaps offering advantages such as better glycaemic control, maintenance of weight, higher digestibility and increased performance during exercise.

An initial study was carried out in working dogs at maintenance fed either a high-carbohydrate (low-protein) or high-protein (low-carbohydrate) diet. The results of this study demonstrated the high-protein diet was more digestible, produced a slower, more sustained release of glucose thus affording better glycaemic control, and was therefore likely to be of benefit to working dogs and also to pet dogs suffering from diabetes mellitus. This diet was therefore deemed suitable to use in further trials to investigate if similar results could be obtained in cats and if measurable performance benefits could be achieved in exercising dogs fed this diet.

The trial in cats used a newly developed marginal ear vein prick technique for blood sampling to measure glucose and this study showed similar results to those seen in the dog study, with the high-protein diet being more digestible and producing a slower steadier release of glucose compared to the low-protein diet. Due to the promising results of this initial cat study, a second longer duration trial was conducted using cats where a high-protein, low-carbohydrate wet diet was also fed alongside these two dry diets to compare the effect not only of macronutrient proportion but also dietary form on glycaemic responses and weight maintenance in these animals. The results of this study showed that the high-protein, low-carbohydrate wet diet produced the smallest glycaemic response of the three diets. The cat's body weights throughout the study period illustrated that the cats fed both of the dry diets (high-protein and low-protein) gained weight over the course of the trial whereas those fed the wet diet lost weight, despite the mean caloric intakes (kcal/kg/day) for each of three diets not being significantly different at 77.3, 73 and 77.5 gross energy (GE). Interestingly the energy digestibility and digestible energy intake was lowest for the wet diet which would have contributed to this lack of weight gain in these cats. This result illustrates that the high-protein dry diet needs to be fed using restricted feeding rather than *ad libitum* feeding and also that a wet high-protein, low-carbohydrate diet may be more successful for cats as it would not rely on strict compliance of owners in regards to the amount fed and feeding method used.

Two treadmill exercise studies were then performed in working dogs to investigate the effects of dietary macronutrients on metabolism and performance during exercise over a 56 day period, where the dogs were tested before and after exercise (1 hour) on days 0, 14, 28 and 56. In the initial exercise study the high-protein (low-carbohydrate) diet showed clear benefits with higher digestibility of protein and fat and lower rectal temperatures and heart rates before and/or after each exercise test, both of which are advantageous to exercise performance. The

plasma glucose concentrations were also lower before and after exercise for the dogs fed this diet on the majority of test days. On day 56 after exercise, the plasma triglyceride concentration decreased in the dogs fed the high-protein diet and increased in the dogs fed the low-protein diet, which may be in part due to the fact that the ambient temperature was highest on this day and probably increased the intensity of the exercise. Following each exercise test the serum free fatty acid concentrations of the dogs fed both diets increased significantly. Taken together these results reflect an increased mobilisation of free fatty acids into circulation from fat sources including triglycerides, for use by the muscles for energy in the high protein diet.

In the second study, which used a more intensive exercise test with the aim of producing clearer differences with diet and exercise, similar performance advantages were shown for the high-protein (low-carbohydrate) diet with higher protein, fat and energy digestibility and lower pre-exercise heart rates for the dogs fed this diet. Serum free fatty acid concentrations in both groups increased significantly following exercise, suggesting an increase in their mobilisation for uptake and use by muscles for energy generation. This is an advantage as it results in the sparing of more limited muscle glycogen and blood glucose stores. No detrimental effects of the diets on the immune system were determined, however the phagocytic activity of the dogs fed the low-protein diet were higher on day 56 and the response of the dogs fed the high-protein diet to the lymphocyte mitogen phytohaemagglutinin (PHA) increased greatly from day 28 to day 56, possibly indicating that changing the macronutrient proportions in the diet may impact on certain aspects of immune function however, more work is needed to confirm this finding. Following these two studies in exercising dogs, a final study measuring a more novel marker in dogs, but one which is commonly used in humans; respiratory exchange ratio (RER) was conducted to determine whether by changing the dietary macronutrient profile, the metabolism and exercise physiology in the working dog can be manipulated.

This final trial investigated the effects of three diets (3x3 crossover design) on apparent digestibility, various measures of performance, immune parameters and respiratory exchange ratios. The high-protein, high-fat, low-carbohydrate diet (Diet 2) conferred performance advantages as evidenced by the higher digestibility and the predominant use of fat sources for fuel during exercise, thus sparing more limited energy stores and delaying the onset of fatigue. In comparison, when the dogs were fed the low-protein, low-fat, high-carbohydrate

diet (Diet 3), they used predominantly carbohydrate as fuel sources and when fed the high-protein, low-fat, low-carbohydrate diet (Diet 1) they used a mixture of carbohydrate and fat sources. Increases in free fatty acid concentrations with exercise were greatest when the dogs were fed Diet 2, which supports the breath hydrogen results indicating the predominant use of fat as the energy source by dogs fed Diet 2. Unlike Diet 2, when Diets 1 and 3 were fed, glucose concentrations increased significantly with exercise, highlighting the use of glycogen and glucose stores for muscular energy in these dogs. All facets of the immune system that were measured (level of expression of CD4, CD8, B cells, and CD14, phagocytic activity, cell proliferation using ConA, PHA and LPS) remained unchanged during the trial.

These findings indicate that by altering the macronutrient profile of the diet, the metabolism of dogs during exercise and dogs and cats at rest can be manipulated. In particular a high-protein, low-carbohydrate diet, may offer working dogs, and cats and dogs with diabetes mellitus and obesity the advantages of better glycaemic control and less large intestinal fermentation of carbohydrate. For working dogs, the high-protein, low-carbohydrate diets fed during the two treadmill exercise tests also appear to be advantageous to these dogs as they were more efficiently utilised, resulting in smaller volumes needing to be fed and reduced faecal output. The results obtained from the final study illustrate how pre-feeding diets differing in macronutrient proportions can profoundly affect fuel utilisation during exercise. The implications of this are that diet can be used to improve efficiency of fuel utilisation, performance and endurance in working dogs. Results from these studies indicate a high-protein, high-fat, low-carbohydrate diet may be of great benefit to farm and hunt dogs, being highly digestible, energy dense, and allowing the dog to rely predominantly on fat as a fuel for exercise it may therefore be closer to the ideal diet for these working dogs than those currently available.



## Abbreviations

$\omega$ -3	Omega 3 fatty acid
$\omega$ -6	Omega 6 fatty acid
GLUT-2	Glucose transporter type 2
SGLT-2	Sodium-glucose co-transporter type 2
GLUT-5	Glucose transporter type 5
Acetyl-CoA	Acetyl co-enzyme A
ATP	Adenosine tri phosphate
ME	Metabolisable energy
kcal	Kilocalorie
BW	Body weight
VCO <sub>2</sub>	Volume of carbon dioxide produced
VO <sub>2</sub>	Volume of oxygen consumed
RQ	Respiratory quotient
RER	Respiratory exchange ratio
NEFA	Non-esterified fatty acid
HCO <sub>3</sub> <sup>-</sup>	Bicarbonate ion
CK	Creatine kinase
AST	Aspartate aminotransferase
MJ	Mega joule
ppm	Parts per million
SEM	Standard error of the mean
MER	Maintenance energy requirements
kJ	Kilo joule
TiO <sub>2</sub>	Titanium dioxide
rpm	Revolutions per minute
mmol	Millimoles
$\mu$ U	Microunits
AUC	Area under the curve
C <sub>max</sub>	Maximum concentration
T <sub>max</sub>	Time of maximum concentration
GI	Glycaemic index
BMI	Body mass index

DEXA	Dual energy x-ray absorptiometry
bpm	Beats per minute
ConA	Concanavalin A
PHA	Phytohemagglutinin
LPS	Lipopolysaccharide
mph	Miles per hour
G	Gauge
EDTA	Ethylene diamine tetraacetic acid
pg	Picogram
fL	Femtoliter
IU	International unit
CBC	Complete blood count
WBC	White blood cell
MCHC	Mean corpuscular haemoglobin concentration
MPV	Mean platelet volume
RBC	Red blood cell
HGB	Haemoglobin
HCT	Haematocrit
MCV	Mean corpuscular volume
MCH	Mean corpuscular haemoglobin
CHCM	Cell haemoglobin concentration mean
CH	Cell haemoglobin
RDW	Red blood cell distribution width
HDW	Haemoglobin distribution width
PLT	Platelet count
µg	Microgram
cpm	Counts per minute
µl	Microlitres
µM	Micromolar
µgml <sup>-1</sup>	Micrograms per millilitre
Uml <sup>-1</sup>	Units per milliliter
mM	Millimolar
mM-L <sup>-1</sup>	Millimolar per litre

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# Statement of Research Contribution

by **Shay Rebekah Hill**

This thesis includes work which has been published in a peer-reviewed journal. This work was conducted as part of the PhD candidature.

Chapter 2 was published in December 2009, in *The New Zealand Veterinary Journal* as “**Hill SR, Rutherford-Markwick KJ; Ravindran G; Ugarte CE; Thomas DG.** (2009). The effects of the proportions of dietary macronutrients on the digestibility, post-prandial endocrine responses and large intestinal fermentation of carbohydrate in working dogs. *New Zealand Veterinary Journal* 57(6): 313-318”.

The candidate was the principal investigator for all studies and held the majority of the responsibility for all aspects of these studies. The candidate planned, conducted, interpreted and wrote up all of the studies. The candidate was responsible for the majority of sample collection and preparation of samples for laboratory analyses (including collection of blood samples, centrifugation of blood and collection of serum or plasma, analysis of lactate, collection of breath samples, gas analysis, faecal collections, sampling and grinding). The candidate also designed and constructed the masks developed for the final trial and was responsible for all manuscript preparations. Input from co-authors was of an advisory, mentorship and critiquing nature.

Signed

DG Thomas, Chief Supervisor

# **Chapter One**

## **Review of Literature**

Canids represent one of the most diverse mammalian species, with a large range of sizes and breeds; therefore the large number of different types of canine athletes that exist with a wide range of abilities is of no surprise. Traditionally dogs were used for controlling farm animals, hunting and warfare, but today they are used for many more activities including companionship, leisure and events such as agility trials and frisbee, and more specialist work tasks such as customs and border control (Kronfeld *et al.* 1994). Canine athletes therefore have an impressive work capability and although the individual dog's genetics dictate its structural and metabolic characteristics, a great deal can be expected from all of these animals if they are fed and trained correctly. All of the different types of work the dog partakes in place enormous demands on the dog's body, however when considering the optimum diet for these animals, the type, intensity and duration of the work, ambient temperature, terrain and dog's living conditions must be taken into account.

Diet, along with genetics, training and psychology are the basis of a working dog's performance. Of these, diet is the one that has generated the most research interest, probably because of its potential economic effects and the desire among breeders, trainers and owners for greater knowledge of and optimum performance from their animals (Reynolds *et al.* 1999; Ahlstrom *et al.* 2006). Despite the importance of working dogs worldwide, most of the research carried out to date has focussed on sled dogs and greyhounds, which can be considered the two extreme examples of working dogs; the greyhound partakes in short intense bouts of sprinting exercise and the sled dog partakes in endurance exercise which can last for many days. Very little information is available on other types of working dog such as farm dogs here in New Zealand.

## **1.1 Evolution of the dog**

The dog has been a human companion for many thousands of years, becoming fully integrated into our lives and becoming one of the most popular companion animals in the world today. Caring properly for dogs encompasses not only attention to animal health and disease treatment but also providing optimum nutrition. The main goals of canine nutrition include maintaining health, promoting growth, supporting pregnancy and lactation and promoting optimum performance in working dogs.

The domestic dog (*Canis familiaris*) is a member of the order Carnivora and family Canidae, which also includes the wolf, coyote, dingo, fox and jackal (Bradshaw 2005; Ostrander and Wayne 2005; Wayne and Ostrander 2007). The taxonomy of the dog is shown in Figure 1.1.

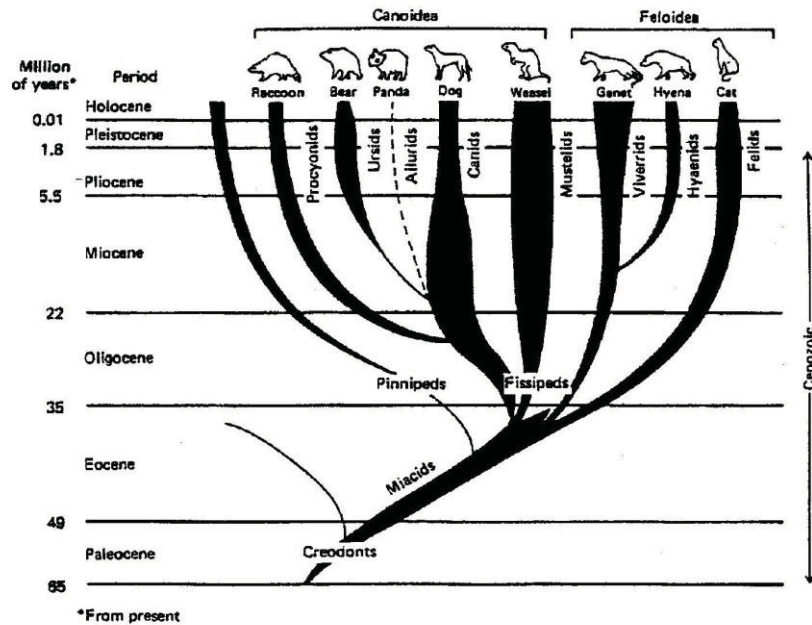


Figure 1.1. Taxonomy of the dog (Morris and Rogers 1989)

The dog is believed to be the first species to be domesticated, but exactly when this occurred is still unclear (Bradshaw 2006). Reports indicate that the history of the domestic dog reaches back at least 15,000 years and possibly as far back as 100,000 years (Lindblad-Toh *et al.* 2005).

The common wild ancestor to the dog is also still debated. One opinion is that the dog descended from the interbreeding of ancestral wolves, coyotes and jackals (Clutton-Brock 1984), while others have theorised that some dog breeds descended from the golden jackal and others descended from the wolf (Coppinger and Coppinger 2001). Furthermore, others suggested that the dog descended from the now extinct European dingo like dog (Fox 1978; Honeycutt 2010). Despite all of these theories, current evidence supports the idea that the gray wolf (*Canis lupus*) is in fact the closest relative of the dog (Wayne and Ostrander 2007; Case 2008; Honeycutt 2010). Therefore, the wolf is most likely to be the modern domestic



dog's closest ancestor and the dog appears to have inherited its feeding habits, biology and behaviour from this wild relative.

The body structure of dogs is adapted for speed and endurance, explaining their excellent capabilities for use as working animals. The general biology of the dog reflects its adaptations to its carnivorous lifestyle. Their eyes are adapted to detecting prey; being very sensitive to movement and having excellent dim light vision (Gazit and Terkel 2003; Viranyi *et al.* 2004). The dog's olfactory and auditory senses are also highly developed and specialised for locating and chasing prey (Bradshaw 2005; Tartaglia and Waugh 2005).

The dentition of the dog appears to reflect both its carnivorous and partly omnivorous diet. The dog has 42 permanent teeth which are all completely erupted by six months of age; 12 incisors, four canines, 16 premolars and 10 molars (Case 2005). The incisors are used for holding prey and the canines are used for shearing and tearing prey, whilst the molars are used for crushing and grinding. Dogs have more of these teeth (molars) than other obligate carnivorous species such as the cat, reflecting their more omnivorous diet.

Since the dog's domestication, and as human populations have increased, a large number of breeds of dogs have been developed for increasingly diverse purposes including companionship, hunting, herding, guarding and racing. It has been estimated that the intentional selective breeding of dogs started 3,000-5,000 years ago, but the greatest changes have only occurred relatively recently (Wayne and Ostrander 2007) and today, dogs are not only kept worldwide as pets but are also used extensively for work.

## **1.2 History of dog food and feeding regimens**

The order Carnivora includes obligate carnivores such as the cat and more omnivorous species like the dog (Bradshaw 2006). Within the dog family (Canidae), most species primarily eat meat. The diet of the ancestral wolf (*Canis lupus*) consisted predominantly of meat; however, the dentition of today's wolf is consistent with a more omnivorous diet (Legrand-Defretin 1994).

Early in the domestication of the dog it was believed that you didn't need to feed them a lot of food as they could forage for themselves like their wild ancestors (McNamara 2006). In 1860

however, the first commercial dog food (a dry kibble), was developed by James Spratt and sold in the United Kingdom (Wortinger 2007). Later, Spratt began selling his products in the United States, and in the late 1950s he became part of General Mills (Wortinger 2007). General Mills was purchased in turn by Spillars Dog Food Company in 1960, which was a British subsidiary of Purina, now known as Nestlé Purina Pet Care. Early in the 1900s other pet foods began to be made and sold. FH Bennett developed a milk bone dog biscuit in 1907 in New York, and in 1922 the Chappel brothers made the first canned dog food, followed by their own brand of dry dog food (Wortinger 2007). Samuel and Clarence Gaines began selling a new dog food or 'meal' in 1925 and by the 1930s many new dog food brands were emerging on the market, including Cadet and Snappy. During the Second World War, dry dog food began to dominate the pet food market as the tin used to make the dog food cans was diverted to the war effort, and by 1946 dry pet foods made up close to 85 % of the total pet food market in the United States (Wortinger 2007). In 1957 Purina dog chow became available, and at a similar time General Foods developed Gaines Burger, the first semi-moist dog food (Wortinger 2007). Hills Science Diet™ became the first product line for different pet life stages when it was released in 1968 (Wortinger 2007).

Today the canine pet food industry continues to develop and grow, with consumers demanding higher quality, more convenient products and subsequently researchers seeking a greater knowledge of canine nutrition. Dog owners are spending more money on their pets and are seeking longer healthier lives for their dogs (Bontempo 2005). For this reason, there has been huge investment of both time and money into canine nutrition, leading to greater understanding of the dog's nutritional needs/requirements and the development of a vast array of new products.

The wide range of complete and balanced dog food products available to dog owners today includes dry, semi moist and moist products. The pet food industry is worth US \$52.25 billion, with the Australasia section worth US \$1.61 billion or 3.1 % of the world market (Euromonitor International 2009).

Dry dog foods (e.g. kibbles or biscuits) are either extruded or baked, and contain between 6 and 10 % moisture (Costa 1997; Wortinger 2007). Semi moist products (mostly snacks), generally contain between 14 and 30 % moisture and are closer to dry foods than moist foods in their nutrient content (Wortinger 2007). Moist products generally contain between 72 and

85 % moisture and are manufactured in cans, pouches or rolls, as loaves, chunks in gravy or chunks in loaves (Wortinger 2007). They are very palatable but can vary greatly in nutrient content and digestibility.

The dog's sense of taste is less refined than humans, which can be seen by the fact that dogs only have about 1,700 taste buds on the tongue, whereas humans have about 9,000 (Case 2005). The process of mastication is also thought to be less important in the dog as they tend to eat rapidly, swallowing pieces of food whole rather than chewing them, which is probably a reflection of their days in the wild (Burger 1993; Tartaglia and Waugh 2005). The most abundant groups of taste buds in the dog are those that respond to sugars and compounds including phosphoric acid, carboxylic acid, histidine and other amino acids (Bradshaw 2005). These latter compounds can be found in meat and products containing meat.

Dogs have a reputation for eating large meals very quickly, which may be due to the competitive feeding habit in the wolf or an adaptation to scavenging during early domestication (Bradshaw 2006). It is believed that during free choice feeding (surplus food is available at all times) dogs tend to eat small meals throughout the day (Wortinger 2007). Free choice feeding (*ad libitum*) is generally discouraged however, as it relies on the dog's ability to self regulate its food intake, and most dogs will overeat and become overweight when fed in this manner (Bradshaw 2005; Wortinger 2007).

Other methods of feeding include time restricted feeding, where the dogs are allowed to eat to appetite in a predetermined amount of time, a set number of times a day, and food restricted feeding where the amount of food offered to the animal is calculated to meet the animal's maintenance requirements. This final method is usually the recommended method for dogs as it allows the owner the greatest control over the pet's diet and it doesn't rely on the animal self regulating their feed intake (Bradshaw 2005; Wortinger 2007).

Generally it is recommended that non-working and non-lactating medium sized dogs be fed once a day; working dogs, lactating bitches, giant breeds, toy breeds and puppies older than three months of age be fed two meals per day; growing pups between six weeks and three months of age be fed three meals per day and puppies less than six weeks of age be fed *ad libitum* (Guilford 1993).

## **1.3 Macronutrients in the dog's diet**

### **1.3.1 Carbohydrates**

Dietary carbohydrates are important as they provide the energy required for a range of processes in the body and provide several molecules needed for basic cell functions such as hormones, and molecules involved in immune function (McNamara 2006). Today, carbohydrates are used widely in pet food manufacturing, often making up 30-60 % of the dry matter in the diet (Murray *et al.* 1999), primarily because of their cost effectiveness in supplying energy when compared to protein and fat. The most common types of carbohydrates used in pet foods are glucose (through the inclusion of starch in the diet), lactose, maltose and sucrose and sources used include cereals, legumes, wheat, barley, soybean, oats, rye, vegetables and corn and potato starches (NRC 2006).

The monosaccharides ('simple sugars') are the carbohydrates that can be found in the blood and are the main type that are used by the body for energy (Wortinger 2007). Glucose is the third most important nutrient for animals, after water and oxygen and is required as an energy source by the central nervous system, red blood cells, nervous tissue, liver and kidneys (Wortinger 2007).

Polysaccharides can be found in plants, where they are used for cell wall material (cellulose) and energy storage (starch) (Wortinger 2007). Examples of starches are amylose and amylopectin and common sources used in commercial canine diets are whole grains of corn, rice, oats, barley and wheat (Fortes *et al.* 2010). Dietary fibre (cellulose, hemicellulose, pectin, gums, mucilages) are also types of polysaccharides found in plants. Sources of these used in canine diets include wheat middling, rice bran, beetpulp, soybean and peanut hulls.

### **1.3.2 Proteins**

Dietary proteins used in the pet food industry are generally provided from sources such as meat, meat and bone meal, fish and fish meal, eggs, casein, soybean and corn.

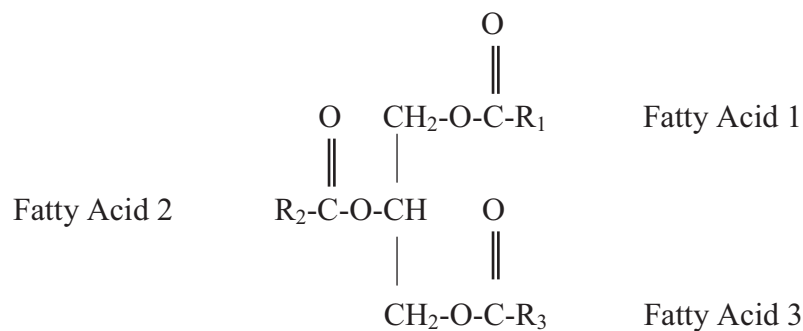
Proteins are vital dietary components in canine diets and are broken down to give amino acids which are then used to synthesise other proteins required for a number of functions including muscle and tissue growth and repair, muscle contraction, enzymes, hormones (e.g. insulin and

glucagon), transport of nutrients (e.g. haemoglobin for oxygen and transferrin for iron), antibodies and integral membrane proteins (Agar 2001; Dust *et al.* 2005; Wortinger 2007).

Essential amino acids for the dog that must to be supplied in the diet are arginine, histidine, isoleucine, leucine, lysine, methionine, tryptophan, threonine, valine and phenylalanine (Laflamme 2008). Non-essential amino acids (which do not need to be supplied in the diet and can be manufactured by the dog) for the dog are alanine, aspartine, aspartic acid, cystine, glutamine, glutamic acid, glycine, hydroxyproline, hydroxylysine, proline, serine and tyrosine (Laflamme 2008).

### 1.3.3 Fats

Triglycerides are the most common form of fat in the diet and their basic composition is shown in Figure 1.2.



**Figure 1.2. Triglyceride structure (Case *et al.* 2000)**

Specific fatty acids, including omega three ( $\omega$ -3) and omega six ( $\omega$ -6) fatty acids, are classified as essential for the dog as they must be provided in the diet because the dog's body cannot make them (Wortinger 2007). Fatty acids play important roles in the body; they are major constituents of cell membranes, are involved in the normal growth and maintenance of the skin and coat, and lipid transport in the blood, therefore deficiencies can lead to a dry coat, scaly skin and can impact negatively on wound healing (Abba *et al.* 2005; Wortinger 2007; Greco 2008).

Of the  $\omega$ -3 fatty acids, the most important is alpha linolenic acid which can be found in soybean, canola, flaxseed and coldwater fish oils. The most important and only  $\omega$ -6 fatty acid

required in the diet of the dog is linoleic acid (Abba *et al.* 2005). In contrast to the cat, if the dog has sufficient linoleic acid in the diet, arachidonic acid can be synthesised from linoleic acid in the body (Case *et al.* 2000). The best sources of linoleic acid include corn, soybean, safflower, cottonseed and sunflower oils, although pork and poultry fats also contain considerable amounts.

The omega fatty acids are precursors of eicosanoids; a group of active, short lived compounds that are involved in inflammatory reactions, immunoregulation and epidermal cell proliferation (Abba *et al.* 2005). Examples include prostaglandins, prostacyclin, thromboxanes, lipoxins and leukotrienes (NRC 2006). The eicosanoids produced by the metabolism of  $\omega$ -3 fatty acids have less potential to produce inflammatory reactions than those produced by the  $\omega$ -6 fatty acids, which can be important in situations where a decreased inflammatory response is desirable (Wortinger 2007).

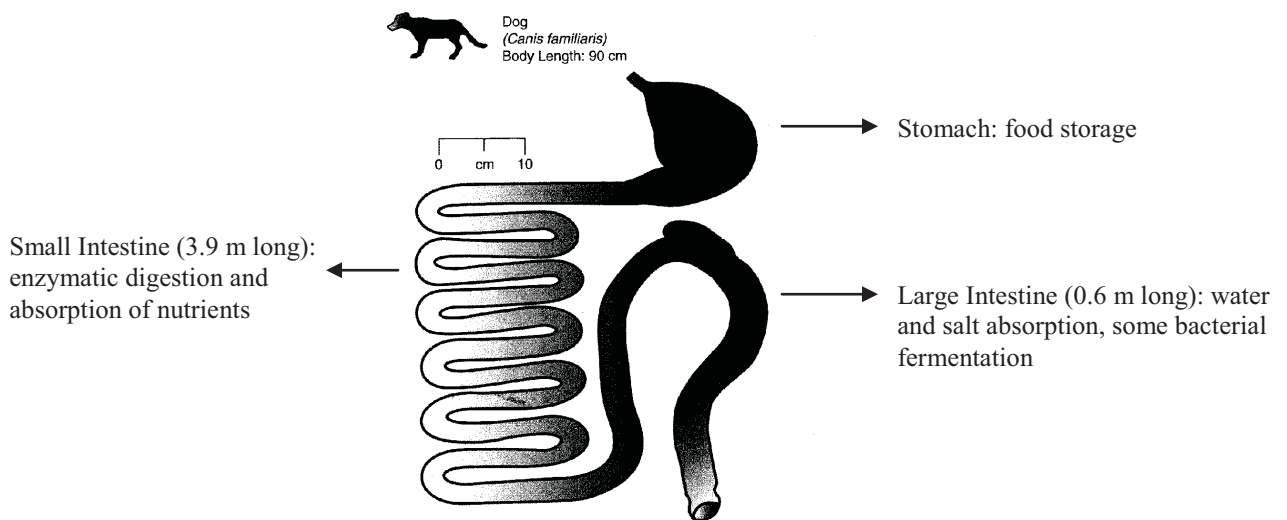
Both the inclusion of  $\omega$ -3 and  $\omega$ -6 fatty acids in the diet and also the ratio of  $\omega$ -6 to  $\omega$ -3 fatty acids are important to the dog. By manipulating the dietary ratio of  $\omega$ -6 to  $\omega$ -3 fatty acids, it has been shown that the ratio in the tissues can also be manipulated, ultimately influencing the inflammatory response in the body (Lands 1996). For example, a high ratio, such as 25 to one, causes an increased production of pro-inflammatory eicosanoids in the body (Abba *et al.* 2005).

Dietary fats provide the most concentrated form of energy, releasing approximately double the amount of energy per unit weight than either carbohydrates or proteins. They also provide the essential fatty acids, function as a form of energy storage, aid in absorption of the fat soluble vitamins (e.g. A, D, E and K), improve palatability and are required for waterproofing, insulation, protection of internal organs and the manufacture of some hormones (e.g. prostaglandin and aldosterone) (Agar 2001; Wortinger 2007).

A wide range of fat sources are used today in the pet food industry, including meat fats, coconut, copra and palm oils, sunflower, soybean and corn oils and purified fish oils (Thompson 2008).

## 1.4 Digestion, absorption and metabolism

Digestion involves breaking up nutrients into their simplest forms so that they can be absorbed and used by the body. This involves mechanical (mastication, muscular contractions), chemical (enzymes in digestive juices) and microbial (bacteria in the digestive tract) activities along the gastrointestinal tract (Figure 1.3).



**Figure 1.3. Dog Gastrointestinal Tract (Stevens and Hume 1995)**

Saliva which aids in lubrication, is secreted into the mouth by four pairs of salivary glands; parotid glands in the front of each ear, sublingual glands under the tongue, zygomatic glands in the upper jaw below the eyes and mandibular (submaxillary) glands on each side of the lower jaw (Burger 1993). The sight and smell of food causes an increase in saliva secretion which is composed of approximately 99 % water and 1 % mucus, inorganic salts and enzymes (Burger 1993).

Before swallowing, food is formed into a bolus by the tongue and in the oesophagus, mucus helps with further lubrication and peristalsis propels the food along the digestive tract. At the end of the oesophagus, the cardia allows the food to enter the stomach (Wortinger 2007). The stomach is used to temporarily store food and is also responsible for controlling the entry of digesta into the small intestine. Mucus, hydrochloric acid, pepsinogen and gastrin are all secreted in the stomach. Gastrin stimulates the secretion of hydrochloric acid, mucus and

pepsinogen and its secretion is controlled by the pH of the stomach (if the pH is below three gastrin release is inhibited) (Burger 1993). Gastrin is produced by the gastric mucosa in response to the same stimuli that increase the flow of saliva (Burger 1993). Mucus helps control motility to ensure the movement of the food away from the mouth and also helps promote mixing and grinding of the food to form a semi fluid mass called chyme (Burger 1993). Hydrochloric acid is important to the stomach because it maintains the low pH required for the optimal activity of digestive enzymes. Pepsinogen is the inactive precursor of the enzyme pepsin; a protein digesting enzyme. It is secreted in its inactive form in order to prevent the active form of the enzyme from damaging the tissues of the stomach (Burger 1993). The stomach also secretes mucoprotein intrinsic factor which is needed to bind vitamin B<sub>12</sub> so it can be absorbed (Burger 1993).

Eventually the chyme formed in the stomach is passed through the pyloric sphincter into the small intestine. The small intestine can be divided into three sections; the duodenum, jejunum and ileum. It is 3.9 m long in the dog, compared to 1.7 m in the cat and 7.0 m in humans (Burger 1993). The large intestine consists of the caecum, colon and rectum and is relatively short in the dog (only 0.6 m) and cat (0.4 m) compared to humans (1.8 m) (Burger 1993; NRC 2006). The ratio of intestinal length to body length is larger in herbivorous species compared to carnivores, reflecting the difference in the digestion of vegetable matter and animal protein. The dog has a total intestinal length to body length ratio of 6:1 whereas the cat has a ratio of 4:1; a reflection that the dog's diet contains more plant material than that of the cat (NRC 2006; Wortinger 2007).

In the small intestine, further mechanical digestion occurs through muscle contractions and enzymatic (chemical) digestion is completed, resulting in the release of amino acids, dipeptides, glycerol, fatty acids and monosaccharides which can be absorbed (Burger 1993; Wortinger 2007). The presence of partially digested food in the small intestine stimulates the release of pancreaticozymins which then stimulates the release of enzyme rich juices (Burger 1993). In the duodenum, the chyme is mixed with a variety of enzymes which are secreted into the lumen by the pancreas and glands in the mucosa (Wortinger 2007). The pancreas has two functions; as an exocrine gland it secretes enzymes such as proteases, lipases and amylases into the gut, and as an endocrine gland it secretes hormones such as insulin, glucagon and somatostatin into the blood (NRC 2006). The acidity in the small intestine also promotes the release of secretin which in turn stimulates the pancreas to increase the secretion



of bicarbonate, thereby optimising the pH for the digestive enzymes (Burger 1993; Wortinger 2007).

Nutrients are then absorbed through a wide array of processes, some active and therefore requiring energy and others not. Absorption involves the transfer of digested nutrients from the intestinal lumen into the blood or lymphatic system for delivery and use throughout the body. The main site of absorption is the small intestine, however, any unabsorbed or incompletely digested nutrients including indigestible fibre leaves the small intestine and enters the large intestine via the ileocaecal valve, where some degree of bacterial fermentation occurs. The products of this fermentation (lactate, carbon dioxide, hydrogen and the short chain fatty acids acetate, butyrate and propionate) can provide energy to the cells lining the large intestine (NRC 2006). Logically, the relatively small size of both the caecum and large intestine should limit fermentative capacity. However, some research has shown that short chain fatty acids produced by the bacterial fermentation of dietary fibre in the dog are an important source of energy and may even contribute to the intestinal health of dogs (Reinhart *et al.* 1994; Sunvold *et al.* 1994). The main function of the large intestine however is the absorption of water and salt (Burger 1993).

#### **1.4.1 Digestion of carbohydrates**

Carbohydrate digestion in the dog does not begin until the digesta reaches the small intestine. Even though food leaving the stomach is in a semi solid state (chyme), the carbohydrate it contains is almost unchanged because most of the digestion thus far is only mechanical (Wortinger 2007), and dogs unlike other species (e.g. humans) do not have any amylase in their saliva to begin chemical digestion.

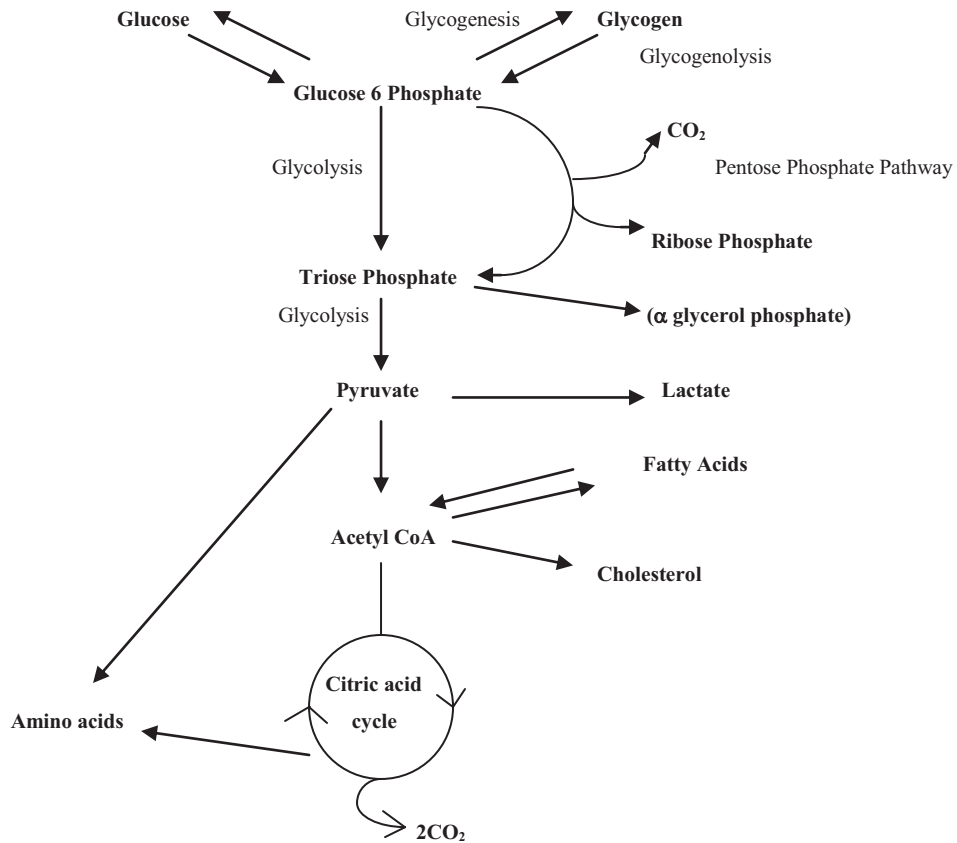
Cells of the small intestine secrete the carbohydrate digesting enzymes maltase, lactase, sucrase and isomaltase which degrade the disaccharides maltose, lactose and sucrose to the monosaccharides; glucose, fructose and galactose respectively (Wortinger 2007). The pancreas secretes the enzyme amylase which is responsible for the hydrolysis of dietary starches (Wortinger 2007). Starch is initially broken down by alpha amylase and by the time it reaches the terminal duodenum most starches have been degraded to oligosaccharides, such as disaccharides, trisaccharides and alpha dextrans (Burger 1993). These products of luminal digestion must then be further degraded to monosaccharides so they can be absorbed. Starches are digested at varying rates depending on the source and type of processing. Any

starches and resistant starches that are not completely digested in the small intestine enter the large intestine where they are fermented to at least some degree by the colonic bacteria to gases (carbon dioxide, hydrogen and methane), short chain fatty acids (acetate, butyrate and propionate) and lactate. Because of this colonic fermentation, total tract digestion of monosaccharides is usually close to 100 % in dogs (NRC 2006).

Absorption of monosaccharides and sugar alcohols into enterocytes occurs directly, and requires no digestive enzymes (Burger 1993), however, the absorption of other carbohydrates requires specific transport mechanisms, many of which are active in nature and therefore require energy to pump the sugars across the brush border membrane (Burger 1993; Wortinger 2007). For example, most glucose and galactose use the same carrier mechanism; an active sodium dependent transporter (GLUT2, SGLT2). Fructose is absorbed in humans by a sodium independent glucose transporter (GLUT5) which does not require energy and it is believed this is probably the same mechanism utilised by the dog (NRC 2006).

Once absorbed into enterocytes, carbohydrates are rapidly released into capillaries, either by diffusing down their own concentration gradient or by a separate carrier system on the luminal membrane which allows facilitated diffusion, and are then taken to the liver (Wortinger 2007). In the liver, monosaccharides arriving through the portal vein are metabolised and those not in the form of glucose already, are converted to glucose or fructose in the liver cells (Wortinger 2007).

Glucose can have several metabolic fates as illustrated in Figure 1.4. It can be stored as glycogen in the liver and muscles, converted to blood glucose and used by all cells to produce energy, catabolised to pyruvate or lactate, used to provide alpha-glycerophosphate for lipid synthesis, hydrolysed to form acetyl-CoA which is then oxidised to water and carbon dioxide via the citric acid cycle or used as a substrate for the synthesis of fatty acids, or degraded via the pentose phosphate pathway to form ribose or triose phosphate (Summers 2002; Wortinger 2007). Intermediates of carbohydrate metabolism can also provide carbon skeletons for the synthesis of non essential amino acids.



**Figure 1.4. Carbohydrate metabolism in animal cells (Reece 2004)**

### 1.4.2 Digestion of proteins

The digestion of protein in the diet begins in the stomach through the secretion of hydrochloric acid and pepsinogen. The enzyme pepsin is responsible for around 10 % of protein digestion (Burger 1993). The hydrochloric acid secreted by the stomach converts pepsinogen into pepsin and then once pepsin is formed; this in turn also converts more pepsinogen to pepsin. Pepsin splits the ingested proteins at specific points, making the internal peptide bonds of the protein more accessible to subsequent digestive enzymes (McNamara 2006).

Once chyme leaves the stomach and enters the duodenum, the proteolytic activity of pepsin stops and other proteolytic enzymes such as trypsin, chymotrypsin and nuclease are secreted by the pancreas to complete the enzymatic digestion of the dietary proteins (Burger 1993; McNamara 2006). The pancreas secretes proteolytic enzymes (proteases) into the small intestine as inactive proenzymes, however the presence of bile in the small intestine then

stimulates the mucosa to release the brush border enzyme enterokinase which converts inactive trypsinogen into trypsin (Burger 1993). Trypsin then activates more trypsinogen to trypsin and also activates the other pancreatic enzymes so the dietary protein can be cleaved to small peptides and amino acids.

The pancreatic enzymes can be divided into two groups; the endopeptidases and exopeptidases. The endopeptidases hydrolyse protein at centrally located peptide bonds and include trypsinogen (the inactive form of trypsin), chymotrypsinogen (the inactive form of chymotrypsin) and proelastase (the inactive form of elastase) (McNamara 2006). The exopeptidases cleave only the terminal bonds of the proteins or peptides and include procarboxypeptidase A (the inactive form of carboxypeptidase A) and procarboxypeptidase B (the inactive form of carboxypeptidase B) (McNamara 2006).

The end products of protein digestion in the small intestine are amino acids, dipeptides and tripeptides. Generally, free amino acids are absorbed directly into enterocytes lining the small intestine by diffusion. However, some single amino acids, dipeptides and tripeptides need to be absorbed using active energy requiring processes that are closely linked to sodium transport (Burger 1993). There are several different carriers used that function as sodium symport systems, similar to those previously mentioned in section 1.4.1 for absorption of glucose and galactose (McNamara 2006). Most amino acids can be absorbed by more than one transport system and amino acids in any one uptake group compete for transport. Di and tripeptides are also absorbed by sodium symport systems but they bypass the brush border peptidases and are hydrolysed by the intracellular peptidases in the absorptive cell (McNamara 2006).

Once amino acids are absorbed into the enterocytes they are transported via the portal vein to the liver for metabolism. A few peptides also enter the portal system intact (McNamara 2006). Some amino acids are taken up into the blood where they become available for absorption into body cells and for use in protein synthesis. Others are synthesised into liver and plasma proteins, catabolised resulting in ATP production, or converted to nucleotides, non essential amino acids and other products (McNamara 2006). This process produces ammonia as a waste product, which is toxic and is therefore converted to urea and excreted via the kidneys.

In the large intestine there may be a very small amount of protein digestion by microbial enzymes with the remaining protein escaping degradation and excreted in the faeces (McNamara 2006). Proteins that are degraded by microbes in the large intestine are re-synthesised into microbial protein (McNamara 2006).

### **1.4.3 Digestion of fats**

In the stomach, fat digestion in mammals is generally initiated by secretion of lingual and gastric lipases which are active at the low pH of the stomach (NRC 2006). Once fats and fatty acids leave the stomach and enter the small intestine, they stimulate the release of cholecystokinin from the duodenal mucosa (Burger 1993). Cholecystokinin then stimulates the contraction of the gallbladder, releasing bile into the intestinal lumen and the release of pancreatic enzymes (pancreatic lipase, colipase, carboxyl ester hydrolase and phospholipase A) (Burger 1993; Wortinger 2007). Bile released from the gallbladder has a vital role in promoting emulsification and solubilisation of the lipids through the action of the bile salts it contains. The fat, digestive enzymes and bile salts form an emulsion of fat droplets called micelles (Wortinger 2007), which are then able to be digested and absorbed.

Pancreatic lipase is responsible for most fat digestion, acting on dispersed lipids and hydrolysing triacylglycerols to form free fatty acids and two-mono-acylglycerols. Procolipase is also secreted from the pancreas and activated by trypsin to its active form colipase. Its purpose is to anchor pancreatic lipase to the substrate in the presence of bile salts, so it can carry out its role in digestion.

All biological membranes in the body have a layer of relatively unstirred water which solutes must diffuse through. The thickness and surface area of this layer provides resistance to passive diffusion. Therefore for absorption to occur, lipids must diffuse across this unstirred water layer where they are then protonated before moving through the brush border membrane by either passive diffusion or active processes (NRC 2006). Once in the cytosol of the enterocytes, fatty acids are bound to fatty acid binding proteins and are transported to the endoplasmic reticulum where re-esterification of free fatty acids and re-assembly of triacylglycerols occurs. Some fatty acids may pass directly into the portal circulation, however most attach to lipoproteins with cholesterol, phospholipids and enterocytes, forming chylomicrons (approximately 84 % triglycerides, 7 % phospholipids, 7 % cholesterol and

cholesterol esters and 2 % protein), which are released into lacteals for transportation to the liver and the rest of the body (Wortinger 2007).

Triglycerides are transported through the lymphatic system to the liver or other cells depending on the body's needs (Wortinger 2007). In the liver, fatty acids are metabolised and distributed to the rest of the body. The fatty acids and free fatty acids from this metabolism in the liver may have a number of fates including oxidation to carbon dioxide with ATP production, ketone formation, synthesis to cholesterol and bile salts, synthesis to plasma lipoproteins or formation into plasma free fatty acids. Fatty acids from circulating chylomicrons may also be taken up by the adipose tissue from the lymphatic system and be stored as triglycerides for later use as an energy source (NRC 2006).

## **1.5 Life stage requirements**

Two of the most important considerations when selecting an appropriate dog food are the dog's life stage and lifestyle. The diet fed must provide the correct quantities and proportions of all nutrients required by the dog at its particular life stage in order for optimum health to be maintained (Burger 1993). Nutritional goals vary slightly for each stage of life, for example the nutritional demands increase during growth, reproduction and physical work and may decrease as the dog reaches adulthood and as they age.

### **1.5.1 Growth**

For newborn pups, the first week of life is the most critical for their survival as they are physiologically immature and have a low percentage of body fat (1-2 % compared to adults that have 12-35 %) (Wortinger 2007). For the next few weeks after this, pups should be nursed *ad libitum*, at least four to six times a day. Puppies should not be fully weaned from their mother until at least six weeks of age (Ackerman 1999), and preferably at seven to eight weeks (Wortinger 2007).

With the exception of peak lactation, post-weaning growth is the most nutritionally demanding stage of a dog's life (Wortinger 2007), and therefore the appropriate feeding of a high quality, highly digestible diet is vital. Because growth and skeletal development are at a peak during the first six months of a pup's life, any deficiencies or imbalances in the diet can be devastating and can result in impaired or abnormal skeletal growth, the development of

skeletal diseases (e.g. hip dysplasia and osteochondrosis) and a predisposition to obesity in later life (Greco 2008).

Energy requirements during growth can vary greatly because the dog has the widest range of normal adult bodyweight (0.6-41.2 kg) of any species (Wortinger 2007). Puppies have higher energy requirements than adult dogs and may require up to 1.5 to 2 times adult maintenance (Guilford 1993). Generally, it is recommended that pups should be gaining approximately 2 to 4 g per day per kg of expected adult weight during the first five months of age (Agar 2001).

While it is important to meet the energy requirements for growing dogs it is also important not to overfeed as this can also cause skeletal problems, especially in large breeds, and may also encourage adult obesity in all breeds (Agar 2001). Therefore free choice feeding is not recommended, and the best feeding method is portion controlled feeding, where the desired amount of diet for optimum and appropriate growth is measured out and given to each pup. The daily ration should be split into three to four meals per day for pups up to three months of age, three meals per day for pups older than three months and two meals per day for pups aged 12 to 15 months (Ackerman 1999). It has been found that although severe underfeeding prevents dogs reaching their full adult size, slight underfeeding (calorie restriction) can increase the lifespan of dogs (Agar 2001). Adult size is reached by small breeds at 8 to 12 months of age, by medium breeds at 12 to 18 months of age and by large and giant breeds at up to 18 to 24 months of age (Wortinger 2007).

Protein requirements for pups are higher than adult dog maintenance levels because protein is needed for both normal body maintenance and also to build the new tissues associated with growth (Agar 2001; Garnsworthy and Wiseman 2002; Wortinger 2007). The minimum protein level recommended for pups is 22 % metabolisable energy (ME) per day (Greco 2008) and the optimum level reported is 25 to 29 % (Wortinger 2007; Greco 2008). This protein should be of a high quality and be highly digestible. The NRC (2006) estimated the metabolic requirement of newly weaned puppies as 1800 g kg<sup>-1</sup> BW of a diet containing 4.0 kcal ME g<sup>-1</sup>. Growing dogs also have different dietary amino acid requirements than adult dogs (Ackerman 1999). The minimum amino acid requirements for growing pups have been published by the NRC (2006).

Puppies have no obligate requirement for carbohydrate as long as there is sufficient protein in the diet to meet the needs of glucose precursors. However, high levels of insoluble carbohydrate in puppy diets are inappropriate due to their higher energy requirements, and therefore their requirement for a diet with a high energy density. The dietary fat requirements of puppies are also higher than for dogs during maintenance. The NRC (2006) has set a recommended allowance for growth of 8.5 % on a dry matter (DM) basis or 18 % on a ME basis.

Puppies also have increased vitamin and mineral requirements. For example they require more dietary calcium than adult dogs (minimum requirement for pups is 2.0 g per 1000 kcal ME<sup>-1</sup> and for adult dogs is 0.5 g per 1000 kcal ME<sup>-1</sup>) (Agar 2001, NRC 2006). The appropriate calcium to phosphorous ratio is vital for pups to ensure correct skeletal development, with the optimal ratio currently set at between 1:1 and 1.5:1 (Agar 2001). The recommended allowance for growth for calcium set by the NRC (2006) is 3.0 g per 1000 kcal ME<sup>-1</sup>. The NRC (2006) has set the requirement for pups for phosphorous as 2.5 g per 1000 kcal ME<sup>-1</sup>.

### **1.5.2 Maintenance**

The maintenance stage of a dog's life is the least nutritionally demanding and occurs after the dog stops growing and before the changes due to ageing begin to appear (Agar 2001). Highly dependant on the dog's breed, this stage is usually between one and seven years of age (Wortinger 2007). Generally smaller breeds of dog mature earlier than large breeds and also age slower. Subsequently the maintenance stage of life for small breeds makes up the majority of their lives (Agar 2001).

Ideally, during maintenance, diets should be chosen based on the dog's individual preference, level of activity and other factors such as level of stress. However, certain dietary requirements for nutrients such as protein, fat, vitamins and minerals must still be met. Generally once a day feeding is sufficient for dogs during this life stage. For an adult dog, the recommended allowance for protein set by the NRC (2006) is 100 g kg<sup>-1</sup> diet, while others have documented the protein requirement as 18 % on a DM basis (Guilford 1993; Case 2005). The essential amino acids that must be provided in the diet are the same across all life stages; arginine, histidine, isoleucine, leucine, lysine, methionine, tryptophan, threonine, valine and phenylalanine.



All animals have a metabolic requirement for glucose which can be met through either endogenous synthesis or dietary sources. In the dog, there is convincing evidence that dietary carbohydrate isn't needed if there is sufficient protein and fat in the diet to provide the necessary glucose precursors (Case 2005). However, the NRC (2006) still recommends 10 to 13.4 g carbohydrate per 1000 kcal ME (37 to 53 % of DM) for maintenance dog diets. Generally for economic reasons, most commercial diets contain at least moderate levels of carbohydrate (30-60 % of DM), often in the form of starch (Murray *et al.* 1999). Dietary fibre is also not required in maintenance diets; however dry foods typically have a crude fibre content of 2.5 to 4.5 % because of its role in the maintenance of gut health (NRC 2006).

A wide range of dietary fat levels appear to maintain good health in adult dogs. Beyond the need to provide essential fatty acids, the exact requirement for total fat in maintenance diets is unknown (NRC 2006). However, a recommended allowance for fat in adult dog diets has been set by the NRC (2006) at 11.7 % on a ME basis, with a safe upper limit of 70 % (ME basis).

### **1.5.3 Pregnancy and lactation**

Both pregnancy and lactation are unique situations which place huge demands on the bitch's body and therefore a good quality, complete and balanced, highly digestible diet must be fed to best meet these needs.

During the first half of pregnancy, there is not a big increase in foetal growth and the bitch therefore only requires a slightly higher energy intake during this time. After this however, the energy required will increase dramatically and a pregnant bitch will require 40-60 % more energy by the time she is whelping than before pregnancy (Ackerman 1999; Agar 2001; Wortinger 2007). One problem often seen with this large increase in energy requirements is that because the pups are taking up more and more space *in utero*, it is difficult for the bitch to consume sufficient food, highlighting the importance of a high quality, palatable, energy dense diet. Therefore, feeding diets containing significant amounts of carbohydrate during late pregnancy and lactation should definitely be avoided, due to the resulting decrease in energy density. Another strategy during this time is to feed the bitch frequent small meals, so that she can still meet her own, and the foetus' requirements.

Lactation places even greater demands on the bitch's body than pregnancy, since she has to satisfy her own maintenance needs and be able to make enough good quality milk for her growing puppies (Ackerman 1999; Buffington *et al.* 2004; Wortinger 2007). The bitch's peak energy requirement occurs when the pups are three to four weeks old, during which time she will require two to four times the calories needed when she was pregnant (or three to four times her own maintenance requirements) (Ackerman 1999; Agar 2001). Because of these enormous demands, *ad libitum* feeding is the preferred method of feeding during this time. Once the pups are introduced to solid food, their demand for the bitch's milk begins to decrease, and once the pups are fully weaned at approximately six weeks of age, the bitch's energy requirements are reduced to approximately 50 % above maintenance requirements. By the time the pups are approximately eight weeks of age the energy requirement of the bitch has returned to pre-pregnancy (maintenance) levels (Ackerman 1999).

It has been reported that the only obligate requirement for carbohydrate in dogs is during pregnancy and lactation (Guilford 1993), however this is an area of debate in the literature. All animals have a metabolic requirement for glucose but this can be supplied through endogenous synthesis during growth and maintenance, provided there is sufficient fat and protein in the diet to provide the necessary precursors, or directly from dietary carbohydrate sources (Greco 2008). Therefore, if there is enough protein and fat in the diet there may not be a need for carbohydrate in the diet during pregnancy and lactation either.

During gestation, the bitch's need for glucose increases because glucose is the major energy source for fetal development, and similarly during lactation extra glucose is required for the synthesis of lactose in the milk. One early study in dogs showed that bitches fed a carbohydrate free diet throughout gestation became hypoglycaemic, hypoalbuminemic and ketotic toward the end of pregnancy, with only 63 % of pups born alive and the mortality of these pups shortly after birth was high (Romsos *et al.* 1981). However, a subsequent experiment found a carbohydrate free diet did not affect the duration of gestation, litter size or pup weight or mortality (Blaza and Burger 1989). The different outcomes of these two studies were believed to relate to the higher protein levels of the diets fed in the second trial. The higher protein levels were thought to have given the bitches enough gluconeogenic amino acids to maintain plasma glucose levels and maintain pup viability. Based on these studies, it appears that although carbohydrate is physiologically essential it isn't an indispensable part of the diet

even during pregnancy and lactation, provided there is sufficient protein in the diet (NRC 2006).

Along with the increased requirements for energy during pregnancy and lactation the requirements for other nutrients is similarly high (Agar 2001). Of most importance is water, which must be available at all times. Dietary fat requirements are thought to be greater than that for maintenance, however as previously mentioned in section 1.5.2 studies that precisely establish the requirement for total fat have not been carried out (NRC 2006). For this reason the NRC (2006) has set the recommended allowance for dietary fat for pregnancy and lactation as 8.5 % on a DM basis or 18 % on a ME basis.

Protein and amino acid requirements for this particular life stage have also not been well defined for dogs, but generally it is assumed that the crude protein requirement for growth would be sufficient to meet the needs of pregnant and lactating dogs (NRC 2006). The recommended allowance for growth set by the NRC (2006) is 200 g kg<sup>-1</sup> of a diet containing 4.0 kcal ME g<sup>-1</sup>.

In terms of micronutrient requirements, the NRC (2006) has also recommended various levels for pregnant and lactating bitches. Two of the most important of these are calcium and phosphorous. The recommended allowances for calcium during the last three weeks of pregnancy and for a 22 kg lactating bitch with eight puppies (assuming a bioavailability of 40 %) are 160 mg kg BW<sup>-1</sup> d<sup>-1</sup> and 360 mg kg BW<sup>-1</sup> d<sup>-1</sup>, respectively (NRC, 2006). However, to ensure adequate calcium intake for lactating bitches of all breeds, the recommended allowance is 380 mg kg BW<sup>-1</sup> d<sup>-1</sup> (NRC 2006). Phosphorous allowances during the last five weeks of pregnancy also for a 22 kg lactating bitch with eight puppies are 133 mg kg BW<sup>-1</sup> d<sup>-1</sup> and 145 to 290 mg kg BW<sup>-1</sup> d<sup>-1</sup> (NRC 2006).

#### **1.5.4 Geriatric**

Dogs can be considered as geriatric or elderly when they have achieved approximately 75 % of their anticipated lifespan, which as previously mentioned in section 1.5.2 varies between breeds (Ackerman 1999). Generally, small breeds of dog are considered geriatric at approximately eleven years of age, medium breeds at ten years of age, large breeds at eight or nine years of age and giant breeds at approximately seven years of age (Wortinger 2007).

As dogs age, their metabolism slows and there is a decrease in the functional capacity of all organs. For example, there can be changes in tissue structure and composition, cardiovascular and pulmonary function, renal and gastrointestinal excretion, the senses, skin and reproductive system (Wortinger 2007).

When dogs reach the geriatric stage it is preferable to consider their feeding requirements on an individual basis taking into account their usual diet, health status and body condition. Generally elderly dogs require fewer calories to maintain their bodyweight than adults due to their slower metabolism and often decreasing level of physical activity. However, geriatric dogs that lose weight and body condition may require more calories. Measured feeding is possibly the best feeding regimen for this life stage, ensuring a target steady bodyweight is maintained (Wortinger 2007). Many older dogs will also become very fussy eaters and may be unwilling to try new foods (Wortinger 2007), further supporting the idea of treating geriatric dogs as individuals.

Geriatric dogs need a high quality, highly digestible diet and benefit particularly from higher quality dietary proteins (Ackerman 1999). The amount of protein required has been widely discussed in the literature, and much controversy still exists regarding the dietary protein requirements of aging animals. In the past, investigators have recommended that all elderly dogs be fed a diet containing decreased protein levels, based on the normal decrease in renal function seen with aging in order to prevent or minimise the progression of kidney dysfunction (Branam 1987). However, today most believe a better strategy is to feed healthy older dogs optimal and unrestricted levels of high quality dietary protein to minimise losses in body protein reserves and to satisfy their maintenance needs (NRC 2006; Laflamme 2008). Only when or if problems are seen in kidney function should dietary protein levels be restricted as required (Laflamme 2008).

Dietary fats for geriatric dogs should contain high levels of essential fatty acids. Recent evidence suggests that aging is associated with a gradual decrease in the dog's ability to desaturate essential fatty acids and therefore increased levels of essential fatty acids may be of benefit to them (Ackerman 1999). One important essential fatty acid affected by this decrease that should be included in the diet is gamma linolenic acid, because the geriatric dog may have a diminished ability to synthesise it from dietary linoleic acid (Frantz *et al.* 2007).

As with adult dogs, geriatric dogs have no obligate carbohydrate requirements, however low fibre diets are generally advised as many dogs in this life stage find fermentation of fibre difficult (Ackerman 1999). The NRC (2006) recommends carbohydrate levels for senior dogs of 11.8 to 17.6 g per 1000 kcal ME<sup>-1</sup> (29 – 62 % ME). This is compared to maintenance recommendations for carbohydrate of between 37 and 53 % ME. As dogs age, their glucose tolerance generally decreases, meaning they take longer for blood glucose concentrations to return to baseline following a meal compared to younger dogs (NRC 2006). Often this change in glucose tolerance can contribute to the development of diabetes mellitus in older dogs.

Overall, relatively little is known about the micronutrient requirements of elderly dogs, compared to the other life stages, but researchers have suggested increasing the levels of various specific dietary minerals and vitamins in line with other nutrients as a safeguard to improve the health and increase the lifespan of these dogs.

## **1.6 Working/Performance dogs**

Of the large number of dog breeds worldwide, the most comprehensive reference of dog breeds lists 91 breeds of hounds, 44 herding breeds, 49 gun breeds and 31 terrier breeds (Palmer 2006). The American Kennel Club on the other hand, describes 24 sporting breeds, 16 herding breeds, 20 working breeds, with 80 breeds of hound worldwide, 13 breeds of sight hound, 49 herding or shepherd dogs and 31 breeds of terrier (Toll and Reynolds 2000).

As previously mentioned dogs have been selectively bred for thousands of years to perform various types of work in different environments and living situations, with domestication contributing to canine athletic prowess amongst other attributes (Snow 1985). Initially, working dogs were used in hunting, farming and warfare, but today working dogs participate in a much wider range of activities.

Today, most owners keep dogs essentially as companions, but many still expect them to perform other duties or activities such as hunting, racing, guiding (for blind owners), agility competitions, police duty, customs and border control, vermin control, pulling loads (sled dogs), search and rescue, frisbee competitions and herding and guarding stock.

Depending on the breed and function they are utilised for, working dogs will have different training, working and rest schedules and will perform different types of exercise. At one extreme of this exercise scale are the sprinters, such as greyhounds, where the exercise is of high intensity but short duration. The greyhound is the fastest dog breed, running races of between 200 - 800 m at speeds of up to  $64.8 \text{ km hr}^{-1}$  (Dobson *et al.* 1988; Rovira *et al.* 2007). Greyhounds are bred for speed, strength and keen eyesight needed for chasing game animals (Dobson *et al.* 1988; Marcellin-Little *et al.* 2005). At the opposite end of this spectrum are the endurance athletes, (e.g. sled dogs such as huskies), which can be considered equivalent to human marathon runners except for the fact they perform in extremely cold temperatures. These dogs partake in less intense exercise than the greyhound, however the exercise is of much longer duration, with dogs commonly covering more than 1000 miles over 14 days, running 10 to 12 hours per day on consecutive days and reaching speeds of 15 to 20 km per hour (Snow 1985; Murphy *et al.* 1997; McKenzie *et al.* 2005; Rovira *et al.* 2007). Sled dogs also run in harsh arctic conditions and are often kept outside in these conditions (Wyatt 1963). They are bred for muscle and cardio respiratory endurance (Marcellin-Little *et al.* 2005). Intermediate to these two extremes (sprinting and endurance) are working dogs that perform a range of activities such as hunt dogs, farm dogs and search and rescue dogs. These dogs still have an extraordinary work capacity and generally work for several hours per day over a week or more and cover large distances, using a mix of high and low intensity exercise (Ahlstrom *et al.* 2006). These dogs are bred for specific characteristics including muscle endurance, strength, agility, balance and speed depending on the specific type of work they perform (Marcellin-Little *et al.* 2005).

### **1.6.1 Sources of energy for muscles**

Exercise requires chemical energy to be converted into physical work and there are several sources of chemical energy for muscle which are called on by the dog (Murphy *et al.* 1997). Adenosine triphosphate (ATP) is the immediate energy source for muscles, however there is only enough ATP stored in each muscle fibre to allow it to contract for a matter of seconds (Burger 1993; Murphy *et al.* 1997; Toll and Reynolds 2000). Because of this low muscular ATP concentration compared to the needs for exercise, ATP has to be formed from other metabolic fuels stored in the muscle (endogenous source) or other parts of the body (exogenous sources). One such source of ATP is the phosphagen system, also known as alactic anaerobiosis (Grandjean and Paragon 1992). Creatine phosphate is an endogenously stored fuel in the muscle cell cytoplasm which is used to rapidly release ATP using the

creatine phosphate shuttle (Toll and Reynolds 2000). This energy source replenishes ATP at a rapid rate, however, it is only sufficient for a few more seconds (5 to 15) because the stores of creatine phosphate in the muscles are also very limited (Burger 1993; Toll and Reynolds 2000).

Another source of anaerobic ATP (which does not require oxygen) is via the metabolism of carbohydrate, known as lactic anaerobiosis (Grandjean and Paragon 1992). This process breaks down glucose and/or glycogen to lactate with the release of ATP. Glycogenolysis is the breakdown of glycogen stored in the muscles to glucose which can then produce ATP and release lactic acid (Grandjean and Paragon 1992; Murphy *et al.* 1997). Glycolysis refers to the catabolism of glucose in the muscle cell cytoplasm to form lactic acid and the release of ATP (Murphy *et al.* 1997; Toll and Reynolds 2000). Glycogenolysis and glycolysis both produce ATP at very fast rates (up to 6 times faster than aerobic metabolism) but both mechanisms only produce a low yield of ATP because production can only be maintained for a relatively short length of time (Grandjean and Paragon 1992; Wortinger 2007). In addition, the lactic acid that accumulates during anaerobic carbohydrate metabolism must eventually be removed from the body otherwise fatigue occurs (Rovira *et al.* 2008).

A further source of ATP for the dog is the metabolism of fats, amino acids and glucose. This aerobic (requiring oxygen) process occurs in the mitochondria and is also referred to as aerobiosis (Grandjean and Paragon 1992; Wortinger 2007). Aerobic metabolism of fat, amino acids and glucose produces ATP at a much slower rate than during anaerobic metabolism, but produces a much higher yield of ATP, for example beta oxidation of palmitate produces 106 molecules of ATP (Grandjean and Paragon 1992). Fats, as fatty acids, are an important muscular fuel for most vertebrates including dogs, with fat stores sufficient to sustain exercise for several days (McClelland *et al.* 1995). These fatty acids, along with triglycerides, are oxidised to produce ATP during exercise. Glucose is metabolised aerobically to carbon dioxide and water, with the release of ATP. The oxidation of protein during exercise only supplies a very small proportion of the energy used, and this occurs mostly from the oxidation of the branched-chain amino acids leucine, isoleucine and valine (Grandjean and Paragon 1992; Mero 1999; Hargreaves and Snow 2001; Tarnopolsky 2004). The amino groups of these amino acids are transferred to pyruvate and glutamic acid, to form alanine and glutamine which are then used for the synthesis of glucose. This glucose is then available for oxidation and results in the production of ATP.

A technique commonly used by researchers to estimate the relative contribution of carbohydrate, fat and protein to energy metabolism is to measure the ratio of the volume of CO<sub>2</sub> produced (VCO<sub>2</sub>) to the volume of O<sub>2</sub> consumed (VO<sub>2</sub>) (Powers and Howley 2004). This ratio is known as the respiratory exchange ratio (RER). During a period of steady state exercise, this ratio is known as the respiratory quotient (RQ) and specifically is the number of moles of CO<sub>2</sub> produced, divided by the number of moles of O<sub>2</sub> consumed (Reynolds *et al.* 1996). Therefore, by measuring the RQ, the relative amounts of carbohydrate, fat and protein burned during steady state exercise can be determined (Reynolds *et al.* 1996). When carbohydrate is burned, the resulting RQ is close to 1.0, whereas during fat oxidation the resulting RQ is less than 1.0 and as low as 0.7 (Reynolds *et al.* 1996). The role protein contributes to energy metabolism is generally ignored as it is less than 2 % of the substrate used during periods of exercise of less than one hour (Powers and Howley 2004).

The relative amounts of ATP produced using the different metabolic processes described above is outlined in Table 1.1.

**Table 1.1. Adenosine triphosphate production in dogs using different metabolic fuels for exercise (Grandjean and Paragon 1992)**

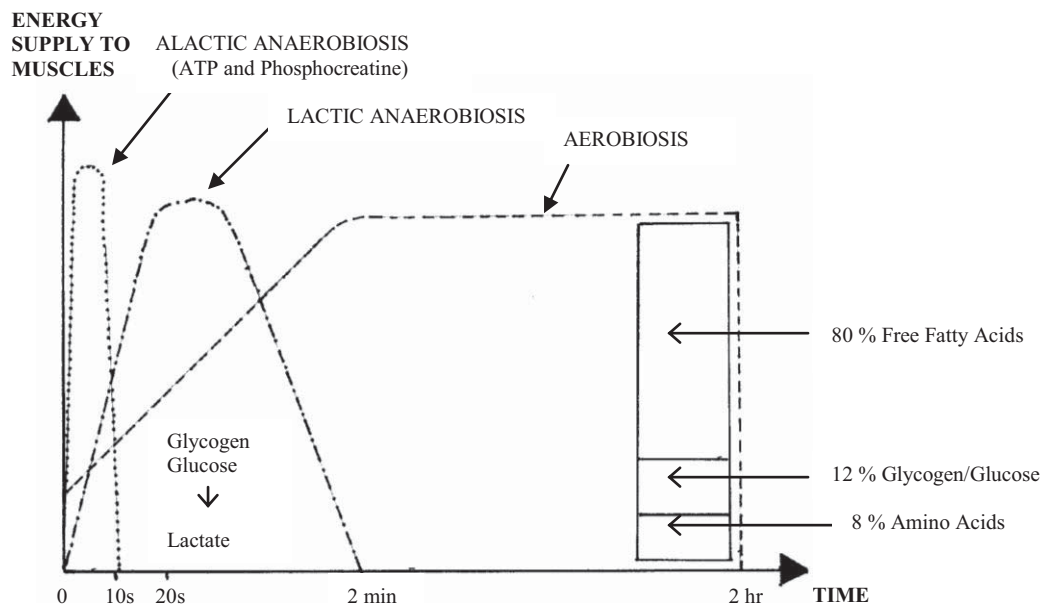
Reaction	ATP Produced		
	Per mole substrate	Per mole O <sub>2</sub>	RQ
Glycogen to lactate	3	-	-
Glucose to lactate	2	-	-
Lactate to CO <sub>2</sub> + H <sub>2</sub> O	17	5.7	1.0
Glycogen to CO <sub>2</sub> + H <sub>2</sub> O	37	6.2	1.0
Glucose to CO <sub>2</sub> + H <sub>2</sub> O	36	6.0	1.0
Free fatty acids to CO <sub>2</sub> + H <sub>2</sub> O	138	5.6	0.71
Acetoacetate to CO <sub>2</sub> + H <sub>2</sub> O	23	5.7	0.73
β-hydroxybutyrate to CO <sub>2</sub> + H <sub>2</sub> O	26	5.8	0.80

RQ= Respiratory Quotient



### 1.6.2 Types of exercise and energy sources used

The proportion of each energy source used by dogs during exercise is determined by the duration and intensity of the exercise as well as the nutritional status of the animal (Wortinger 2007). In general, dogs rely more heavily on free fatty acids at all exercise intensities compared to humans (Wortinger 2007). However, the source of energy used for muscle work is also dependent on the duration of exercise as shown in Figure 1.5.



**Figure 1.5. Sources of energy for muscle work (Grandjean and Paragon 1992)**

Endurance and intermediate canine athletes undergo predominantly aerobic exercise which is usually of a long duration but lower intensity than sprinters such as greyhounds (Grandjean and Paragon 1992; Burger 1993). These dogs primarily use free fatty acid oxidation (aerobiosis) to provide the ATP required for exercise (Grandjean and Paragon 1992; Burger 1993; Toll and Reynolds 2000). Dogs performing more intermediate endurance exercise may also use energy from the oxidation of glucose (and possibly glycogen), if required during periods of increased intensity. However, as the duration of exercise increases, free fatty acid oxidation becomes more important.

Prolonged exercise results in an increase in plasma free fatty acid levels, which in humans and dogs can be used as an index of non-esterified fatty acid mobilisation (McClelland *et al.* 1995). It has not been established whether all individual non-esterified fatty acid levels increase in parallel or whether certain fatty acids are preferentially mobilised. However, McClelland and co-workers (1995) showed that dogs mobilise 18:1 and 16:0 fatty acids preferentially compared to others, as the concentration of these two non-esterified fatty acids were highest at rest and showed the largest relative increase during exercise. The highest plasma free fatty acid concentration occurs slightly after completion of exercise in the dog (15 minutes after the end of a 40 %  $\text{VO}_2$  max run) and this high level after exercise may have a role in the regulation of fuel oxidation by inhibiting muscle glycolysis and promoting the resynthesis of muscle glycogen due to an increase in the availability of glucose (McClelland *et al.* 1995). Free fatty acid oxidation in dogs allows glycogen stores to be spared, which is beneficial given that depletion of muscle glycogen is a primary factor in the onset of fatigue and therefore reduced performance.

Sprinters such as greyhounds undergo anaerobic exercise which is very intense but only lasts for a few minutes and they rely heavily on the anaerobic metabolism of carbohydrates (glycogen and glucose) for fuel during this period (Burger 1993; Toll and Reynolds 2000). However, one of the by products of anaerobic metabolism of carbohydrates is lactic acid. This accumulates in the muscle as exercise progresses and is then passed into the bloodstream and must eventually be eliminated, as lactate accumulation in the blood (hyperlactacemia) is associated with the onset of fatigue. Blood lactate concentrations usually peak several minutes after exercise finishes and the concentration after exercise provides an indication of the intensity level (Grandjean and Paragon 1992; Burr *et al.* 1997; Kelley *et al.* 2002). In sled dogs serum lactate concentrations can be up to five times higher and in greyhounds they can be up to twenty times higher than baseline after exercise (Burr *et al.* 1997).

Muscle enzyme activity is very pH sensitive therefore pH must be precisely regulated. Some of the short term effects of the increasing concentrations of lactate and  $\text{CO}_2$  during exercise can be reduced by intracellular buffering. However, to avoid more long-term detrimental decreases in pH, acids such as lactic acid must be eliminated (Toll and Reynolds 2000). Both during and after exercise, lactic acid can be eliminated in the cardiac muscle, inactive and active skeletal muscle and the liver (Kelley *et al.* 2002). During recovery after exercise the skeletal muscle can eliminate lactate by glyconeogenesis, oxidation to  $\text{CO}_2$  and water,

transamination to alanine and subsequent incorporation into proteins (Kelley *et al.* 2002). Generally, mammalian muscles are believed to dispose of lactate by oxidation to produce energy in the muscle or conversion back to glucose in the liver through the Cori cycle (Toll and Reynolds 2000). Carbon dioxide has less of an effect on muscle pH than lactate, and is eliminated via the respiratory system or by renal excretion of  $\text{HCO}_3^-$  (Toll and Reynolds 2000). The relative importance of the different metabolic pathways for different types of canine exercise is shown in Table 1.2.

**Table 1.2. Relative importance of different metabolic pathways in the energy generation for canine exercise (Grandjean and Paragon 1992)**

Type Sport	Alactic anaerobiosis	Lactic anaerobiosis	Aerobiosis
Jumping	+++	+	O
Short attack	++	++	+
Greyhound race	+	+++++	++
Agility	O	++++	++
Ring Competition	O	+++	+++
Field Trial	O	++	+++
Sled race	O	O	+++++

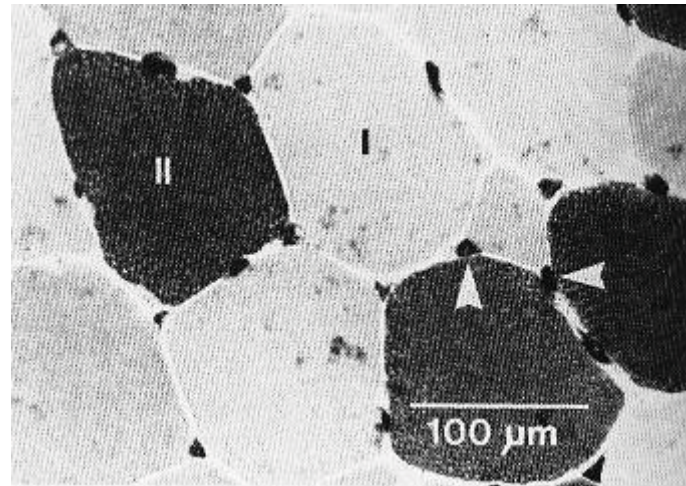
O = relatively unimportant

+ to +++++ = of increasing importance

### 1.6.3 Muscle fibres

The skeletal muscle of dogs is composed of two types of muscle fibre; type I and type II (Figure 1.6). Type I fibres are smaller than type II fibres; they have a higher capillary density, a higher number of mitochondria and are highly oxidative (Toll and Reynolds 2000). These fibres are considered slow twitch fibres and they have a higher capacity for aerobic metabolism, primarily using fat as an energy source (Murphy *et al.* 1997; Wortinger 2007). Type II fibres are larger, contain more glycolytic enzymes, have less oxidising capacity and are stronger than type I fibres (Toll and Reynolds 2000). These are the fast twitch fibres and

rely more on anaerobic metabolism of glucose and glycogen as an energy source (Murphy *et al.* 1997).



**Figure 1.6. Transverse section of semitendinosus muscle. Types I (I) and II (II) fibres and capillaries (arrows) are shown. (Rosenblatt *et al.* 1988)**

Endurance athletes generally have more type I slow twitch muscle fibres which are utilised during the long periods of low to moderate intensity exercise that they perform (Toll and Reynolds 2000; Wortinger 2007). Sprinting dogs on the other hand have a higher proportion of type II fast twitch fibres which are adapted to the short, intense exercise they perform (Toll and Reynolds 2000; Wortinger 2007). It has been hypothesised that intermediate athletes have a muscle fibre profile type more similar to endurance athletes than sprinters (Toll and Reynolds 2000; Wortinger 2007).

#### **1.6.4 Changes with exercise**

Training and exercise cause various physiological changes in the dog. The primary by-product of exercise is heat, with approximately 75-80 % of the energy used during exercise being converted to heat. The main way the dog rids itself of this heat is by increasing respiration through the mouth (panting) and nose, with approximately 60 % of heat being lost this way (Young *et al.* 1959; NRC 2006). Panting in dogs involves rapid breathing through the open mouth during which evaporation occurs from the oral surfaces and the tongue. Unlike species such as the horse and humans, dogs do not sweat through the majority of their

skin and the only sweat glands that are used for heat loss by the dog are eccrine sweat glands located between their toes (Case 2005).

Often during intense exercise, the heat produced exceeds the ability of the dog to dissipate it, causing the body temperature to increase (Toll and Reynolds 2000). The rectal temperatures of greyhounds after a 30 second race have been shown to increase more than 1°C (Toll *et al.* 1995) and those of retrievers by up to 3°C during a training drill (Steiss *et al.* 2004). During exercise the dog's heart rate also increases from a resting level of approximately 60 to 150 beats per minute (bpm) (Steiss *et al.* 2004), to rates as high as 300 bpm, however, within a minute of exercise ceasing this has usually decreased to approximately 150 bpm (Hammel *et al.* 1977).

Post exercise blood glucose levels remained unchanged from baseline levels in several studies using beagles, sled dogs and mongrel dogs (Nazar *et al.* 1992; Hinchcliff *et al.* 1993; Burr *et al.* 1997; Chanoit *et al.* 2002); however they have been shown to increase during sprint exercise in greyhounds (Snow *et al.* 1988; Ilkiw *et al.* 1989; Rose *et al.* 1989). Concentrations of free fatty acids and triglycerides have also been shown to increase after exercise in some sled dog and greyhound studies (Hammel *et al.* 1977; Snow *et al.* 1988) and these may indicate the degree of lipid metabolism (Rovira *et al.* 2007).

Serum lactate concentrations following exercise may provide an indication of the exercise intensity for the dog, with more intense exercise resulting in higher lactate concentrations (Burr *et al.* 1997). This, along with differences in energy generation for muscles (i.e. aerobic or anaerobic), may explain why dramatic increases in lactate concentrations are not generally seen in sled dogs undergoing endurance exercise of lower intensity but large increases are often seen after high intensity, short exercise in greyhounds (Snow *et al.* 1988; Ilkiw *et al.* 1989; Burr *et al.* 1997).

Free radicals and reactive oxygen species increase with exercise and the concentrations of antioxidants in the blood decrease, both of which occur as a result of the increase in oxidative stress caused by exercise (Baskin *et al.* 2000; Marshall *et al.* 2002; NRC 2006). This oxidative stress can also lead to muscle damage. This damage can be assessed by measuring plasma creatine kinase (CK) which is found mainly in skeletal muscle, myocardium and brain tissue (Burr *et al.* 1997). High levels of aspartate aminotransferase (AST) can also indicate

muscle damage but this enzyme is less organ specific than creatine kinase as it is also found in the liver (Burr *et al.* 1997). Various studies of sled dogs have shown increased serum activities of CK and AST after exercise (Hinchcliff *et al.* 1993; Burr *et al.* 1997; Hinchcliff 1998; McKenzie *et al.* 2007). However a study using beagles found no significant changes in the activities of these enzymes after a 60 minute run (Chanoit *et al.* 2002). It is generally well accepted that high levels of CK and AST indicate muscle damage however, it is not known what levels of the two markers are correlated to fatigue or impaired performance (Burr *et al.* 1997).

Other changes that have been reported in the body in response to exercise include variations in plasma volume, red blood cell numbers, packed cell volume, capillary density, mitochondrial volume, muscle fibre volume and the levels of haemoglobin, haematocrit, serum protein, creatinine, electrolytes and the activities of various enzymes (Dobson *et al.* 1988; Snow *et al.* 1988; Ilkiw *et al.* 1989; Sneddon *et al.* 1989; Toll *et al.* 1995; Reynolds *et al.* 1996; Burr *et al.* 1997; Hinchcliff *et al.* 1997; Matwichuk *et al.* 1999; McKenzie *et al.* 2007; Rovira *et al.* 2007; Wortinger 2007). The majority of the studies that determined some or all of these variables were conducted in greyhounds and sled dogs and the results are inconsistent and conflicting. For example, significant increases in total plasma protein concentration following exercise have been reported in greyhound studies (Snow *et al.* 1988; Ilkiw *et al.* 1989; Rose and Bloomberg 1989) and a study using Labrador Retrievers (Matwichuk *et al.* 1999), however significant decreases following exercise have been reported in sled dog studies (Hinchcliff *et al.* 1997; Hinchcliff *et al.* 1998; McKenzie *et al.* 2007) and no changes following exercise were reported in studies using sled dogs and agility dogs (Hinchcliff *et al.* 1993; Rovira *et al.* 2007).

The results of studies investigating the above changes with exercise in various types of working dogs are varied, with some reporting increases, some reporting decreases and others finding no significant differences. Therefore, it would be hugely beneficial if more reliable biochemical markers associated with exercise in dogs could be identified, that are indicative of fatigue or relative performance, however to date none have been identified.

### **1.6.5 Feeding regimens**

When feeding the working dog there are many factors to consider, including the type and frequency of the work, level and severity of exercise, distance covered, terrain, environmental conditions, age, bodyweight and body condition.

Many different feeding regimens have been recommended for working dogs, with the actual regimen used ultimately dependant on owner preference. However, most use the portion control method where a specific amount of food is given to each dog at each meal. This method allows the owner to control the amount fed, and to rectify any changes seen in the dog's appetite, bodyweight or condition.

Ultimately working dogs should be fed when the food will increase energy production during exercise and will not affect performance; therefore it is unwise to feed large amounts of food immediately before exercise. In fact, working dogs should not be fed for at least four hours before they are worked (Toll and Reynolds 2000; Wortinger 2007) to ensure changes caused by eating have time to normalise. Bowel emptying is also promoted, which will minimise extra faecal weight. Dogs ideally should be fed their main meal within two hours after work is completed in order to replenish muscle glycogen stores (Toll and Reynolds 2000; Wortinger 2007).

The exact number of meals fed to working dogs each day varies greatly, however a lot of owners tend to feed their dogs their daily requirements in one or two meals depending on their preference, schedule and the intensity and duration of work the dog is undertaking. Ideally working dogs should be fed several small meals per day, as this prevents them having to eat large volumes of food at once when they are often too tired to eat and only have a limited stomach capacity.

### **1.6.6 Dietary requirements**

Water is crucial to working dogs because dogs, unlike humans, do not sweat to any great extent; and instead they lose water through respiration (Case 2005). During exercise water losses can increase by ten to twenty times and even mild dehydration in working dogs can affect their performance (Wortinger 2007). Therefore, dogs should have plenty of opportunity to drink before, during and after exercise to ensure adequate hydration. One study carried out in dogs by Young and co-workers (1960) demonstrated that to exploit the

dog's full potential, between 1.2 and 1.6 litres of water was required during exhaustive treadmill exercise ranging from 175 to 920 minutes at 5.84 km/hr.

Due to their enormous energy expenditure, working dogs require a highly palatable, energy dense, complete and balanced diet (Burger 1993; Grandjean and Paragon 1993). They also require one which is highly digestible; minimum levels of digestibility suggested for working dogs range from 82 % (Guilford 1993) to 90 % (Burger 1993).

The exercise working dogs undergo, causes physical and psychological stress which increases these dog's requirements for energy and various other nutrients. Dogs exercising in adverse weather conditions can have even higher requirements than other working dogs (Agar 2001). Stressed dogs have normal or slightly enhanced appetites but unfortunately the amount of food they can eat is limited by their gastrointestinal capacity. Many dogs may also be too tired to eat large amounts of food, further supporting the idea of needing to feed an energy and nutrient dense highly digestible diet (Agar 2001).

#### **1.6.6.1 Energy**

The amount of extra energy working dogs require is different for each individual dog and is dependant on the duration and intensity of exercise, environmental conditions and characteristics of the dog such as age, sex and bodyweight.

Dogs taking part in intermediate type work such as hunting and herding have energy requirements that are much greater than adult maintenance requirements. Studies have reported a range of appropriate energy levels for these dogs; from 1.5 to 2.5 times (Case 2005) to 1.5 to 6 times maintenance levels (Buffington *et al.* 2004). Guilford (1993) suggested 2 to 3 times maintenance, or feeding 70.4 to 110 kcal of food per kilogram of bodyweight per day. Due to the lack of research regarding these types of canine athletes, more studies are required to accurately define the energy needs for the various working dogs that fall into this intermediate workload category.

Endurance dogs, and dogs working in harsh climatic conditions, such as sled dogs, have the highest energy requirements of all working dogs, with their requirements estimated to be 2 to 5 times (Wortinger 2007), to up to 10 times maintenance requirements (Hill 2006; Buffington *et al.* 2004). Male dogs with a mean body weight of 40.2 kg running 10.9 km per day for a



total of 932.4 km required an intake of between 18 and 25 MJ (GE) per day (Campbell and Donaldson 1981). Hinchcliff and co-workers (1997) reported dogs (male and female) with a mean bodyweight of 23 kg running a 490 km race at 7 km per hour (in temperatures of between -10 and -35 °C ) had an energy expenditure of approximately 47.1 MJ per day.

Sprinting dogs that only race short distances once or twice a week (e.g., greyhounds) have been reported to have energy requirements only slightly above those of moderately active pet dogs (Buffington *et al.* 2004). Other reports recommend greyhounds receive between 1.5 and 3 times maintenance energy requirements (Buffington *et al.* 2004; Hill 2006; Wortinger 2007).

A general rule for the energy requirements of working dogs proposed by Ackerman (1999) is that dogs require an extra 10 % calories for every hour of work they do. Other authors suggest that dogs greater than 10 kg in weight require approximately 1 kcal ME per kg of body weight and smaller dogs require 1.6 to 1.9 kcal ME per kg of bodyweight for each km travelled, on top of normal maintenance requirements (Hill 2006). Using these figures, a 12 kg dog would require an extra 120 kcal ME above maintenance requirements for a 10 km race, while a 6 kg dog would require between 96 and 114 kcal ME above maintenance requirements. These requirements are increased in cold temperatures because the dog needs extra energy to keep warm in addition to the extra energy they require for work. The accuracy of these or the previous recommendations for the different types of working dogs requires further investigation, and similarly more work needs to be carried out specifically on intermediate working dogs.

#### **1.6.6.2 Carbohydrates**

Although there are little published data on the dietary carbohydrate requirements of dogs compared to other species, most literature reports that there are no absolute dietary requirements for carbohydrate in pet dog or working dog diets provided there is enough protein in the diet to supply the precursors required for gluconeogenesis (Burger 1993; Ackerman 1999; Wortinger 2007).

One study using sled dogs did however demonstrate that the performance of the dogs improved when the dietary carbohydrate content was decreased from 22 % to 0 %, suggesting the optimal carbohydrate intake for these working dogs is relatively low, possibly

approaching zero (Kronfeld 1973). This study showed that dogs fed a zero carbohydrate diet experienced no health problems and could obviously synthesise the glucose required from amino acids and glycerol despite the increased glucose synthesis and use caused by exercise (Kronfeld 1973).

Starch sources can be beneficial in working dog diets, maintaining normal muscle and liver glycogen levels (Case 2005). However, when carbohydrate is included in the working dog diet it should be highly digestible and concentrated, as excess undigested carbohydrate can increase water loss, the production of colonic gas and faecal bulk, adding unneeded weight to the dog.

Despite these studies, the National Research Council (2006) has set a maximum dietary carbohydrate level for dog diets of 65 % on a DM basis or 52 % ME of the diet.

### **1.6.6.3 Proteins**

Various studies in working dogs have suggested they may have a higher requirement for dietary protein due to the increased anabolism (synthesis) and catabolism (degradation) of protein and amino acids caused by exercise (Reynolds *et al.* 1999; Case 2005; NRC 2006). Synthesis of protein increases with exercise due to the changes that occur with training, which include increases in total plasma protein, plasma volume, capillary density, mitochondrial volume, muscle fibre volume and the total mass and activity of many of the enzymes involved in energy generating reactions (Reynolds *et al.* 1999; Case *et al.* 2000). Protein synthesis also increases to replace proteins and amino acids catabolised during exercise to produce energy and those lost as precursors of gluconeogenesis (NRC 2006). Studies have shown that protein utilization is not only greater in exercising versus sedentary subjects but also increases with the duration of the exercise.

Exercise stimulates amino acid production, predominantly branched chain amino acids. Of these the most investigated is leucine because its oxidation rates are higher than isoleucine or valine (Mero 1999; Hargreaves and Snow 2001; Tarnopolsky 2004). Leucine stimulates protein synthesis in the muscle (Mero 1999) and is closely associated with the release of gluconeogenic precursors such as alanine from the muscle (Mero 1999). Studies have shown that after exercise there is a significant decrease in plasma and serum levels of leucine; 11-33 % for aerobic exercise, 5-8 % for anaerobic exercise and 30 % for strength exercise (Mero

1999). Also during exhaustive aerobic exercise there is a decrease in leucine and glycogen levels in skeletal muscle (Mero 1999; Tarnopolsky 2004). An increase in lysine oxidation has also been reported in endurance exercise (Tarnopolsky 2004). It has been suggested that consumption of branched chain amino acids, particularly leucine before or during endurance exercise may decrease the net rate of protein degradation and may increase performance and spare muscle glycogen stores. Further studies are required to establish this however.

Following exercise the synthesis of protein rebounds for less than 48 hours, however protein catabolism remains elevated and therefore a positive balance of protein can only be achieved if amino acid availability is increased also (Tarnopolsky 2004).

Endurance dogs, such as sled dogs have been shown to require high levels of dietary protein ( $\geq 32$  % on an ME or 42 % on a DM basis) because anaemia develops during training when these dogs are fed a lower protein diet. A study by Toll and Reynolds (2000) also illustrated that a minimum of 25 to 30 % of the calories from dietary protein may help prevent or reduce the number of injuries in working dogs. In contrast, a study in greyhounds showed they ran more slowly when fed a high protein diet (36 % on an ME basis) compared to a lower protein diet (24 % on an ME basis) and therefore it has been suggested that protein levels should be less for sprinting dogs than for endurance dogs. Regardless of the precise level of protein included in working dog diets, all protein must be of a high quality to ensure a high digestibility and allow the dog to utilise this protein fully. Furthermore, it is vital that the absorbed amino acid pattern from the protein fed meets the needs of the animal. During exercise the amino acid requirements of the animal change due to changes associated with exercise including increased glucose requirements and the increased oxidation of branched chain amino acids such as leucine.

#### **1.6.6.4 Fats**

Dietary fat is important for working dogs as it increases the palatability and dietary energy density of the food, allowing the dog to eat enough calories without having to ingest large volumes of food. Fat, because of its high palatability also encourages eating in times of stress and fatigue, particularly for endurance and intermediate athletes (Toll and Reynolds 2000). Increasing the level of fat in dog food usually increases its digestibility and may also enhance fatty acid availability (Buffington *et al.* 2004; Wortinger 2007).

High fat diets are recommended for working dogs, and current recommendations are for 49 % ME from fat as adequate for endurance working dog diets (NRC 2006; Wortinger 2007). Generally, as the duration of exercise increases so should the fat content of the diet, indicating that greyhounds do not require as much fat in the diet as endurance dogs such as sled dogs (Grandjean and Paragon 1993). In fact sprinting dogs such as greyhounds may only require approximately 10 % dietary fat on a DM basis, and current recommendations for dietary fat levels for sprinting dogs such as greyhounds is 30 % on a ME basis.

## **1.7 Dietary strategies for working dogs**

There has been significant debate surrounding the best way to provide the energy required in working dog diets and therefore many different dietary strategies have been proposed by academics, owners, breeders and trainers. The diet must provide the optimal mixture of fuels and optimal level of nutrients to maintain health, minimise injury and produce optimum performance (Kronfeld *et al.* 1994, Toll and Reynolds 2000).

To complicate matters, the dog is one of the most diverse mammalian species and therefore dogs have a wide range of athletic abilities and perform very different types of work. For the dog to perform to its genetic potential it is important that the dog's nutrition is matched to the type of work (i.e., intensity, duration and frequency) they undergo (Toll and Reynolds 2000), therefore the optimal diet for farm dogs may be very different to that for greyhounds or search and rescue dogs.

It is also important to ensure that the diet does not impact on the animal's immune system in a negative way. To date there is limited work on immunity in the dog, and what is available tends to focus on the addition of nutraceuticals to enhance immunity and the effects of aging on the canine immune system (Greeley *et al.* 1996; Greeley *et al.* 2001; Heaton *et al.* 2002; Hayek *et al.* 2004). The lack of information regarding the effects of changes in dietary macronutrient proportions on any facets of the canine immune system means it is important to establish if any effects exist or not. Although theoretically if the diet provides adequate nutrition and meets the dog's requirements there should be no reason for any detrimental immune effects, unless perhaps there is a problem with the animals' health to begin with, or if the dietary changes were to create problems with absorption of vital nutrients.

### 1.7.1 Carbohydrate loading

In humans, muscle glycogen content has been shown to be an important determinant of stamina, and the idea of carbohydrate loading was developed from this by Swedish physiologists and is now practiced widely amongst human athletes (Burger 1993). The aim of carbohydrate loading is to maximise muscle glycogen stores before exercise, through feeding a high carbohydrate diet combined with training (Burger 1993).

In the late 1960s, muscle glycogen concentrations in humans were shown to increase significantly in response to feeding a high carbohydrate diet during a sub-maximal training regimen (Bergstrom *et al.* 1967; Reynolds *et al.* 1997). Early in the 1980s it was further shown that muscle glycogen concentrations return to pre-exercise levels within 24 hours of exercise if humans ate at least 500 g of carbohydrate per day (Costill *et al.* 1981; Reynolds *et al.* 1997), and that the rate of muscle glycogen repletion after exercise was significantly influenced by the time of carbohydrate ingestion (Ivy *et al.* 1988; Reynolds *et al.* 1997). Carbohydrate loading both before and carbohydrate ingestion during moderate intensity exercise (70 to 75 % maximum oxygen uptake) have been reported to increase the time to exhaustion (Laurie Rauch *et al.* 1998) in humans.

Humans like dogs, use carbohydrates, fats and to a much lesser extent proteins as fuels for exercise. However, in humans, unlike in dogs, despite fatty acids stored in adipose tissue and fats entering circulation after meals serving as potential energy sources, fatty acid oxidation is limited and therefore carbohydrates represent the major fuel source for exercise (Hawley *et al.* 1998). A study by Christensen and Hansen (1939) was probably the first to show when humans are fed high fat diets they become exhausted earlier compared to when they are fed high carbohydrate diets (88 minutes versus 210 minutes). The main issue with this study however was that the high fat diet used was very extreme (94 % MJ), and therefore likely deficient in protein. Another study demonstrating negative effects of feeding high fat diets to humans exercising was reported by Bergstrom and co-workers in 1967. They demonstrated that time to fatigue was impaired by 66 % after three days of feeding a high fat diet (54 % MJ) compared to a high carbohydrate diet. In contrast, one study did report a positive effect of humans consuming high fat diets. In this study five out of the six subjects ran approximately 32 % longer after eating the high fat diet (38 % fat, 50 % carbohydrate) compared to eating their habitual diet (24 % fat, 61 % carbohydrate) and approximately 20 % longer compared to eating a high carbohydrate diet (15 % fat, 73 % carbohydrate) (Muoio *et al.* 1994).

Carbohydrate loading has been suggested for use with canine athletes as a means of improving stamina. However, it does not appear beneficial and appears to risk a variety of abnormalities for this species. In dog studies (particularly endurance dog), carbohydrate loading has been shown to have varying results. Downey and co-workers found dietary carbohydrate intake was negatively correlated with time to fatigue in Beagles that were run to exhaustion on a treadmill (Downey *et al.* 1980) and attempts to carbohydrate load sled dogs has been shown to produce tying up and coprophagy (Kronfeld 1973).

The carbohydrate feeding regimen may seem more suited to greyhounds than endurance dogs because anaerobic sources of energy (i.e. carbohydrates) are more important to sprinters than to endurance athletes such as sled dogs (NRC 2006). However, the point at which the possible benefits of increasing carbohydrate levels in the greyhound diet are outweighed by the need to decrease dietary fat levels still needs to be investigated (NRC 2006).

### **1.7.2 Fat adaptation**

Fat adaptation (feeding a high fat diet during training) has been found to improve aerobic performance in dogs by enhancing fatty acid oxidation, thus sparing the use of muscle glycogen and glucose and decreasing lactate accumulation (Kronfeld *et al.* 1977; Reynolds *et al.* 1995).

Endurance and intermediate athletes who partake in prolonged exercise of low to moderate intensity rely heavily on fat as a fuel, with muscle fibres providing energy through the aerobic oxidation of fatty acids (Orme *et al.* 1997). The intermediate athlete is thought to get the majority of its energy (approximately 70 to 90 %) from the metabolism of fat, and only a very small proportion from carbohydrate metabolism (NRC 2006).

Therefore, for these two types of canine athletes, feeding a high fat diet will prepare the muscles to efficiently mobilise and use free fatty acids for energy (Burger 1993; Ackerman 1999). This in turn, spares the use of muscle glycogen and glucose, prolonging muscle glycogen stores and thus increasing stamina (NRC 2006). There is also an adaptive increase in free fatty acid oxidation during training which is associated with an increase in the number of mitochondria and again a decrease in the use of muscle glycogen and blood glucose (Burger 1993).

Various studies have been published which support the benefits of this feeding regimen for intermediate and endurance dogs. Kronfeld and co-workers (1994) found that a carbohydrate free, high fat diet produced advantages for prolonged running in sled dogs. A champion team of Huskies that had shown 'tying up' using a carbohydrate loading strategy displayed better performance as the carbohydrate in the diet was replaced by dietary fat and protein.

For dogs participating in endurance exercise it is suggested a high fat (>50 % fat on an ME basis), low carbohydrate diet can increase stamina and maximise energy production (Hill 1998), however more work is needed to confirm this.

### **1.7.3 Fuel mixes for different canine athletes**

Many studies have been carried out in dogs feeding diets varying in quantities of fat, carbohydrate and protein (NRC 2006). However, just how the energy in the diet should be partitioned between the three macronutrients has been debated for the past 25 to 30 years (Reynolds *et al.* 1996).

Various recommended proportions of macronutrients in sled dog diets have been published, but nearly all of them suggest a diet high in fat, high in protein and low in carbohydrate (Burger 1993; Grandjean and Paragon 1993; Ackerman 1999; Toll and Reynolds 2000; Hill *et al.* 2001; Buffington *et al.* 2004; McKenzie *et al.* 2005). Burger (1993) reported that the "ideal" diet for sled dogs contains maximum fat consistent with optimal protein requirements for stress and work and some complex carbohydrates for 'feed-ability'. The National Research Council (2006) recommends 35 % of dietary ME as protein and 49 % ME as fat for dogs partaking in endurance exercise.

Other reports for sled dogs recommend fat levels of 50 % or more of dietary ME (Burger 1993; Hill 1998; Toll and Reynolds 2000; Buffington *et al.* 2004; McKenzie *et al.* 2005), protein levels greater than 25 % and as high as 40 % of dietary ME (Hill 1998; Toll and Reynolds 2000; Hill *et al.* 2001; Buffington *et al.* 2004; McKenzie *et al.* 2005). Recommended carbohydrate levels are between 0 and 20 % of dietary ME (Toll and Reynolds 2000; Buffington *et al.* 2004; McKenzie *et al.* 2005).

As with sled dogs, a number of different macronutrient proportions have been suggested for sprinting dogs such as greyhounds, but most studies agree that diets for these dogs should

contain moderately high levels of fat, moderate levels of protein (high levels have been shown by Hill (1998) and Hill *et al.* (2001) to be detrimental to the performance) and higher levels of carbohydrate than in endurance diets because as previously shown in humans it enables quick restoration of muscle glycogen after anaerobic exercise (Ackerman 1999).

The NRC (2006) recommends a diet containing approximately 24 % ME as protein and 30 % ME as fat for greyhounds. Other authors suggest fat levels of between 30 and 50 % on an ME basis (Hill 1998; Toll and Reynolds 2000), protein levels of between 24 and 27 % ME (Hill 1998; Ackerman 1999; Toll and Reynolds 2000) and carbohydrate levels of between 38 and 46 % ME (Toll and Reynolds 2000).

## 1.8 Comparisons with the Cat

Like dogs, cats are one of the most popular companion animals today and rely on their owners to provide them with an optimal source of nutrition. The goals of feline nutrition are also very similar to those of canine nutrition, with the exception of the need to support performance.

The domestic cat (*Felis catus*) is a member of the order Carnivora and family Felidae, which also includes the lion, tiger and puma (MacDonald and Rogers 1984). The taxonomy of the cat is also shown in Figure 1.1.

Cats and their wild ancestors have been in existence for a very long time with fossil records suggesting they appeared 35 million years ago (O'Brien *et al.* 2008). However, like the dog, there is much debate surrounding the exact date cats were domesticated. Despite nearly 40 % of felid species having been tamed on all continents except Europe and Oceania, only 1 species was domesticated; *Felis silvestris* (Faure and Kitchener 2009). The hypothesised ancestor of the domestic cat is the African wildcat (*Felis silvestris lybica*) (Agnarsson *et al.* 2010), and recent genetic and archaeological evidence supports the domestic cats origin in Mesopotamia. This domestication probably coincided with the development of agricultural villages here about 10,000 years ago (Driscoll *et al.* 2007). However, it is believed full domestication may only have occurred in ancient Egypt and that early domestic cats here were probably tamed wildcats imported from Mesopotamia (Faure and Kitchener 2009).

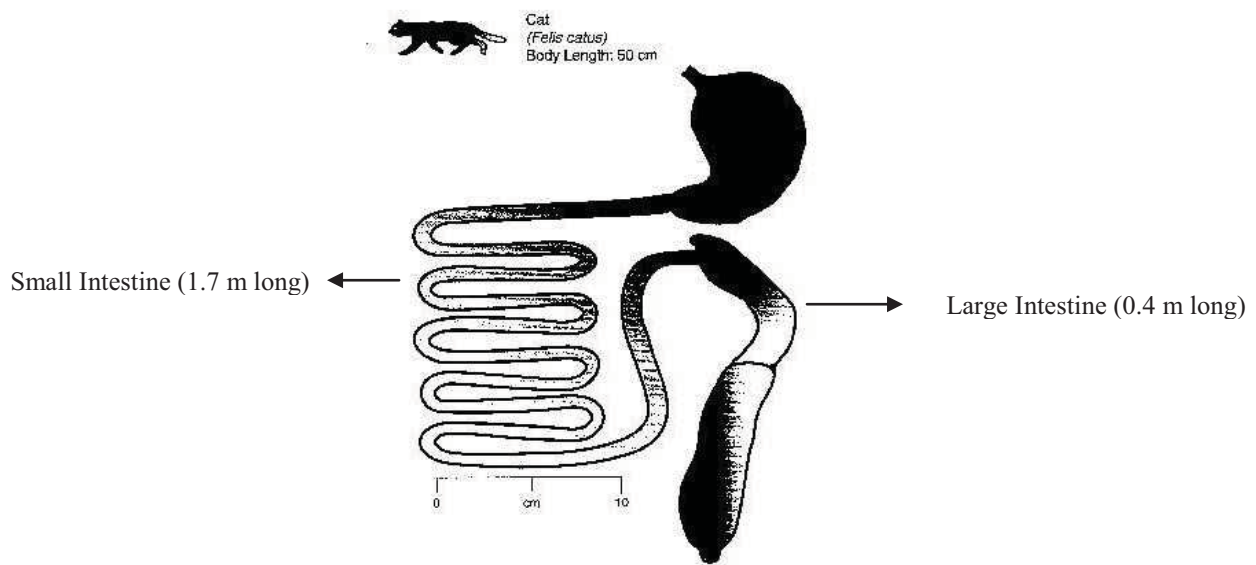


The relationship between humans and cats has changed from being exploitative through to utilitarian as a pest controller and even executioner to objects of strong cultural and emotional importance as gods or pets (Faure and Kitchener 2009). Unlike other species domesticated for agricultural or transport purposes, the cat's relationship with people was predominantly commensal with cats feeding on pests infesting the grain stores of the first farmers (Clutton-Brock 1999).

The family Felidae shows much less diversity than the Canidae. Whereas wild dogs hunt in packs, with the exception of lions, wild felids are solitary hunters. Domestic cats can be described as opportunistic predators which can exploit a wide range of prey depending on their environment, however generally mammals are the most frequently eaten prey (Jones and Coman 1981). Cats if allowed free access to food typically eat 12 to 20 smaller meals per day during both day and night time, whereas in contrast dogs eat approximately 8 meals per day predominantly during day time (Mugford and Thorne 1980). The dentition of the cat reflects both its strictly carnivorous diet; the incisors and canines are used to apprehend and kill prey, the premolars are pointed for piercing and holding prey and the molars are used for slicing and crushing prey (Van Valkenburgh 2007).

In the cat, the most abundant groups of taste buds are those that respond to amino acids, followed by those that respond to acids such as phosphoric and carboxylic acids, and nucleotide tri phosphate. Cats prefer amino acids which are described as 'sweet' by humans such as proline, cysteine, lysine, histidine and alanine that stimulate these groups and reject 'bitter' amino acids such as arginine, isoleucine and phenylalanine which inhibit them (Bradshaw *et al.* 1996).

The cat's digestive tract is very similar to that of the dog except it is shorter (Figure 1.7); the intestines are 2.1 metres long compared to 4.5 metres in the dog, and the caecum in the cat is not coiled like in the dog (Stevens and Hume 1995).



**Figure 1.7. Cat Gastrointestinal Tract (Stevens and Hume 1995)**

In contrast to the dog's more omnivorous diet, cats are strict carnivores, relying on the nutrients found in animal tissues to meet their unique requirements (Zoran 2002). Due to this meat based diet, cats are metabolically adapted to preferentially use protein and fat as energy sources rather than carbohydrate (Kienzle 1993 a; Kienzle 1993 b; Zoran 2002). This unique metabolism mandates that regardless of the protein content of the diet, cats use protein to maintain blood glucose concentrations and therefore have a limited ability to spare protein utilisation. Subsequently a high protein diet is required.

Despite the cat relying on gluconeogenesis to meet its daily energy requirements (Tartellin 1991), there is evidence cats can effectively utilise dietary glucose from starch digestion (Kienzle 1993 a; Kienzle 1993 b). However the rate to which starch is digested by the cat and the resulting glycaemic and insulinaemic responses vary greatly depending on the starch source and the degree of food processing (Englyst *et al.* 1999). The cat liver exhibits no activity of glucokinase; one of the rate limiting enzymes in glycolysis. Glucokinase in the liver plays a key role in regulating glucose uptake and release and therefore the feline liver can take up and utilise less glucose than the dog because of this lower glucokinase (Washizu *et al.* 1999).

Cats also have increased requirements for amino acids and require the same essential amino acids as the dog, except they are unique in that they also require a dietary source of the

sulphur containing amino acid taurine (Baker 1991). Taurine is important for vision, cardiac muscle function and for the functioning of the reproductive, nervous and immune systems (Zoran 2002). The reasons for this are because cats have both an inadequate synthesis of taurine from methionine and cysteine and also exhibit a high physiological demand for taurine (Zoran 2002). The enzymes required for the synthesis of taurine are only minimally active in cats. The high demand in cats is because unlike most animals which can respond to changes in dietary taurine and glycine concentrations by adjusting the ratio of the bile acid conjugates containing these two amino acids, cats only conjugate bile acids with taurine, regardless of the concentration of taurine in the diet (Baker 1991; Zoran 2002).

Although arginine is essential for both cats and dogs, the consequences of arginine deficiency are much more dramatic in the cat. This can be attributed to the cats' inability to synthesis ornithine or citrulline from glutamine or glutamic acid, for conversion to arginine (Baker 1991; Zoran 2002). This inability is due to the enzymes involved in this synthesis; pyrroline 5-carboxylate synthase, ornithine aminotransferase, carbamoyl phosphate synthase and ornithine carbamoyl transferase being very low in activity in the cat. In addition, cats constantly use large quantities of arginine in the urea cycle because this cycle isn't down-regulated during periods of food deprivation or when the diet consumed is low in protein.

Domestic cats also have higher methionine and cysteine requirements compared to the dog. Methionine and cysteine are gluconeogenic amino acids that are catabolised to pyruvate and oxidised to provide energy in the cat (Zoran 2002).

Most animals can make vitamin A from precursors such as beta carotene, however cats lack the enzyme that begins the conversion of carotenoids to retinol and therefore require a source of preformed vitamin A in the diet (Schweigert *et al.* 2002).

Cats can tolerate and digest high levels of dietary fat and like the dog, require certain fatty acids are included in their diet. Linoleic acid and alpha linolenic acid are essential for all animals and most animals convert these through the action of elongase and desaturase to arachidonic acid and EPA and DHA which can only be found in food of animal origin. Cats however only have little activity of desaturase and therefore require foods containing these fatty acids.

All of these biological and metabolic peculiarities in the cat strictly relate to their carnivorous nature and their subsequent need for a high protein diet.

## **1.9 Where to from here?**

Research ascertaining the effects of different diets on companion animals such as dogs and cats at both rest and with exercise could be beneficial in preventing and treating health issues and also for the owners, breeders and trainers of working dogs. The ability to alter the metabolism of animals by manipulating their diet could have large implications worldwide in a number of species. Such information would enable owners to tailor their animal's diet to their specific needs, such as to improve the health of dogs and cats with certain medical conditions such as diabetes mellitus and to optimize the performance of working dogs. Research of this kind may also lead to possibilities for research in the future including studies examining the mechanisms by which diet can be used to alter an animal's metabolism and how the diet can affect muscle and tissue metabolism specifically.

Today there is a high rate of incidence of feline diabetes mellitus and a large number of dogs and cats which are obese. Despite this, and the fact that cats evolved on a strictly carnivorous diet which contains negligible quantities of carbohydrates (Rand and Marshall 2005) and dogs also evolved on a meat based diet (Legrand-Defretin 1994), today's commercially available cat and dog foods are relatively high in carbohydrates (Appleton *et al.* 2004), diets which are probably not ideal for these species given the prey they have evolved eating. It is also possible that these high carbohydrate diets could be at least partially responsible for the increase in the rates of diabetes and obesity in cats and dogs that have been observed over the past few decades. For this reason it would be of great interest to establish if altering the proportions of these macronutrients in the diet can alter cat's and dog's metabolism and whether this would be advantageous to these species in any way.

In addition, due to the clear lack of information on working dogs other than greyhounds and sled dogs, clearly a large amount of research needs to be conducted into all aspects of working dog nutrition and exercise physiology. Studies are required on all aspects of exercise and nutrition for intermediate canine athletes such as farm dogs, agility dogs and police dogs, as their nutritional needs may be quite different to those of the extremes of greyhounds and sled dogs. The research into farm dog requirements would be especially relevant for New

Zealand, where farming is a large and important industry and farm dogs play vital roles in daily farm management.

The exact energy requirements for different working dogs should be ascertained, as should the absolute minimum carbohydrate requirement for these dogs and optimal levels of dietary protein, fat and carbohydrate for different working dogs. The effect of different dietary fat types on performance needs to be studied and whether certain fatty acids are preferentially mobilised during canine exercise. In addition to this, optimal levels of other nutrients, such as vitamins and minerals for different levels of exercise need to be determined in the dog.

Establishing a standard protocol for aerobic and anaerobic exercise tests in dogs would also be beneficial as it would enable direct comparison of results from multiple trials. Also of great importance is determining a reliable, easy to measure and repeatable parameter (or parameters) to use to measure fitness, performance and stamina and to determine the onset of fatigue in these animals.

This thesis was therefore conducted to evaluate the effects of dietary macronutrients on animal metabolism by determining apparent digestibility, post-prandial glycaemic and insulinaemic responses, large intestinal carbohydrate fermentation, weight maintenance, exercise performance, immunity and fuel utilisation during exercise in working dogs (exercise or at rest) and/or cats (at rest). A high-protein, low-carbohydrate diet was expected to prove to be beneficial for dogs at rest and exercising, and cats because of their naturally carnivorous diets and the literature currently available.

## **Chapter Two**

### **The effects of the proportions of dietary macronutrients on the digestibility, post-prandial endocrine responses and large intestinal fermentation of carbohydrate in working dogs**

Chapter 2 has been published as a scientific article (Appendix 1): **Hill SR, Rutherford-Markwick KJ; Ravindran G; Ugarte CE; Thomas DG.** (2009). The effects of the proportions of dietary macronutrients on the digestibility, post-prandial endocrine responses and large intestinal fermentation of carbohydrate in working dogs. *New Zealand Veterinary Journal* 57(6): 313-318.

## 2.1 Introduction

A large proportion of the canine population in New Zealand is working dogs, partaking in long bouts of exercise requiring sustained energy. Working dogs, such as those used for herding and hunting, have much greater energy requirements than pet dogs at the same stage of life (Case 2005c). As a result, larger amounts of food are needed; food that must be highly digestible, highly palatable and, more importantly, energy dense (Toll and Reynolds 2000).

The dog's recommended allowance of dietary protein and fat for maintenance is well established as 100 g/kg and 55 g/kg of diet, respectively (NRC 2006). Working dogs have higher requirements for dietary protein than those reported for maintenance due to the increased synthesis and degradation of protein caused by exercise (Case 2005c); however, an exact minimum requirement has not been set. It has also been suggested that high-protein diets may help reduce the occurrence of musculoskeletal injuries in working dogs and therefore may be advantageous for these animals (Reynolds *et al.* 1996). A recommended carbohydrate allowance for dogs in general, has also not been established; however, the National Research Council in 2006 did set a maximum level of carbohydrate in dog diets as 52 % of the energy in the diet.

Despite the widespread belief that a source of carbohydrate is not necessary in the adult dog's diet, as long as there is enough protein and fat to provide the necessary glucose precursors (Toll and Reynolds 2000; Case 2005b), most commercial pet foods contain significant quantities of carbohydrate due to their low cost. Energy from carbohydrates is rapidly released for utilisation by animals, which means that carbohydrates may not be the optimal source of energy for these working dogs that require a steady supply of energy over an extended period of time. Furthermore, diets that help to minimise large fluctuations in blood glucose, thus improving the glycaemic response and controlling hyperglycaemia, may not only help working dogs performing long bouts of exercise by providing them with a sustained energy source, but may also help many breeds of dogs with health issues including obesity and diabetes mellitus (Case 2005d).

Digestibility is a measure of the proportion of nutrients in the diet that are actually available for metabolic use by the animal. A highly digestible diet contains a low proportion of indigestible ingredients and therefore less diet will need to be fed to meet the animal's

nutritional requirements. The apparent digestibility of a food does not consider endogenous losses of nutrients and can be determined using either of two standard methods, the use of an indigestible marker, or a total faecal collection (AAFCO 2009). Studies comparing these two methods in both working and pet dogs are limited. However, a study by Carciofi and associates (2007) showed that the results obtained from both methods agree relatively closely, ( $p > 0.05$ ). It was of interest to see if the two methods would produce similar results in the current study in this group of working dogs as the indigestible marker method may be more practical for studies using working dogs in the field, where total faecal collection is not possible.

Large intestinal fermentation of carbohydrate is another important issue to assess in working dogs as it results in inefficient use of the diet, and commonly causes watery diarrhoea due to the osmotic movement of fluid into the small intestine, decreased intestinal transit time, and reduced colonic absorption of fluid (Washabau *et al.* 1986a, b). Breath-hydrogen tests have been widely used to evaluate large intestinal fermentation of carbohydrate and oro-caecal transit time in humans, dogs and cats (Papasouliotis *et al.* 1995; German *et al.* 1998; Spohr *et al.* 1999). In normal healthy fasted cats and dogs, when there is complete absorption of carbohydrate, there are minimal amounts of hydrogen excreted in the breath (<5 ppm) (German *et al.* 1998). However, when carbohydrate absorption is incomplete, the unabsorbed carbohydrate is fermented by colonic bacteria to produce hydrogen and some of this is released in the breath (Washabau *et al.* 1986a, b; Papasouliotis *et al.* 1995; Spohr *et al.* 1999). The rate of production of breath hydrogen can be influenced by the type and number of colonic bacteria, and the amount of carbohydrate available in the diet (Muir *et al.* 1991).

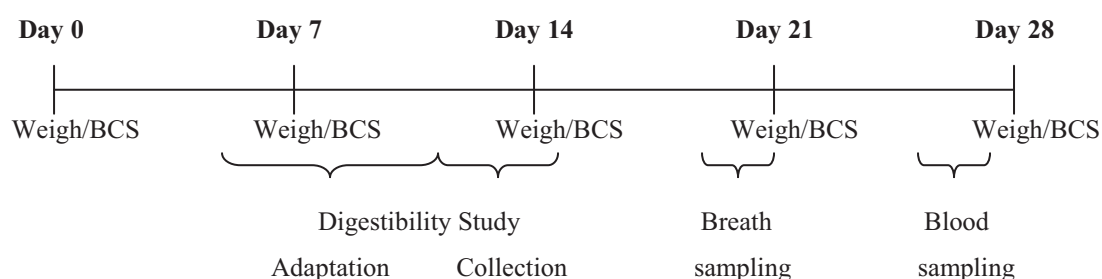
This study was undertaken to compare the effects of dietary macronutrient proportions on the metabolism of working dogs at rest. In particular the effects on apparent digestibility, glycaemic and insulinaemic responses, and large intestinal fermentation of carbohydrate were investigated. The apparent digestibility of both diets was determined using two standard methods in an attempt to identify the degree of variation associated with each, and to establish which method is best suited for future studies with working dogs. It was hypothesized that the macronutrient profiles of the two diets would effect the dog's digestion, absorption and metabolism differently, with the high-protein, low-carbohydrate diet tested being more digestible and providing better glycaemic control and therefore a more sustained source of energy to these working dogs.



## 2.2 Materials and methods

### 2.2.1 Animals and housing

Twelve (n=5 females and n=7 males) healthy entire adult Harrier Hounds (*Canis familiaris*), aged from 2 to 8 (mean 4.17, SEM 0.54) years, were obtained from the Manawatu Hunt Club, Palmerston North, New Zealand, and used in a 4-week trial (Figure 2.1). Harrier hounds were selected because they are a genetically similar population of dogs, the work they partake in is very similar to that of farm dogs, and therefore it was believed they were a good model of the working farm dog in New Zealand.



**Figure 2.1. Time-line of the study to determine the effects of the proportions of dietary macronutrients on the digestibility, post-prandial endocrine responses and large intestinal fermentation of carbohydrate of two diets fed to 12 working dogs.**

The dogs were considered to be physically fit, having just completed a hunt season. The dogs used in this study had been used in previous studies and were therefore familiar with the environment and management procedures at the unit. All of the dogs were trained to be handled, walked on a leash, restrained for sampling procedures and exercised on a treadmill.

At the beginning of the trial, and at weekly intervals until the end of the trial the dogs were weighed (Tru-test AG500 series scales, Tru-test Pty Limited, Sydney, Australia), and their body condition score assessed subjectively by the same personnel, using a standard scale (Laflamme 1997). The dogs were housed in pairs, except for during the digestibility study and breath-hydrogen collection when they were housed individually, in purpose-built concrete pens measuring approximately 2.9 x 5.5m (Figure 2.2), at the Canine Unit, Massey University, Palmerston North, New Zealand. The pens were cleaned daily, and all faeces

were scored, to aid in the monitoring of animal health, using a 5-point scale, where 1 indicated liquid faeces with no shape, and 5 well-formed faecal pellets. All dogs were exercised daily for approximately 30 minutes, and had free access to fresh water at all times. All management and experimental procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.



**Figure 2.2. Purpose-built concrete pens where the dogs were housed during the study to determine the effects of the proportions of dietary macronutrients on the digestibility, post-prandial endocrine responses and large intestinal fermentation of carbohydrate of two diets fed to 12 working dogs.**

### **2.2.2 Diets and feeding**

On arrival the dogs were randomly assigned to either Diet 1 or Diet 2, with equal numbers of dogs on each diet (n=6), and the groups were balanced as close as possible for gender. The dogs were fed the assigned diet for the duration of the study.

Diet 1 comprised of a low-carbohydrate, high-protein dry baked biscuit diet manufactured specifically for the study by Heinz Watties Limited (Hastings, New Zealand), and Diet 2 comprised a high-carbohydrate, low-protein dry baked biscuit diet available commercially

(Champ Max; Heinz Watties Limited, Hastings, New Zealand). The daily food allowance (adult maintenance energy requirements; MER) for each dog was determined using the equation:  $MER = 132 \times \text{bodyweight (kg)}^{0.67}$  (Case 2005b). The food allowance for each dog was increased or decreased by 10 % per one point change in body condition score, to maintain individual ideal body condition. The diets were weighed daily, and any food refusals were recorded daily.

The diets were analysed for moisture, using a convection oven at 105°C; ash, using a furnace at 550 °C; crude protein, using the leco total-combustion method; and fat, using acid hydrolysis/Mojonnier extraction (AOAC 1995). Gross energy (kJ/g) was determined using bomb calorimetry (Leco AC350, Leco Corporation, St Joseph, MI, USA). Insoluble, soluble and total dietary fibres were determined, using the enzymatic-gravimetric method (AOAC 1995). Carbohydrate was calculated by difference: (100 %-moisture %-fat %-protein %-ash %) (Table 2.1). Digestible energy (DE) was calculated using the equation outlined by AAFCO (2009), and then converted to kJ/g:

$$DE = 1 - \left( \frac{\text{Gross energy of faeces} \times \text{Titanium dioxide (TiO}_2\text{) in food}}{\text{Gross energy of food} \times \text{TiO}_2 \text{ in faeces}} \right) \times \text{Gross energy of food}$$

**Table 2.1. Dietary analysis<sup>a</sup> of Diet 1 and Diet 2 fed to 12 Harrier Hounds, in order to determine the effects of the proportions of dietary macronutrients on the digestibility, post-prandial endocrine responses and large intestinal fermentation of carbohydrate.**

<b>Nutrient</b>	<b>Diet 1</b>	<b>Diet 2</b>
Moisture (%)	10.3	6.5
Crude protein (%)	49.4	21.6
Crude fat (%)	17.7	14.9
Total dietary fibre (%)	8.3	10.4
Insoluble fibre (%)	7.2	8.9
Soluble fibre (%)	1.1	1.5
Ash (%)	21.0	18.9
Carbohydrate <sup>b</sup>	13.3	45.3
Gross energy (kJ/g)	21.7	18.8
Digestible energy (kJ/g)	19.0	15.5

<sup>a</sup> All results are on an ‘as received’ basis

<sup>b</sup> Calculated by difference

### **2.2.3 Digestibility of the diets**

During the digestibility study an indigestible marker, TiO<sub>2</sub>, was added to the diets at an inclusion rate of 0.3 % of total daily food allowance (Rutherford *et al.* 2007). Five percent of the dogs’ daily allowance of diet was ground, mixed with the required TiO<sub>2</sub> and enough water to form a thick paste. The paste was then poured over the remainder of the dog’s daily allowance of biscuits (95 %) and offered to the dogs. The diets were fed for 10 days, with the first 6 days serving as an adaptation period, and the final 4 days for the total collection of faeces. The faeces produced by each dog, including any excreted during exercise, were collected daily, using gloves and metal scrappers, weighed (Mettler Toledo SB32000, Mettler Toledo Incorporated, Columbus, USA), and frozen at -20°C.

The faeces were freeze-dried (FD18 freeze dryer, Cuddon Engineering, Christchurch, NZ) for approximately 190 hours, reweighed and ground using an ultracentrifugal mill (Retsch ZM100, F.Kurt Retsch GmbH & Co., Haan, Germany) to pass through a 0.5 mm screen, pooled for each dog, and analysed for dry matter, crude protein, crude fat, ash and gross energy, using the methods described previously (AOAC 1995).

The percentage TiO<sub>2</sub> in the faeces was measured using colourimetry, where the sample was ignited at 500°C to burn off all organic material and the remaining minerals digested to release titanium which was then determined using a colorimetric assay at 410nm (Short *et al.* 1996). The apparent dry matter, crude protein, fat, energy and carbohydrate (by difference) digestibility of the two diets, using the two methods (total collection and indigestible marker), were calculated using the equation outlined by AAFCO (2009):

Apparent digestibility (total-collection method) = (total intake-total excreted)/total intake

Apparent digestibility (indigestible-marker method) = (nutrient in diet-nutrient in faeces)/nutrient in diet x 100

where nutrient in faeces = (amount in faeces/TiO<sub>2</sub> in faeces) x TiO<sub>2</sub> in diet.

#### **2.2.4 Postprandial changes in concentrations of glucose and insulin**

The glucose and insulin concentrations in plasma were determined from all animals, over a two day period (n=3 animals on each diet per day), after the consumption of a single meal. The time between the last meal and the baseline sample was 24 hours. One hour before feeding, a 2 ml baseline blood sample was obtained from the jugular vein of each dog. A single meal consisting of their full day's allowance (determined using the equation for MER) was fed, and then further 2 ml blood samples were collected 10, 20, 30, 45, 60, 75, 90, 120, 240 and 480 minutes after consumption of the meal (all the dogs consumed the entire meal). Sample collection was staggered for each dog (2.5 minutes apart) so all six dogs were sampled within a 15 minute window.

The 2 ml blood samples were split into vacutainers; 0.5 ml into a vacutainer containing fluoride oxalate, and 1.5 ml into a vacutainer containing heparin (Becton-Dickinson, Franklin Lakes NJ, USA), for glucose and insulin analysis, respectively. Immediately after mixing, the samples were refrigerated on ice, then centrifuged at 3,000 rpm for 15 minutes, to separate the plasma, which was stored at -20 °C until it was analysed. Concentrations of glucose in plasma (mmol/L) were analysed using the hexokinase method, using a Gluco-quant kit (Roche Diagnostics, Basel, Switzerland). Concentrations of insulin in plasma (μU/ml) were

analysed using the Linco HI-14HK insulin kit (Linco Research Incorporated, St Charles MO, USA). All samples were analysed in a single run of the respective assays.

The integrated area under the glucose and insulin curves (AUC) were calculated using the trapezoidal method, for the 480-minute period after the meal, using the Microsoft Excel-based program PK functions (Usansky JI, Desai A, Tang-Liu D, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine CA, USA).

### **2.2.5 Post-prandial changes in the concentration of breath hydrogen**

The dogs were trained to accept an anaesthetic mask placed over their muzzle during a seven day period prior to sampling. During this period, the dogs were fed their daily MER in a single meal in the morning. Breath samples were collected one hour before feeding (baseline), and then 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 14 hours after feeding. Samples were collected using the same apparatus and protocol used for previous studies in dogs (Washabau *et al.* 1986a, b; Papasouliotis *et al.* 1995) where an anaesthetic mask was connected to a 2-L latex bag with a three-way stopcock attached to the base via a one-way valve (Figure 2.3).



**Figure 2.3.** Apparatus used to collect post-prandial breath samples, in order to determine the effects of the proportions of dietary macronutrients on large intestinal fermentation of carbohydrate when Diet 1 and Diet 2 were fed to 12 Harrier Hounds.

The dog was allowed to breathe into the mask (Figure 2.4) until the bag was full, before the bag was detached, emptied of all air, re-attached and the dog filled the bag for a second time. This process minimised any contamination from the previous dog's sample and dead space. Once the bag was filled for a second time, the mask was removed, and two samples were drawn from the bag into 20 ml syringes, using the three-way stop cock.



**Figure 2.4. Collection of post-prandial breath samples to determine the effects of the proportions of dietary macronutrients on large intestinal fermentation of carbohydrate when Diet 1 and Diet 2 were fed to 12 Harrier Hounds.**

The samples were analysed for hydrogen concentration (ppm), using an exhaled hydrogen monitor (GMI Medical Limited, Renfrew, UK) (Figure 2.5). An average hydrogen concentration for each dog for each sampling time was determined from the two samples collected.



**Figure 2.5. Analyser used to establish the concentration of hydrogen in the breath of 12 Harrier Hounds fed Diet 1 and Diet 2 to determine the effects of the proportions of dietary macronutrients on large intestinal fermentation of carbohydrate.**

### **2.2.6 Statistical analysis**

The apparent digestibility of the two diets, determined using the indigestible-marker and total-collection methods, was compared using two-way ANOVA. Comparisons were included in the model to analyse differences between male and female dogs within and between dietary groups. The mean concentration of breath hydrogen, concentration of glucose and insulin in plasma  $\pm$  SEM were calculated at each time-point. Changes in the concentrations of glucose and insulin in plasma were calculated separately for each post-meal period by using the plasma concentration before the meal as a baseline. Post-prandial responses were compared for maximum increase, time to peak increase, and incremental area under the glucose and insulin curves for both diets, and sex of dogs.

These parameters were then analysed using ANOVA, to determine any significant differences between the diets or sexes, and between baseline and maximum values for each variable. Differences between diets at each of the time-points for concentration of hydrogen, glucose and insulin were analysed using Repeat Measures ANOVA, to determine any differences between the diets at any of the time-points. The ratio of concentrations of glucose-to-insulin was also calculated for the dogs fed the two diets at each of the time-points, and these, as well as the differences between the sexes, were compared using repeat measures ANOVA. All analyses were conducted using SAS v 9.1 (SAS Institute Inc, Cary NC, USA).



## 2.3 Results

All dogs remained in good health throughout the trial, and maintained ( $p>0.05$ ) their initial bodyweights (Diet 1: mean 24.4 (SEM 1.8) kg versus Diet 2: mean 26.6 (SEM 1.1) kg). The faecal scores observed during the study were not different for dogs fed the two diets ( $p>0.05$ ); with the mean faecal scores for dogs fed Diet 1 and Diet 2 being 3.1 and 3.2, respectively. However, the dogs fed Diet 2 produced more ( $p<0.01$ ) faeces compared to those fed Diet 1 ( $267.2 \pm 11.4$  g/day vs  $206.2 \pm 12.5$  g/day respectively). This was not due to differences in the volume fed as the dogs fed Diet 2 actually consumed slightly less than those fed Diet 1 ( $396.0$  g/day vs  $398.7$  g/day respectively). However, this difference in intake was not significant ( $p>0.05$ ).

### 2.3.1 Digestibility of the diets

The apparent nutrient digestibility coefficients of the two diets determined using the indigestible-marker and total-collection methods are summarised in Table 2.2. The effect of the type of diet was significant for all parameters ( $p<0.001$ ). The apparent digestibility values for dry matter, crude protein, fat and energy were higher ( $p<0.05$ ) for Diet 1 than Diet 2, however the apparent carbohydrate digestibility was lower ( $p<0.001$ ) for Diet 1 than for Diet 2. The apparent crude protein and fat digestibility values were not different between the two methods of determination ( $p>0.10$ ), but the values determined using the total-collection method were lower for carbohydrates ( $p=0.03$ ), and tended to be lower for dry matter ( $p=0.08$ ) and energy ( $p=0.07$ ), than for the indigestible-marker method within diets. No significant effects of gender were observed for any of the parameters ( $p>0.05$ ).

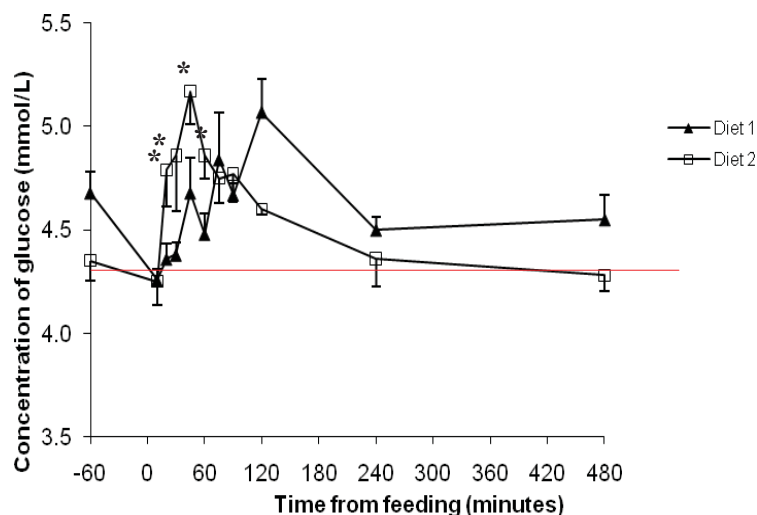
**Table 2.2. Mean apparent nutrient digestibility coefficients of two diets fed to 12 Harrier Hounds (n=6 per diet), determined using the indigestible-marker (Marker) and total-collection (Total C) methods. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 was a high-carbohydrate, low-protein diet.**

Coefficient	Diet 1		Diet 2		Pooled SEM
	Marker	Total C	Marker	Total C	
<b>Dry matter</b>	0.818	0.807	0.767	0.752	0.016
<b>Crude Protein</b>	0.888	0.874	0.795	0.769	0.017
<b>Fat</b>	0.976	0.973	0.960	0.956	0.004
<b>Carbohydrate<sup>a</sup></b>	0.639	0.594	0.814	0.797	0.014
<b>Energy</b>	0.885	0.871	0.841	0.824	0.012

<sup>a</sup> Calculated by difference

### **2.3.2 Post-prandial changes in concentrations of glucose and insulin**

The changes in plasma glucose concentrations in response to the consumption of the two diets are shown in Figure 2.6. These indicate real differences between the dogs fed the two diets because the baseline values were similar ( $p>0.05$ ). The majority of the glucose concentrations were within the normal glucose range (Figure 2.6), with none above the upper limit of this range and only at time 0 and 480 minutes did any value come close to falling or fall below the lower limit of this range.



**Figure 2.6. Mean ( $\pm$  SEM) concentration of glucose in plasma determined from serial blood samples ( — lower limit of normal glucose range for dogs, upper limit is greater than the y axis), collected from 12 Harrier Hounds before and after a single meal of either Diet 1 or Diet 2 (\* $p < 0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

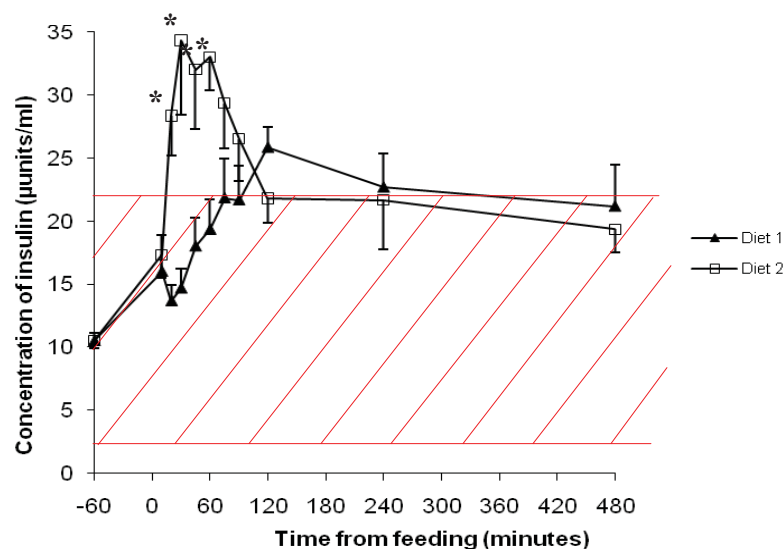
Peak concentrations of glucose in plasma occurred earlier ( $p < 0.01$ ) in dogs fed Diet 2 compared with those fed Diet 1 (Table 2.3). However, the maximum concentration ( $C_{max}$ ) of glucose and the total amount of glucose released by the dogs fed the two diets were not significantly different ( $p > 0.05$ ).

**Table 2.3. Characteristics of plasma glucose curves determined from serial blood samples collected from 12 Harrier Hounds, following a single meal of either two diets. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

Parameter	Diet 1	Diet 2	Pooled SEM
Peak concentration (mmol/L)	5.30	5.38	0.11
Time to peak (minutes)	101.62*	35.83	6.96
Area under curve [(mmol/L)/minutes]	2,216	2,151	25.12

\*Difference between diets ( $p < 0.01$ )

The changes in concentrations of insulin in plasma for the two diets are shown in Figure 2.7. The mean values for dogs consuming both diets rose above the normal range. For dogs consuming Diet 2 concentrations of insulin in plasma were well above the normal range at every time-point from 20 to 120 minutes after feeding, while for dogs on Diet 1, concentrations of insulin peaked above the normal range at 120 and 240 minutes after feeding. The insulin concentrations determined for dogs fed Diet 2 were significantly ( $p<0.05$ ) higher compared to those for the dogs fed Diet 1, 20, 30, 45 and 60 minutes after feeding.



**Figure 2.7.** Mean ( $\pm$  SEM) concentration of insulin in plasma determined from serial blood samples ( normal insulin range for dogs), collected from 12 Harrier Hounds before and after a single meal of either Diet 1 or Diet 2 ( $*p<0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.

The peak concentrations of insulin in plasma was reached earlier ( $p<0.0001$ ) in the dogs fed Diet 2 compared with those fed Diet 1 (Table 2.4). The  $C_{max}$  of insulin of dogs on Diet 2 was also higher ( $p<0.05$ ) than that of Diet 1 (Table 2.4). However, there was no difference in the total amount of insulin released, as determined by AUC, by dogs fed the two diets over the time measured.

**Table 2.4. Characteristics of plasma insulin curves determined from serial blood samples collected from 12 Harrier Hounds, following a single meal of either two diets. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

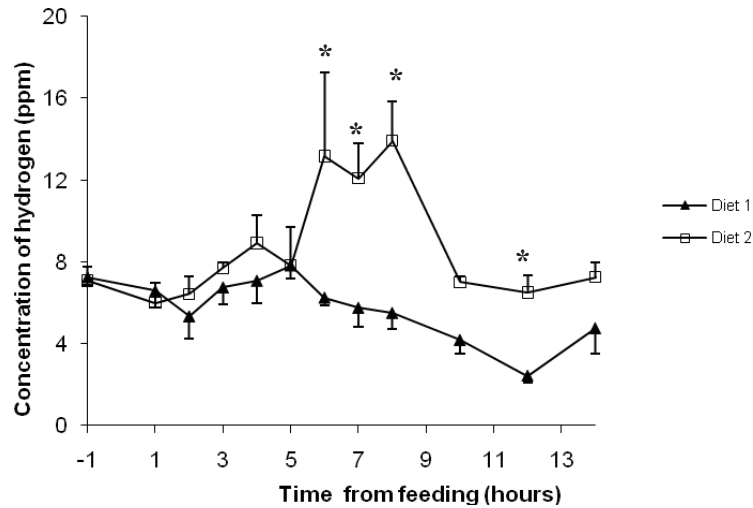
<b>Parameter</b>	<b>Diet 1</b>	<b>Diet 2</b>	<b>Pooled SEM</b>
Peak concentration ( $\mu\text{U/ml}$ )	27.94	40.00*	2.63
Time to peak (minutes)	104.71*	40.83	5.33
Area under curve [ $(\mu\text{U/ml})/\text{minutes}$ ]	10,532	11,132	860

\* Difference between diets ( $p < 0.05$ )

The ratio of glucose-to-insulin concentration in plasma was significantly higher for Diet 1 than Diet 2 at 20 and 30 ( $p < 0.001$ ), 45 ( $p < 0.01$ ), and 60 ( $p < 0.05$ ) minutes after feeding. No significant effects of gender were determined for any of the glucose or insulin parameters ( $p > 0.05$ ).

### **2.3.3 Post-prandial changes in the concentration of breath hydrogen**

Changes in the concentration of breath hydrogen for dogs fed the two diets are shown in Figure 2.8. For Diet 2, the concentration of breath hydrogen increased rapidly from 5 hours after feeding, and reached a peak concentration of 19.17 ppm approximately 8 hours after the meal. This  $C_{\text{max}}$  was significantly higher than the mean baseline concentration of 7.08 ppm ( $p < 0.01$ ). The  $C_{\text{max}}$  for Diet 1, reached approximately 3 hours after feeding, was much lower (8.44 ppm) but was still significantly higher than the mean baseline level of 7.25 ppm ( $p < 0.05$ ).



**Figure 2.8.** Mean ( $\pm$  SEM) concentration of breath hydrogen determined from serial breath samples, collected from 12 Harrier Hounds before and after a single meal of either Diet 1 or Diet 2 (\* $p < 0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.

The  $C_{max}$  of breath hydrogen occurred significantly ( $p < 0.01$ ) earlier following the consumption of Diet 1 than Diet 2, but the  $C_{max}$  and AUC were significantly greater for Diet 2 than Diet 1 (Table 2.5). There were no significant effects of gender for any of the parameters ( $p > 0.05$ ).

**Table 2.5.** Characteristics of breath-hydrogen curves determined from serial breath samples collected from 12 Harrier Hounds, following a single meal of either two diets. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.

Parameter	Diet 1	Diet 2	Pooled SEM
Peak concentration (ppm)	8.44	19.17*	1.46
Time to peak (hours)	3.24	7.00*	0.43
Area under curve (ppm/hour)	73.46	139.58*	8.19

\* Difference between diets ( $p < 0.01$ )

## 2.4 Discussion

Establishing any effects of different dietary macronutrient profiles on metabolism in working dogs is of interest because of the need to meet their energy and nutrient requirements, as well as to maximise their performance. In the present study, feeding a high-protein, low-carbohydrate (49 % protein, 13 % carbohydrate) dry biscuit diet (Diet 1) compared with a commercial high-carbohydrate (22 % protein, 45 % carbohydrate) dry diet (diet 2) demonstrated that the high-protein diet offered higher apparent nutrient digestibility, a slower release of glucose into the bloodstream, and a reduction in large intestinal carbohydrate fermentation.

The higher apparent digestibility of Diet 1 in this study, for all nutrients except carbohydrate, suggested that it conferred significant advantages over Diet 2, meaning the dogs could absorb a greater concentration of nutrients and thus requiring less of the diet to meet their nutrient requirements, and producing less excrement (Grandjean and Paragon 1993). In line with this, in this study the dogs consuming Diet 1 produced significantly less excrement than those consuming Diet 2 (206.2 g/day vs 267.2 g/day respectively), despite consuming slightly more (398.7 g/day vs 396.0 g/day respectively). Based on examination of the pens, faeces and trends in faecal weights for each of the dogs, there didn't appear to be any coprophagia occurring with these dogs. However, without having someone watching all of the animals 24 hours a day, this can not be excluded as a factor contributing to the differences seen between the two methods of digestibility determination observed. The digestibility of Diet 2 may have been reduced compared with that of Diet 1 due to poorer-quality ingredients, differences in processing techniques or simply the different composition of the diet. The similar digestibility results for the two methods of determination obtained in this study indicated that either the indigestible-marker method or total-collection method is suitable for use in research into the health of working dogs, although the indigestible-marker method may be more practical for use in field situations. The total-collection method did produce lower carbohydrate digestibility values for both diets; however, the reason for this difference remains unclear. It may possibly be due to some level of coprophagy occurring that was not detected or issues with the ability to collect absolutely all of the faeces produced.

Carbohydrates fermented in the large intestine are less available to the animal, and together with poorly digested carbohydrate result in the diet being utilised less efficiently. Thus, the

extent of large intestinal fermentation of carbohydrate, as indicated by breath hydrogen concentrations, as well as carbohydrate digestibility, was examined during this study. The carbohydrate digestibility of Diet 2 was significantly higher than that of Diet 1, possibly due in part to Diet 2 containing a much higher proportion of carbohydrate. The fasted breath-hydrogen values determined in this study were slightly higher (between 5 and 9 ppm) than those reported by both Washabau *et al.* (1986a) of <1 ppm, and German *et al.* (1998) of <5 ppm. However, the present study showed a clear peak in breath hydrogen concentration in dogs consuming Diet 2 that was much higher, and produced a larger AUC, compared with dogs consuming Diet 1. This indicates that significantly more large intestinal fermentation of carbohydrate occurred after feeding Diet 2.

The normal concentration range for plasma glucose in dogs is between 4.34 and 6.93 mmol/L (Plumb 2002). Glucose concentrations determined during the current study were generally within this range irrespective of diet. However, some values were slightly, but not significantly ( $p>0.05$ ) below the lower limit. The normal concentration range for plasma insulin in dogs is 2 - 22  $\mu\text{U}/\text{mL}$  (Plumb 2002). The mean values for dogs on either diet in the current study rose above this normal range. Our findings showed that the insulin response was higher and occurred earlier in dogs consuming Diet 2. This was expected as the peak plasma glucose concentrations also occurred earlier in this diet and insulin should peak shortly after glucose to lower blood glucose levels. The higher ratio of glucose-to-insulin concentrations determined for the dogs fed Diet 1, indicated that these animals had better glucose tolerance than the dogs fed Diet 2. It was believed the effect of stress from the dogs being restrained and blood-sampled would not have had a significant effect on the concentrations of glucose and insulin in plasma determined during this study, as all dogs were handled daily and had been de-sensitised to restraint. Whether the differences seen in this study could be attributed specifically to changes in the protein and carbohydrate levels of the diets, or to a difference in some other ingredient or in the quality of any ingredient is uncertain. However, large quantities of carbohydrate and also highly digestible carbohydrates have previously been reported to produce a rapid increase in blood glucose levels, and therefore a large increase in the demand for insulin (Case 2005d). It is also possible that the increasing amount and type of dietary protein could alter post-prandial responses.

From this study, as seen by the similar results obtained using the two methods of digestibility determination, it also appears that either of the two methods of determination of digestibility



would be suitable for use in studies on working dogs; however the indigestible-marker method obviously would be more appropriate for studies in the field where total faecal collection is not practical or possible. Further research is required to test these theories, and to identify the exact dietary change responsible for the differences seen between these two diets. It would be of benefit, in future studies, to alter only the carbohydrate and protein levels in the diets, keeping everything else constant, to allow for a clearer comparison and to also conduct further glucose and insulin tolerance tests, to determine the effectiveness of the glucose/insulin axis.

In conclusion, as indicated by the time to peak glucose and insulin concentrations and the glucose to insulin ratio, the low-carbohydrate, high-protein Diet 1 developed for this study has the potential to be beneficial to working dogs subjected to long strenuous exercise that requires a sustained release of energy (Nguyen *et al.* 1998; Bennett *et al.* 2006; Carciofi *et al.* 2006). This diet, being high in protein and low in carbohydrate, is likely to be not only closer to the predatory diet the dog evolved on and that was eaten by its wild ancestors (Case 2005a) but also appears to confer advantages to the dogs in this study, including higher digestibility of protein and fat, slower release of glucose into the bloodstream, and less large intestinal fermentation of carbohydrate. These benefits, particularly the indication of better glycaemic control, as hypothesised before the study, may be especially useful for dogs with diseases such as diabetes mellitus that require tight blood glucose control. It is possible that similar changes in macronutrient proportions may be advantageous to other companion animals including the cat, as the incidence of diabetes mellitus and obesity is increasing worldwide in this species (Sloth 1992; German 2006). Unlike the dog, cats have evolved on a strictly carnivorous diet, yet as is the case with dog foods, many of the commercially available cat diets are high in carbohydrates. It would therefore be of interest to see if a diet similar to the one developed for this study (i.e. high-protein, low-carbohydrate) can provide similar benefits to cats by minimising fluctuations in blood glucose levels, thereby helping to prevent and control feline diabetes mellitus.

## **Chapter Three**

**The effects of the proportions of dietary  
macronutrients on the digestibility and post-prandial  
endocrine responses in cats**

### 3.1 Introduction

Cats as obligate carnivores evolved to eat meat-only diets containing high levels of protein, moderate levels of fat and negligible levels of carbohydrate (Zoran 2002; Biourge 2005; Rand and Marshall 2005; Kirk 2006; Backus *et al.* 2007). The cat, mink and fox display the highest requirements for protein; with the adult cat requiring approximately four to five times the protein of humans, dogs and rats (Rogers and Morris 1982).

The cat has developed several adaptations that reflect their carnivorous diet and subsequently low carbohydrate intake (Kienzle 1993 a; Kienzle 1993 b; Zoran 2002). Like dogs, cats lack the enzyme responsible for initiating carbohydrate digestion in the mouth (salivary amylase) (Kienzle 1993 a; NRC 2006). Cats also possess low activities of intestinal and pancreatic amylases and a reduced activity of intestinal disaccharidases. These enzymes are responsible for the breakdown of carbohydrates in the small intestine (Kienzle 1993 a; Kienzle 1993 b; Zoran 2002). In the liver of the cat, there is also only minimal function of hepatic glycogen synthetase and hepatic glucokinase and furthermore the activity of this latter enzyme is not adaptive when diets varying in carbohydrate content are fed (Morris and Rogers 1982; Zoran 2002).

Cats are therefore metabolically adapted for higher metabolism of protein and lower utilisation of carbohydrate. Subsequently they use dietary protein to maintain blood glucose concentration even when dietary protein sources are limiting (Rogers and Morris 1982; Hoenig 2002; Zoran 2002). Despite the lack of carbohydrates in the natural carnivorous diet of the cat (e.g., a rat carcass only contains approximately 2 % calories from carbohydrate) (Rand and Marshall 2005), today commercial cat diets typically contain large quantities of carbohydrates due to their low cost (often greater than 50 % of total calories) (Zoran 2002; Appleton *et al.* 2004; Rand and Marshall 2005). Therefore, except for their nutritional completeness in terms of vitamin and mineral content, today's commercial pet foods convey little likeness to the natural diet of domestic cats (Bradshaw 2006).

In humans and dogs, levels and sources of certain dietary elements, including carbohydrates, have been shown to stimulate different postprandial endocrine responses than other dietary components such as proteins (Crappo *et al.* 1977; Crappo *et al.* 1981; Coulston *et al.* 1984; Sunvold and Bouchard 1998; Bouche *et al.* 2002; Appleton *et al.* 2004). For example in a

study using dogs, a rice based diet was found to elicit greater postprandial glucose and insulin concentrations compared to sorghum or corn based diets (Sunvold and Bouchard 1998). Similarly, in cats, diets differing in composition can also alter blood glucose and insulin responses post-prandially (Nguyen *et al.* 1998; Rand *et al.* 2004; Biourge 2005; de-Oliveira *et al.* 2008). It may therefore be possible to formulate cat foods to minimise these responses which may aid in the prevention and management of health issues such as obesity and diabetes mellitus (Case 2003; Rand *et al.* 2004).

Today obesity is one of the most common nutritional diseases of domestic cats, and is also a major risk factor for the development of various health issues including feline diabetes mellitus (Butterwick 2000; Zoran 2002; Appleton *et al.* 2004; Hoenig *et al.* 2007; Vester *et al.* 2009). The incidence of feline obesity continues to increase and with it so does the incidence of diabetes mellitus. Therefore prevention of both obesity and diabetes are of particular importance in the cat (Hoenig 2002; Vester *et al.* 2009). A study by Scarlett and colleagues (1994) reported 20 % of cats as overweight and 5 % as obese, whereas a more recent study by Courcier and colleagues (2010) reported a much higher incidence of overweight or obese cats (39 %). Cases of diabetes mellitus in cats have also increased from 8 cases per 10,000 cats in 1970 (Prahl *et al.* 2007) to 124 cases per 10,000 cats in 1999 (Prahl *et al.* 2007). Numerous factors contribute to the development of obesity and diabetes mellitus in the cat, including sex, age, level of activity, diet composition and feeding regimen (Zoran 2002; Kirk 2006; Laflamme 2006) however the role diet plays is of particular interest.

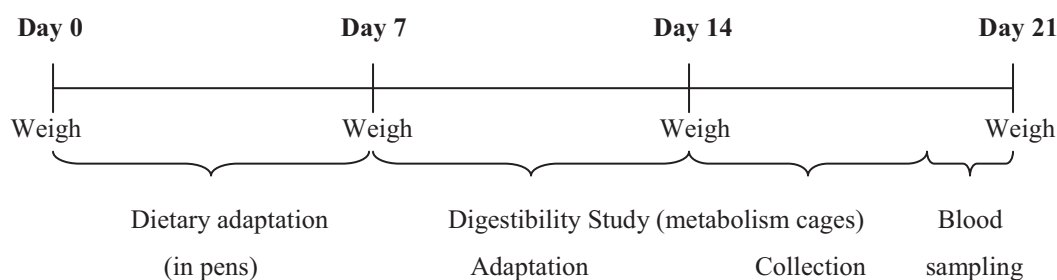
Previous dietary recommendations for the management of obesity and diabetes mellitus in cats were extrapolated from humans and dogs and comprised feeding high fibre, high carbohydrate diets (Zoran 2002; Mazzaferro *et al.* 2003; Biourge 2005; Bennett *et al.* 2006). However, based on the cats nutritional peculiarities and more recent research (Zoran 2002; Biourge 2005; Bennet *et al.* 2006; Kirk 2006), it is believed low carbohydrate, high protein diets (which are also more closely aligned with the composition of the cat's natural diet) may be more beneficial by promoting better glycaemic control, a decreased demand for insulin secretion from the pancreas, maintenance of lean body mass during weight loss and increased satiety (Appleton *et al.* 2004; Biourge 2005; Rand and Marshall 2005; Kirk 2006; Laflamme 2006; Hoenig *et al.* 2007; Vesta *et al.* 2009).

Based on the previous study in working dogs at rest and the strictly carnivorous nature of the cat, it was expected that the glycaemic response observed from feeding a high-protein diet to cats would be smaller compared to a high-carbohydrate diet. The purpose of this study was therefore to compare the effects of different dietary macronutrient proportions on the metabolism of cats at maintenance. The digestibility of the diets and glycaemic responses resulting from their consumption were determined in a group of domestic cats. The apparent digestibility of the high-carbohydrate, low-protein and low-carbohydrate, high-protein diets were determined using the total-collection method. Digestibility measures the concentration of nutrients absorbed from the diet and therefore influences the amount of the diet the animal needs to consume in order to meet its daily energy and nutrient requirements. Serial blood samples were collected using the marginal ear vein prick method and analysed for blood glucose concentrations to compare glycaemic responses between the cats fed the two different diets.

## 3.2 Materials and methods

### 3.2.1 Animals and housing

Sixteen (n=6 entire females and n=10 neutered males) healthy adult domestic short haired cats (*Felis catus*), aged from 1 to 9 (mean 4.56, SEM 2.79) years, were used in the 3-week study (Figure 3.1).



**Figure 3.1. Time-line of the study to determine the effects of the proportions of dietary macronutrients on the digestibility and post-prandial endocrine responses of two diets fed to 16 cats.**

The cats were familiar with the environment and management at the Centre for Feline Nutrition (Massey University, Palmerston North, New Zealand), having been born and raised there. In addition, as part of the management of the Centre for Feline Nutrition all of these cats were trained to be handled, weighed and restrained for sampling procedures. Therefore this study was not expected to adversely affect their wellbeing.

The cats were weighed (Mettler Toledo PM11-N, Mettler Toledo Incorporated, Columbus, USA), at the beginning of the trial, and at weekly intervals until the end of the trial to monitor their health and body condition. The cats were housed in two purpose-built colony cages at the Centre for Feline Nutrition, Massey University, Palmerston North, New Zealand in groups of eight (n=3 females and n= 5 males). The cages were cleaned daily as part of normal management procedure and fresh water (changed daily) was available at all times. All management and experimental procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

### 3.2.2 Diets and feeding

The two pens were randomly assigned to either Diet 1 or Diet 2, i.e. one colony cage on each diet. Diet 1 comprised a low-carbohydrate, high-protein dry extruded diet manufactured specifically for the study by Heinz Watties Limited (Hastings, NZ), and Diet 2 comprised a high-carbohydrate, low-protein dry extruded diet available commercially (Friskies; Nestle Purina Petcare, Blayney NSW, Australia).

During the first week of the study, the cats were gradually introduced and adapted to the assigned diets in their colony cages. The daily food allowance (adult maintenance energy requirement; MER) for each cat was determined using the equation:  $MER = 70 \text{ kcal} \times \text{bodyweight (kg)}$ . These requirements were then pooled to give a total daily food allowance for each pen (n=8 cats) (Case 2003; NRC 2006). The food allowance for each colony cage was adjusted weekly based on the cat's bodyweights. The diets were weighed daily (Mettler Toledo PM11-N, Mettler Toledo Incorporated, Columbus, USA), and any refusals were recorded daily. The cats were allowed free access to water throughout the study.

The diets were analysed for moisture, ash, crude protein, fat, gross energy, fibre and carbohydrate using the methods described in Chapter 2, section 2.2.2 (Table 3.1).

**Table 3.1. Dietary analysis<sup>a</sup> of Diet 1 and Diet 2 fed to 16 cats, in order to determine the effects of the proportions of dietary macronutrients on the digestibility and post-prandial endocrine responses.**

Nutrient	Diet 1	Diet 2
Moisture (%)	5.0	7.8
Crude protein (%)	54.3	26.6
Crude fat (%)	13.1	9.3
Crude fibre, %	2.6	2.2
Ash (%)	7.1	7.6
Carbohydrate <sup>b</sup> (%)	20.6	48.7
Gross energy (kJ/g)	21.0	18.0

<sup>a</sup> All results are on an 'as received' basis

<sup>b</sup> Calculated by difference

### 3.2.3 Digestibility of the diets

Once the cats had adapted to their assigned diets, the digestibility of these diets was determined. During the digestibility study, the cats were housed individually in metabolism cages measuring 1.1 m x 0.8 m x 0.8 m and were fed their assigned diet to individual maintenance requirements, using the equation in section 3.2.2 to determine the digestibility of the diets. Any food refusals were recorded to determine daily food intakes. The diets were fed for 12 days, with the first seven days serving as an adaptation period to the metabolic cages, and the final five days for collection of faeces and urine.

Collection of faeces and urine was made using specially designed litter trays (Figure 3.2) which separated the urine and faeces (Hendriks *et al.* 1999) and the faeces were collected daily and weighed (Mettler Toledo PM11-N scales, Mettler Toledo Incorporated, Columbus, USA) and frozen at -20°C. The urine was also collected daily and weighed, before a 10 % sub-sample was removed and frozen. The remaining urine was analysed for pH using an Orion 250A pH meter (Orion Pacific Pty Limited, NSW, Australia) and specific gravity using an Atago UG-1 digital refractometer (Atago Company Limited, Tokyo, Japan) before being discarded.



**Figure 3.2.** Specially designed litter trays used to collect faeces and urine separately, to determine the apparent digestibility of Diet 1 and Diet 2 fed to 16 cats.



The faeces were freeze-dried ( FD18 freeze dryer, Cuddon Engineering, Christchurch, NZ) for approximately 190 hours, reweighed and ground using an ultracentrifugal mill (Retsch ZM100, F.Kurt Retsch GmbH & Co., Haan, Germany) to pass through a 0.5 mm screen. The faeces were analysed for dry matter, crude protein, crude fat, ash and gross energy, using the methods described in Chapter 2, section 2.2.2. The apparent dry matter, crude protein, fat, energy and carbohydrate (by difference) digestibility of the two diets, were calculated using the following equation:

Apparent digestibility = (total intake-total excreted)/total intake (AAFCO 2009)

#### **3.2.4 Post-prandial changes in concentrations of glucose**

Following the digestibility part of this study, post-prandial changes in glucose concentrations were determined in the cats fed both diets. During this time, the cats were housed individually in metabolism cages and were fed their assigned diet to individual maintenance requirements, using the equation in section 3.2.2 to determine the digestibility of the diets.

Glucose concentrations in blood were determined from the cats, after the consumption of a single meal. The time between the last meal and the baseline sample was 16 hours. One hour before feeding, a baseline blood sample was obtained from each cat using the marginal ear vein prick technique (Casella *et al.* 2002). Using the technique, the pinna was first warmed (Figure 3.3), petroleum jelly was smeared over the puncture site and a needle lancet (each cat had its own lancet) was used to pierce the skin and produce a small drop of blood.



**Figure 3.3. Warming the cat's pinna prior to taking a blood sample using the marginal ear vein pick technique, to determine the effects of the proportions of dietary macronutrients on glycaemic responses when Diet 1 and Diet 2 were fed to 16 cats.**

The blood was applied to a hand held glucometer (Accucheck, Roche Diagnostics, Auckland, NZ) to determine blood glucose concentration (mmol/L) (Figure 3.4). Pressure was then applied to the puncture site to prevent further bleeding. Samples were taken alternately from the left and right ears to minimise any damage to the veins. The concentrations determined by the glucometer were multiplied by a correction factor of 1.3 (Cave, NJ, personal communication) which was determined at the Institute of Veterinary, Animal and Biomedical Sciences, Massey University (Palmerston North, New Zealand). Serial dilutions of a blood sample were spiked with glucose to create a standard curve which was then compared to the same samples submitted to the New Zealand Veterinary Pathology laboratory (Palmerston North, New Zealand). The glucometer reading was consistently 1.3 times less than the true value.



**Figure 3.4. Application of blood to the hand held glucometer for glucose determination, to determine the effects of the proportions of dietary macronutrients on glycaemic responses when Diet 1 and Diet 2 were fed to 16 cats.**

A single meal consisting of 25 % of their daily maintenance energy requirement (calculated as outlined in section 3.2.2) was fed, and the cats were allowed 10 minutes to eat the meal, after which time any refusals were recorded. Additional blood samples were then collected 30, 60, 90, 120, 180, 240, 300, 360 and 420 minutes after consumption of the meal. After all the samples had been collected the cat was returned to its normal colony cage and offered the remaining 75 % daily allowance of food.

### **3.2.5 Statistical Analysis**

Two of the eight cats receiving Diet 2 exhibited soft faeces and intermittent diarrhoea during the study, therefore it was impossible to make a total collection and no digestibility results were determined for these two animals. No such problems were observed with the eight cats fed Diet 1.

All analyses were conducted using SAS v9.1 (SAS Institute Inc, Cary NC, USA). The apparent digestibility of the two diets, determined using the total-collection methods, was compared statistically using simple ANOVA analyses. Comparisons were included in the model to analyse differences between male and female cats within and between dietary groups. This would determine any differences between the sexes but also, as all of the males were neutered and all of the females were entire, it would determine any neutering effects on the results obtained. The urine pH and specific gravities were compared statistically using

simple ANOVA and the model used included comparisons between the male and female cats within and between dietary groups.

Due to differences between the baseline concentrations for the cats fed the two diets, the data was re-adjusted to a consistent baseline and presented as changes from baseline concentrations for each diet so differences between the dietary treatments could be seen. The mean corrected concentrations of glucose in blood  $\pm$  SEM were calculated at each time-point. Changes in concentration of glucose in blood were calculated separately for each post-meal period by using the blood concentration before the meal as a baseline. Post-prandial responses were compared for maximum increase (C<sub>max</sub>), time to peak increase (T<sub>max</sub>), and incremental area under the glucose curves (AUC) for both diets, and gender of cats. The AUC were calculated using the trapezoidal method, for the 420 minute period after the meal, using the Microsoft Excel-based program PK functions (Usansky JI, Desai A, Tang-Liu D, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine CA, USA). These parameters were then analysed using ANOVA, to determine any significant differences between the diets or gender, and between baseline and maximum values for each variable. Differences between diets at each of the time-points for concentration of glucose were analysed using repeat measures ANOVA, to determine any differences between the diets at any of the time-points.

## 3.3 Results

### 3.3.1 Weight maintenance

The mean caloric intakes (GE) were not significantly different ( $p>0.05$ ) between the two diets and between female and male cats fed any of the diets (Table 3.2).

**Table 3.2. Mean ( $\pm$ SEM) caloric intakes (GE) of 14 cats fed two diets (n=8 Diet 1 and n=6 Diet 2). Diet 1 was a low-carbohydrate, high-protein dry diet and Diet 2 was a high-carbohydrate, low-protein dry diet.**

Mean caloric intake	Diet 1	Diet 2	Pooled SEM
(kcal/kg/day)	66.5 ( $\pm$ 1.6)	63.9 ( $\pm$ 2.3)	1.4

The mean body weights of the cats fed Diet 1 and Diet 2 at the start of the study were 4.44 kg and 4.64 kg, respectively. The cats fed Diet 2 gained a small amount of weight over the study with a mean body weight at the end of the study of 4.71 kg. In comparison the mean body weight of the cats fed Diet 1 at the end of the study was 4.41 kg, indicating a small decrease in body weight during the study period. These differences between the two diets at the start and end of the study were not significant ( $p>0.05$ ). However, within each dietary group an effect of time on bodyweight was evident ( $p<0.05$ ).

The changes in bodyweight measured from baseline values for each diet were significantly ( $p<0.01$ ) different between the two diets and also between the female and male cats ( $p<0.01$ ).

### 3.3.2 Digestibility of the diets

The mean faecal output from the cats fed Diet 1 was significantly ( $p<0.01$ ) lower than that from the cats fed Diet 2 (35.78 g versus 56.62 g, respectively). There was no effect ( $p<0.05$ ) of sex on the faecal output for either of the diets.

The apparent nutrient digestibility coefficients of the two diets determined using the total-collection methods are summarised in Table 3.3. The effect of diet was significant for all parameters measured ( $p<0.05$ ). The apparent digestibility values for dry matter, crude protein, fat and energy of Diet 1 were higher ( $p<0.05$ ) than Diet 2. However, the apparent

carbohydrate digestibility was lower ( $p < 0.001$ ) in Diet 1 compared to Diet 2. No significant effects of diet x sex interactions were observed for any of the digestibility parameters ( $p > 0.05$ ).

**Table 3.3. Mean apparent nutrient digestibility coefficients of two diets fed to 14 cats (n=8 Diet 1 and n=6 Diet 2), determined using the total-collection method. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 was a high-carbohydrate, low-protein diet.**

<b>Coefficient</b>	<b>Diet 1</b>	<b>Diet 2</b>	<b>Pooled SEM</b>	<b>Diet</b>
<b>Dry Matter</b>	0.689	0.606	0.013	<0.050
<b>Crude Protein</b>	0.813	0.613	0.014	<0.0001
<b>Fat</b>	0.880	0.803	0.013	<0.050
<b>Carbohydrate<sup>a</sup></b>	0.478	0.664	0.015	<0.001
<b>Energy</b>	0.748	0.650	0.011	<0.010

<sup>a</sup> Calculated by difference

### 3.3.3 Drinking water intakes and urine characteristics

The average daily drinking water intakes (Table 3.4) for the cats fed the two diets were similar ( $p > 0.05$ ).

**Table 3.4. Mean ( $\pm$ SEM) daily drinking water intakes of 14 cats fed two diets (n=8 Diet 1 and n=6 Diet 2). Diet 1 was a low-carbohydrate, high-protein dry diet and Diet 2 was a high-carbohydrate, low-protein dry diet.**

<b>Mean water intake</b>	<b>Diet 1</b>	<b>Diet 2</b>	<b>Pooled SEM</b>
<b>(g/day)</b>	118.92 ( <b><math>\pm</math>5.00</b> )	135.78 ( <b><math>\pm</math>9.08</b> )	5.19

The urine pH for the cats fed Diet 1 was lower ( $p < 0.001$ ) than for those fed Diet 2 (Table 3.5). There were no significant ( $p > 0.05$ ) effects of sex or day on the urine pH. The average pH

over the five days for the cats fed Diet 1 and Diet 2 were significantly ( $p<0.001$ ) different; 7.28 and 7.69, respectively.

**Table 3.5. Urine pH values from 14 cats over five consecutive days fed two diets (n=8 Diet 1 and n=6 Diet 2). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 was a high-carbohydrate, low-protein diet. Results are presented as means and pooled SEM.**

Day	Diet 1	Diet 2	Pooled SEM
1	7.31	7.55	0.09
2	7.65	8.01	0.14
3	7.05	7.31	0.12
4	7.32	7.91	0.11
5	7.07	7.62	0.12

The urine specific gravity for the cats fed Diet 1 was higher ( $p<0.01$ ) than for those fed Diet 2 for all days except day 1 (Table 3.6). The values for the cats fed both diets were significantly different on day one compared to the values obtained on all of the other days. However, no effects ( $p>0.05$ ) of sex were seen.

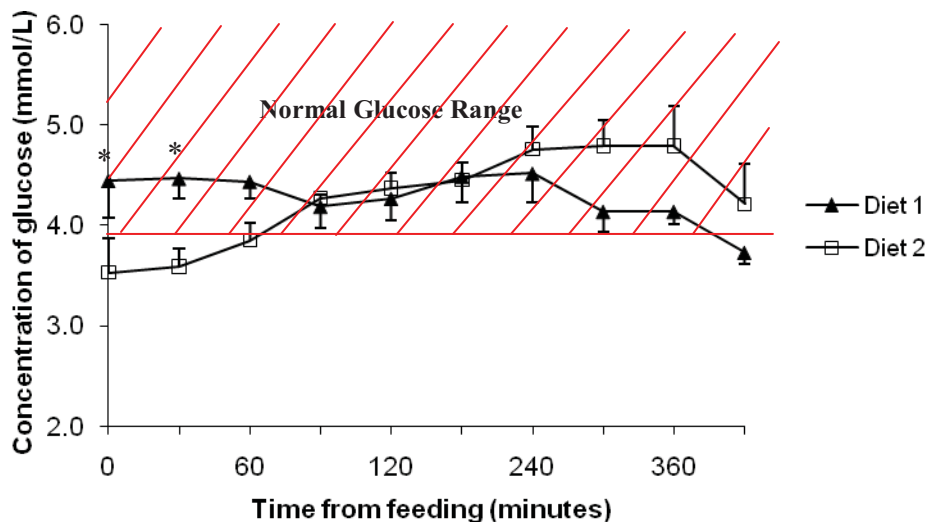
**Table 3.6. Urine specific gravity values from 14 cats over five consecutive days fed two diets (n=8 Diet 1 and n=6 Diet 2). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 was a high-carbohydrate, low-protein diet.**


Day	Diet 1	Diet 2	Pooled SEM
1	1.050	1.051	0.0007
2	1.070*	1.057	0.0025
3	1.065*	1.059	0.0022
4	1.070*	1.058	0.0020
5	1.067*	1.062	0.0025
<b>Mean (<math>\mu</math>)</b>	<b>1.064*</b>	<b>1.058</b>	<b>0.0011</b>

\* Higher than Diet 2 ( $p<0.01$ )

### 3.3.4 Post-prandial changes in concentrations of glucose

The post-prandial changes in plasma glucose concentrations in response to consumption of the two diets are shown in Figure 3.5. The diets were significantly different at baseline and 30 minutes after consumption of the meal, making it difficult to ascertain real differences in post-prandial glucose responses between the diets.



**Figure 3.5.** Mean ( $\pm$  SEM) concentration of glucose determined from serial blood samples (  normal glucose range for cats), collected from 16 cats before and after a single meal of either Diet 1 or Diet 2 (\* $p < 0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 was a high-carbohydrate, low-protein diet.

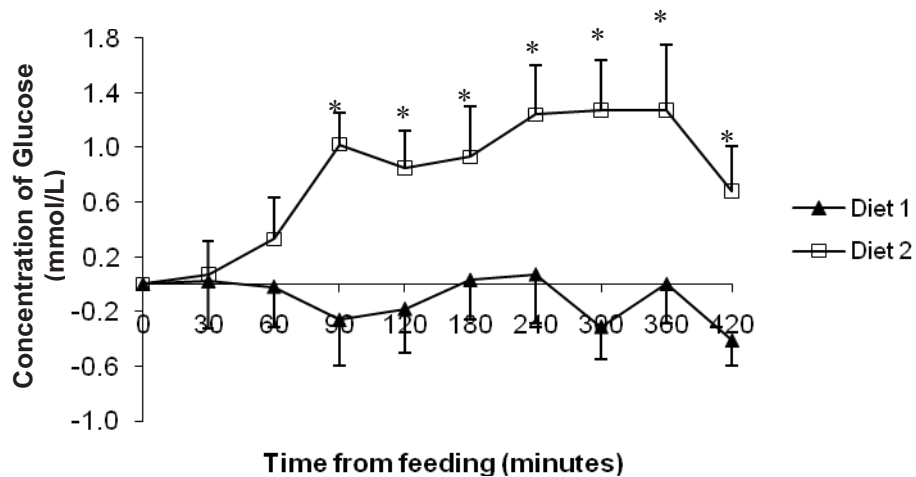
The AUC, Cmax and Tmax were not different ( $p > 0.05$ ) between the two diets (Table 3.7). There were also no age or gender effects seen.



**Table 3.7. Characteristics of blood glucose curves determined from serial blood samples collected from 16 cats, following a single meal of either Diet 1 (low-carbohydrate, high-protein diet), or Diet 2 (high-carbohydrate, low-protein diet).**

Parameter	Diet 1	Diet 2	Pooled SEM
Peak concentration (mmol/L)	4.97	5.35	0.20
Time to peak (minutes)	292.5	303.75	23.75
Area under curve [(mmol/L)/minutes]	1585	1862	83.66

The changes in blood glucose concentration from baseline values were much greater for the cats fed Diet 2 compared to Diet 1 (Figure 3.6). These increases were significant ( $p < 0.05$ ) from baseline 90 minutes after feeding Diet 2, and continued to increase further ( $p < 0.01$ ) between 240 and 360 minutes after the meal. In contrast, no significant changes from baseline were seen for the cats fed Diet 1 ( $p > 0.05$ ). No effects of age or gender were observed.



**Figure 3.6. Mean ( $\pm$  SEM) glucose concentration adjusted to a common initial glucose concentration in 16 cats fed a single meal of either Diet 1 or Diet 2 (\* $p < 0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 was a high-carbohydrate, low-protein diet.**

### 3.4 Discussion

It is of interest to determine the effects of feeding diets varying in macronutrient profiles to cats because they are strict carnivores and therefore are adapted to high-protein, low-carbohydrate diets, rather than the high-carbohydrate diets currently available. In this study, feeding a high-protein, low-carbohydrate (54 % protein, 21 % carbohydrate) dry diet (Diet 1) compared with a commercial high-carbohydrate, low-protein (27 % protein, 49 % carbohydrate) dry diet (Diet 2) demonstrated that as hypothesized, the high-protein diet offered higher apparent nutrient digestibility, smaller fluctuations in blood glucose concentrations and therefore a slower, steadier release of glucose into the bloodstream.

In this study, the higher apparent digestibility of Diet 1 for all nutrients except carbohydrate, indicated that it provided advantages over Diet 2, meaning cats may have absorbed a greater concentration of nutrients from the diet. Only six of the eight cats fed Diet 2 participated in the digestibility study because the remaining two animals exhibited consistent soft faeces and intermittent diarrhoea which made it impossible to undertake a total collection. This is possibly due to the much higher carbohydrate content of this diet, which probably leads to greater hind gut fermentation and therefore loose stools and diarrhoea. All eight cats fed Diet 1 had well-formed stools and participated in the digestibility study.

The incidence of feline diabetes mellitus is increasing worldwide and because of the negative effects of this disease on animal health, its management and prevention are important to veterinarians and pet owners alike (Hoenig 2002; Vester *et al.* 2009). Recommendations for the optimum diet for animals suffering from diabetes mellitus have recently changed from a high-carbohydrate high-fibre diet to a low-carbohydrate high-protein diet (Zoran 2002; Biourge 2005; Bennett *et al.* 2006). It has been theorised that a low-carbohydrate, high-protein diet enhances glycaemic control in both healthy and diabetic cats and may even contribute to remission in some cats with diabetes mellitus (Biourge 2005; Kirk 2006).

The marginal ear vein prick technique was chosen because of its speed, non-evasiveness and the minimal stress it causes to the animal. The alternative method of fitting cats with catheters in order to take repeated blood samples is a costly and evasive procedure. It was hoped by using the marginal ear vein prick technique in this study we could evaluate its effectiveness for use in future studies with these animals. This technique proved to be highly

effective for determining blood glucose concentrations in this study, causing the cat's minimal stress, providing repeated samples quickly and effectively and therefore offering an excellent alternative to catheterisation in future studies.

The normal range for blood glucose for cats reported by the National Research Council of the National Academies (NRC 2006) is 3.9 to 6.7 mmol/L. During this study none of the cats had blood glucose concentrations exceeding the upper limit of this range. However, some of the cats in both dietary groups had values below the lower limit of this range at one or more of the sampling points.

The only significant differences in blood glucose curves determined between the two diets in the present study were at baseline and 30 minutes after feeding. The differences between the diets at baseline clearly illustrate that the cats in the two groups were not the same before the start of the blood collection. This may be due in part to the random allocation of cats to the treatments, meaning the two groups were not balanced for the cat's normal glucose responses and also due to the effect of the diets fed before the 16 hour fast on the cat's initial baseline glucose concentrations. However, the differences between the baseline concentrations for the cats fed the two diets masked the diet effect and it was only after re-adjusting the data to a consistent baseline and presenting it as a change from baseline concentrations, that differences between the dietary treatments could be seen. The changes in concentrations of blood glucose from baseline were much greater for the cats fed Diet 2, with the change from baseline significant 90 minutes after feeding. In contrast, there were no significant changes in blood glucose concentration from baseline for the cats fed Diet 1. This suggests there is a slower, steadier release of glucose into the bloodstream following consumption of Diet 1.

Other researchers have had varying results for demonstrating the effects on glycaemic and insulinaemic responses of feeding high-protein, low-carbohydrate diets. Hoenig *et al.* (2000) tested a high-protein (58.2 % on a dry matter basis) and a low-protein (33.1 % on a dry matter basis) diet and found no significant difference in postprandial glucose responses between the two diets when fed to cats. A study by Farrow *et al.* (2002) fed a high -protein (46 % on an energy basis), a high-fat (47 % on an energy basis) and a high-carbohydrate (47 % on an energy basis) diet to cats for four weeks before conducting *ad libitum* and meal response tests and discovered that the high-carbohydrate diet produced significantly higher mean and

maximum glucose concentrations and AUC compared to both the high-protein and high-fat diets. Unlike the study by Farrow *et al.* (2002), the results of the present study found no difference in Cmax and AUC between the high- carbohydrate and high-protein diets.

More recent studies have demonstrated improved glycaemic control in healthy and diabetic cats fed low-carbohydrate, high-protein diets (Frank *et al.* 2001; Mazzaferro *et al.* 2003; Bennett *et al.* 2006). These studies reported feeding diabetic cats a low-carbohydrate diet improved hyperglycemia, led to a decrease in insulin dosage and an increase in the rate of diabetic remission (Frank *et al.* 2001; Mazzaferro *et al.* 2003; Bennett *et al.* 2006). Frank *et al.* (2001) fed a high-protein (56.9 % on a dry matter basis), low-carbohydrate (8.1 % on a dry matter basis) diet to diabetic cats for three months and after this time, eight of the nine cats which completed the study successfully required reduced levels of insulin, and three cats no longer required insulin at all. Similarly, Mazzaferro *et al.* (2003) fed 24 diabetic cats a low-carbohydrate (6.9 % on a dry matter basis), high-protein (49 % on a dry matter basis) diet for four months and also observed improved glycaemic control leading to a decrease in insulin dosage when the cats were fed this diet. Finally, a study by Bennett *et al.* (2006) involved feeding a moderate-carbohydrate (26 % of ME) and protein (40 % of ME) diet and a low-carbohydrate (12 % of ME) and protein (37 % of ME) diet to 63 diabetic cats. As 68 % of the cats fed the low-carbohydrate diet reverted back to a non-insulin dependent state, compared to only 41 % for the moderate-carbohydrate diet, these authors concluded that the low-carbohydrate diet produced better glycaemic control. The current study also demonstrated that a low-carbohydrate, high-protein diet produced better glycaemic control.

Feline urological syndrome has presented a problem in cats for a number of years (Gaskell 1989). Various factors are believed to contribute to its development including the moisture content and mineral concentration in the diet (Osborne 1984; Gaskell 1989). A urine pH of greater than 7.0 has been identified as a risk factor for the development of struvite induced feline urological syndrome (Osborne and Polzin 1986; Osborne 1999). The average urine pH for the cats on both diets in the current study were higher than 7.0, and were also higher than the reported optimum range for urine pH in cats (6.0 to 6.4, Cottam *et al.* 2002). The mean urine pH for the cats fed Diet 1 was significantly lower than for those fed Diet 2 (7.28 and 7.68, respectively). However if these diets are to be sold commercially, the formulation of both diets should be re-evaluated to produce lower urine pH values when fed in order to avoid health problems. Because the exact ingredients in these diets are unknown, it is difficult to

make recommendations as to the exact changes that are required in order to decrease the pH, however ultimately the balance of cationic and anionic minerals included in the diet needs to be adjusted. The cats in this study also exhibited urine specific gravity values at the higher end of the reported healthy range of 1.001 to 1.070, encouraging an environment conducive to the formation of struvite crystals and feline urological syndrome (Gaskell 1989). However, this was somewhat expected because it is known that cats consuming dry diets produce smaller volumes of more concentrated urine compared to those consuming wet foods (Gaskell 1989; Zoran 2002).

In conclusion, the high-protein low-carbohydrate Diet 1 developed for this study is beneficial for blood glucose control in cats. This diet, being high in protein and low in carbohydrate, is more aligned to their natural carnivorous diet (Zoran 2002; Kirk 2006; Backus *et al.* 2007) and also conferred advantages to the cats in this study, including higher digestibility, smaller fluctuations in blood glucose concentrations and therefore a slower, steadier release of glucose into the bloodstream. As these benefits may also be useful for overweight cats, it would be of interest to conduct a study to determine the effects of this high-protein, low-carbohydrate diet on weight maintenance using *ad libitum* feeding. In addition due to the high urine pH and specific gravities determined for the two dry diets in this study, these parameters need to be re-evaluated in another cat study to confirm the diets need re-formulation to produce lower urine pH and specific gravities. It would also be interesting to feed a wet diet alongside these two dry diets to compare blood glucose responses and weight maintenance from diets in different forms, having undergone different processing. Ideally the individual glycaemic responses of the cats used in the subsequent study should be determined in a pre-trial period. This would ensure each dietary group is balanced with cats displaying a similar range of glycaemic responses.

## **Chapter Four**

**The effects of the proportions of dietary macronutrients and diet form on weight maintenance, digestibility and post-prandial endocrine responses in cats**

## 4.1 Introduction

Obesity can be defined as an accumulation of excessive amounts of adipose tissue in the body and cats with more than 15 % excess bodyweight are considered obese (German 2006; Colliard *et al.* 2009). Obesity is increasing in cats, and today is the most common form of malnutrition seen in these animals in the western world (Sloth 1992; Burkholder and Bauer 1998; Zoran 2002; German 2006). It is associated with a number of health conditions including diabetes mellitus, osteoarthritis, dermatological diseases and urinary tract disease (Butterwick 2000; Zoran 2002; Laflamme 2006; Hoenig *et al.* 2007), and also creates physical problems, reduced mobility and grooming. Because of these reasons prevention and treatment of obesity is of utmost importance today.

Numerous factors contribute to the development of obesity in cats including sex, neutering, age, activity, diet and feeding style (Zoran 2002; Laflamme 2006). However, diet and feeding style represent the most important means of preventing and treating obesity in cats. Part of the reason for this increase in obesity may be because unlike their wild ancestors, today's domestic cats no longer have to hunt for food and many have become relatively inactive, tend to overeat and subsequently put on weight (Zoran 2002). Much attention has also been focussed on the role high-carbohydrate diets play in the incidence of feline obesity (Zoran 2002; Kirk 2006; Hoenig *et al.* 2007), because often these diets provide more carbohydrate than the cat can utilise, with the excess carbohydrate being stored as fat.

Calorie restriction, through feeding less of the diet, decreasing fat levels in the diet or feeding a diet higher in water content (i.e. canned rather than dry diets) is the most obvious means of promoting weight loss or maintaining an ideal bodyweight, however it is also important to use a diet which promotes fat loss rather than lean tissue loss (Zoran 2002; Kirk 2006; Laflamme 2006). Feeding a diet which minimises postprandial glucose and insulin responses in not only overweight or obese cats but also in healthy cats is also an important consideration as this may help in improving glycaemic control, improving feelings of satiety and avoiding the development of diabetes mellitus (Zoran 2002; Kirk 2006; Laflamme 2006; Vester *et al.* 2009). Several human studies have shown that low-carbohydrate, high-protein diets can help promote weight loss whilst preserving lean body mass (Volek and Westman 2002; Foster *et al.* 2003) and there is some suggestion this may also be the case in cats (Kirk 2006; Laflamme 2006; Vesta *et al.* 2009). Low-carbohydrate diets are also believed to produce a low

glycaemic load and have even been suggested as preventative for obesity and diabetes mellitus (Frank *et al.* 2001; Rand and Marshall 2005; Bennett *et al.* 2006; Kirk 2006).

Formulation of both dry and canned high-protein, low-carbohydrate diets is possible (Biourge 2005), therefore it would be worthwhile comparing any differences in the effects of these dietary forms on glycaemic control, weight maintenance and weight loss. Many pet owners today prefer feeding dry diets due to their minimal mess and ease of feeding, however these diets are often very energy dense and highly palatable and if the quantity fed is not correctly controlled, the pet will gain weight rapidly (Zoran 2002; Kirk 2006; Backus *et al.* 2007). In contrast, although wet diets (e.g. canned) typically are higher in fat and calorie content on a dry matter basis, per volume they have much lower calorie density compared to dry foods and are therefore an effective way of diluting calories in the diet (Kirk 2006; Laflamme 2006).

It was hypothesized that high-protein diets of any form (i.e. wet or dry or semi-moist) would produce more beneficial effects on blood glucose responses and weight maintenance compared to high-carbohydrate diets. When considering weight control, it was believed that high-carbohydrate diets would also lead to significant increases in weight compared to high-protein diets.

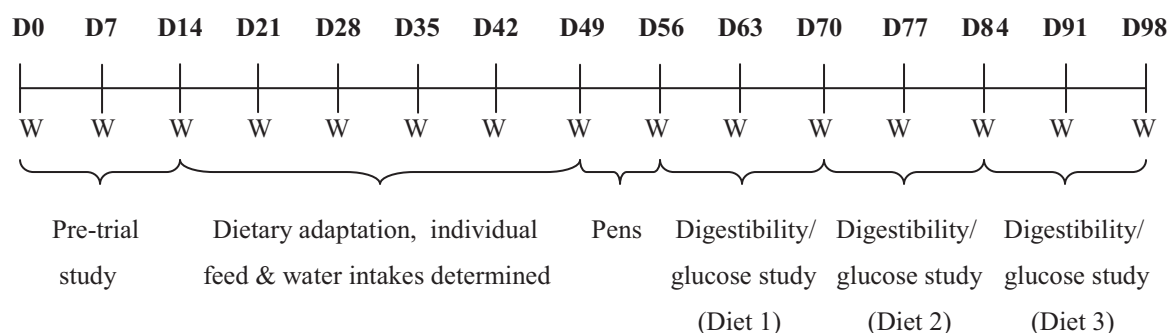
Therefore this study was undertaken to compare the effects of feeding diets varying in proportions of macronutrients (carbohydrate and protein) and dietary form on metabolism, particularly weight maintenance, apparent digestibility, and post-prandial glycaemic responses in cats.



## 4.2 Materials and methods

### 4.2.1 Animals and housing

Twenty four (n=7 entire females and n=17 neutered males) healthy adult domestic short haired cats (*Felis catus*), aged from 2 to 10 (mean 4.67, SEM 2.16) years, were used in the 14-week study (Figure 4.1).



**Figure 4.1. Time-line of the study to determine the effects of the proportions of dietary macronutrients and diet form on weight maintenance, digestibility and post-prandial endocrine responses of three diets fed to 24 cats (D= day, W=weigh).**

As outlined in Chapter 3, section 3.2.1, the cats were born and raised at the unit and therefore adapted to the environment and trained to be handled, weighed and restrained for blood sampling. The cats (n=8) were housed in three purpose-built colony pens at the Centre for Feline Nutrition, Massey University, Palmerston North, New Zealand. The pens were cleaned daily as part of normal management procedure. Fresh water was available at all times. All management and experimental procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

### 4.2.2 Diets and feeding

During a two week pre-trial period, all 24 cats were gradually introduced and adapted to the Diet 2 (high-carbohydrate, low-protein dry diet) in their colony cages and their glycaemic responses determined. The cats were then randomly assigned to either the Diet 1, Diet 2 or Diet 3 based on the glycaemic responses determined in the pre-trial period, so there were equal numbers of cats on each diet (n=8), i.e. one colony cage per diet. This ensured that each

dietary group had a pool of cats with a similar range of glycaemic responses. The cats were fed the assigned diet for the duration of the study.

Diet 1 comprised a low-carbohydrate, high-protein dry extruded biscuit diet manufactured specifically for the study by Heinz Watties Limited (Hastings, NZ). Diet 2 comprised a commercial high-carbohydrate, low-protein dry extruded biscuit diet (Chef Chicken and Fish; Heinz Watties Limited, Hastings, NZ) and Diet 3 comprised a low-carbohydrate, high-protein commercial wet canned diet (Chef Jellimeat; Heinz Watties Limited, Hastings, NZ).

Each pen of cats (n=8) were fed fresh food *ad libitum* once a day. The daily food allowance offered to each cage (adult maintenance energy requirement (MER) plus 15 %) was determined using the equation:  $MER = 70 \text{ kcal} \times \text{bodyweight (kg)}$  (NRC 2006) plus 15 %. The food allowance for each colony cage was adjusted weekly based on each cage's total bodyweight. The diets were weighed daily (Mettler Toledo PM11-N, Mettler Toledo Incorporated, Columbus, USA) and any refusals were recorded daily so actual daily intakes could be calculated for each pen of cats.

At weekly intervals, two different animals from each dietary group were removed from the colony cages and housed in individual metabolism cages measuring 1.1 m x 0.8 m x 0.8 m for two days. During this time they were fed their assigned diet (as calculated above) and allowed free access to water. Food refusals and water bowls were weighed back each day to establish the typical individual daily food and drinking water intakes for each cat.

The diets were analysed for moisture, ash, crude protein, fat and gross energy content and for carbohydrate content by difference using the methods described in Chapter 2, section 2.2.2 (Table 4.1).

**Table 4.1. Dietary analysis<sup>a</sup> of Diet 1, Diet 2 and Diet 3 fed to 24 cats, in order to determine the effects of the proportions of dietary macronutrients and diet form on weight maintenance, digestibility and post-prandial endocrine responses.**

<b>Nutrient</b>	<b>Diet 1 (High P; dry)</b>	<b>Diet 2 (High CHO; dry)</b>	<b>Diet 3 (High P; wet)</b>
Moisture (%)	6.5	8.5	82.0
Crude protein (%)	48.0	30.3	9.4
Crude fat (%)	18.5	10.1	5.2
Ash (%)	4.7	6.0	1.9
Carbohydrate <sup>b</sup> (%)	22.3	45.2	1.6
Gross energy (kJ/g)	22.5	18.3	5.1

<sup>a</sup> All results are on an ‘as received’ basis

<sup>b</sup> Calculated by difference

P = protein

CHO = carbohydrate

#### **4.2.3 Weight maintenance**

In order to determine the changes in bodyweight, the cats were weighed (Mettler Toledo PM11-N scales, Mettler Toledo Incorporated, Columbus, USA), at the beginning of the trial, and at weekly intervals until the end of the 14-week trial.

#### **4.2.4 Digestibility of the diets**

During the last six weeks of the study, at different times eight of the cats at a time were housed individually in metabolism cages and fed as previously outlined in section 4.2.2 to determine the digestibility of the diets. The digestibility protocol required the cats to be housed for 12 days, with the first seven days serving as an adaptation period to the new environment, and the final five days for collection of faeces and urine. The faeces and urine produced by each cat was collected daily as outlined in Chapter 3, section 3.2.3. Any food refusals were recorded to determined daily food intakes.

The urine samples were analysed for specific gravity using an Atago UG-1 digital refractometer (Atago Company Limited, Tokyo, Japan) before being discarded. The faeces were freeze-dried, ground, and analysed as described in Chapter 3, section 3.2.3. The dry

matter, protein, fat, and energy digestibility of the three diets were calculated using the equations in Chapter 3 (section 3.2.3).

#### **4.2.5 Post-prandial changes in concentrations of glucose**

Immediately after the digestibility study was completed, concentrations of glucose in blood were determined from all of the cats, after the consumption of a single meal. A baseline blood sample was obtained from each cat 16 hours after their last meal and one hour before feeding of the test meal using the marginal ear vein prick method used in Chapter 3 (section 3.2.4). A single meal consisting of 25 % of their daily maintenance energy requirements (calculated as outlined in Chapter 3; Section 3.2.2) was fed, and the cats were allowed 10 minutes to consume it, after which time any refusals were recorded. Further blood samples were then collected 15, 30, 45, 60, 90, 120 and 180 minutes after consumption of the meal, using the ear prick technique and correction methods outlined in Chapter 3.

#### **4.2.6 Statistical analysis**

All of the statistical analyses were conducted using SAS v9.1 (SAS Institute Inc, Cary NC, USA). The apparent digestibility of the three diets, determined using the total-collection method, specific gravity and blood glucose data was compared statistically as outlined in Chapter 3 (Section 3.2.5). The model included comparisons to analyse differences between male and female cats within and between dietary groups and therefore also examine any effects of neutering. The mean caloric and water intakes for each dietary group were compared using ANOVA. The mean bodyweights and changes in bodyweights  $\pm$  SEM were plotted for each dietary group in time series graphs. The mean bodyweights and mean changes in bodyweights for each dietary group were compared statistically using repeat measures ANOVA. Comparisons were included in the model to analyse differences between male and female cats.

## 4.3 Results

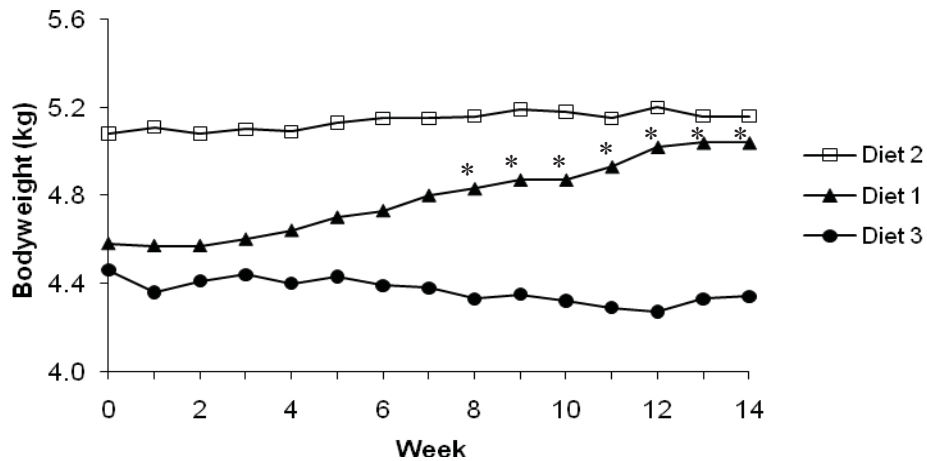
### 4.3.1 Weight maintenance

The mean caloric intakes (GE) determined from the individual metabolism cages were similar ( $p>0.05$ ) between dietary groups, and between female and male cats fed any of the diets (Table 4.2). All of the cats consumed more than their energy requirements, according to work carried out by Kienzle and colleagues (2006); ( $60 \pm 18$  kcal per kg of bodyweight) while being fed the diets *ad libitum*.

**Table 4.2. Mean ( $\pm$ SEM) caloric intakes (GE) of 24 cats fed three diets (n=8 per diet). Diet 1 was a low-carbohydrate, high-protein dry diet, Diet 2 was a high-carbohydrate, low-protein dry diet and Diet 3 was a wet low-carbohydrate, high-protein diet.**

Mean caloric intake	Diet 1	Diet 2	Diet 3	Pooled SEM
(kcal/kg/day)	77.3 ( $\pm$ 1.6)	73.0 ( $\pm$ 2.6)	77.5 ( $\pm$ 2.6)	1.3

The cats fed Diet 1 showed the most variation in bodyweight in response to *ad libitum* feeding compared to the other two groups (Figure 4.2). The cats fed Diet 2 and Diet 3 maintained a more constant bodyweight during the trial, however none of these differences between the diets were significant ( $p>0.05$ ). There were significant differences ( $p<0.05$ ) between the body weights of the male and female cats in both groups. An effect of time on bodyweight was also evident ( $p<0.05$ ).



**Figure 4.2. Mean bodyweights of 24 cats fed three diets (n=8 per diet). Diet 1 was a low-carbohydrate, high-protein dry diet, Diet 2 was a high-carbohydrate, low-protein dry diet and Diet 3 was a low-carbohydrate, high-protein wet diet (Diet 1 higher (\*p<0.05) than Diet 3).**

The changes in bodyweight measured from baseline values (Figure 4.3) were significantly different between Diet 1 and Diet 3 from week 8 to 14 of the study, with the cats fed Diet 1 gainind weight and those fed Diet 3 losing weight. The changes in bodyweight observed in cats fed Diet 2 were not significantly different from those in the cats fed Diet 1 or Diet 3. No effects (p>0.05) of sex were seen in the change in body weight during the trial.

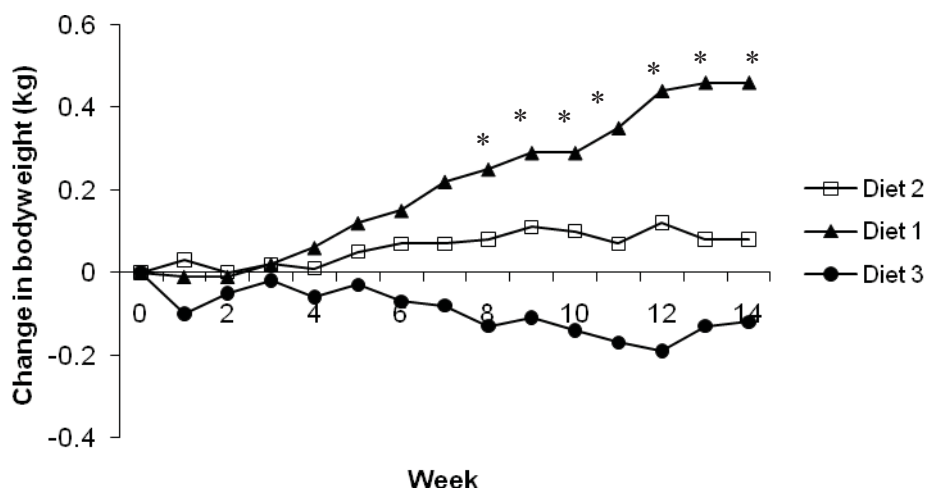


Figure 4.3. Change in bodyweight of 24 cats fed three diets (n=8 per diet). Diet 1 was a low-carbohydrate, high-protein dry diet, Diet 2 was a high-carbohydrate, low-protein dry diet and Diet 3 was a low-carbohydrate, high-protein wet diet (\*  $p < 0.05$ , higher than Diet 3).

#### 4.3.2 Digestibility of the diets

The apparent nutrient digestibility coefficients of the three diets determined using the total-collection method are shown in Table 4.3. The effect of the type of diet was significant for all parameters ( $p < 0.0001$ ). The apparent digestibility values for dry matter, crude protein, fat and energy of Diet 1 were higher ( $p < 0.001$ ) than Diet 2 and Diet 3. However, the values for Diet 2 and Diet 3 were only significantly ( $p < 0.001$ ) different for apparent crude protein and fat digestibility. The apparent dry matter and energy digestibility values were not different ( $p > 0.05$ ) between Diet 2 and Diet 3. No significant effects of gender were observed for any of the parameters ( $p > 0.05$ ).

**Table 4.3. Mean apparent nutrient digestibility coefficients of the three diets fed to 24 cats (n=8 per diet), determined using the total-collection method. Diet 1 was a low-carbohydrate, high-protein dry diet, Diet 2 was a high-carbohydrate, low-protein dry diet and Diet 3 was a low-carbohydrate, high-protein wet diet.**

<b>Coefficient</b>	<b>Diet 1</b>	<b>Diet 2</b>	<b>Diet 3</b>	<b>Pooled SEM</b>	<b>Diet</b>
<b>Dry matter</b>	0.888	0.710	0.675	0.008	<0.0001
<b>Crude Protein</b>	0.936	0.738	0.801	0.004	<0.0001
<b>Fat</b>	0.956	0.821	0.886	0.006	<0.0001
<b>Energy</b>	0.916	0.728	0.686	0.009	<0.0001

#### **4.3.3 Drinking water intakes and urine characteristics**

The average daily drinking water intakes for the cats fed Diet 3 were significantly ( $p<0.0001$ ) lower compared to those for the cats fed the other two diets, which were not significantly ( $p>0.05$ ) different (Table 4.4).

**Table 4.4. Mean ( $\pm$ SEM) daily drinking water intakes of 24 cats fed three diets (n=8 per diet). Diet 1 was a low-carbohydrate, high-protein dry diet, Diet 2 was a high-carbohydrate, low-protein dry diet and Diet 3 was a low-carbohydrate, high-protein wet diet.**

<b>Mean water intake</b>	<b>Diet 1</b>	<b>Diet 2</b>	<b>Diet 3</b>	<b>Pooled SEM</b>
<b>(g/day)</b>	132.88 ( $\pm$ 12.41)	126.00 ( $\pm$ 14.14)	30.63 ( $\pm$ 13.01) *	16.32

\* Lower than Diet 1 and Diet 2 ( $p<0.0001$ )

The urine specific gravity of Diet 3 was lower ( $p<0.0001$ ) than both of the other diets, which were not different ( $p>0.05$ ) (Table 4.5). The specific gravity of the urine collected from the cats fed Diet 1 and Diet 3 was not significantly ( $p>0.05$ ) different between the five days. However, the specific gravity of the urine from the cats fed Diet 2 was significantly different between collection days.



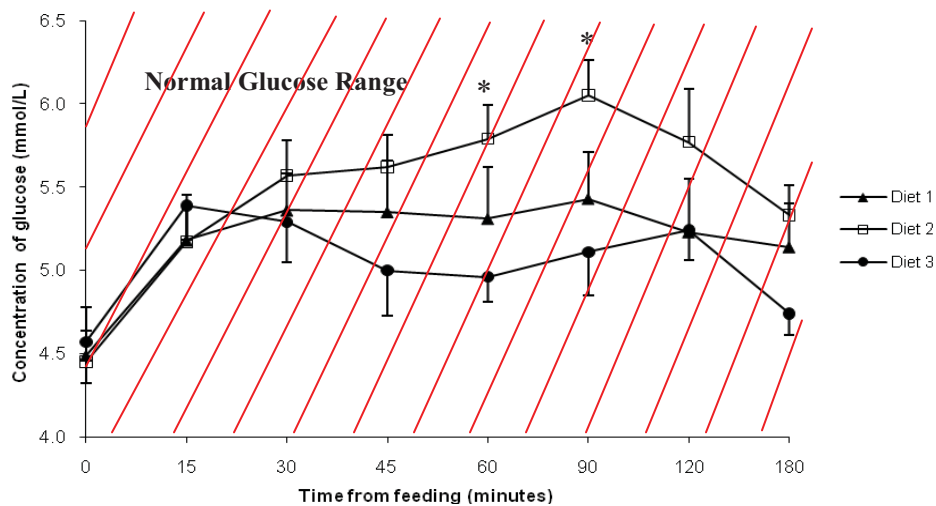
**Table 4.5. Mean urine specific gravity values from 24 cats fed three diets (n=8 per diet). Diet 1 was a low-carbohydrate, high-protein dry diet, Diet 2 was a high-carbohydrate, low-protein dry diet and Diet 3 was a low-carbohydrate, high-protein wet diet.**

Day	Diet 1	Diet 2	Diet 3	Pooled SEM
1	1.081	1.086	1.033	0.0030
2	1.081	1.081	1.034	0.0025
3	1.074	1.065	1.036	0.0018
4	1.069	1.068	1.034	0.0011
5	1.074	1.069	1.036	0.0014
<b>Mean (<math>\mu</math>)</b>	1.076*	1.074*	1.035	0.0013

\* Higher than Diet 3 ( $p < 0.0001$ )

#### 4.3.4 Post-prandial changes in concentrations of glucose

The changes in post-prandial plasma glucose concentrations in response to consumption of the three diets are shown in Figure 4.4. The diets were significantly ( $p < 0.05$ ) different 60 and 90 minutes after the meal.



**Figure 4.4. Mean ( $\pm$  SEM) concentration of glucose determined from serial blood samples ( normal glucose range for cats), collected from 24 cats before and after a single meal of one of three diets (n=8 per diet). Diet 1 was a low-carbohydrate, high-protein dry diet, Diet 2 was a high-carbohydrate, low-protein dry diet and Diet 3 was a low-carbohydrate, high-protein wet diet (\*  $p < 0.05$ , higher than Diet 3).**

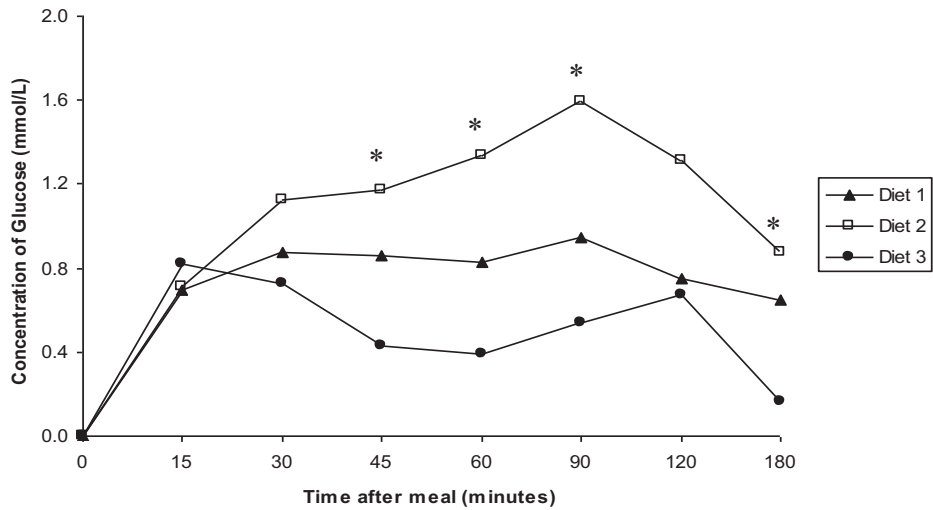
The AUC, and Cmax were not significantly ( $p>0.05$ ) different between the diets (Table 4.6). However, the Tmax was significantly shorter for Diet 1 ( $p<0.05$ ) and Diet 3 ( $p<0.01$ ) compared to Diet 2. The Cmax for Diet 2 was also higher than for either of the other two diets although not significantly ( $p>0.05$ ).

**Table 4.6. Characteristics of blood glucose curves determined from serial blood samples collected from 24 cats, before and after a single meal of one of three diets (n=8 per diet). Diet 1 was a low-carbohydrate, high-protein dry diet, Diet 2 was a high-carbohydrate, low-protein dry diet and Diet 3 was a low-carbohydrate, high-protein wet diet.**

Parameter	Diet 1	Diet 2	Diet 3	Pooled SEM
Peak concentration (mmol/L)	5.64	6.24	5.58	0.12
Time to peak (minutes)	56.25	105.00*	42.86	8.32
Area under curve (mmol/L)minutes	726	777	701	16.00

\* Higher than Diet 1 and Diet 3 ( $p<0.05$ )

The increases in blood glucose concentrations from baseline values were clearly greater in the cats fed Diet 2 compared to those fed the other two diets (Figure 4.5). However, these differences were only significantly higher ( $p<0.05$ ) at 45, 60, 90 and 180 minutes after consumption and were only significantly different between Diet 2 and Diet 3. The peak increase in glucose concentration occurred 15 minutes after the meal for Diet 3 and 90 minutes after the meal for both Diets 1 and 2.



**Figure 4.5. Mean glucose concentration adjusted to a common initial glucose concentration in 24 cats fed three diets (n=8 per diet). Diet 1 was a low-carbohydrate, high-protein dry diet, Diet 2 was a high-carbohydrate, low-protein dry diet and Diet 3 was a low-carbohydrate, high-protein wet diet (\* p<0.05, higher than Diet 3).**

## 4.4 Discussion

The incidence of overweight and obese cats is increasing worldwide and dietary strategies, coupled with owner education appear to be the main options for combating this issue (Sloth 1992; Butterwick 2000; German 2006; Laflamme 2006). As in humans, obesity in cats has a number of detrimental effects on cat health, including the development of skin problems and diabetes mellitus, and also impacts negatively on the animal's quality and length of life (Butterwick 2000; Zoran 2002; Laflamme 2006). A recent study found that overweight cats were 3.8 times more likely to develop diabetes mellitus than cats of a healthy bodyweight (Biourge 2005), so strategies to prevent and manage obesity are therefore of great interest.

Strategies for weight maintenance and weight loss in cats are mainly centred on limiting caloric intake but increasing interest has also been expressed in formulating diets that contain an ideal ratio of macro- and micronutrients to promote fat loss, whilst minimising the loss of lean tissue (Zoran 2002; Vester *et al.* 2009). This ratio is yet to be definitively established, however, in recent years, a diet high in protein and low in carbohydrate has been promoted for use in feeding obese and diabetic animals (Hoenig *et al.* 2000; Frank *et al.* 2001; Zoran 2002; Mazzaferro *et al.* 2003). It has been suggested such a diet leads to better glycaemic control, an increased perception of satiety and promotes weight loss whilst preserving lean body tissue (Zoran 2002; Kirk 2006; Laflamme 2006; Vester *et al.* 2009). In addition to the quantity of protein and carbohydrate in the diet, the sources of these macronutrients and overall form of the diet are also probably important aspects to consider as these may also affect the metabolism of the animal.

In human nutrition, foods with a low glycaemic index (GI) are promoted as producing greater satiety after eating while high GI foods which produce a rapid and high postprandial response, are said to lead to lower satiety, increased hunger and overeating and therefore weight gain (Appleton *et al.* 2004; Rand *et al.* 2004). Although no such index is available for companion animals, a food with a low glycaemic load may also be beneficial for cats. Therefore, a high-protein, low-carbohydrate dry diet (Diet 1) was formulated and fed during this trial alongside a commercially available high-carbohydrate, low-protein dry diet (Diet 2) and a commercially available high-protein, low-carbohydrate wet diet (Diet 3).

The higher apparent digestibility of Diet 1 (low-carbohydrate, high-protein dry diet), for dry matter, energy, crude protein and fat, indicated a greater concentration of the nutrients from the diet were absorbed by the cats and therefore less of this diet needs to be fed for the cat's requirements to be met. The higher apparent crude protein and fat digestibility of Diet 3 (wet diet) compared to Diet 2 (high-carbohydrate, low-protein dry diet) also suggested a greater concentration of the protein in this diet was apparently absorbed by the cats.

The cats assigned to Diet 1 and Diet 2 exhibited urine specific gravity values outside the range of 1.001 and 1.070 previously reported for healthy cats (Gaskell 1989), with mean values of 1.076 and 1.074 respectively. In contrast, the mean value for the cats fed Diet 3 was well within the healthy range (1.035). The differences in urine specific gravity between the three diets are as expected because of the differences in dietary form and water content. The results are supported by the large difference observed in the daily drinking water intakes of the cats fed the three diets, with the cats fed the two dry diets drinking more than four times the quantity of water of the cats fed the wet diet. It is common knowledge that cats fed dry diets tend to produce smaller amounts of more concentrated urine compared to wet diets and therefore it is logical to assume from this that specific gravity values would be higher for dry diets compared to those for wet diets (Gaskell 1989), as was determined in this study. Canned (wet) diets increase the volume of water the cat consumes (from the diet and drinking) and therefore also increases the volume of urine produced and decreases its concentration (Gaskell 1989; Zoran 2002). Subsequently wet foods are rarely implicated in the development of Feline Urological Syndrome (Gaskell 1989, Rowe 2002; Zoran 2002). However, in this study it would be expected that the increased daily drinking water intakes for the two dry diets would make up for the difference in total water consumption (from diet and drinking water) between the dry and wet diets. Regardless of this, the two dry diets tested in this study did have high specific gravity values and therefore need to be reformulated to reduce these urine specific gravity values within the healthy range so there are no risks of urinary tract issues developing with their consumption over time.

High-protein, low-carbohydrate diets have also been reported to help with weight loss by stimulating fat loss, whilst preserving lean mass (Hoenig *et al.* 2000; Biourge 2005; Laflamme and Hannah 2005; Laflamme 2006; Vester *et al.* 2009). One well designed study tested high-protein and low-protein diets in a cross-over design and found that the cats maintained their bodyweights and body mass index (BMI) over the trial on both diets,

however, the amount of body fat (as measured by DEXA) was higher when the cats were fed the low-protein diet than the high-protein diet (Hoenig *et al.* 2000). Another study by Laflamme and Hannah (2005) determined that increasing the protein in the diet from 35 % of energy to 45 % of energy resulted in more than 10 % greater fat loss (1.2 kg and 1.4 kg respectively) despite nearly an identical total amount of weight loss for the cats in the two dietary groups. Additionally the absolute loss of lean tissue was reduced by approximately 50 % in the cats that were fed the higher protein diet (Laflamme and Hannah 2005). However, other authors have seen no effects of this kind of diet in overweight or obese animals (Hoenig *et al.* 2007), with one study reporting feeding a low-carbohydrate, high-protein diet only induced weight loss when total calories were also restricted (Michel *et al.* 2005). During the course of the study reported here, no such determination of body composition was conducted; however this would be an important factor to consider in further studies.

It has been reported that the maintenance energy requirements of adult cats ranges from 20 to 100 kcal metabolizable energy (ME) per kg of bodyweight (Kienzle *et al.* 2006), with the mean energy requirements of the cats determined in the study of Kienzle and colleagues (2006) being  $60 \pm 18$  kcal ME per kg of bodyweight. On average, the cats fed each of the diets used in this study consumed more than this (73.01 - 77.48 kcal ME per kg bodyweight). As a result of this, the cats fed the two dry diets (Diet 1 and Diet 2) gained bodyweight over the course of the trial. The cats fed Diet 1 (low-carbohydrate, high-protein) gained the most weight, this may be due to their much higher digestible energy (DE) intake of this diet compared to the other two (72.34 kcal DE/kg body weight/day compared to 53.66 (Diet 2) and 47.95 (Diet 3)). Interestingly, the cats fed the wet diet (Diet 3) actually lost a little weight during the study. These results further support reports that cats fed dry diets often overeat and as a result put on weight (Zoran 2002; Kirk 2006; Hoenig *et al.* 2007) and that due to their lower calorie density, wet (canned) foods are a better option for weight regulation in cats (Kirk 2006; Laflamme 2006). Some of the increased food intake seen in this study may be due to the cats' environment and the increased requirements this demanded. These cats are housed in partially outdoor cages and therefore they would have increased energy requirements in order to maintain their normal body temperatures in these conditions.

It appears the high-protein, low-carbohydrate dry diet needs to be tested using restricted feeding methods and if commercialised its success in maintaining bodyweight would depend largely on the compliance of the owner to use this feeding regimen. Therefore a wet high-

protein, low-carbohydrate cat food may be more successful, because as shown in this study, even with *ad libitum* feeding the cats were able to either maintain their weight or lose a little weight while being fed this diet. A long term weight loss study using overweight or obese cats fed the three diets used in this study would also be worthwhile conducting, to provide further support of the use of a high-protein, low-carbohydrate diet (whether wet or dry) in feline weight loss programs.

All of the cats in the current study had blood glucose concentrations below maximum levels in the healthy range reported by the National Research Council in 2006 (6.7 mmol/L). However some cats in all three dietary groups had values below minimum levels in the healthy range (3.9 mmol/L) at one sampling point or more. The high-protein low-carbohydrate dry diet (Diet 1) produced intermediate glycaemic responses when compared to Diet 2 and Diet 3, however there were no differences ( $p>0.05$ ) between the AUC and Cmax values between the three diets. In contrast to these results, the peak glucose concentration was attained much later for the cats fed Diet 2 compared to the other two diets and this maximum was also numerically higher than for the other two diets, suggesting a larger postprandial response to the meal and therefore less glycaemic control. The high-protein, low-carbohydrate diets (wet and dry) would therefore be beneficial in cats with a risk of developing diabetes mellitus or with diabetes as minimising post-prandial endocrine responses is vital in these situations. Future studies using these two diets should also assess insulin responses post-prandially as insulin sensitivity is strongly associated with obesity and the development of diabetes in both cats and humans (Mazzaferro *et al.* 2003; Rand and Marshall 2005; Hoenig *et al.* 2007)

The high-protein low-carbohydrate dry diet (Diet 1) produced a smaller glycaemic response than the commercial high-carbohydrate dry diet (Diet 2). However unexpectedly, due to its high energy digestibility, it also produced more weight gain than the other two diets, illustrating that this diet needs to be fed using restricted (calorie controlled) rather than *ad libitum* feeding. Part of the reason for the inability for this particular group of cats to control their intake may be related to the fact they have been bought up on wet diets and therefore this may have predisposed them to respond poorly to the dry diets in this study and overeat. This theory would need to be tested however, what is interesting is that the cats fed the wet diet (Diet 3) in-fact maintained their weights or lost a minimal amount of weight with *ad libitum* feeding during this study. Therefore the problem seems to lie with the dietary energy content.

The results of this study also highlighted the potential of a high-protein, low-carbohydrate wet diet as the wet diet tested also produced smaller glycaemic responses than the high-carbohydrate diet and it had the extra benefits, unlike the dry high-protein diet of producing healthy values for urine specific gravity and being able to be fed *ad libitum* without the cats putting on weight.

Due to the practicalities of testing these two high-protein diets further, including running such a long term trial on weight maintenance and the ability to access diabetic cats, it was decided to focus on the use of these types of diets in dogs, particularly working dogs because they play such a vital role in New Zealand's farming industry and their diets are often substandard for the large amount of work they partake in on an often daily basis.



## **Chapter Five**

**The effects of the proportions of dietary  
macronutrients on digestibility and exercise in  
working dogs**

## 5.1 Introduction

One of the oldest uses of dogs is for controlling and moving farm animals in the cattle and sheep industries (Feldman and Lessard 1992; Kronfeld *et al.* 1994). However, despite this vital role in both New Zealand and Australia, very little research has been carried out regarding the exercise they partake in and their specific dietary requirements (Hampson and McGowan 2007). This is also true of other canine athletes that participate in a wide range of activities, such as hunt dogs and search and rescue dogs. In fact, the majority of the research available on the working dog has been conducted on the two extremes of the spectrum; the greyhound and the sled dog (Grandjean and Paragon 1993; Reynolds *et al.* 1995; Hill *et al.* 2000; Hill *et al.* 2001; McKenzie *et al.* 2005; Rovira *et al.* 2007) and these other working dogs (e.g. hunt dogs, farm dogs etc) are likely intermediate to these extremes.

Methods of improving the performance of human athletes using dietary manipulations have been actively sought for many decades (Reynolds *et al.* 1999). Similarly, interest is growing in finding a way of improving the performance and wellbeing of working dogs using nutrition. Often these animals are subjected to less than optimal care and although they will still work under these conditions, their performance, stamina and lifespan are decreased, ultimately disadvantaging both the dog and their owner (Guilford 1997).

Exercise places extraordinary demands on the dog's body, and diet is one of the basic foundations of performance and should be matched to the specific type of exercise being undertaken (Toll and Reynolds 2000). Providing the dog with the correct amount of energy is somewhat dictated by the quantity of food fed and the method of feeding, however, the macronutrient profile (protein, carbohydrate and fat) of the diet affects the maximum caloric consumption possible (Toll and Reynolds 2000). Exactly how these macronutrients should be partitioned in the diet of working dogs is the subject of much debate.

Dietary protein is used during exercise to meet structural, biochemical and energy requirements. There is substantial evidence that exercise increases an athlete's protein requirement and may reduce the incidence of injury (Reynolds *et al.* 1996; Reynolds *et al.* 1999). However, the magnitude of this increase and the best way of meeting it are still unknown. It is not only important to increase the protein levels in working dog diets but also to ensure there are enough calories from fats and carbohydrates in the diet so that the protein

provided is used primarily for tissue synthesis and other protein functions and not for energy (Reynolds *et al.* 1996).

When formulating working dog diets it is important to consider the ability of the macronutrients to increase the calorie density of the diet because working dogs frequently have trouble ingesting sufficient quantities of food to meet their energy requirements (Kronfeld 1973; Toll and Reynolds 2000). Fat can be used to increase the calorie density of the diet however carbohydrate contains relatively little energy and therefore can not be used to increase the energy density of the diet to any appreciable extent (Reynolds *et al.* 1995, Toll and Reynolds 2000). Despite this, carbohydrates are commonly used in working dog diets in large quantities due to their ability to provide bulk at a low cost compared to other ingredients such as meats and vegetables (Murray *et al.* 1999). These high-carbohydrate diets result in working dogs having to eat large volumes of food to meet their calorie requirements and therefore do not seem well suited for this application. The development of a diet more suited to these dogs' requirements; one which provides long lasting energy and meets the increased nutrient requirements exercise demands is of great interest.

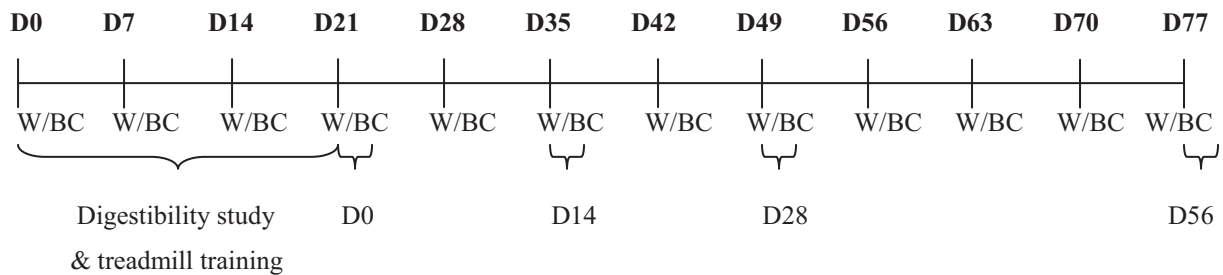
The purpose of this study was therefore to compare the effects of feeding diets with different macronutrient profiles on metabolism and performance in exercising dogs. The digestibility of the two diets were determined as part of this study because a highly digestible diet is of utmost importance for working dogs as it minimises the volume of the intestinal bolus, which is added weight for the animal to carry during exercise (Grandjean and Paragon 1992) and ensures the energy contained in the diet is readily available and quickly metabolised by the body for use (Grandjean and Paragon 1993; Toll and Reynolds 2000). Subsequently a poorly digestible diet has been reported to negatively impact on stamina and performance in working dogs (Downey *et al.* 1980). It was hoped the parameters measured during the exercise tests would allow a comparison to be made between the diets and would allow differences in performance to be determined. It was hypothesized that the high-protein diet would, as in the previous studies reported here in both cats and dogs at rest, have positive effects on exercising dogs also.

## 5.2 Materials and methods

### 5.2.1 Animals and housing

All management and experimental procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Eighteen (n=9 females and n=9 males) healthy entire adult Harrier Hounds (*Canis familiaris*), aged from 2 to 9 (mean 4.44, SEM 1.69) years, were obtained from the Manawatu Hunt Club, Palmerston North, New Zealand, and used in the 11-week study (Figure 5.1). Harrier hounds were selected because the work they partake in is very similar to that of farm dogs and therefore it was believed they would be a close model of the New Zealand working farm dog.



**Figure 5.1. Time-line of the study to determine the effects of the proportions of dietary macronutrients on digestibility and exercise of two diets fed to 18 working dogs (D= day, W/BC=weigh and body condition score).**

The dogs had been involved in previous studies at the unit (over half had been used in the study outlined in Chapter 2) and were therefore already trained to be walked on a leash, handled and restrained for sampling. However, all of the dogs needed to be trained to exercise on the treadmills before the first sampling day. All of the dogs were considered to be physically fit, having just completed a hunt season.

The dogs were weighed (Tru-test AG500 series scales, Tru-test Pty Limited, Sydney, Australia), and their body condition score assessed subjectively by the same personnel, using a standard scale (Laflamme 1997), at the beginning of the trial, and at weekly intervals until the end of the trial. The dogs were housed in purpose-built concrete pens measuring

approximately 2.9 x 5.5 m, at the Canine Unit, Massey University, Palmerston North, New Zealand. The pens were cleaned daily and all faeces scored, as outlined in Chapter 2, section 2.2.1.

All dogs were exercised daily for approximately 30 minutes, and had access to fresh water at all times. Twice a week throughout the 11-week study the dogs' daily exercise entailed treadmill training, where the dogs were run on the treadmill for a set length of time (30 minutes on Tuesdays and 15 minutes on Fridays) and intensity (4.6 ° slope on Tuesdays and 6.3 ° slope on Fridays). The speed of the treadmill was 3.5 ms<sup>-1</sup> for the female (n=9) dogs and 3.7 ms<sup>-1</sup> for the male (n=9) dogs. The reason for using different speeds for the two sexes was because of the difference in size between the male and female dogs and subsequently their difference in gaits.

### **5.2.2 Diets and feeding**

On arrival the dogs were randomly assigned to either Diet 1 or Diet 2, with 10 dogs receiving Diet 1 and eight dogs receiving Diet 2. The dietary groups were balanced for gender and the dogs were fed the assigned diet for the duration of the 11-week study.

Diet 1 comprised a low-carbohydrate, high-protein dry extruded diet manufactured specifically for the study by Heinz Watties Limited (Hastings, NZ), and Diet 2 comprised a high- carbohydrate, low-protein dry extruded diet available commercially (Pedigree Meaty Bites; Mars Incorporated, Bathurst NSW, Australia). The daily food allowance (adult maintenance energy requirement; MER) for each dog was determined and weighed as detailed in Chapter 2, section 2.2.2. The food allowance for each dog was adjusted to maintain individual ideal body condition.

The diets were analysed for moisture, ash, crude protein, fat, gross energy, crude fibre, carbohydrate and digestible energy as described in Chapter 2, section 2.2.2 (Table 5.1).

**Table 5.1. Dietary analysis<sup>a</sup> of Diet 1 and Diet 2 fed to 18 Harrier Hounds, in order to determine the effects of the proportions of dietary macronutrients on digestibility and exercise.**

<b>Nutrient</b>	<b>Diet 1 (High P)</b>	<b>Diet 2 (High CHO)</b>
Moisture (%)	4.8	11.3
Crude protein (%)	54.9	13.4
Crude fat (%)	11.4	12.2
Crude fibre (%)	2.5	3.2
Ash (%)	10.8	5.8
Carbohydrate <sup>b</sup> (%)	18.0	57.4
Gross energy (kJ/g)	20.3	17.9
Digestible energy (kJ/g)	17.2	16.3

<sup>a</sup> All results are on an ‘as received’ basis

<sup>b</sup> Calculated by difference

### **5.2.3 Digestibility of the diets**

The dogs were randomly assigned to three groups (n=6 per group), with each group participating in the digestibility study consecutively. During the digestibility study, the dogs were housed and fed as outlined in Chapter 2, section 2.2.3. The diets were fed for nine days, with the first four days serving as an adaptation period, and the final five days for collection of faeces. The faeces produced by each dog, including any excreted during exercise, were collected, weighed and handled as described in Chapter 2, section 2.2.3.

The apparent dry matter, crude protein, fat and energy digestibility of the two diets were calculated using the equation for the total collection method described in Chapter 2, section 2.2.3.

### **5.2.4 Treadmill study**

Following the digestibility study, a treadmill study was conducted over eight weeks. The dogs were introduced to the treadmills (DCG model, Jog-a-dog Ltd., Ottawa Lake, MI, USA) and trained to run on them over a three week period before the treadmill study using the exercise regime described in section 5.2.1.

Prior to the sampling days (day 0, 14, 28 and 56 of the treadmill part of the study), the dogs were shaved to reveal both jugular veins and assist with blood sampling. They were fed approximately 2 hours before the exercise test, and a 6 ml blood sample was collected from the jugular vein of each dog using Becton-Dickinson plastic 10 ml syringes and 23 G  $\frac{3}{4}$  needles (Becton-Dickinson, New Jersey, USA). The dog's rectal temperature was taken and they were fitted with a Polar FS1 heart rate monitor (Polar Electro Oy, Helsinki, Finland), which had been soaked with water along with the dog's fur to allow maximum conductivity. The watch for the heart rate monitor was attached to the side of the dog's treadmill and an initial heart rate was then recorded.

The dogs ran for 60 minutes on a 4.6 ° slope (Figure 5.2), at a speed of 3.5 ms<sup>-1</sup> for the female (total distance 12.4 km) and 3.7 ms<sup>-1</sup> for the male dogs (total distance 13.4 km), with a 5 minute break after 30 minutes. Heart rate readings were taken every 5 minutes during the exercise test and during the 5 minute break, the rectal temperature of the dog was taken. At the end of the exercise test, a final heart rate and rectal temperature were recorded. A second 6 ml blood sample was then taken from each of the dogs from the alternate jugular vein not used for the pre-exercise sample (to minimise any damage to the veins).



**Figure 5.2. Treadmill exercise test to determine the effects of the proportions of dietary macronutrients on exercise when Diet 1 and Diet 2 were fed to 18 Harrier hounds.**

The 6 ml blood samples were split into vacutainers; 1.0 ml a vacutainer containing fluoride oxalate for glucose analysis, 3.0 ml into a vacutainer containing heparin for triglyceride analysis, and 2.0 ml into a vacutainer free of additive for free fatty acid analysis (Becton-Dickinson, Franklin Lakes NJ, USA). The vacutainers were inverted and placed on an electric mixer for approximately 15 minutes to thoroughly mix, before being centrifuged (Labofuge 200, Heraeus Septatech, Osterode, Germany) at 3000 rpm for 10 minutes. The plasma or serum was then collected and frozen at -20 °C until it was analysed.

Concentrations of glucose in plasma (mmol/L) were analysed using the hexokinase method, using a Gluco-quant® kit (Roche Diagnostica, Basle, Switzerland). Concentrations of triglycerides in plasma (mmol/L) were analysed using the lipase/glycerol kinase method, and concentrations of free fatty acids in serum (mmol/L) were analysed using the ACS/ACOD enzymatic method.

#### **5.2.5 Statistical analysis**

One female dog could not be trained to exercise adequately on the treadmill and therefore only 17 of the dogs participated in this part of the study. All analyses were conducted using SAS v9.1 (SAS Institute Inc, Cary NC, USA).

The apparent digestibility of the two diets was compared statistically using simple ANOVA. Comparisons were included in the model to analyse differences between male and female dogs within and between dietary groups.

The mean ambient temperatures on each sampling day were also compared statistically using simple ANOVA. Differences between diets at each of the time-points for rectal temperature and heart rate were analysed using repeat measures ANOVA, to determine any differences between the diets at any of the time-points. The differences between pre and post exercise values for each dog on each diet were also compared using ANOVA.

The concentrations of glucose, triglycerides and free fatty acids before and after exercise, on each sampling day were compared using repeat measures ANOVA. The mean concentrations of blood glucose, triglyceride and free fatty acids  $\pm$  SEM were calculated for each diet before and after exercise. Differences between diets before and after exercise for glucose, triglycerides and free fatty acids were analysed using simple ANOVA, to determine any



significant differences between the diets, and between pre and post exercise values for each variable.

## 5.3 Results

### 5.3.1 Digestibility of the diets

The apparent nutrient digestibility coefficients of the two diets determined are summarised in Table 5.2. The effect of the type of diet was significant for all parameters ( $p < 0.01$ ) except energy. The apparent digestibility values for crude protein and fat of Diet 1 were higher ( $p < 0.01$ ), but the apparent dry matter digestibility was lower ( $p < 0.0001$ ), than for Diet 2. The apparent energy digestibility values were not different between the two diets ( $p > 0.05$ ). No significant effects of gender or diet-by-gender interactions were observed for any of the parameters ( $p > 0.05$ ).

**Table 5.2. Mean apparent nutrient digestibility coefficients of two diets fed to 18 Harrier Hounds (n=10 Diet 1 and n=8 Diet 2). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 was a high-carbohydrate, low-protein diet.**

Coefficient	Diet 1	Diet 2	Pooled SEM	Diet Significance
Dry Matter	0.731	0.792	0.005	<0.0001
Crude Protein	0.847	0.779	0.005	<0.0001
Fat	0.943	0.903	0.006	0.005
Energy	0.806	0.809	0.005	0.722

### 5.3.2 Ambient temperature

Despite the same procedure being used on each of the sampling days, the ambient temperature in the treadmill building was different between the four sampling days ( $p < 0.01$ ) (Table 5.3). The mean temperature on day 0 was lower than that on day 28 ( $p < 0.01$ ) and the mean temperature on day 56 was higher than on days 0 ( $p < 0.001$ ) and 14 ( $p < 0.01$ ).

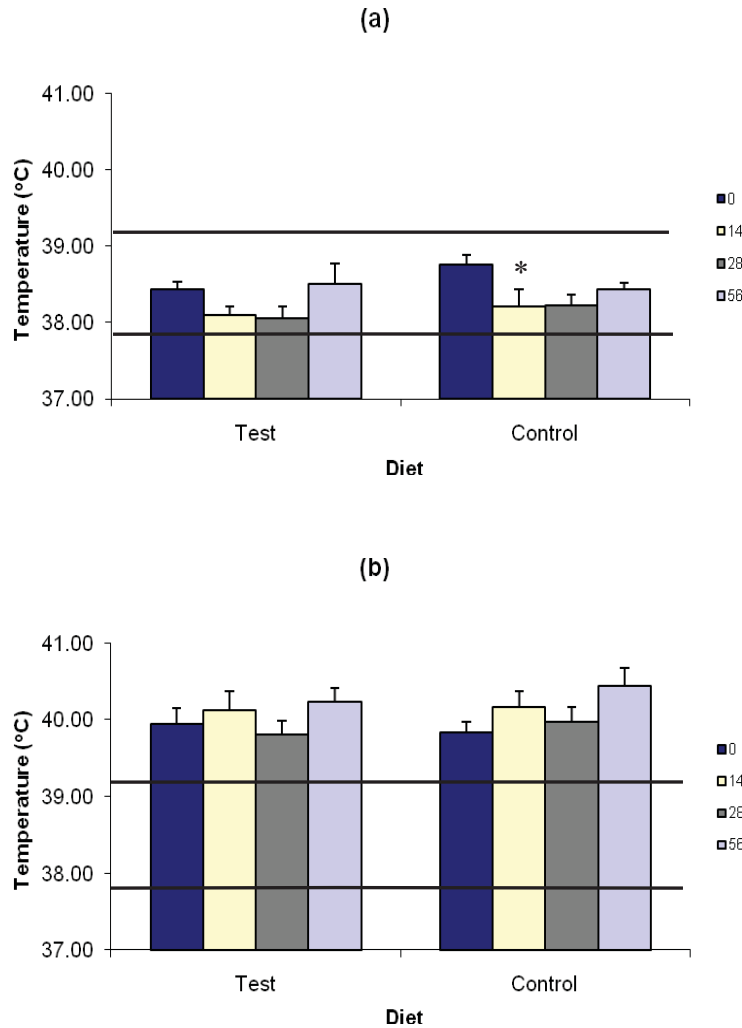
**Table 5.3. Mean ambient temperatures during the treadmill exercise tests of 17 Harrier Hounds fed Diet 1 and Diet 2.**

<b>Day</b>	<b>Ambient Temperature (°C)</b>
0	14.7
14	17.3
28	19.7
56	22.3

### **5.3.3 Rectal temperature**

Before the exercise tests, the dogs fed both of the diets displayed resting rectal temperatures within this range (Figure 5.3). As expected, after each exercise test the dogs fed both of the diets exhibited rectal temperatures above the upper limit of this resting range.

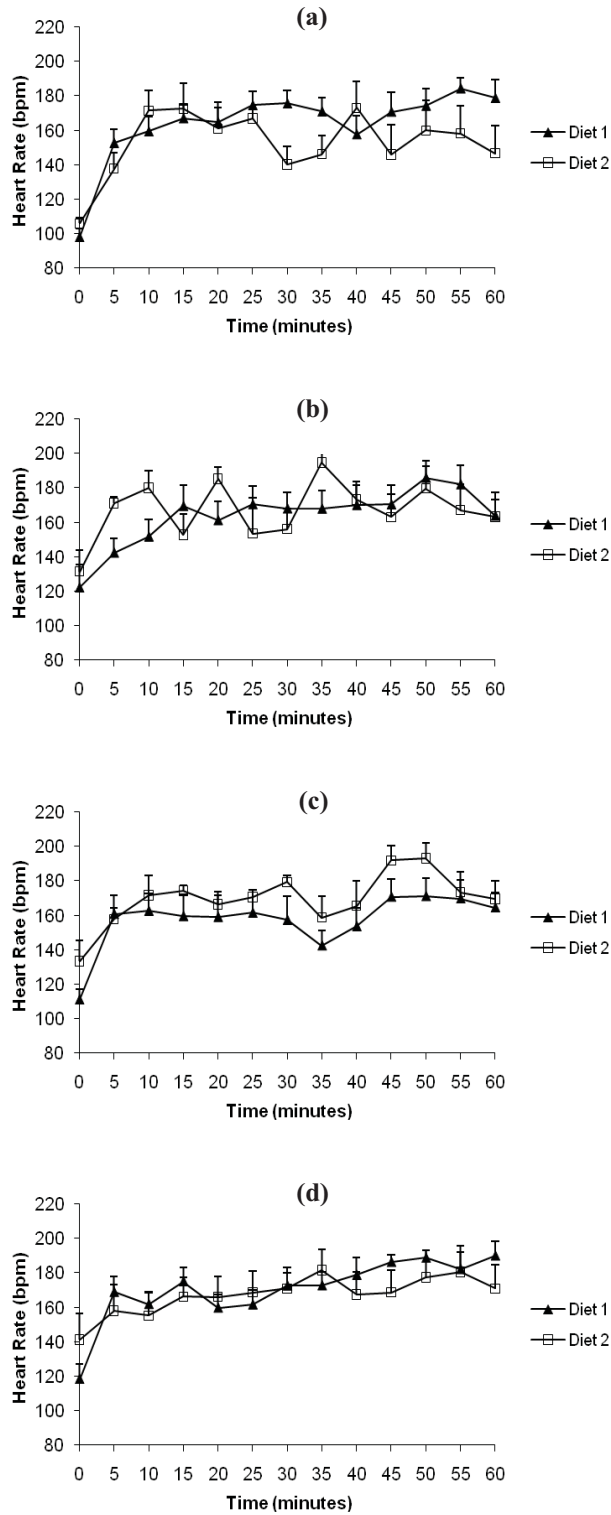
The mean rectal temperature of the dogs fed Diet 1 were consistently numerically lower than those of the dogs fed Diet 2, both before and after each of the exercise tests however, this difference was only significant ( $p < 0.05$ ) on day 14 before exercise. Following each exercise test the rectal temperature for the dogs fed both diets increased from the pre exercise values ( $p < 0.001$ ).



**Figure 5.3. Mean ( $\pm$  SEM) rectal temperatures from 17 Harrier Hounds fed either Diet 1 or Diet 2 (a) before, (b) after exercise tests ( — normal range,\*  $p < 0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

### 5.3.4 Heart rate

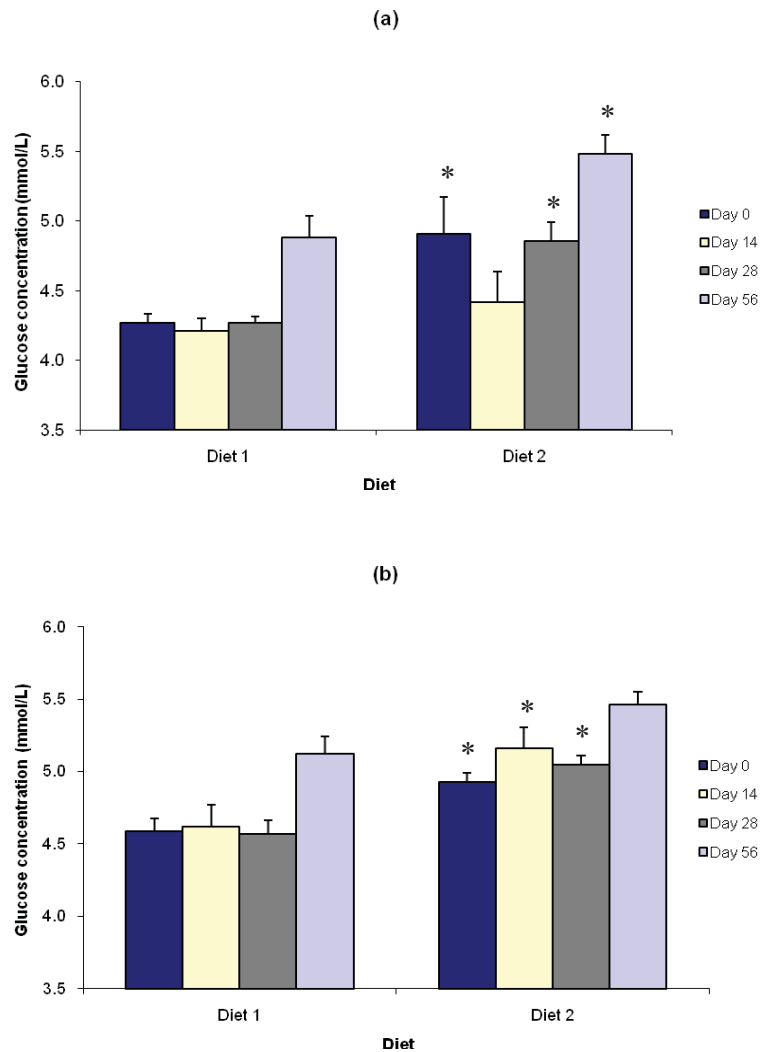
Heart rates were elevated in both the dogs fed Diet 2 ( $p < 0.05$ ) and Diet 1 ( $p < 0.001$ ) following each exercise test (Figure 5.4). The heart rates of the dogs fed Diet 1 were lower ( $p < 0.05$ ) than the dogs fed Diet 2 before exercise on each of the four test days (Day 0, 14, 28, 56) however this effect was not seen at any of the other collection times on any of the four sampling days ( $p > 0.05$ ).



**Figure 5.4. Mean ( $\pm$  SEM) heart rates from 17 Harrier Hounds fed Diet 1 or Diet 2 on (a) day 0, (b) day 14, (c) day 28 and (d) day 56. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

### 5.3.5 Concentration of plasma glucose

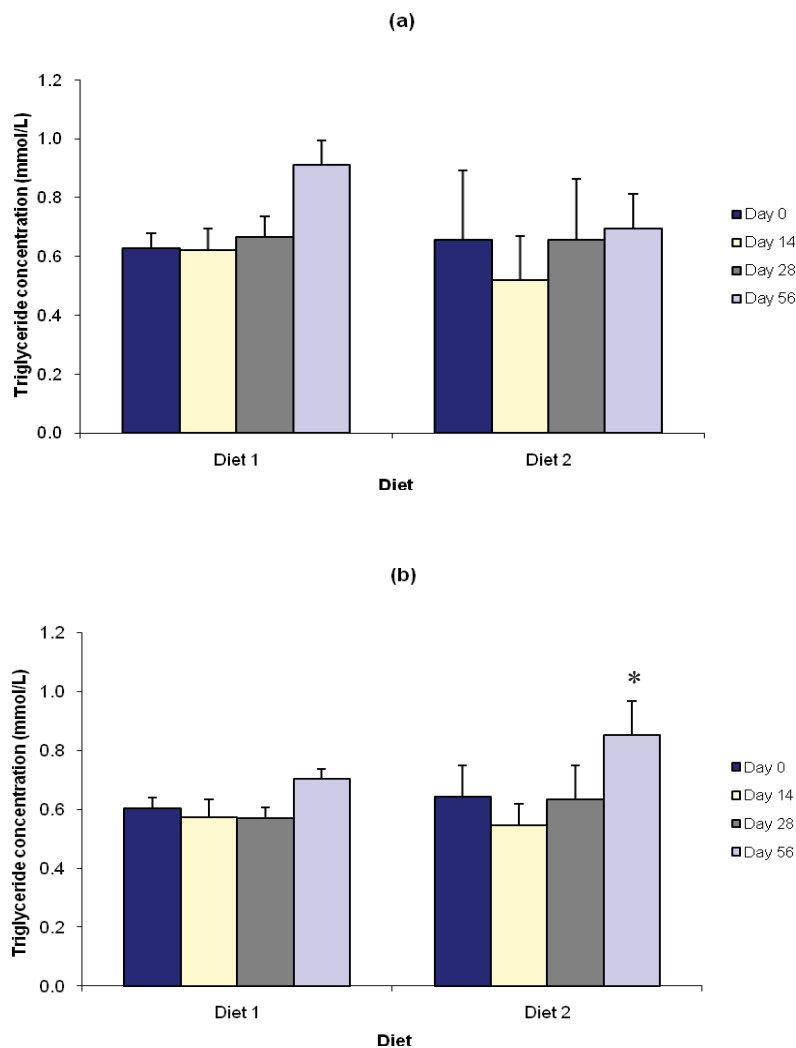
The concentrations of plasma glucose for dogs fed Diet 1 were lower than those fed Diet 2 on the majority of sampling days ( $p < 0.05$ ) (Figure 5.5a and 5.5b). There was no difference ( $p > 0.05$ ) in concentrations before and after exercise on any of the days for either diet.



**Figure 5.5. Mean ( $\pm$  SEM) plasma concentration of glucose of 17 Harrier Hounds fed Diet 1 and Diet 2 (a) before, (b) after exercise tests (\*  $p < 0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

### 5.3.6 Concentration of plasma triglycerides

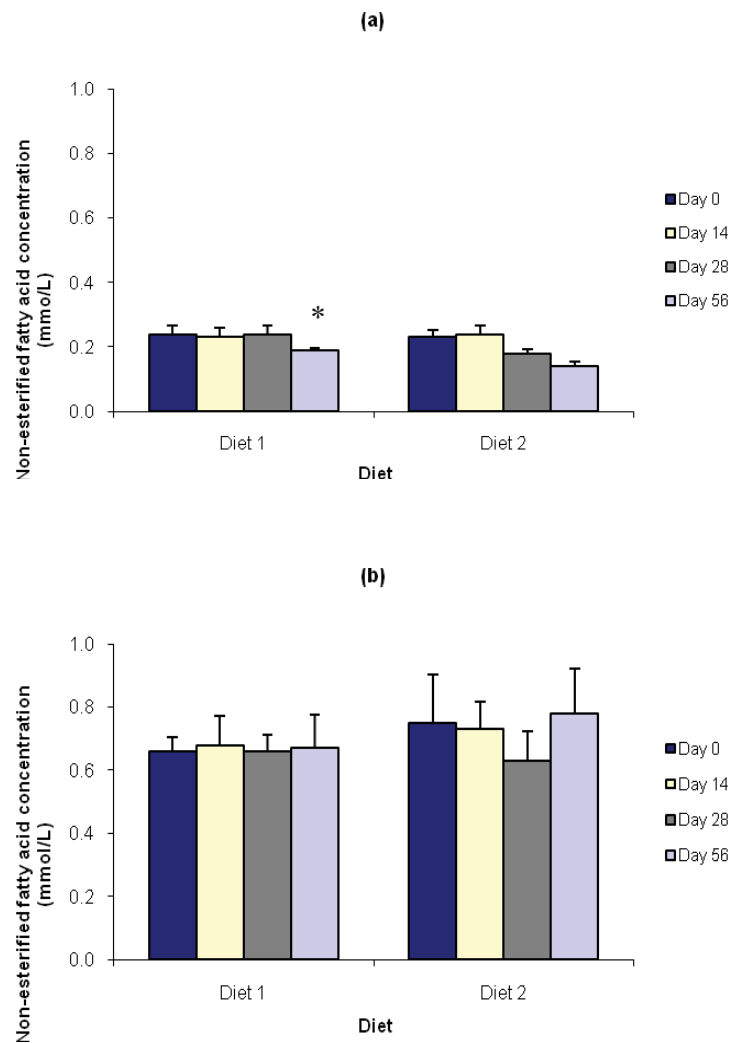
Triglyceride concentrations declined from baseline (i.e. before exercise ) after exercise for Diet 1 on day 56 ( $p>0.05$ ), but increased from baseline after exercise for Diet 2 on day 56 ( $p>0.05$ ) (Figure 5.6a and 5.6b). There were no significant ( $p>0.05$ ) differences in pre-exercise triglyceride concentrations between the 2 diets however, after exercise on day 56, the mean for Diet 2 was higher than that for Diet 1 ( $p<0.05$ ).



**Figure 5.6.** Mean ( $\pm$  SEM) plasma concentration of triglycerides from 17 Harrier Hounds fed Diet 1 and Diet 2 (a) before, (b) after exercise tests (\*  $p<0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.

### 5.3.7 Concentration of serum free fatty acids

Serum free fatty acid concentrations before exercise were only different between the two diets on day 56 ( $p < 0.01$ ), and there was no difference between the diets after exercise on any of the sampling days ( $p > 0.05$ ) (Figure 5.7a and 5.7b). Compared to pre-exercise values, free fatty acid concentrations were significantly elevated in dogs fed both of the diets following each exercise test ( $p < 0.0001$ ).



**Figure 5.7.** Mean ( $\pm$  SEM) serum concentration of free fatty acids from 17 Harrier Hounds fed Diet 1 and Diet 2 (a) before, (b) after exercise tests (\*  $p < 0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.



## 5.4 Discussion

Today the majority of farmers in New Zealand still feed home killed meat to their dogs, however, these are deficient or marginal in many micronutrients and a large quantity must be ingested to meet the working dogs increased energy needs (Guilford 1997). Furthermore, the majority of dry diets that are available for working dogs today are high in carbohydrates and the carbohydrate sources are often not of a high quality. The result of feeding these high carbohydrate diets is that a large amount of food needs to be consumed to meet their energy demands, which is limited by the dog's stomach capacity. This excessive carbohydrate intake can also lead to increased water loss in the stools, increased gas production and increased faecal bulk (Grandjean and Paragon 1993; Toll and Reynolds 2000).

In the present study, the effects of feeding a high-protein, low-carbohydrate (55 % protein, 18 % carbohydrate) dry diet (Diet 1) were compared with the effects of feeding a commercial high-carbohydrate (13 % protein, 57 % carbohydrate) dry diet (Diet 2) on digestibility and exercise in working dogs. Hunting dogs were used in the present study as it was believed the results obtained could be extrapolated to farm dogs because they perform similar exercise (primarily endurance with bouts of sprinting), and both can be considered intermediate in the exercise they do and their nutrient requirements compared to the extremes of greyhounds and sled dogs (Toll and Reynolds 2000; Ahlstrom *et al.* 2006; Rovira *et al.* 2007). There is very scarce information on these intermediate athletes, especially farm dogs and therefore it is vital because of their important roles in farm management that more research is conducted on them.

The higher apparent crude protein and fat digestibility of Diet 1 in this study suggested that greater concentrations of these nutrients were available by the dogs fed this diet compared to Diet 2. This confers an advantage because diets with a high digestibility and energy density have been shown to positively affect stamina (Downey *et al.* 1980). Although the dry matter digestibility of Diet 2 was higher than Diet 1, the values for both diets were slightly lower than the 80 % minimum recommended for working dogs (Toll and Reynolds 2000; Guilford 1997).

The reported normal resting temperature range for dogs is between 37.8 °C and 39.2 °C (Carlson and Griffin 1992). The mean rectal temperatures of the dogs fed Diet 1 were

consistently lower (only significant on day 14 before exercise) than those fed Diet 2 before and after each of the exercise tests. As expected the temperatures after exercise for the dogs fed both diets were significantly elevated from pre exercise values. This is because a portion of the energy in the diet is converted into heat and therefore the animal's body temperature increases as the dog can not dissipate all of this extra heat (Guilford 1997). The mean temperatures of the dogs in this study before and after exercise are similar to those previously reported for labrador retrievers, sled dogs and greyhounds (Downey *et al.* 1980; Matwichuk *et al.* 1999; Steiss *et al.* 2004).

The resting heart rates of the dogs fed Diet 1 were numerically lower than those fed Diet 2, indicating these animals were physically fitter. In humans, training and therefore increased fitness results in lower resting heart rates and lower resting blood pressure (Wyatt and Mitchell 1974; Dickhuth *et al.* 2004). The heart rates of the dogs fed both diets were significantly higher than their resting rates following exercise, as has been reported in other studies of working dogs (Ilkiw *et al.* 1989; Steiss *et al.* 2004). The mean resting heart rates of the dogs during this study (104 to 141 bpm) are similar to those reported for sled dogs and labrador retrievers (Hammel *et al.* 1977; Downey *et al.* 1980; Steiss *et al.* 2004), but are much higher than those reported for greyhounds (Taylor 1988) which may be an indication that these dogs are more similar to the sled dog and Labrador retriever than greyhounds in terms of physiology. Following exercise the heart rates of the dogs involved in this study (147 to 196 bpm) were also similar to those reported for sled dogs (Hammel *et al.* 1977; Downey *et al.* 1980), but were much lower than the maximum heart rates published by Hampson and McGowan in 2007 for sled dogs, greyhounds and mongrel dogs (300, 318 and 301 bpm respectively).

Studies using sled dogs have highlighted that changes occurring with exercise can be measured in the blood. However, the importance of these changes, their relationship to performance and the actual level of exercise required to demonstrate these changes is very much unknown (Burr *et al.* 1997). It appears from previous work and the present study that measurements such as heart rate and rectal temperature may be more sensitive than such blood biochemical parameters (Sneddon *et al.* 1989).

This study attempted to use blood glucose, free fatty acid and triglyceride concentrations as parameters for distinguishing physiological differences between the dogs fed the two diets,

producing mixed results. All the dogs fed Diet 2 had concentrations of glucose within the range (3.9 to 6.7 mmol/L) reported by the National Research Council of the National Academies in 2006. One dog fed Diet 1 had two pre-exercise concentrations below the lower limit of this range. The blood glucose concentrations of the dogs fed Diet 1 were significantly lower than those fed Diet 2 before and after exercise on nearly all sampling days, which may be an early indication that the high-protein diet releases glucose at a slower steadier rate than the high-carbohydrate diet. Alternatively, it could suggest that the dogs fed Diet 2 were relying more heavily on the utilisation of carbohydrates for muscle fuel, hence the higher concentrations of glucose in the blood, however these theories require further investigation. Interestingly, on day 56, the glucose concentrations for the dogs fed Diet 1 were much higher than on the other days. The reason for this is unknown, however, it may relate to the higher ambient temperature and therefore the exercise intensity being such that sensitive changes in could be observed.

The only significant difference in triglyceride concentrations between the diets was after exercise on day 56, with decreases determined for the dogs fed Diet 1 and increases determined for the dogs fed Diet 2. Interesting this was once more when the ambient temperature was also higher compared to the other sampling days and therefore may be related to an increase in intensity of exercise. This result was surprising because it has been reported circulating triglyceride concentrations may indicate the mobilisation of fat for use by the muscles for energy (Rovira *et al.* 2007) and based on the glucose results and speculation regarding their explanation, one would therefore expect the triglyceride concentrations to be higher in Diet 1 than Diet 2. However, it has also been reported that the extent of the change in triglyceride concentrations following exercise depends largely on the exercise intensity and activity of lipolysis (Poso *et al.* 1989), therefore once more the level of intensity used in the study reported here may not have been enough.

The post-exercise free fatty acid concentrations of the dogs fed the two diets were significantly elevated from pre-exercise concentrations, indicating an increase in free fatty mobilisation during this exercise (Hammel *et al.* 1977; McClelland *et al.* 1995; Reynolds *et al.* 1996; Murphy *et al.* 1997) and therefore a greater reliance on fat sources to fuel muscular work. This is beneficial because total stores of fatty acids in the body represent a much larger energy potential than carbohydrate stores, such as muscle glycogen (Sahlin 1986). Therefore using these sources spares more limited carbohydrate stores, delays the onset of fatigue and

may improve stamina (Reynolds *et al.* 1994). The free fatty acid concentrations were only significantly different between the two dietary groups before exercise on day 56 but the reason for this difference is unclear.

Due to the relative newness of this area of research for the university (this is only the second exercise study of this nature carried out at this university with these harrier hounds) and lack of any kind of data specific to such hounds, the physical capacity of these dogs is still somewhat unknown. Therefore it may be that the amount of exercise these dogs were subjected to during the exercise tests was not enough to push the dogs and produce differences in the biochemical parameters selected. This is supported by the fact that on day 56 when the ambient temperature increased significantly some differences were seen, possibly due to the increased intensity the higher temperature created. Alternatively, it may be that the parameters chosen are not easily altered during even the most intense exercise, as relatively little work has been carried out to establish reliable sensitive markers to use in such exercise studies. A further explanation is that there simply were no differences to be seen between the two diets tested. It would be very beneficial to identify some reliable biochemical markers associated with exercise in the dog and then establish a set of reference values for different types of exercise before trying to evaluate the effects of factors such as diet on performance or exercise intolerance in the working dog. It would be worthwhile carrying out another exercise study using these dogs and make key changes to the study protocol and treadmill exercise to establish if by increasing the intensity of exercise, clearer differences in exercise performance between the two diets can be established.

In conclusion, the high-protein, low-carbohydrate Diet 1 formulated for this study may be beneficial to intermediate working dogs such as those used for farming and hunting, however further research is needed to confirm this as the results of this study were not definitive. The logical next step would be to increase the intensity of exercise used in the treadmill exercise, attempt to better control the temperature of the treadmill room over the 56 day study and measure some additional markers, to observe if these factors allow clear dietary differences to be determined in these exercising dogs.

## **Chapter Six**

**The effects of the proportions of dietary  
macronutrients on digestibility, exercise and  
immunity in working dogs**

## 6.1 Introduction

It is widely accepted that working dogs require a diet with a high digestibility and high energy density (Hammel *et al.* 1977; Toll and Reynolds 2000) and ideally one which maintains their energy levels throughout periods of work. There is also an increasing amount of evidence available that athletes require more dietary protein than non-athletes, due to an increased level of protein synthesis and catabolism (Hammel *et al.* 1977; Reynolds *et al.* 1999). For example, a study using sled dogs determined that 18 % protein in the diet was insufficient as it resulted in a significant decrease in VO<sub>2</sub>max, suggesting this diet failed to meet the animal's requirements, and a greater rate of soft tissue injuries for these animals during a 12 week training program involving runs of 10 to 15 km four times a week and maximal treadmill tests (Reynolds *et al.* 1999). These authors subsequently recommended a level of 35 % would be more suitable because this diet was found to enhance performance by increasing plasma volume and resulting in fewer soft tissue injuries (Reynolds *et al.* 1999).

On the other hand, high-carbohydrate diets, despite being prevalent on the commercial dog market, have been shown to be detrimental to some working dogs, in particular sled dogs. One particular study showed that a team of sled dogs fed a high carbohydrate diet exhibited poor performance, a stiff gait and coprophagy (Kronfeld 1973; Hammel *et al.* 1977). When this diet was changed in three steps by decreasing the proportion of carbohydrate and increasing the proportion of protein and fat, the coprophagy stopped and the dog's performance and stamina improved (Kronfeld 1973; Hammel *et al.* 1977; Kronfeld 1994). Working dogs in New Zealand include farm dogs, hunt dogs, gun dogs and those used for a range of other leisure activities. Studies of the effects of high-carbohydrate diets when fed to these specific types of dogs are lacking, however, it is believed the exercise these athletes perform is closer to the sled dog than the greyhound and therefore the results of studies with sled dogs may be able to be extrapolated to these animals.

There is a dearth of information available relating specifically to the effects of diet and exercise on immunity in working dogs, with the majority relating to the impacts of nutraceuticals and aging on the immune system (Greeley *et al.* 1996; Greeley *et al.* 2001; Heaton *et al.* 2002; Hayek *et al.* 2004). Over and under nutrition as well as exercise, due to the increased release of stress hormones, may impact on the immune function in a negative manner (Gleeson 2006). Subsequently until this area is investigated more widely, it is

important to at least ensure that no particular diet or exercise regime is impacting negatively on the dog's immune system. Lymphocyte (T and B cell) proliferation assays have previously been used as indicators of immune function (Campbell *et al.* 1988; Davis *et al.* 2008) and such assays were subsequently utilised during this study.

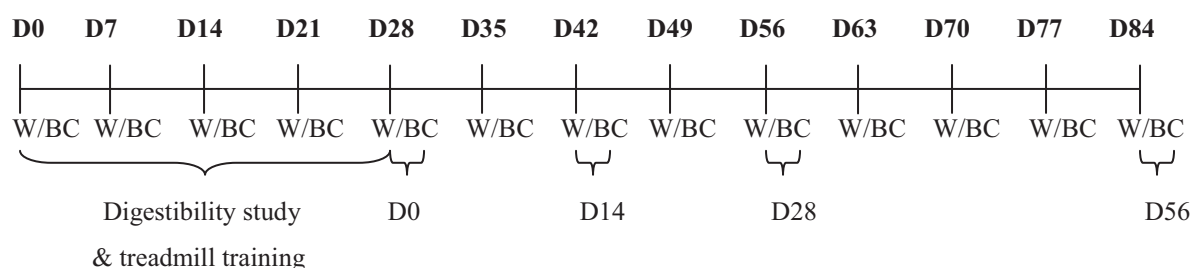
High-protein, low-carbohydrate diets are of great practical interest for working dogs such as those used in New Zealand. Hunt dogs were the subjects of this trial and it was hoped they could be used as a model for working farm dogs as both are likely to do similar work; mainly endurance with bouts of sprinting. The purpose of this study was to further compare the effects of feeding working dogs (*Canis familiaris*) diets differing in macronutrient proportions on exercise physiology and performance. In order to do this the same high-protein, low-carbohydrate diet used in Chapter 5 (Diet 1) and a different commercially available high-carbohydrate diet (Diet 2) than that used in Chapter 5 were fed and their effects on digestibility, exercise and immunity determined. The key differences between this and the previous study were the dogs underwent an initial one month training program, on the training and sampling days the intensity of exercise was increased by increasing the slope of the treadmill to 6.3 ° and in addition to the blood parameters measured in the previous study, lactate and various immune variables were also determined. It was hypothesized that with these changes, differences in the metabolism of the dogs fed the two diets would be able to be more clearly seen in the variables measured.

## 6.2 Materials and methods

### 6.2.1 Animals and housing

All management and experimental procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Thirteen (n=7 females and n=6 males) healthy entire adult Harrier Hounds (*Canis familiaris*), aged from 1 to 6 (mean 3.42, SEM 0.47) years, were obtained from the Manawatu Hunt Club, Palmerston North, New Zealand, and used in the 12-week study (Figure 6.1), with one female serving as a spare in case of injuries or illness.



**Figure 6.1. Time-line of the study to determine the effects of the proportions of dietary macronutrients on digestibility, exercise and immunity of two diets fed to 12 working dogs (D=day, W/BC=weigh and body condition score).**

Over half of the dogs used in this study had been involved in the study outlined in Chapter 5 and were therefore already trained to be handled, restrained and exercised on the treadmills. The remainders were trained during the four week period before the first exercise test. All of the dogs were considered to be physically fit, having just completed a hunt season.

The dogs were weighed, and their body condition score assessed as described in Chapter 5, section 5.2.1. The dog's housing and daily management procedures were the same as described in Chapter 5, section 5.2.1.

Twice a week the dog's daily exercise entailed treadmill training, where the dogs were run on the treadmill for 30 minutes on Tuesdays and 15 minutes on Fridays on a 6.3 ° slope



(previously 4.6 ° on Tuesdays and 6.3 ° on Fridays). The speed of the treadmill was 3.5 ms<sup>-1</sup> for the female dogs and 3.7 ms<sup>-1</sup> for the male dogs.

### 6.2.2 Diets and feeding

The dogs were all fed Diet 2 for the one month training period before the main study. Following the baseline sampling day (day 0), the dogs were randomly assigned to either Diet 1 or Diet 2 (n=7 on Diet 1 and n=5 on Diet 2), which they were fed for the remainder of the study. One of the original dogs on Diet 2 was removed from the study due to illness and the replacement dog was unfortunately already being fed Diet 1.

Diet 1 comprised a low-carbohydrate, high-protein (as compared to the other diet fed) dry extruded diet manufactured specifically for the study by Heinz Watties Limited (Hastings, New Zealand), and Diet 2 comprised a high-carbohydrate, low-protein dry extruded diet available commercially (Pedigree Naturals; Mars Incorporated, Bathurst NSW, Australia). The daily food allowance for each dog was determined and weighed as detailed in Chapter 2, section 2.2.2, and food allowances were adjusted to maintain individual ideal body condition.

The diets were analysed for moisture, ash, crude protein, fat, carbohydrate, ash, crude fibre and gross energy (Table 6.1), as described in Chapter 2, section 2.2.2.

**Table 6.1. Dietary analysis<sup>a</sup> of Diet 1 and Diet 2 fed to 12 Harrier Hounds, in order to determine the effects of the proportions of dietary macronutrients on digestibility, exercise and immunity.**

<b>Nutrient</b>	<b>Diet 1</b>	<b>Diet 2</b>
Moisture, %	10.6	11.0
Crude protein, %	43.0	17.2
Crude fat, %	10.8	9.5
Crude fibre, %	2.2	2.7
Ash, %	4.7	6.9
Carbohydrate <sup>b</sup> , %	30.9	55.5
Gross energy (kJ/g)	19.3	17.3

<sup>a</sup> All results are on an ‘as received’ basis

<sup>b</sup> Calculated by difference

### **6.2.3 Digestibility of the diets**

The digestibility assay was carried out as described in Chapter 5, section 5.2.3, except there were two groups of six dogs and the dogs had already been adapted to these diets prior to the digestibility assay commencing. Therefore, the dogs were only housed individually for five days to allow for total faecal collection. The faeces produced by each dog in these five days, were collected, weighed and handled as outlined in Chapter 2, section 2.2.3. The apparent dry matter, crude protein, fat, energy and carbohydrate (by difference) digestibility of the two diets, were calculated using the equation for the total collection method described in Chapter 2, section 2.2.3.

### **6.2.4 Treadmill study**

The dogs were trained to run on the treadmills (DCG model, Jog-a-dog Ltd., Ottawa Lake, MI, USA) during the one month pre-study period. However, because most of the dogs had been involved in the previous treadmill trial (Chapter 5), no problems with dogs adapting to the treadmill exercise were encountered. Throughout the three month treadmill period of the study the dogs were exercised on the treadmills as described in section 6.2.1.

The preparation of the dogs for the sampling days and the sampling day (day 0, 14, 28, 56) protocol was similar to that used in Chapter 5, but with the following key differences. An 8 ml blood sample was collected from the jugular vein of each dog before exercise and a 6 ml blood sample after the exercise test. The exercise test was again 60 minutes long with a 5 minute break after 30 minutes, however, the dogs were run on a 6.3 ° slope (previously 4.6 °), at a speed of 3.5ms<sup>-1</sup> for the female and 3.7 ms<sup>-1</sup> for the male dogs in order to increase the exercise intensity from that used in Chapter 5. During the 5 minute break, the dogs were also offered water equivalent to an hour of their daily water requirements, calculated using the equation 60 ml x bodyweight (kg) / 24 (Schaer 1989). Any water refused was recorded. During these days the ambient temperature in the treadmill room was also more controlled using heaters and thermometers to try to maintain the temperature at 20 ± 4 °C.

Blood lactate was assayed immediately using a hand held lactate analyser (Accutrend lactate analyser, Roche, Basel, Switzerland) and the remainder split into Becton-Dickinson vacutainers; 1.0 ml into a vacutainer containing fluoride oxalate for glucose analysis, 3.0 ml into a vacutainer containing heparin for triglyceride analysis, 2.0 ml into a vacutainer free of additive for free fatty acid analysis, and for the pre-exercise samples, 2.0 ml into a second

vacutainer containing heparin for the immunological assays. The vacutainers for glucose, triglyceride and free fatty acid analysis were inverted ten times, placed on an electric mixer for approximately 15 minutes to thoroughly mix, before being centrifuged (Labofuge 200, Heraeus Septatech, Osterode, Germany) at 3000 rpm for 10 minutes. The plasma or serum was then removed and handled as described in chapter 5.2.4. The vacutainers for immunological assays were inverted ten times, and placed on the electric mixer until they were collected for analysis. Blood glucose, triglyceride and non-esterified fatty acid concentrations (mmol/L) were analysed using the methods detailed in Chapter 5.2.4.

A number of assays were carried out to ensure there were no detrimental effects of the diets or exercise on the immune system (Table 6.2).

**Table 6.2. Specificity of CD markers and role of the labelled cell type**

<b>CD Marker</b>	<b>Type Cell</b>	<b>Role</b>
<b>CD4</b>	<b>T-helper cell</b>	Secrete factors to activate white blood cells to fight infection
<b>CD8</b>	<b>Cytotoxic T cell</b>	Directly kill tumour cells, viral infected cells, parasites
<b>B cell</b>	<b>B cell</b>	Differentiate into plasma cells which make antibodies to foreign bacterial proteins, viruses, tumour cells
<b>CD14</b>	<b>Monocyte</b>	Phagocytes which circulate in the blood

The following analyses were conducted to investigate any impacts of the diets and/or exercise on the immune system. Two colour flow cytometric analysis was used to determine the level of expression of CD4<sup>+</sup> (T-helper cells), CD8<sup>+</sup> (cytotoxic T-cells), B cells and CD14<sup>+</sup> (monocytes) antigens on peripheral blood leucocytes and granulocytes. Antibodies obtained from Serotec (Raleigh, NC) were either canine-specific (B cells, CD4 and CD8) or human-specific (CD14). Immunolabelling was performed according to the method of Gill *et al.* (2000), and samples were analysed using a FACSCalibur flow cytometer (Becton Dickinson Instruments, Cambridge, MA). For each sample, data was collected for 10,000 gated events.

In order to measure the stimulation index for ConA and PHA in whole blood, a proliferation assay was modified from methods previously reported (Hiraga *et al.* 1981; Weiss 1992; Saker *et al.* 2001). Firstly, lithium heparin-treated peripheral whole blood was diluted 1:4 in complete RPMI-1640 medium (RPMI-1640 medium supplemented with 10 % foetal calf serum, 10 mM HEPES, 2 mM-L-glutamine, 100 Uml<sup>-1</sup> penicillin, 100 µgml<sup>-1</sup> streptomycin sulphate and 50 µM 2-mercaptoethanol; all reagents from Gibco, Poole, UK). 150 µl of this diluted blood was then added in quadruplicate to the wells of a 96-well, flat-bottomed tissue culture plate (Greiner, Neuberger, Germany) and cultured in the presence of either 2.5 µgml<sup>-1</sup> Concanavalin A (Con A) (Sigma, USA), 1:100 diluted Phytohaemagglutinin (PHA) (Gibco, Poole, UK), or complete RPMI-1640 in place of the mitogen (control wells). The cells were cultured for 48 hours at 37 °C in a 5 % humidified CO<sub>2</sub>-air atmosphere, before being pulsed for 18 hours with 0.5 µCi methyl-<sup>3</sup>H-thymidine (Amersham Biosciences, UK) per well. Each plate was then harvested onto a 96-well glass fibre mat using a Tomtek cell harvester 96 (Hamden, CT, USA) and counted using a Wallac MicroBeta Trilux 1450 liquid scintillation and luminescence counter (Turku, Finland). Stimulation index was calculated as counts per minute (cpm) in wells with mitogen divided by cpm in wells without mitogen.

Assessment of the phagocytic capacity of peripheral blood leucocytes by flow cytometry was based on the method of Rutherford-Markwick *et al.* (2005). Briefly, 5 µl of FITC-labelled *Escherichia coli* bacteria (1x10<sup>9</sup>/ml) (Molecular Probes Incorporated, Oregon, USA) was mixed with 100 µl of whole blood and was incubated for 30 minutes at 37 °C. Immediately following incubation the cells were fixed with paraformaldehyde and the erythrocytes lysed by the addition of 1 ml of ice-cold water. Following centrifugation, the pellet was resuspended in 500 µl of PBS, and 50 µl of 4 % Trypan blue added to quench extraneous fluorescence. The phagocytic activity was determined using a FACSCalibur flow cytometer (Becton Dickinson Instruments, Cambridge, MA).

### **6.2.5 Statistical analysis**

All analyses were conducted using SAS v9.1 (SAS Institute Inc, Cary NC, USA). The apparent digestibility of the diets was compared statistically using simple ANOVA, where comparisons in the model analysed differences between male and female dogs. Simple ANOVA was also used to compare the mean ambient temperatures on the sampling days.

Repeat Measures ANOVA was used to analyse any differences between the diets for rectal temperature and heart rate. Differences between pre and post exercise heart rates and rectal temperatures were calculated and compared using ANOVA.

The mean concentrations of glucose, triglycerides and free fatty acids before and after exercise, on each sampling day were compared using repeat measures ANOVA. Differences between the diets before and after exercise for glucose, triglycerides and free fatty acids were analysed using simple ANOVA, to determine any significant differences between the diets, and between pre and post exercise values for each variable. Lactate concentrations determined before and after exercise on Days 28 and 56 were also compared using repeat measures ANOVA and changes in lactate concentration from pre to post exercise compared using simple ANOVA.

The immune data for each dog, for each sampling day was compared using repeat measures ANOVA, to determine differences between the diets, dogs and sampling days.

## 6.3 Results

### 6.3.1 Digestibility of the diets

The apparent nutrient digestibility coefficients of the two diets are summarised in Table 6.3. The effect of the type of diet was significant for all parameters ( $p < 0.01$ ) except dry matter. The apparent digestibility values for crude protein, fat and energy of Diet 1 were higher ( $p < 0.01$ ), but the apparent carbohydrate digestibility was lower ( $p < 0.0001$ ), than for Diet 2. The apparent dry matter digestibility values were not different between the two diets ( $p > 0.05$ ) and no significant effects of gender or diet-by-gender interactions were observed for any of the parameters ( $p > 0.05$ ).

**Table 6.3. Mean apparent nutrient digestibility coefficients of two diets fed to 12 Harrier Hounds (n=7 Diet 1 and n=5 Diet 2). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 was a high-carbohydrate, low-protein diet.**

Coefficient	Diet 1	Diet 2	Pooled SEM	Diet Significance
Dry matter	0.836	0.812	0.006	0.071
Crude Protein	0.908	0.815	0.004	<0.0001
Energy	0.864	0.830	0.005	0.006
Fat	0.964	0.876	0.003	<0.0001
Carbohydrate <sup>a</sup>	0.752	0.838	0.007	<0.0001

<sup>a</sup> Calculated by difference

### 6.3.2 Ambient temperature

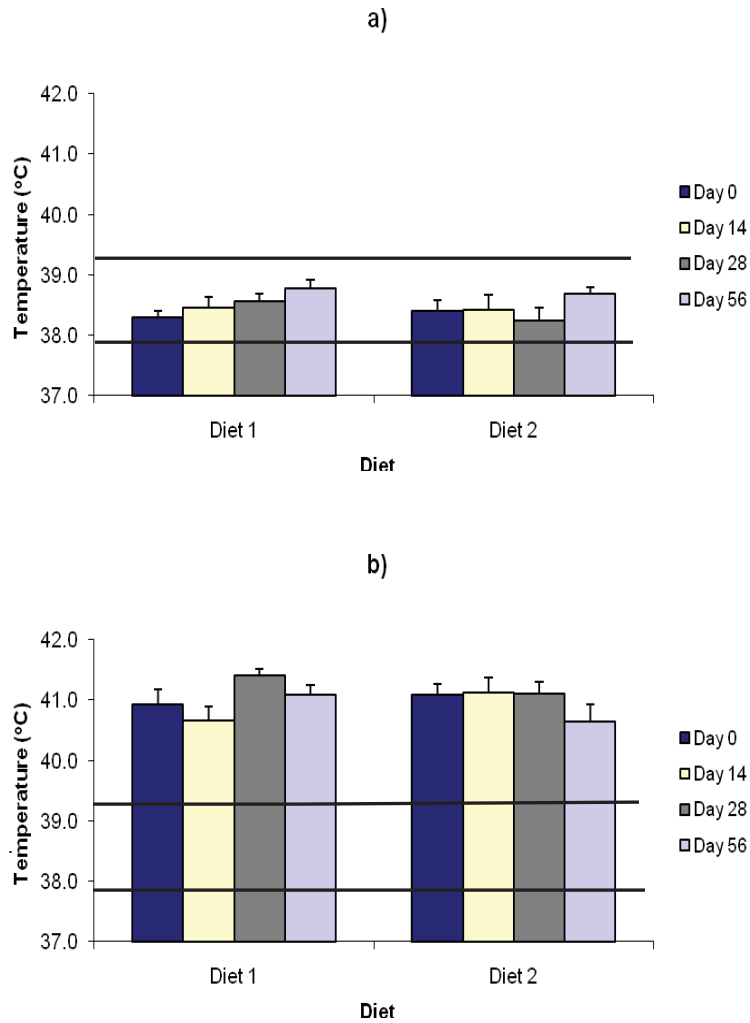
Despite the efforts made to maintain a constant and similar temperature on each of the sampling days by using heaters and thermometers to monitor the temperature and adjust it as needed, the ambient temperature in the treadmill building was still different (Table 6.4). The ambient temperature on day 0 was higher than day 14 ( $p < 0.01$ ) and day 56 ( $p < 0.05$ ), and day 56 was warmer than day 14 ( $p < 0.05$ ).

**Table 6.4. Mean ambient temperatures during the treadmill exercise tests of 12 Harrier Hounds fed Diet 1 and Diet 2.**

<b>Day</b>	<b>Ambient Temperature (°C)</b>
0	19.9
14	18.9
28	19.6
56	19.3

### **6.3.3 Rectal temperature**

There were no significant differences between the rectal temperatures of the dogs fed the two diets on any of the sampling days ( $p>0.05$ ) (Figure 6.2). Following each exercise test, the rectal temperatures of the dogs fed both diets increased significantly ( $p<0.05$ ) from resting values.

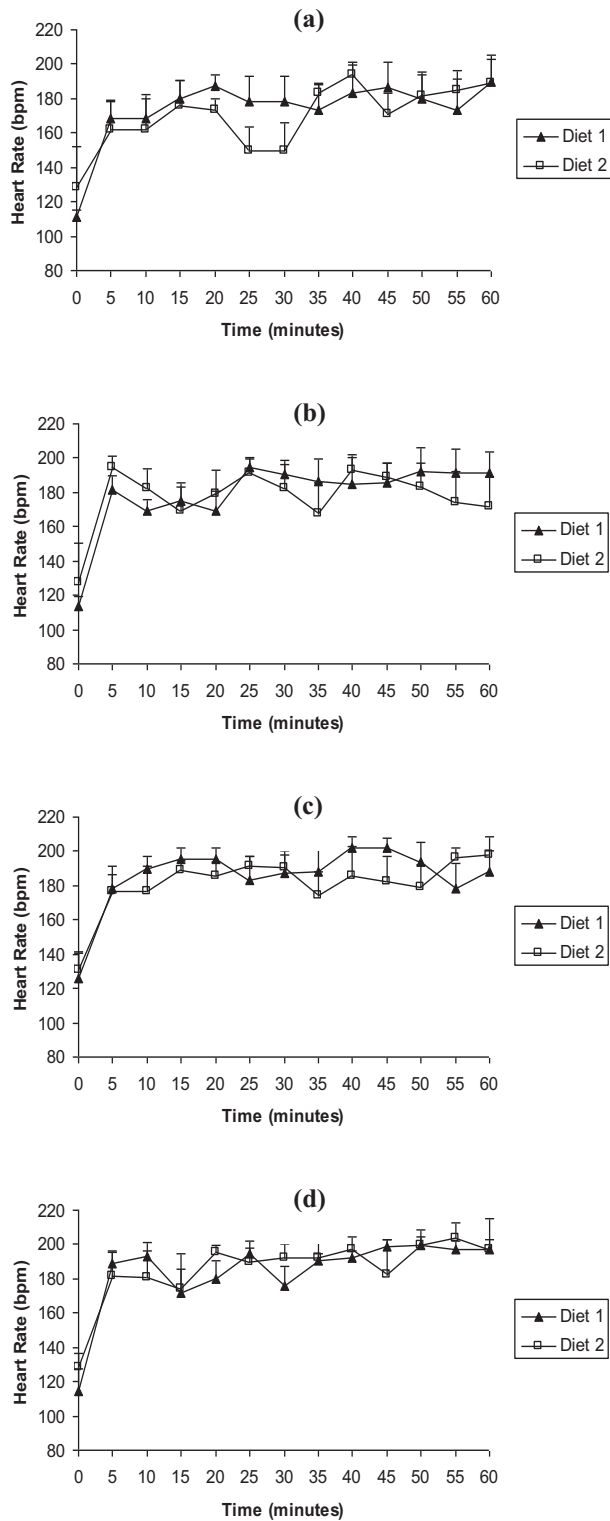


**Figure 6.2. Mean ( $\pm$  SEM) rectal temperatures from 12 Harrier Hounds fed either Diet 1 or Diet 2 (a) before, (b) after exercise tests ( — normal range). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

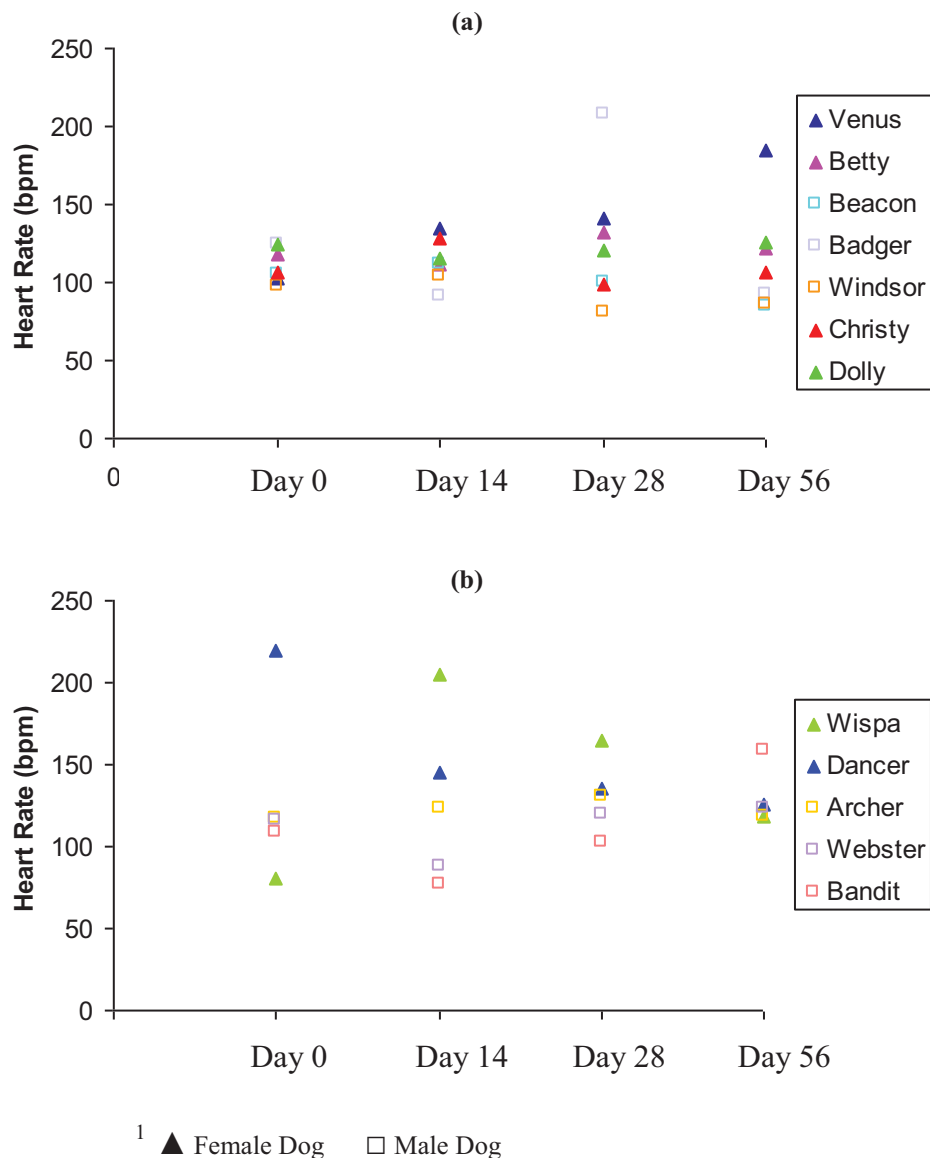
#### 6.3.4 Heart rate

Heart rates were elevated in dogs fed both diets following each exercise test ( $p < 0.001$ ) (Figure 6.3). There were no significant difference between the heart rates of the dogs fed Diet 1 or Diet 2 at any of the time points, on any of the sampling days ( $p < 0.05$ ). The exception was that the mean pre-exercise heart rate of the dogs fed Diet 1 was consistently lower than the dogs fed Diet 2 on all four sampling days ( $p < 0.01$ ) (Figure 6.4).





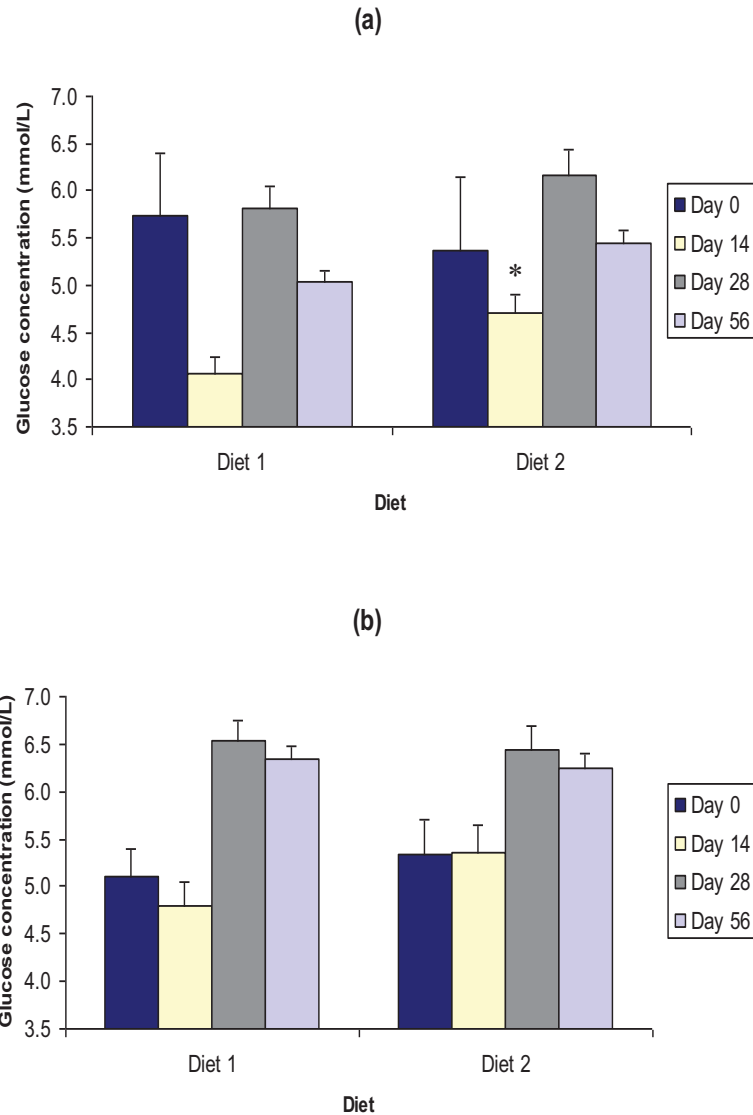
**Figure 6.3. Mean ( $\pm$  SEM) heart rates from 12 Harrier Hounds fed Diet 1 or Diet 2 on (a) day 0, (b) day 14, (c) day 28 and (d) day 56. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**



**Figure 6.4. Baseline heart rates from 12 Harrier Hounds<sup>1</sup> fed either (a) Diet 1 or (b) Diet 2 on sampling days. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

### 6.3.5 Concentration of plasma glucose

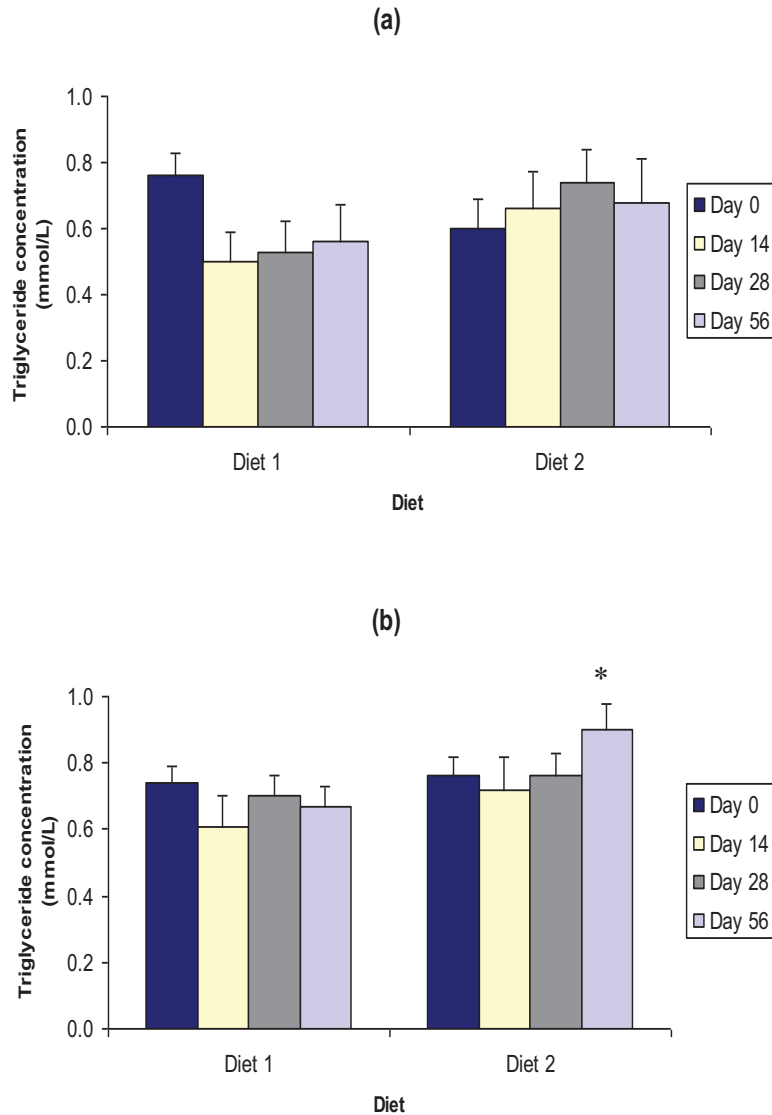
Concentrations of plasma glucose for the dogs fed Diet 1 were numerically lower (only significant on day 14) than those for the dogs fed Diet 2 before all of the exercise tests except day 0 (Figure 6.5). There were no significant differences between the plasma glucose concentrations of the dog fed the 2 diets following the exercise test on any of the sampling days ( $p > 0.05$ ). There were also no significant differences between the pre and post-exercise values for any of the dogs fed either of the diets ( $p > 0.05$ ).



**Figure 6.5. Mean ( $\pm$  SEM) plasma concentration of glucose from 12 Harrier Hounds fed Diet 1 and Diet 2 (a) before, (b) after exercise tests (\*  $p < 0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

### 6.3.6 Concentration of plasma triglycerides

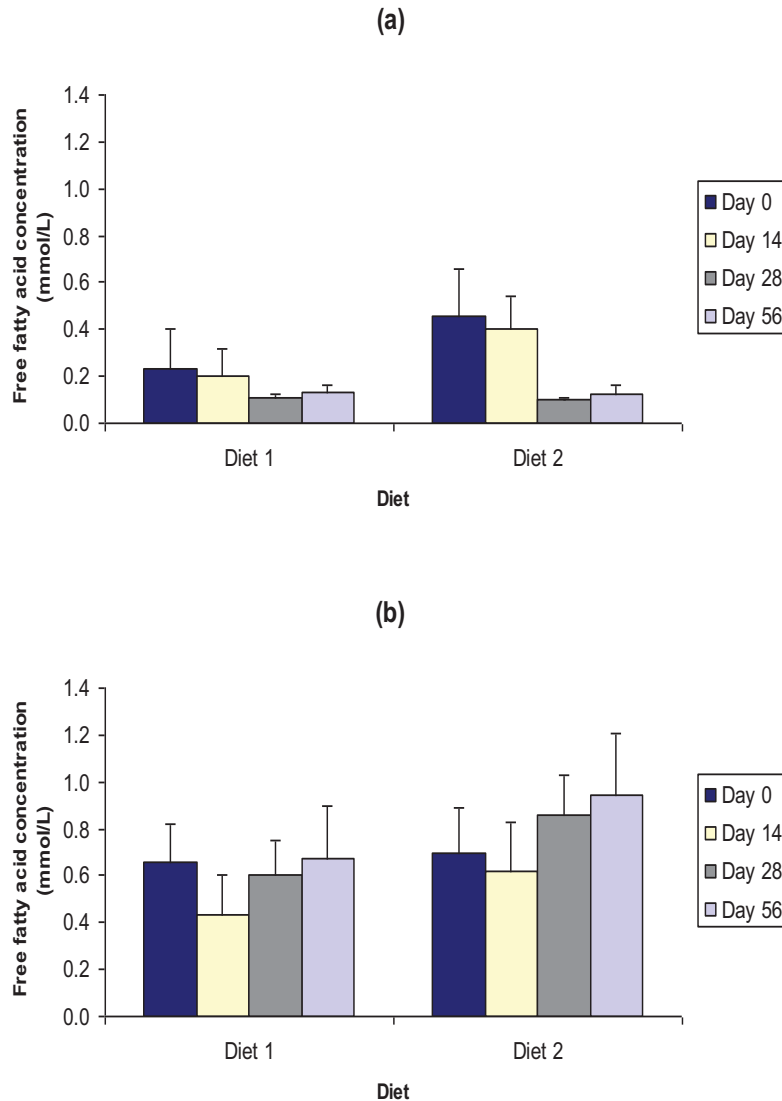
There was no effect ( $p > 0.05$ ) of diet on pre exercise plasma triglyceride concentrations. On day 56, after exercise, the triglyceride concentration was higher for the dogs fed Diet 2 compared to Diet 1 ( $p < 0.05$ ) (Figure 6.6). There were no significant differences ( $p > 0.05$ ) between the pre and post exercise values for each of the diets.



**Figure 6.6.** Mean ( $\pm$  SEM) plasma concentration of triglycerides from 12 Harrier Hounds fed Diet 1 and Diet 2 (a) before, (b) after exercise tests (\*  $p < 0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.

### 6.3.7 Concentration of serum free fatty acids

Free fatty acid concentrations prior to exercise were not significantly different between the two diets ( $p > 0.05$ ). The free fatty acid concentration after exercise was consistently lower for dogs fed Diet 1 compared to Diet 2, however this difference was not significant ( $p > 0.05$ ). Compared to pre-exercise levels, free fatty acid concentrations were significantly ( $p < 0.01$ ) elevated in both groups following each exercise test (Figure 6.7).

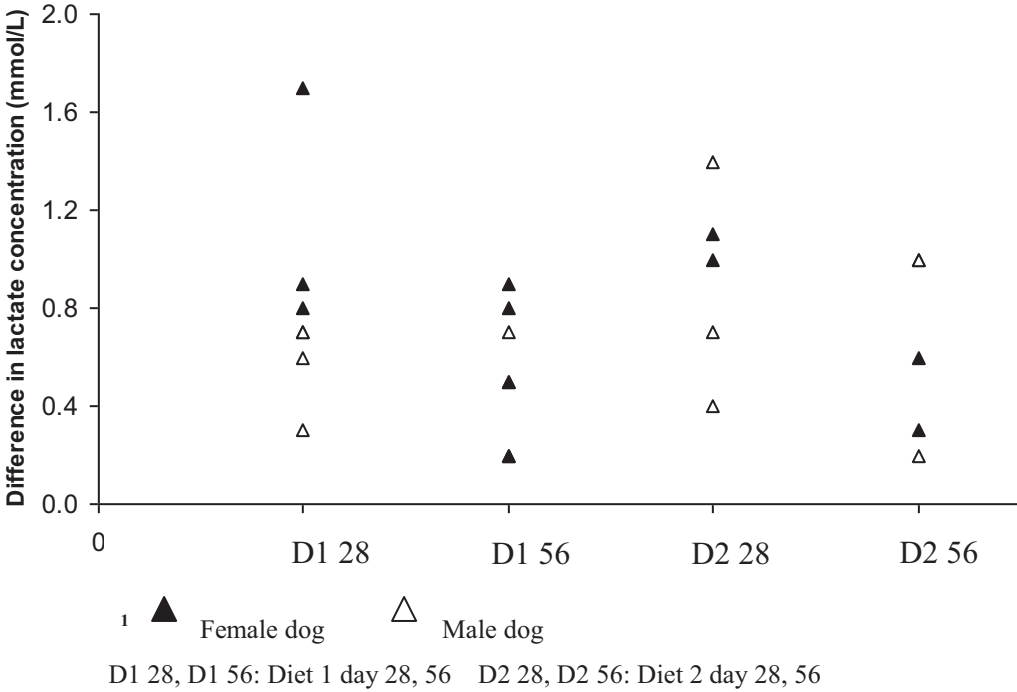


**Figure 6.7. Mean ( $\pm$  SEM) serum concentration of free fatty acids from 12 Harrier Hounds fed Diet 1 and Diet 2 (a) before, (b) after exercise tests. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

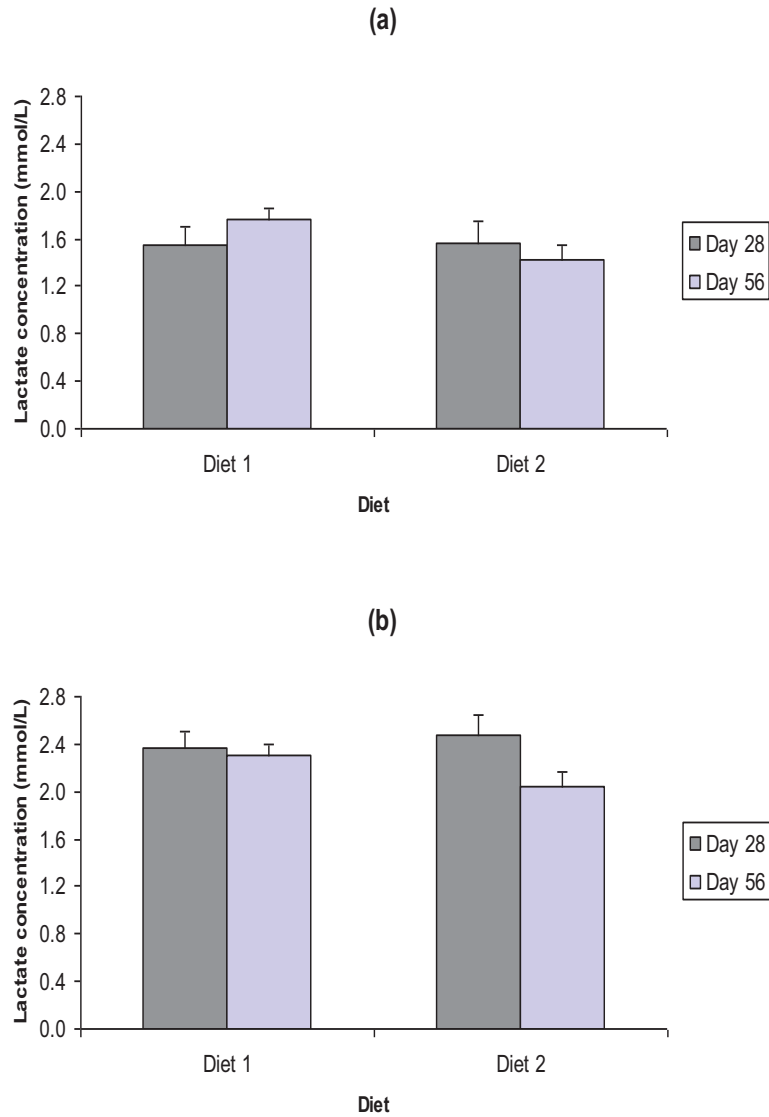
### 6.3.8 Concentration of blood lactate

Differences in blood lactate concentrations before and after the exercise tests on days 28 and 56 are shown in Figure 6.8.1 for each diet. Lactate concentrations before exercise were not significantly different between the dogs fed the two diets ( $p > 0.05$ ). The lactate concentrations after exercise was also not significantly different ( $p > 0.05$ ). Compared to pre-exercise levels, lactate concentrations were significantly ( $p < 0.05$ ) elevated in Diet 1 following each exercise test, however, there was no significant difference between pre and post-exercise lactate

concentrations in Diet 2 (Figure 6.8.2). Unfortunately lactate was not analysed on days 0 and 14 as it was decided to analyse this variable too late for these days.



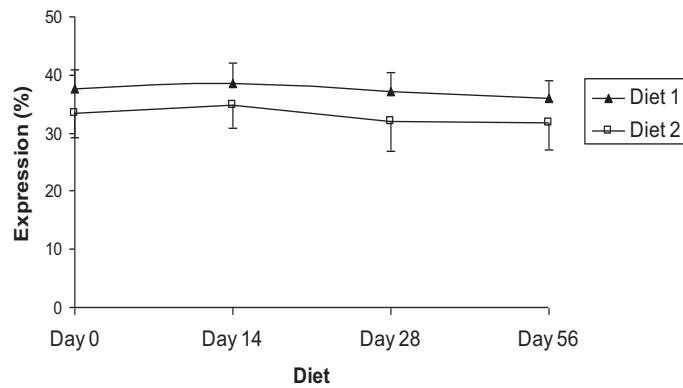
**Figure 6.8.1. Mean ( $\pm$  SEM) differences in blood concentration of lactate from 12 Harrier Hounds<sup>1</sup> fed Diet 1 and Diet 2 before and after exercise tests. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**



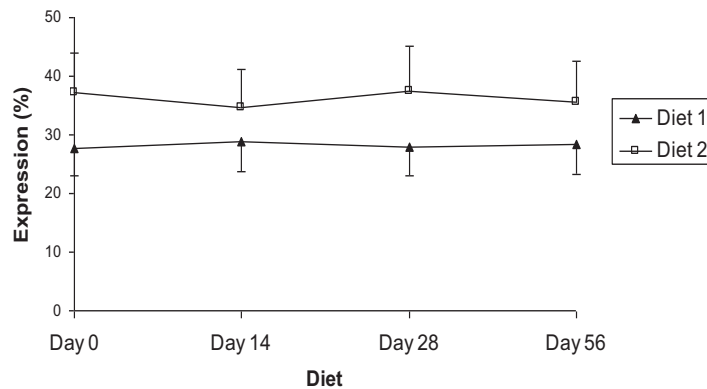
**Figure 6.8.2. Mean ( $\pm$  SEM) blood concentration of lactate from 12 Harrier Hounds fed Diet 1 and Diet 2 (a) before, (b) after exercise tests. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

### 6.3.9 Immune parameters

The expression of CD4 and CD8 was not significantly ( $p > 0.05$ ) different between the two diets at any of the sampling points (Figure 6.9.1 and 6.9.2).



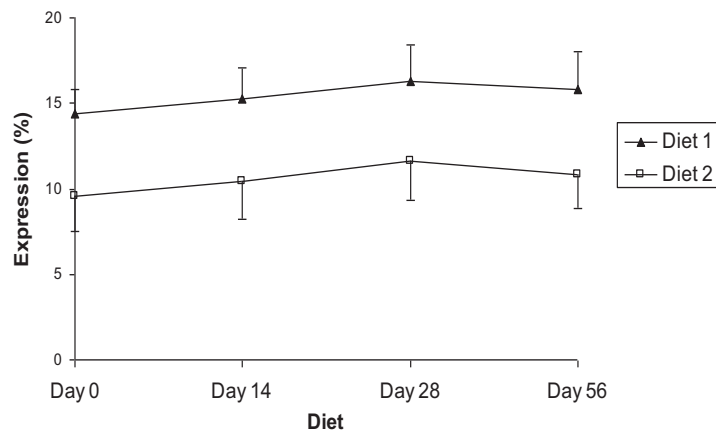
**Figure 6.9.1. Mean ( $\pm$  SEM) expression of cell surface marker CD4 from 12 Harrier Hounds fed Diet 1 and Diet 2 on the sampling days. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**



**Figure 6.9.2. Mean ( $\pm$  SEM) expression of cell surface marker CD8 from 12 Harrier Hounds fed Diet 1 and Diet 2 on the sampling days. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

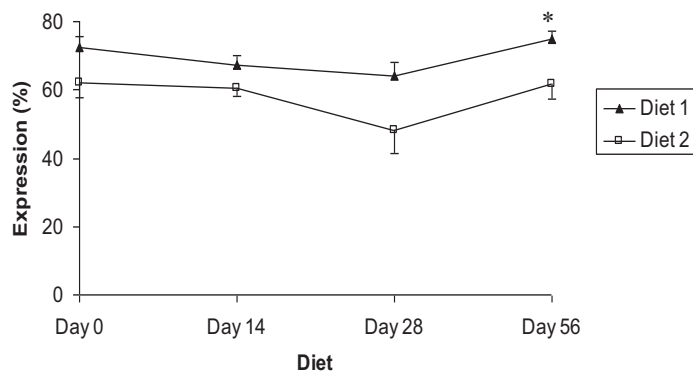
Over the trial the pattern in the expression of B cells was similar in both diets and there were no significant ( $p > 0.05$ ) differences between the two groups on any of the collection days (Figure 6.9.3).





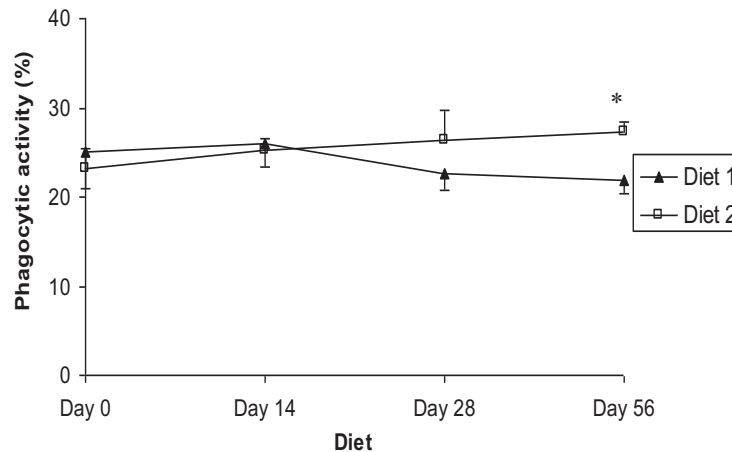
**Figure 6.9.3. Mean ( $\pm$  SEM) expression of B cells from 12 Harrier Hounds fed Diet 1 and Diet 2 on the sampling days. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

There were no significant ( $p > 0.05$ ) differences in the expression of CD14 between Diet 1 and Diet 2 on days 0, 14 and 28, however CD14 expression was significantly ( $p < 0.05$ ) higher in Diet 1 on day 56 (Figure 6.9.4).



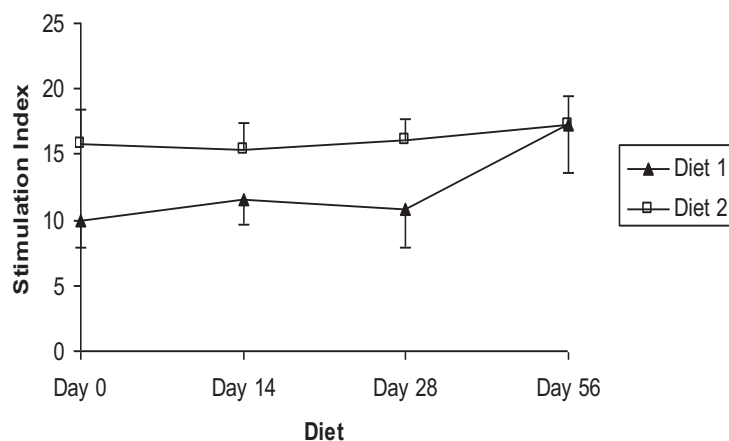
**Figure 6.9.4. Mean ( $\pm$  SEM) expression of cell surface marker CD14 from 12 Harrier Hounds fed Diet 1 and Diet 2 on the sampling days (\* $p < 0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

There was a numerical but non-significant time dependent increase in phagocytic activity in dogs fed Diet 2 (Figure 6.9.5). Although phagocytic activity initially increased in dogs fed Diet 1, it began to decrease after day 14. There were no significant differences in phagocytic activity between the two diets on days 0, 14 and 28, however on day 56 the % phagocytic activity was significantly ( $p<0.05$ ) higher in the dogs fed Diet 2 compared to those fed Diet 1.



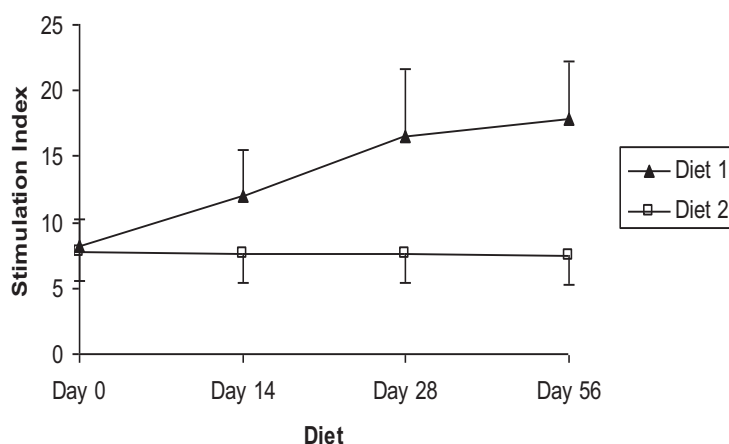
**Figure 6.9.5. Mean ( $\pm$  SEM) peripheral blood phagocytosis from 12 Harrier Hounds fed Diet 1 and Diet 2 on the sampling days (\* $p<0.05$ ). Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

For the dogs fed both diets there was a general, non significant increase in the stimulation index for ConA over the trial period (Figure 6.9.6). The values for diet 2 were higher than those for the dogs fed diet 1 on all days, however these only approached significance on days 0 and 14 ( $p=0.07$  and  $p=0.09$  respectively). Overall the stimulation index values for ConA were more consistent over the trial for the dogs fed Diet 2, whereas for the dogs fed Diet 1 there was a large but insignificant increase from day 28 to day 56.



**Figure 6.9.6. Mean ( $\pm$  SEM) stimulation index for ConA from 12 Harrier Hounds fed Diet 1 and Diet 2 on the sampling days. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

There was a clear time dependent increase in the stimulation index for PHA for the dogs fed Diet 1 over the trial period (Figure 6.9.7). The mean values for these dogs was significantly ( $p < 0.05$ ) higher on day 56 compared to day 0. In contrast, the stimulation index for PHA for the dogs fed Diet 2 was very steady over the course of the trial.



**Figure 6.9.7. Mean ( $\pm$  SEM) stimulation index for PHA from 12 Harrier Hounds fed Diet 1 and Diet 2 on the sampling days. Diet 1 was a low-carbohydrate, high-protein diet, and Diet 2 a high-carbohydrate, low-protein diet.**

## 6.4 Discussion

It is unknown as to the ideal proportions of protein, fat and carbohydrate in the intermediate working dog's diet (e.g. farm dogs, hunt dogs). Much more is known about this for greyhounds and sled dogs (Hammel *et al.* 1977; Grandjean and Paragon 1993; Reynolds *et al.* 1994; Reynolds *et al.* 1995; Hill *et al.* 2000; Hill *et al.* 2001). It is likely intermediate working dogs have requirements closer to the sled dog than the greyhound as they tend to mainly participate in lengthy bouts of exercise with bursts of sprinting. Therefore in this study, the effects of diets differing in protein and carbohydrate on the metabolism of intermediate working dogs, such as those used in farms throughout New Zealand, were examined.

The apparent crude protein, fat and energy digestibility of Diet 1 were significantly higher than Diet 2, indicating higher quantities of these nutrients were available by the animals fed Diet 1 compared to Diet 2. However, the carbohydrate digestibility of Diet 2 was higher than Diet 1. The reason for this difference is unclear; however, it may be related to the much higher proportion of carbohydrates in Diet 2, differences in the sources of carbohydrates used in each of the diets or perhaps that some or all of the dogs fed Diet 2 were practicing coprophagia. In addition to this there are some implications of using the total-collection method, including the difficulty with ensuring the entire stool is collected, particularly if the stool is soft to any degree or if coprophagia is present and also the fact that by measuring apparent digestibility any endogenous losses of nutrients are not considered (AAFCO 2009).

The resting rectal temperatures of the dogs were not significantly different between the two diets and were within the normal range reported (Carlson and Griffin 1992). As in the previous treadmill study conducted, the rectal temperatures of the dogs were elevated following each exercise test and the pre and post exercise values were similar to those reported for working dogs by other authors (Downey *et al.* 1980; Matwichuk *et al.* 1999; Steiss *et al.* 2004).

Following each test the heart rate of the dogs fed both diets were raised significantly. The pre-exercise heart rates of the dogs fed Diet 1 were significantly lower than the dogs fed Diet 2 on all sampling days; which may indicate the animals in the Diet 1 group were physically fitter as generally a reduced heart rate for a given intensity of exercise is due to an

improvement in fitness (Wyatt and Mitchell 1974; Sneddon *et al.* 1989; Burr *et al.* 1997). Further factors that may be responsible for these differences in heart rate between the two groups of dogs include dehydration, which can increase heart rate, biological variation between the two groups and heat and humidity which can also increase heart rate. However, this last factor can be excluded as the dogs in both groups were exercised on the same days and therefore this factor was constant for all of the dogs.

Building on the trial carried out in the previous chapter, we measured plasma glucose, plasma triglycerides, serum free fatty acids, blood lactate as well as some immune parameters. We were interested to establish if these diets or this exercise affected any aspects of the immune system in a detrimental manner.

The only significant difference found in plasma glucose concentrations between the two diets and between pre and post exercise was before exercise on day 14. The reason for this is unclear, however the values before exercise were lower for the dogs fed Diet 1 on the other days also but they were not significant. The lack of significant differences in plasma glucose concentrations with exercise indicates a balance between hepatic mobilisation of glucose and peripheral glucose uptake (Rovira *et al.* 2007). Exercise intensity and endocrine factors including insulin primarily determine hepatic glucose mobilisation, therefore the exercise intensity used in this study may have played a part in this.

There were also no real differences found in plasma triglyceride concentrations between the diets except after exercise on day 56 where the concentration was higher for the dogs fed Diet 2. As in the previous study, this may have been caused by differences in ambient temperatures between sampling days and therefore differences in exercise intensity. Because circulating triglycerides may be a potential source of fatty acids for beta oxidation in muscles (Terjung and Kaciuba-Uscilko 1986), it was hypothesised that triglyceride concentrations may increase with exercise, indicating an enhanced mobilisation of fat for utilisation by the muscle; however this was not the case. In line with these results, it has been reported that serum triglyceride concentrations may not be as an effective measure of fat mobilisation during exercise as free fatty acid and intramuscular triglyceride concentrations (Poso *et al.* 1989). However, radioisotope studies have illustrated that serum triglyceride levels may be an important source of free fatty acids for replenishing intramuscular triglyceride stores following exercise (Terjung *et al.* 1982); therefore higher post exercise triglyceride

concentrations on day 56 for Diet 2 in this study would be beneficial by providing these animals more substrate to replenish intramuscular triglyceride stores.

There were no significant differences in serum free fatty acid concentrations seen between the dietary groups, however following each exercise test, the free fatty acid concentrations were significantly elevated from pre-exercise levels in both groups on all days, indicating that free fatty acids are being mobilised from adipose tissue for uptake and use by the muscles for energy (Hammel *et al.* 1977; Grandjean and Paragon 1993; McClelland *et al.* 1995). This utilisation of free fatty acids for energy spares the use of limited muscle glycogen stores and delays fatigue, because fatigue is related to muscle glycogen depletion. Dogs are considered highly aerobic and are known to have a greater ability to transport free fatty acids than less aerobic species such as the goat (McKenzie *et al.* 2005), therefore many other studies of working dogs have, like this study, reported increases in free fatty acid concentrations following exercise (Kronfeld *et al.* 1977; Reynolds *et al.* 1994; McClelland *et al.* 1995). The study by McClelland and associates in 1995 determined much larger increases with exercise compared to those reported in this study; 15 minutes after a run at  $\text{VO}_2\text{max}$  the concentrations of the fatty acids palmitic acid (16:0) and oleic acid (18:1) reached maximum values 60 and 150 % higher than pre-exercise concentrations respectively. From this result, it may have been interesting to take a further sample from the dogs 15 minutes after the end of the exercise test to analyse for free fatty acid concentrations in the study reported here to determine if the concentration increased any further than was determined immediately after the test.

The usefulness of the blood lactate measurements made is limited as these were only made on days 28 and 56 and therefore a full picture over the course of the trial can not be seen. However no significant differences were seen between the diets on these two days. The pre-exercise lactate concentrations of the dogs in both groups were somewhat higher (means ranging from 1.42 to 1.76 mmol/L) than the mean baseline levels reported for retrievers of  $0.90\pm 0.32$  mmol/L (Steiss *et al.* 2004) and  $1.31\pm 0.61$  mmol/L (Matwichuk *et al.* 1999). However the post-exercise values in our study were much lower than those reported for retrievers of  $3.57\pm 2.22$  (Matwichuk *et al.* 1999) and sled dogs of  $11.4\pm 2.78$  (Burr *et al.* 1997). The reasons for these differences between studies may be differences between breeds of dogs used in these studies, in aspects of exercise physiology such as differences in proportions of fibre types, the type and intensity ( $\text{VO}_2\text{ max}$ ) of exercise and also level of training. Canine skeletal muscle is composed of type I and type II fibres. Type I fibres are geared towards

utilising lipids for fuel which results in relatively slow muscle contraction suited to endurance exercise and type II fibres have less oxidising capacity rely on glucose for fuel and contract more quickly (Murphy *et al.* 1997; Toll and Reynolds 2000). These fibres are therefore beneficial for sprinting and also have a greater tendency to accumulate lactic acid. Therefore different breeds of dogs and also individual dogs will have slightly different proportions of each fibre type and a different likelihood of lactic acid accumulation. Increases in lactic acid concentrations with exercise are also somewhat dependant on the duration and intensity of the exercise performed, with strenuous exercise causing larger increases (Ilkiw *et al.* 1989; Matwichuk *et al.* 1999; Toll and Reynolds 2000). Training however can be used to modify the extent of the increase seen for a given exercise intensity (Ilkiw *et al.* 1989).

The expression of CD4, CD8 and B cells were not found to be significantly different between the diets over the study. Similarly there was no difference between the diets in the expression of CD14 cells on days 0, 14 and 28. On day 56 however the expression of CD14 cells was higher in the dogs fed Diet 1. These results are as expected overall and illustrate there are no negative effects of these diets or exercise on the immune system because no large changes in these variables were observed. If large changes were determined in the percentage of B or T cells this may have indicated the potential to lead to a predisposition to certain types of infections, meaning large changes are undesirable.

Phagocytic activity measures the ability of cells to recognise, bind and phagocytose. Despite a numerical time dependent increase in phagocytic activity seen over the trial for the dogs fed Diet 2, this was not significant ( $p>0.05$ ). For the dogs fed Diet 1 there was also initially an increase in this activity but after day 14 this activity began to decrease in these dogs. The only significant difference found between the two groups in phagocytic activity was on the last day (day 56) where the percentage activity was higher in the dogs fed Diet 2, indicating an enhanced ability to combat infection and disease in these animals. Due to the fact the ingredient lists for these products could not be compared, because of proprietary issues, it is hard to explain these differences.

After 56 days of dietary intake, no significant increases in lymphoproliferative responses to the T-cell mitogen ConA were seen for the dogs fed either diet. The responses of the dogs fed both diets did increase over the 56 day period and from day 28 to day 56 there was a large increase for the dogs fed Diet 1. For the dogs fed Diet 1 there was a clear enhanced

lymphoproliferative response to the T-cell mitogen PHA, however no such enhancement was seen for the dogs fed Diet 2. These responses indicate that Diet 1 was exerting more of an influence on the immune system of the dogs compared to Diet 2. Due to the lack of research in the area of immunity in working dogs and its possible ability to influence performance, further work is needed to understand the mechanisms whereby one could stimulate the immune system in these animals using dietary manipulations. This would be beneficial due to the extra stress caused by exercise on all facets of the dog, including the immune system.

It was hoped the changes made to the protocol of this trial would increase the intensity of the exercise sufficiently to allow us to determine differences between the diets in the animal's blood biochemical parameters, physiological responses, exercise performance and immunity. However, these particular dogs are accustomed to running for several hours during a hunt covering considerable distances over mixed terrain and in comparison, during this trial the dogs were subjected to a one hour treadmill run at a relatively low speed and small incline. This does not seem to come close to reflecting the work they are used to, which is potentially a major reason for our lack of clear differences in the parameters measured before and after exercise and between the two diets in this study. Until we can convince the ethics committee that the work we are putting these dogs through is not even close to the work they are accustomed to and that they have the potential to complete much more intense exercise, a similar outcome can be expected from all trials using this protocol. In future studies of this nature at the university it is suggested that these hunt dogs be fitted with actical monitors during a hunt to demonstrate to the animal ethics committee the work these dogs are used to compared to the exercise we are exposing them to.

A further limitation of this type of study is that we have not yet found a reliable marker we can use that is sensitive enough to indicate dietary differences in performance. Other researchers in the field agree with our findings, that physiological parameters such as heart rate and rectal temperature are much more sensitive at showing differences than markers such as blood glucose, free fatty acids, triglycerides, lactate and creatine kinase (Sneddon *et al.* 1989). Perhaps the use of other markers can be explored for working dogs, such as respiratory exchange ratio (RER) or muscle glycogen and fatty acid synthase concentrations from muscle biopsies to see if these are more suitable for use in exercise studies in these animals.



One well known study that did find clear differences between dogs fed a variety of diets clearly highlights the point that the level of exercise must be extreme for differences to be seen in these types of studies (Downey *et al.* 1980). Beagles were run on treadmills on a slope of 7 ° (compared to 6.3 ° in this study) and speed of 9 mph (compared to 7.8 mph for the females and 8.3 mph for the males in this study) and fed diets differing in proportions of fat, protein and carbohydrate. These dogs were run till exhaustion (compared to one hour in this study) and it was found that the dogs fed the cereal diet (high carbohydrate) had a mean exhaustion time of 103 minutes whereas the dogs fed the other diets which were higher in fat and protein and lower in carbohydrate had exhaustion times of 136, 138 and 139 minutes. The authors therefore concluded that stamina was negatively affected by dietary carbohydrate and positively affected by dietary fat, energy density and digestibility (Downey *et al.* 1980). It is unlikely that this type of exercise protocol would be approved by the ethics committee at this university.

In conclusion, the high-protein, low-carbohydrate diet (Diet 1) fed during this study and the previous study needs to be tested more fully, using more intense exercise, perhaps in the field. A reliable, sensitive marker to measure performance differences must also be established before this can happen. Due to ethical limitations, it may be more useful and productive to trial these diets during a hunt season in field conditions, where the dogs are performing the type and intensity of exercise they are accustomed to. This would also provide evidence that may be considered more relevant to working dog owners compared to that obtained under laboratory conditions and therefore encourage the use of such a diet. However a major disadvantage of such a field study is that it is very difficult to have full control of all variables and therefore the reliability of the results is questionable. Alternatively a very different marker needs to be trialled to attempt to indicate differences in exercise physiology and performance when dietary macronutrient proportions are altered. Therefore it may be interesting to consider looking at the fuels the dogs are utilising during exercise by using indirect calorimetry to determine respiratory exchange ratios (RER) and establish if by prefeeding these diets differing in macronutrient proportions, the dog's metabolism can be manipulated. This information could also be compared to blood results to see if they are as expected based on the RERs determined.

## **Chapter Seven**

**The effects of the proportions of dietary  
macronutrients on digestibility and fuel utilisation  
during exercise in working dogs**

## 7.1 Introduction

Canids represent one of the most diverse species, including a vast array of athletic abilities performing a variety of exercise types. Dogs performing different types of work can have very different nutritional requirements (Toll and Reynolds 2000) and it is important that the exercise type is considered when assessing each dog's diets. The requirements of sled dogs and greyhounds have been widely researched, however, research of more "intermediate" athletes, such as farm dogs, hunt dogs, and agility dogs is lacking and therefore their specific requirements are not established. This is partly due to the large variation in this group in terms of age, breed, size and work type (Guilford 1997). These dogs are particularly important in New Zealand, where a large proportion of the canine population is used on farms, and it is therefore vital that more research is conducted in this area.

Despite genetics dictating an individual dog's physiological characteristics, nutrition and training can be used to modify some of these and to also enhance performance (Toll and Reynolds 2000). Nutrition has a profound effect on athletic performance and thus providing optimal nutrition is required to maximise the performance for a working dog at any given level of fitness (Toll and Reynolds 2000).

ATP is the compound used by muscles for energy, however, the concentration in muscles is relatively low and therefore it must be replenished during exercise for work to continue. This replenishment can come from endogenous and exogenous sources and the fuels from these sources can be metabolised with oxygen (aerobic) or without oxygen (anaerobic) (Toll and Reynolds 2000). Aerobic sources of energy include the oxidation of glucose, free fatty acids and amino acids in the mitochondria and anaerobic sources include the creatine phosphate shuttle, and glycolysis in the cytoplasm (Murphy *et al.* 1997; Toll and Reynolds 2000). Anaerobic pathways produce ATP at a very rapid rate but produce small yields, whereas aerobic pathways produce ATP at a slower rate but produce much larger amounts (Powers and Howley 2004; Hargreaves and Spriet 2006).

The proportion of each pathway, and therefore each fuel utilised during exercise is determined by the intensity and duration of work, training and nutrition (Toll and Reynolds 2000). Therefore, a goal for feeding athletic dogs is to provide the correct balance of macronutrients that result in optimal, efficient delivery of energy that matches the rate and duration of ATP

demands by the muscle (Grandjean and Paragon 1992). In fact, research with human athletes has shown that carbohydrate loading is an effective means of improving stamina by increasing muscle glycogen stores and therefore delaying the onset of fatigue (Bergstrom *et al.* 1967). In contrast however, a similar strategy has not proven effective for dogs. A team of sled dogs fed a high carbohydrate diet became coprophagous and their performance decreased (Kronfeld 1973). This was attributed to the excess carbohydrate intake, and the dog's performance improved and coprophagy ceased as the proportion of carbohydrate in the diet was replaced by fat and protein (Kronfeld 1973).

Research conducted on sled dogs has shown that high fat diets may be of more benefit to these working dogs, by enhancing the capacity to oxidise free fatty acids, thus sparing muscle glycogen and blood glucose reserves (Kronfeld *et al.* 1994; Reynolds *et al.* 1995). Sled dogs are endurance athletes, relying mainly on aerobic metabolism and fatty acid oxidation (Toll and Reynolds 2000, McKenzie *et al.* 2005). Higher fat diets are also of benefit to these types of athletes as they increase the palatability and energy density of the food, allowing the animal to obtain its requirements daily by consuming a much smaller volume of food and also encouraging tired, stressed animals to eat (Toll and Reynolds 2000). At the other end of the spectrum, greyhounds perform high intensity, short duration exercise, which is primarily supported by anaerobic metabolism and the exercise these dogs do may therefore be better supported by feeding a higher carbohydrate diet (Toll and Reynolds 2000). It remains to be determined whether the intermediate athlete's requirements for dietary macronutrients and the fuels used for exercise are similar to those of greyhounds, sled dogs, or lies somewhere in between. It could also vary depending on the exact type of intermediate athlete being considered, such as farm dogs versus agility or police dogs (Toll and Reynolds 2000). However, it is believed intermediate athletes; such as those used for farming and hunting, would generally be more similar in needs to sled dogs as they mainly work at low to moderate intensities for long periods of time with some short periods of high intensity work (Toll and Reynolds 2000).

Proteins are not used primarily for energy by the working muscles, although amino acid oxidation may contribute 5 to 15 % depending on the exercise undertaken (Toll and Reynolds 2000). Protein requirements do increase with exercise however, and higher protein diets have been shown to reduce the risk of injury and prevent training induced anemia (Hill 1998; McKenzie *et al.* 2005).

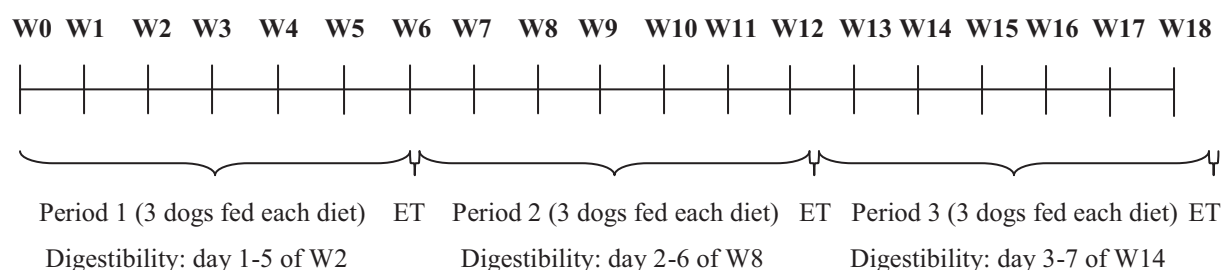
The relative proportions of fat and carbohydrate utilised during exercise can be determined by using indirect calorimetry to calculate the respiratory exchange ratio (RER), which is the ratio between CO<sub>2</sub> exhaled and O<sub>2</sub> inhaled. The respiratory exchange ratio can then be used to estimate the respiratory quotient (RQ); an indicator of the proportions of fuel being metabolised for energy by the body. The respiratory quotient is equal to the number of moles of CO<sub>2</sub> produced, divided by the number of moles of O<sub>2</sub> consumed (at the cellular level) (Reynolds *et al.* 1996). A respiratory quotient of 1.0 indicates that predominantly carbohydrate is being utilised and a respiratory quotient of 0.7 indicates fat oxidation is predominating (Reynolds *et al.* 1996). Therefore an increased utilisation of fat as a muscle fuel is shown by a decreased respiratory quotient for a given exercise intensity (Reynolds *et al.* 1996).

This study was undertaken to use indirect calorimetry and a variety of blood parameters to determine if by prefeeding diets differing in macronutrient profiles, the fuels working dogs utilize during a sub-maximal exercise test can be manipulated. It was also hoped to determine if the different diets elicited any different immune responses in the dogs during the trial, to check the changes in macronutrient proportions didn't cause any detrimental changes in immunity. The hypothesis formulated was that these dietary manipulations would alter the fuel utilization of these dogs slightly and that subtle changes would be seen in the other parameters measured with the diet fed and exercise undertaken. As it is believed that intermediate working dogs, such as farm and hunt dogs are probably more similar to sled dogs than greyhounds it was thought that one of the high protein diets would be the most suitable for these dogs. It was expected that when the high-protein, low-carbohydrate, low-fat and high-protein, low-carbohydrate, high-fat diets were fed, the dogs would use more fatty acids for energy than carbohydrates and that when the low-protein, high-carbohydrate, low-fat diet was fed, the dogs would use more carbohydrates for energy than fatty acids to fuel their exercise. However based on the results obtained from the previous two exercise studies, the difference between the diets was not expected to be significant.

## 7.2 Materials and methods

### 7.2.1 Animals and housing

Ten (n=6 females and n=4 males) healthy entire adult Harrier Hounds (*Canis familiaris*), aged from 2 to 7 (mean 4.20, SEM 0.49) years, were obtained from the Manawatu Hunt Club, Palmerston North, New Zealand, and used in the 18-week (3 x 3 cross-over design) study (Figure 7.1).



**Figure 7.1. Time-line of the study to determine the effects of the proportions of dietary macronutrients on digestibility and fuel utilization during exercise of three diets fed to 9 working dogs (W=week, ET=exercise test).**

Harrier hounds were used in this study for the reasons outlined in Chapter 2, namely as a model for the working farm dog. The dogs involved in this study had all been used in previous treadmill studies and therefore were already familiar with the treadmills and handling procedures. They had also just completed a hunt season and were therefore all in peak physical condition.

The dogs were weighed, and their body condition score assessed subjectively by the same personnel, using a validated scale (LaFlamme 1997), at the start of the trial, and at weekly intervals until the end of the trial. The dogs were housed in pairs in purpose-built concrete pens (2.9 x 5.5 m) at the Canine Unit, Massey University, Palmerston North, New Zealand, except for during the digestibility study, when they were housed individually. The pens were cleaned daily as outlined in Chapter 2, section 2.2.1.

The dogs were exercised daily for approximately 30 minutes, and had free access to fresh water at all times. Twice a week the dogs were run on treadmills for 30 minutes on a slope of 6.3° at speeds of 3.5 ms<sup>-1</sup> for the female dogs and 3.7 ms<sup>-1</sup> for the male dogs to train the dogs on the treadmills and re-familiarise the dogs with them. All management and experimental procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

### **7.2.2 Diets and feeding**

On arrival, nine of the dogs were randomly assigned to either, Diet 1, Diet 2 or Diet 3 (n=3 dogs per diet), with the tenth dog (a spare in case of injuries) being fed Diet 3 throughout the trial.

Diet 1 was a high-protein, low-carbohydrate, low-fat (as compared to the other two diets) dry extruded diet manufactured specifically for the study by Heinz Watties Limited (Hastings, NZ). Diet 2 was a high-protein, low-carbohydrate, high-fat dry extruded diet also manufactured specifically for the study by Heinz Watties Limited (Hastings, NZ), and Diet 3 was a low-protein, high-carbohydrate, low-fat dry extruded diet available commercially (Pedigree Naturals; Mars Incorporated, Bathurst NSW, Australia).

The dogs were fed the assigned diets for six weeks before they underwent a sub-maximal exercise test. Following this, the dogs were randomly re-assigned to one of the other three diets and fed this for six weeks before undergoing a second exercise test. This was then repeated, so the nine dogs had been fed all three diets. For the first two weeks of each six week period, the daily food allowance (adult maintenance energy requirements; MER) for each dog was calculated and weighed as outlined in Chapter 2, section 2.2.2. For the next three weeks, the dogs were fed 1.3 times MER and for the final week they were fed 1.5 times MER based on the dog's bodyweight at the time. Based on previous experience using these dogs, it was believed we could maintain all of the dogs on the same factor of their MER using this feeding schedule. This would avoid having to adjust the allowances of individual dogs.

The diets were analysed for moisture, ash, crude protein, fat, gross energy, crude fibre and carbohydrate as described in Chapter 2, section 2.2.2 (Table 7.1). The percentage of energy from each macronutrient in the 3 diets was also calculated (Table 7.2).

**Table 7.1. Dietary analysis<sup>a</sup> of Diet 1, Diet 2 and Diet 3 fed to 10 Harrier Hounds, in order to determine the effects of the proportions of dietary macronutrients on digestibility and fuel utilisation during exercise.**

<b>Nutrient</b>	<b>Diet 1</b>	<b>Diet 2</b>	<b>Diet 3</b>
Moisture (%)	10.1	4.9	10.1
Crude protein (%)	44.4	48.8	19.3
Crude fat (%)	10.8	18.8	9.7
Crude fibre (%)	2.2	0.9	2.3
Ash (%)	4.8	6.0	6.6
Carbohydrate <sup>b</sup> (%)	29.9	21.5	54.3
Gross energy (kJ/g)	19.6	21.8	17.7

<sup>a</sup> All results are on an ‘as received’ basis

<sup>b</sup> Calculated by difference

**Table 7.2. Macronutrient proportions of Diet 1, Diet 2 and Diet 3 based on percentage of dietary energy, fed to 10 Harrier Hounds, in order to determine the effects of the proportions of dietary macronutrients on digestibility and fuel utilisation during exercise.**

<b>Nutrient</b>	<b>Diet 1</b>	<b>Diet 2</b>	<b>Diet 3</b>
Crude Protein (%)	45.2	42.4	20.4
Fat (%)	26.7	39.7	24.8
Carbohydrate (%)	28.2	17.9	54.8

### **7.2.3 Digestibility of the diets**

To determine total dietary digestibility, the dogs were housed individually, and were fed to the requirements outlined previously (Section 7.2.2). All ten dogs were involved in the digestibility study. The digestibility was determined during the second week of each six week feeding period and because the dogs had already been fed the diet for a week they were already adapted to the diets and were separated only for the five days of faecal collection. The faeces produced by each dog, including any excreted during exercise, were collected, weighed and handled as described in Chapter 2, section 2.2.3.



The apparent dry matter, crude protein, fat, energy and carbohydrate digestibility of the three diets were calculated using the equation for the total collection method described in Chapter 2, section 2.2.3.

#### **7.2.4 Treadmill study**

All of the dogs had previously been trained to run on the treadmills. However, only nine of the dogs participated in this part of the trial, with one female dog being kept as a spare in case of injuries. The dogs were trained to accept the fitting and removal of the calorimetry masks while running without breaking stride during the twice-a-week treadmill exercise described in section 7.2.1.

Upon their arrival, the dogs were washed and shaved to reveal their jugular veins and a 9 ml baseline blood sample was taken from each of the ten dogs using a plastic 10 ml syringe and 23 G  $\frac{3}{4}$  needle (Becton-Dickinson, New Jersey, USA).

A 1-drop aliquot was used to analyse blood lactate concentration immediately after collection using a hand held lactate analyser (Accutrend lactate analyser, Roche, Basel, Switzerland) and the remainder split into vacutainers; 1.0 ml into a vacutainer free of additive for creatine kinase analysis, 4.0 ml into another vacutainer free of additive for glucose and free fatty acid analysis, 1.5 ml each into two vacutainers containing heparin for immune and triglyceride analysis, and the remaining 1.0 ml into a vacutainer containing EDTA for complete blood count analysis. Additional 2.0 ml blood samples were also collected and transferred to a vacutainer containing heparin at weeks two and four of each six week period for immune analysis of CD4, CD8, CD14, B cells, phagocytosis, and lymphoproliferative responses to ConA, PHA and LPS.

The vacutainer for glucose and non-esterified fatty acid analysis and the vacutainer for triglyceride analysis were inverted 10 times, placed on an electric mixer for approximately 15 minutes to thoroughly mix, before being centrifuged (Labofuge 200, Heraeus Septatech, Osterode, Germany) at 3000 rpm for 10 minutes. The plasma or serum was then removed and handled as described in Chapter 5, section 5.2.4. The remainder of the vacutainers were simply placed on the electric mixer until analysis.

Blood glucose, triglyceride and free fatty acid concentrations (mmol/L) were analysed using the methods detailed in Chapter 5.2.4. Creatine kinase concentrations (IU/L) and complete blood counts were analysed by New Zealand Veterinary Pathology (Palmerston North, New Zealand). The level of expression of CD4, CD8, B cells, CD14 and phagocytic activity, were determined using the assays outlined in Chapter 6.2.4. A whole blood cell proliferation assay was performed as described in Chapter 6, section 6.2.4, however, the diluted blood was also cultured in the presence of  $1.25 \mu\text{gml}^{-1}$  lipopolysaccharide (LPS), as well as ConA and PHA. The stimulation index for LPS was also calculated as cpm in wells with mitogen divided by cpm in wells without mitogen.

Following each six week dietary period, the dogs were subjected to a sub-maximal exercise test, where the dogs were exercised on the treadmills for a total of 40 minutes on a  $4.6^\circ$  slope, at a speed of  $3.5 \text{ ms}^{-1}$  for the female dogs and  $3.7 \text{ ms}^{-1}$  for the males. The five female dogs were exercised one day and the four males the following day. During these tests the female dogs ran a total distance of approximately 8.3 km and the male dogs 8.9 km. Before each of the sub-maximal exercise tests, the dogs were washed, dried and shaved to reveal their jugular veins and each dog was fed their daily food allowance 14 hours before their exercise test (they all consumed all that was offered within 5 minutes). On all of the sampling days, the ambient temperature of the room was monitored and maintained at a target temperature of  $16 \pm 2^\circ \text{C}$  using heaters. Immediately before the exercise test a 9 ml blood sample was collected from each dog and handled as outlined above, their rectal temperature was measured, and they were fitted with a heart rate monitor as described in Chapter 5.2.4. An initial resting heart rate was recorded for each dog.

The dogs were fitted with a specially constructed respiratory mask with an adjustable head strap, designed to minimise discomfort and permit opening of the mouth during panting. The mask covered the dog's muzzle, extended well past the angle of the jaw, and a rubber flange effectively sealed the lips within the mask. A 1-way valve (25.4 mm outer diameter, 22 mm inner diameter) was fitted to the top towards the end of the mask to allow inhalation, and a 1-way valve (25.4 mm outer diameter, 22 mm inner diameter) was located at the nasal end of the mask to allow expiration (VacuMed, Ventura CA, USA). The mask was connected to a 100 litre Douglas bag (Figure 7.2) via a 1 m length of aerosol tubing (7/8 inch) and a hose adapter (1 inch x 1-3/8 inch) (VacuMed, Ventura CA, USA). A 3-way valve was also

attached to the douglas bag to allow the bag to be connected to the gas analyser and a sample to be taken directly from the bag and analysed (VacuMed, Ventura CA, USA).



**Figure 7.2. Douglas bag used to determine the effects of the proportions of dietary macronutrients on fuel utilisation during exercise when Diet 1, Diet 2 and Diet 3 were fed to 9 Harrier Hounds.**

A baseline breath sample was collected with the dog standing on the static treadmill by fitting the mask to the dog, opening the valve into the Douglas bag, collecting the exhaled air for 2 minutes, closing the valve and removing the mask (Figure 7.3).



**Figure 7.3. Collection of breath samples, to determine the effects of the proportions of dietary macronutrients on fuel utilisation during exercise when Diet 1, Diet 2 and Diet 3 were fed to 9 Harrier Hounds.**

The Douglas bag was then attached to an AD Instruments gas analyser model ML206 (Figure 7.4). Samples were collected by opening the 3-way valve and analysed immediately for CO<sub>2</sub> and O<sub>2</sub> using powerlab 4.20 model ML840 (AD Instruments Pty Limited, Bella Vista NSW, Australia). The software used for analysis was AD Instruments chart version 5.5 (AD Instruments Pty Limited, Bella Vista NSW, Australia). Once the sample was analysed, the valve was closed and the bag detached. The bag was then emptied of all air and the volume of air in the bag measured using a 12 volt vacuum cleaner (Figure 7.4) adapted to connect to the Douglas bag. At the same time the time, the ambient temperature and barometric pressure were read off a barometer and recorded. The gas analyser was calibrated in the morning and at regular intervals using BOC beta standard calibration gas (8 % CO<sub>2</sub>, 13 % O<sub>2</sub>).



**Figure 7.4. Equipment used for gas analysis, to determine the effects of the proportions of dietary macronutrients on fuel utilisation during exercise when Diet 1, Diet 2 and Diet 3 were fed to 9 Harrier Hounds.**

The dogs then began the exercise test and heart rate readings and breath samples were taken every 5 minutes. At the end of the exercise test a final heart rate and breath sample were taken before the dogs were allowed to cool for 2 minutes. The dogs were then removed from the treadmill, a final rectal temperature was measured and an 8 ml blood sample collected from the jugular vein not used for the pre-exercise sample (to minimise any damage to the veins). This blood sample was smaller than the pre-exercise sample because no immune analysis was required. The dogs were then offered water, taken outside to cool down further before being returned to their pen. One hour after completing their exercise test, the dogs were offered their daily allowance of food.

### **7.2.5 Statistical analysis**

All analyses were conducted using SAS version 9.1 (SAS Institute Inc, Cary NC, USA). The apparent nutrient digestibility of the three diets, determined using the total-collection method, was compared statistically using simple ANOVA. Comparisons were included in the model to analyse differences between the male and female dogs ( $y = \text{diet sex diet} \times \text{sex}$ ). The ambient temperatures over the sampling days were also compared using simple ANOVA. The differences before and after exercise in rectal temperatures of the dogs, glucose, lactate, triglyceride, free fatty acid and creatine kinase concentrations, and complete blood counts between dogs fed the different diets were all analysed using repeated measures ANOVA, to

determine any differences between the diets before and after the exercise test. The immune parameters CD4, CD8, CD14, B cells, phagocytic activity, lymphoproliferative responses to ConA, PHA and LPS were compared for the diets using repeated measures ANOVA and the effects of time and time x diet interactions were included in the model (y=diet time diet x time). The heart rate data for the dogs and respiratory exchange ratios were also analysed using repeated measures ANOVA. Comparisons were included in these models to not only analyse differences between diets but also between sampling points and to determine if there were any significant diet x time interactions (y=diet time diet x time).

## 7.3 Results

### 7.3.1 Digestibility of the diets

The apparent nutrient digestibility coefficients of the three diets are summarised in Table 7.3. The effect of the type of diet was significant for all parameters ( $p < 0.01$ ), however, the effect of sex was only significant for dry matter ( $p < 0.05$ ). The apparent digestibility values for crude protein, energy and fat were significantly different ( $p < 0.001$ ) between all three diets, with the highest values for test diet 2 and lowest for test diet 3. The apparent digestibility for carbohydrate was significantly ( $p < 0.001$ ) lower for test diet 1 compared to the 2 other diets. However, the apparent digestibility of carbohydrate determined for test diet 2 and test diet 3 were not significantly different ( $p > 0.05$ ). The apparent digestibility of dry matter was significantly higher for test diet 2 compared to test diets 1 and 3 ( $p < 0.05$ ) which were not significantly different to each other ( $p > 0.05$ ).

**Table 7.3. Mean apparent nutrient digestibility coefficients of three diets fed to 10 Harrier Hounds, determined using the total-collection method. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

Coefficient	Diet 1	Diet 2	Diet 3	Pooled SEM	Diet Significance
<b>Dry matter</b>	0.815 <sup>c</sup>	0.848 <sup>b</sup>	0.788 <sup>c</sup>	0.007	0.002
<b>Crude Protein</b>	0.910 <sup>b</sup>	0.946 <sup>c</sup>	0.830 <sup>d</sup>	0.002	<0.0001
<b>Energy</b>	0.865 <sup>b</sup>	0.930 <sup>c</sup>	0.825 <sup>d</sup>	0.003	<0.0001
<b>Fat</b>	0.954 <sup>b</sup>	0.980 <sup>c</sup>	0.864 <sup>d</sup>	0.003	<0.0001
<b>Carbohydrate<sup>a</sup></b>	0.739 <sup>b</sup>	0.808 <sup>c</sup>	0.828 <sup>c</sup>	0.004	<0.0001

<sup>a</sup> Carbohydrates were calculated by difference

<sup>b,c,d</sup> Diets with different superscripts are significantly ( $p < 0.05$ ) different from each other

### 7.3.2 Ambient temperature

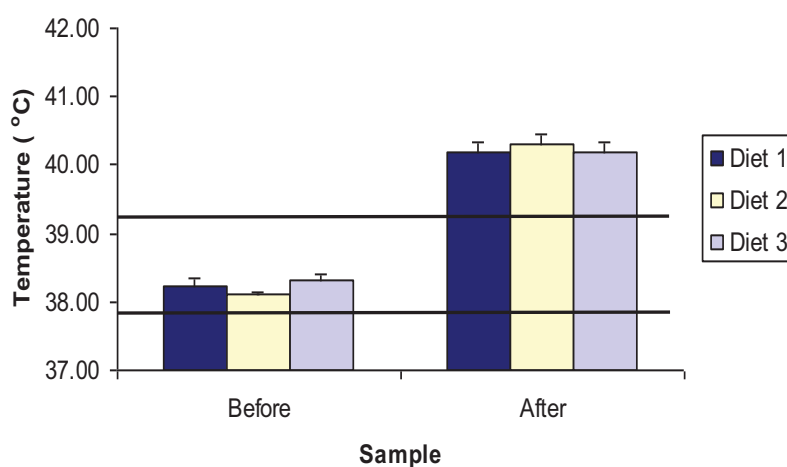
Despite attempts to control the ambient temperature, the temperature was significantly ( $p < 0.001$ ) higher on the sampling days at week 18 compared to weeks 6 and 12 (Table 7.4).

**Table 7.4. Mean ambient temperatures during the treadmill exercise tests of 9 Harrier Hounds fed Diet 1, Diet 2 and Diet 3.**

Week	Ambient Temperature (°C)
6	16.9
12	16.9
18	19.5

### 7.3.3 Rectal temperature

The rectal temperature for the dogs fed all three diets increased significantly from baseline following the exercise test ( $p < 0.001$ ) (Figure 7.5). There were no significant differences between the diets in the mean rectal temperatures either before or following the exercise test ( $p > 0.05$ ). There were also no significant differences in the rectal temperatures before and after exercise, between the sexes for any of the three diets ( $p > 0.05$ ).

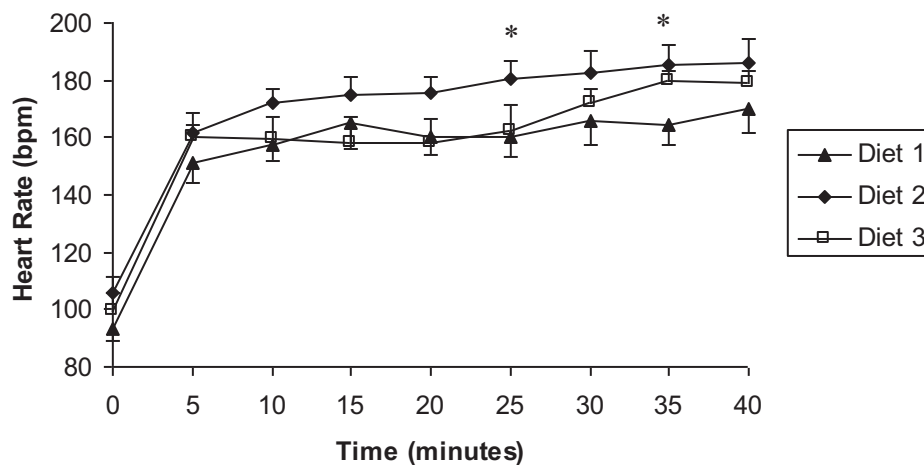


**Figure 7.5. Mean ( $\pm$  SEM) rectal temperatures from 9 Harrier Hounds fed Diet 1, Diet 2 and Diet 3, before and after exercise tests (— normal range). Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**



### 7.3.4 Heart rate

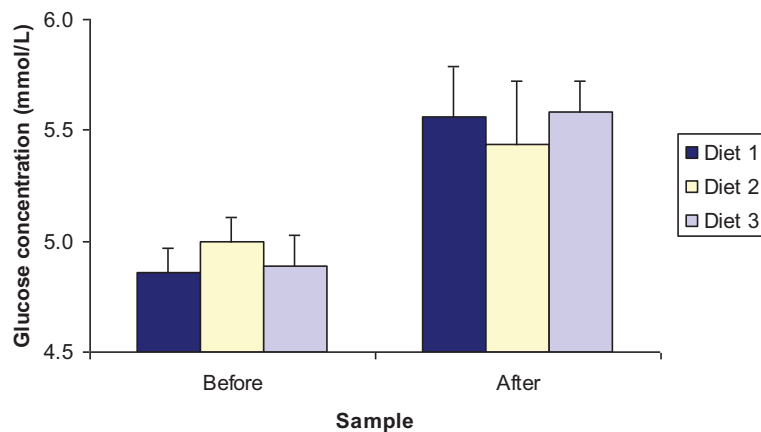
The heart rates were elevated from baseline at the end of the exercise test for the dogs fed all three diets ( $p < 0.001$ ) (Figure 7.6). The mean baseline heart rates were not different between the three diets ( $p > 0.05$ ). In fact, the only differences in heart rates between the diets were at 25 and 35 minutes ( $p < 0.05$ ), where the heart rates of the dogs fed Diet 2 were higher than those fed Diet 1.



**Figure 7.6. Mean ( $\pm$  SEM) heart rates during the exercise test from 9 Harrier Hounds fed Diet 1, Diet 2 and Diet 3 (\* $p < 0.05$ ). Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

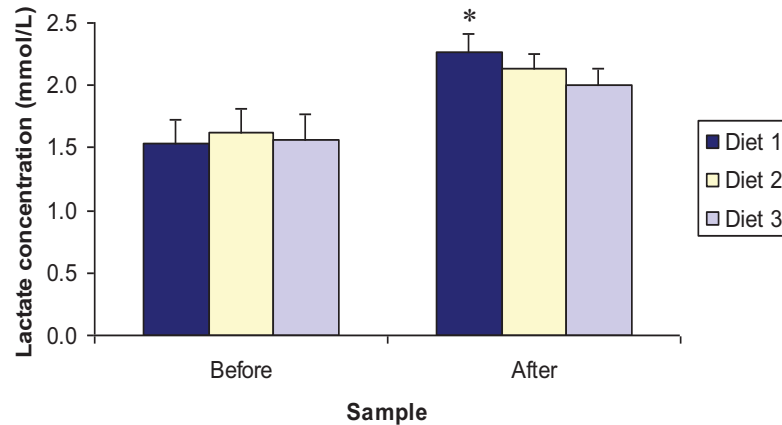
### 7.3.5 Concentrations of plasma glucose and blood lactate

Plasma glucose concentrations increased significantly from baseline following the exercise test for the dogs fed Diet 1 and Diet 3 ( $p < 0.05$  and  $p < 0.01$ ). In contrast, there were no significant differences between the pre and post-exercise glucose concentrations when the dogs were fed Diet 2 ( $p > 0.05$ ) (Figure 7.7.1). The mean glucose concentrations of the dogs fed the three diets were not significantly ( $p > 0.05$ ) different either before or after the exercise tests.



**Figure 7.7.1. Mean ( $\pm$  SEM) plasma concentrations of glucose before and after the exercise test from 9 Harrier Hounds fed Diet 1, Diet 2 and Diet 3. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

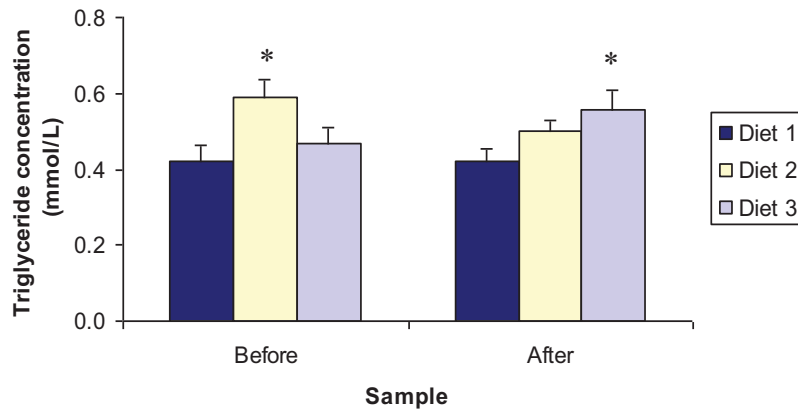
The blood lactate concentrations increased from baseline following the exercise test when the dogs were fed Diet 1 ( $p < 0.01$ ), however, there were no differences between the pre and post-exercise lactate concentrations when the dogs were fed the other two diets ( $p > 0.05$ ) (Figure 7.7.2). The mean blood lactate concentrations of the dogs fed the three diets were not significantly different either before or after the exercise tests ( $p > 0.05$ ).



**Figure 7.7.2. Mean ( $\pm$  SEM) blood concentrations of lactate before and after the exercise test from 9 Harrier Hounds fed Diet 1, Diet 2 and Diet 3. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet (\* $p < 0.05$  higher than before exercise).**

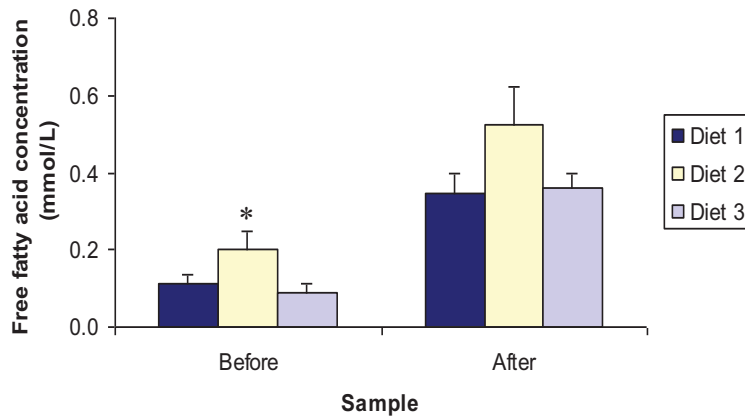
### **7.3.6 Concentrations of plasma triglycerides and serum free fatty acids**

Plasma triglyceride concentrations did not increase significantly from baseline following the exercise test when the dogs were fed any of the three diets ( $p > 0.05$ ) (Figure 7.8.1). The baseline triglyceride concentrations were lower when the dogs were fed Diet 1 compared to Diet 2 ( $p < 0.05$ ). There were no differences between the baseline concentrations when the dogs were fed Diet 1 and Diet 3, or between the baseline values for Diet 2 and Diet 3 ( $p > 0.05$ ). Following exercise, the only difference between the triglyceride concentrations was between Diet 1 and Diet 3 ( $p < 0.05$ ).



**Figure 7.8.1. Mean ( $\pm$  SEM) plasma concentrations of triglycerides before and after the exercise test from 9 Harrier Hounds fed Diet 1, Diet 2 and Diet 3 (\* $p < 0.05$ ). Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

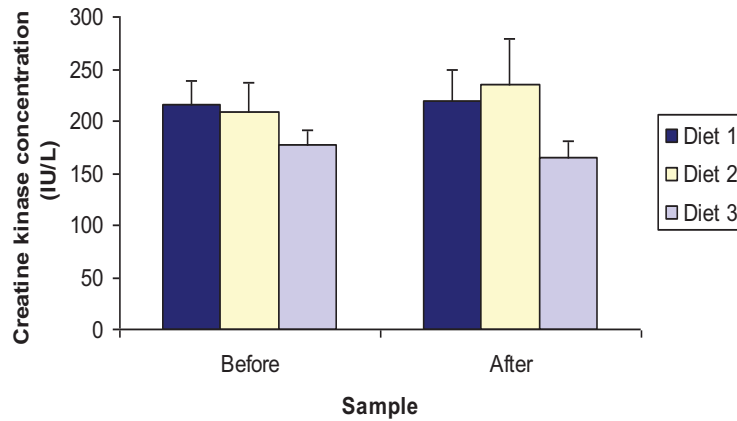
The serum free fatty acid concentrations increased significantly following the exercise test when the dogs were fed all three diets ( $p < 0.01$ ) (Figure 7.8.2). The only difference in baseline concentrations was between Diet 2 and Diet 3 ( $p < 0.05$ ), and there were no differences between the post-exercise concentrations when the dogs were fed any of the three diets ( $p > 0.05$ ).



**Figure 7.8.2.** Mean ( $\pm$  SEM) serum concentrations of free fatty acids before and after the exercise test from 9 Harrier Hounds fed Diet 1, Diet 2 and Diet 3 (\* $p < 0.05$ ). Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.

### 7.3.7 Concentrations of creatine kinase and complete blood counts

The creatine kinase concentrations when the dogs were fed all three diets did not increase significantly from baseline following the exercise test ( $p > 0.05$ ) (Figure 7.8.3). There were also no significant differences in baseline or post-exercise creatine kinase concentrations between any of the diets ( $p > 0.05$ ).



**Figure 7.8.3. Mean ( $\pm$  SEM) concentrations of creatine kinase before and after the exercise test from 9 Harrier Hounds fed Diet 1, Diet 2 and Diet 3. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

The only blood cell parameters which showed any differences were white blood cells (WBC), mean corpuscular haemoglobin concentration (MCHC) and mean platelet volume (MPV) (Table 7.5). The WBC values were significantly lower for Diet 2 compared to Diet 1 before and after the exercise test ( $p < 0.05$ ). The MCHC values for Diet 2 increased significantly from baseline values following the exercise test ( $p < 0.05$ ). Before the exercise test the MPV values for Diet 3 were significantly lower than Diet 2 ( $p < 0.01$ ) and following the exercise test the MPV values for Diet 3 were significantly lower than those for Diets 1 and 2 ( $p < 0.01$ ).

**Table 7.5. Mean complete blood count values of three diets fed to 9 Harrier Hounds. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

Variable	Diet 1		Diet 2		Diet 3	
	Before	After	Before	After	Before	After
WBC (x10 <sup>9</sup> cells/L)	17.70 <sup>x</sup>	17.28 <sup>x</sup>	14.56	13.73	16.09	15.39
RBC (x10 <sup>12</sup> cells/L)	6.84	7.26	6.67	7.18	6.79	7.13
HGB (g/L)	161.56	172.22	156.89	171.67	157.33	166.44
HCT (L/L)	0.47	0.49	0.45	0.48	0.46	0.48
MCV (fL)	68.23	67.78	67.98	67.30	67.23	66.93
MCH (pg)	23.66	23.76	23.53	23.90	23.19	23.34
MCHC (g/L)	346.89	350.44	346.33	355.11 <sup>y</sup>	345.11	348.67
CHCM (g/L)	361.22	366.11	362.33	369.56	361.11	364.33
CH (pg)	24.56	24.71	24.53	24.78	24.20	24.30
RDW (%)	13.41	13.59	13.03	13.29	13.04	13.20
HDW (g/L)	19.86	20.31	20.12	20.98	19.32	19.88
PLT (x10 <sup>9</sup> cells/L)	317.00	313.33	297.00	277.56	334.67	312.78
MPV (fL)	9.60	10.08 <sup>z</sup>	10.77 <sup>z</sup>	10.54 <sup>z</sup>	8.54	8.42

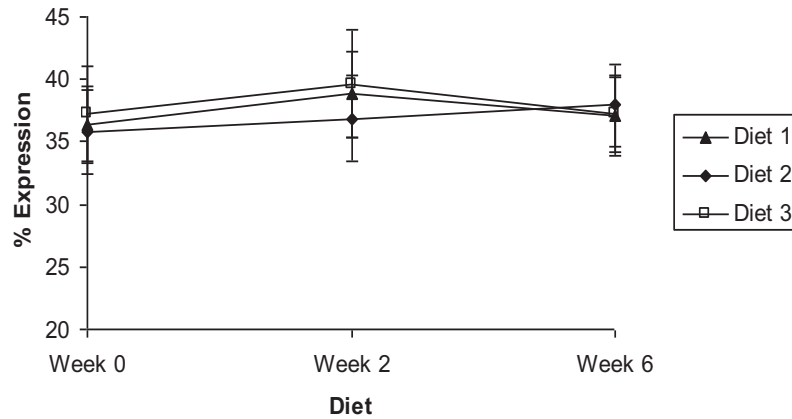
<sup>x</sup> Significantly (P<0.05) higher than the corresponding values for Diet 2

<sup>y</sup> Significantly (P<0.05) higher than the before exercise value for Diet 2

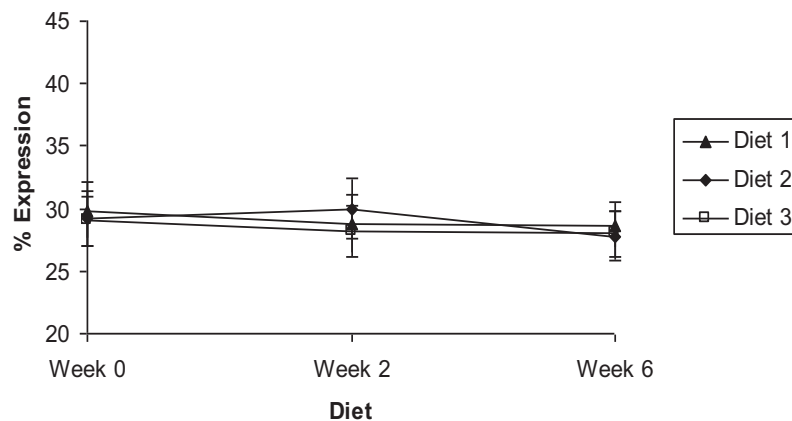
<sup>z</sup> Significantly (P<0.01) higher than the corresponding values for Diet 3

### 7.3.8 Immune parameters

The expression of CD8 and CD4 were not significantly different between the diets at any of the sampling points and there were no time x diet interactions (p>0.05) (Figure 7.9.1 and 7.9.2). There was however an effect of time seen for CD8 expression (p<0.05).



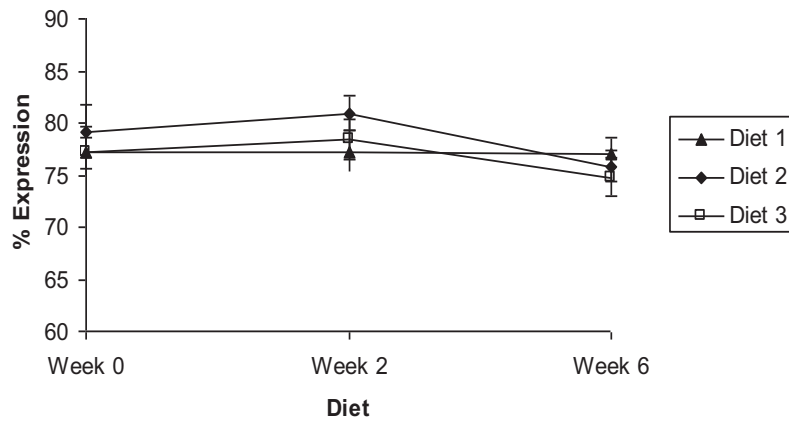
**Figure 7.9.1.** Mean ( $\pm$  SEM) expression of cell surface marker CD8 from 10 Harrier Hounds fed Diet 1, Diet 2 and Diet 3. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.



**Figure 7.9.2.** Mean ( $\pm$  SEM) expression of cell surface marker CD4 from 10 Harrier Hounds fed Diet 1, Diet 2 and Diet 3. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.

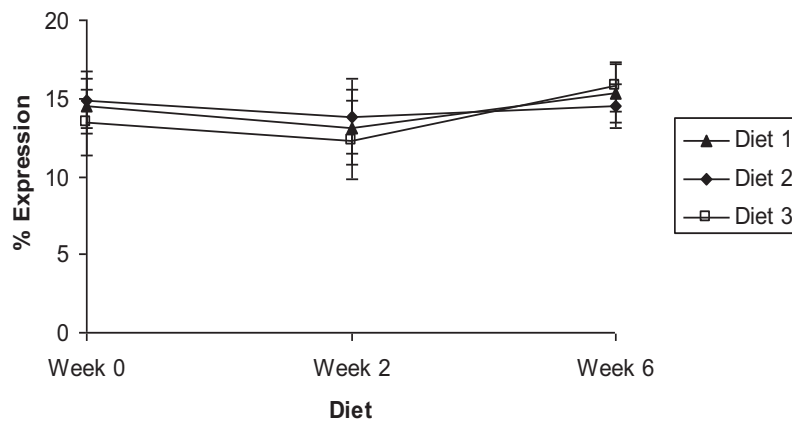
There were no significant ( $P > 0.05$ ) differences in the expression of CD14 between the diets and no time\*diet interactions (Figure 7.9.3). There was an effect of time ( $P < 0.05$ ) seen.





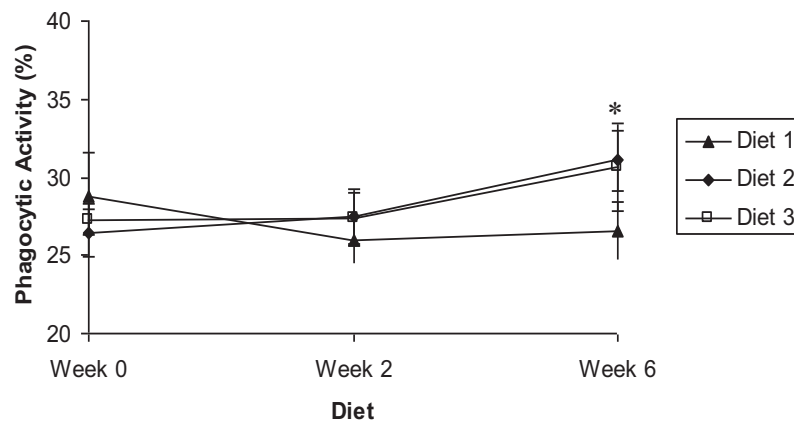
**Figure 7.9.3. Mean ( $\pm$  SEM) expression of cell surface marker CD14 from 10 Harrier Hounds fed Diet 1, Diet 2 and Diet 3. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

Over the trial there were no significant ( $P > 0.05$ ) differences in the expression of B cells between the diets and no time by diet interactions (Figure 7.9.4). There was an effect of time ( $P < 0.01$ ) seen.



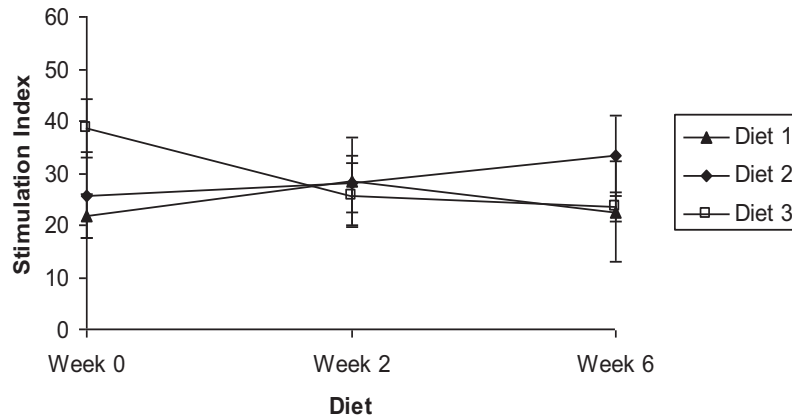
**Figure 7.9.4. Mean ( $\pm$  SEM) expression of B cells from 10 Harrier Hounds fed Diet 1, Diet 2 and Diet 3. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

There was a time dependent increase in phagocytic activity in test diet 2 and 3, however there was a decrease in test diet 1 (Figure 7.9.5). Despite these trends there were no significant ( $P<0.05$ ) differences between the diets at any over the sampling points and no time by diet interactions. The only effect of time was seen for test diet 2, with a significant ( $P<0.05$ ) increase in phagocytic activity seen for test diet 2 between weeks 0 and 6.



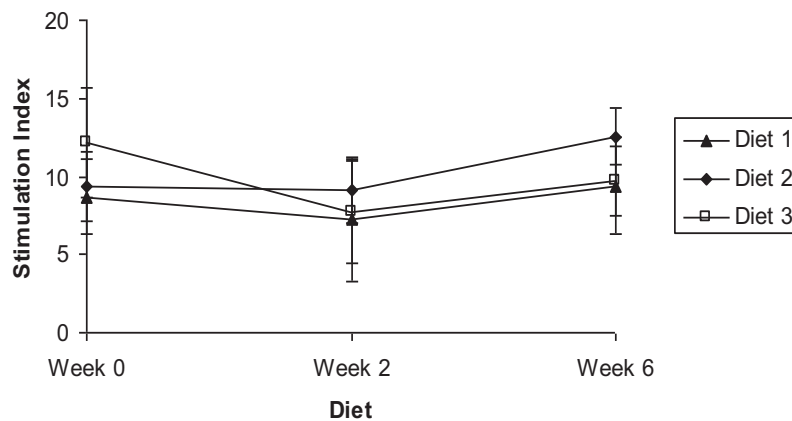
**Figure 7.9.5. Mean ( $\pm$  SEM) peripheral blood phagocytosis from 10 Harrier Hounds fed Diet 1, Diet 2 and Diet 3 (\* $p<0.05$ ). Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

The dogs fed Diet 2 showed a non-significant increase in the stimulation index for ConA during the six week period, while the dogs fed Diet 3 showed a non-significant decrease in the stimulation index for ConA (Figure 7.9.6). There were no differences in the stimulation index for ConA when the dogs were fed the three diets ( $p>0.05$ ) or effects of time or time by diet interactions ( $p>0.05$ ).



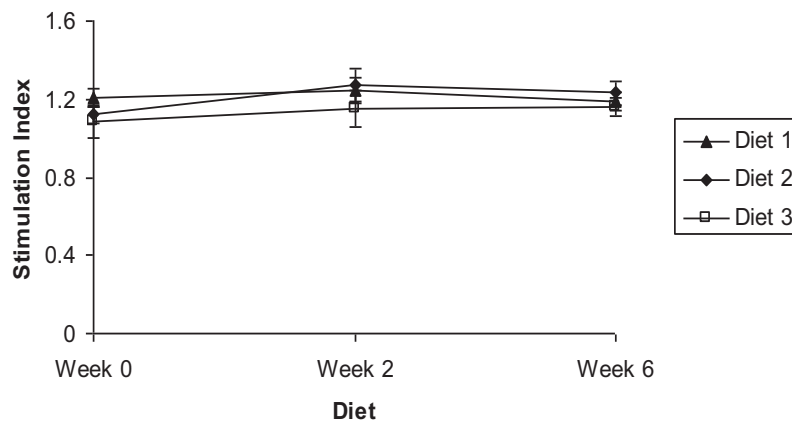
**Figure 7.9.6. Mean ( $\pm$  SEM) stimulation index for ConA from 10 Harrier Hounds fed Diet 1, Diet 2 and Diet 3. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

The dogs fed all three diets showed an initial decrease in the stimulation index for PHA and then an increase during the six week period (Figure 7.9.7). These trends were not significant and there were no differences between the diets ( $p > 0.05$ ). There were no effects of time or time by diet interactions ( $p > 0.05$ ).



**Figure 7.9.7. Mean ( $\pm$  SEM) stimulation index for PHA from 10 Harrier Hounds fed Diet 1, Diet 2 and Diet 3. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

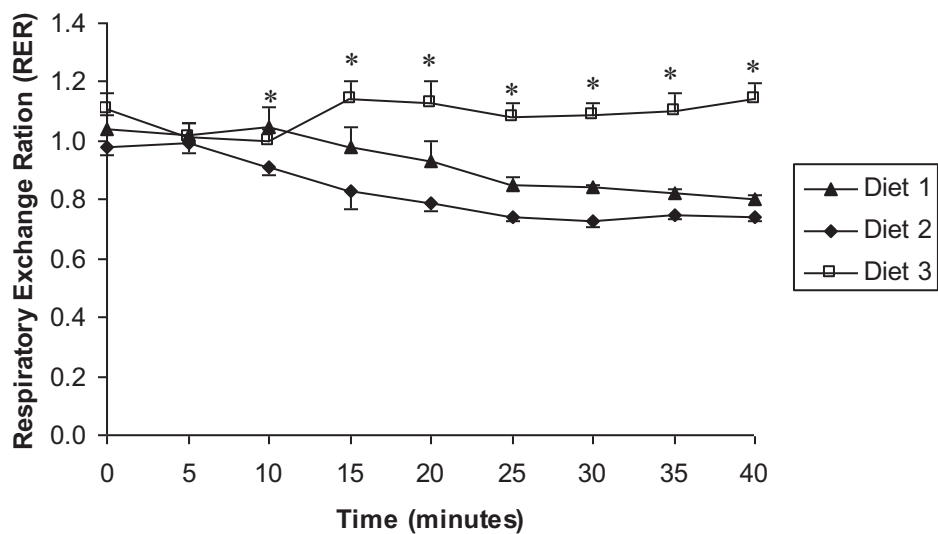
The dogs fed Diet 3 showed a non-significant increase in the stimulation index for LPS over the six week period, whereas the stimulation index for LPS when the dogs were fed the other two diets increased and then decreased (Figure 7.9.8). These trends were not significant and there were no differences between the diets ( $p>0.05$ ). There were also no effects of time or time-by-diet interactions ( $p>0.05$ ).



**Figure 7.9.8.** Mean ( $\pm$  SEM) stimulation index for LPS from 10 Harrier Hounds fed Diet 1, Diet 2 and Diet 3. Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.

### 7.3.9 Respiratory Exchange Ratio (RER)

The respiratory exchange ratios were significantly different when the dogs were fed the three diets at all of the sampling points except 5 minutes (Figure 7.9.9). The RER for Diet 3 was higher than for the other two diets from 15 minutes until the end of the exercise ( $p<0.01$ ). The value for Diet 3 was also higher than that for Diet 2 at baseline ( $p<0.05$ ). At 10, 15 and 20 minutes the RER for Diet 1 were higher than for Diet 2 ( $p<0.05$ ). The effects of diet and time on the RER were highly significant ( $p<0.0001$ ), as was the diet-by-time interaction ( $p<0.0001$ ).



**Figure 7.9.9. Mean ( $\pm$  SEM) respiratory exchange ratios from 9 Harrier Hounds fed Diet 1, Diet 2 and Diet 3 (\* $p < 0.05$ ). Diet 1 was a high-protein, low-carbohydrate, low-fat diet, Diet 2 was a high-protein, low-carbohydrate, high-fat diet and Diet 3 was a low-protein, high-carbohydrate, low-fat diet.**

## 7.4 Discussion

The specific requirements of 'intermediate activity' canine athletes, including farm and hunt dogs, needs considerable research, as to date there is a lack of information available. These dogs may not be receiving the optimum diet to meet their nutrient requirements and support the work they undertake. It is of importance to determine if these types of athletes are more similar to the sled dog or greyhound in the exercise they perform and their nutritional needs, as these two types of dogs have been researched widely and therefore some of these results may be able to be extrapolated to the more 'intermediate' working dog.

The apparent digestibility of Diet 2 was higher than the other two diets for all nutrients except carbohydrate, which was highest in Diet 3. Higher digestibility is advantageous, as it increases the maximum possible delivery of nutrients to the dog's tissues and decreases faecal bulk which is a handicap during exercise (Hammel *et al.* 1977; Toll and Reynolds 2000). Apart from carbohydrate, the apparent digestibility of Diet 3 was the lowest of the three diets fed to the dogs and therefore the least desirable diet in this sense.

The baseline rectal temperatures of the dogs (37.8-39.0 °C ) when they were fed all three diets were within the normal range (Carlson and Griffin 1992) and were comparable to baseline rectal temperatures measured in other studies using Labrador Retrievers and Canaan dogs (Sneddon *et al.* 1989; Steiss *et al.* 2004). As seen in the previous treadmill trials in this study, following exercise the rectal temperatures of the dogs increased (39.4 to 41.3 °C) regardless of the diet. The reported post exercise rectal temperatures for Labrador Retrievers and Canaan dogs are again very similar to the range exhibited in the trial reported here, ranging between 38.6 and 41.8 °C (Sneddon *et al.* 1989; Matwichuk *et al.* 1999; Steiss *et al.* 2004).

The baseline heart rates of the dogs when fed all three diets ranged from 71 to 139 beats per minute, with those reported for Beagles, Labrador Retrievers, Canaan dogs and mixed breeds ranging from 87 to 108 beats per minute (Downey *et al.* 1980; Sneddon *et al.* 1989; Matwichuk *et al.* 1999; Steiss *et al.* 2004; Haskins *et al.* 2005). The higher upper limit of the range seen in this study may have resulted from some dogs becoming excited on staff entering the pen to measure their heart rate or the anticipation of the exercise ahead of them. Following the exercise test, the heart rates increased significantly (118 to 227 beats per minutes). This range is very similar to that reported following exercise in Labrador Retrievers,

Canaan dogs, agility dogs and working farm dogs of 119 to 237 beats per minute (Sneddon *et al.* 1989; Matwichuk *et al.* 1999; Steiss *et al.* 2004; Hampson and McGowan 2007; Rovira *et al.* 2007). This indicates the dogs used in the study reported here were performing similar exercise to these other types of working dogs and they may also be similar in regards to their physiological responses to exercise.

Plasma glucose concentrations increased significantly from baseline following the exercise test when the dogs were fed Diet 1 and Diet 3 but not when they were fed Diet 2. This may be an indication that when the dogs were fed these two diets, carbohydrate utilisation was being promoted, resulting in the increased metabolism of glycogen and glucose for fuel and subsequently higher circulating glucose levels (Murphy *et al.* 1997). The ranges of glucose concentrations determined in this study, both before (4.4- 5.7 mmol/L) and after (3.7-6.6 mmol/L) exercise were very similar to those reported previously for other working dogs of 4.1-5.8 mmol/L (baseline) and 4.1-6.6 mmol/L (post-exercise) (Sneddon *et al.* 1989; Hinchcliff *et al.* 1993; Matwichuk *et al.* 1999; Steiss *et al.* 2004).

Circulating triglycerides are believed to be a source of fatty acids for beta oxidation in muscles (Terjung and Kaciuba-Uscilko 1986), however free fatty acid and intramuscular triglyceride concentrations are considered to be a better indication of fat metabolism in working muscles during exercise (Poso *et al.* 1989; Rovira *et al.* 2007). In studies of working dogs conducted by other researchers, changes in triglyceride concentrations with exercise have been varied, with increases seen in agility dogs but decreases seen in sled dogs (Hinchcliff *et al.* 1993; Rovira *et al.* 2007). In contrast to these findings, in the study reported here there were no differences found between triglyceride concentrations in the blood with exercise for any of the diets which may be evidence that serum triglyceride is relatively insignificant as a fuel for exercise compared to the contributions made by free fatty acids and intramuscular triglycerides (Terjung *et al.* 1982; Reynolds *et al.* 1994).

The majority of studies in working dogs have shown increases in free fatty acid concentrations with exercise (Hammel *et al.* 1977; McClelland *et al.* 1995; Toll and Reynolds 2000). In agreement with these studies and in stark contrast to the triglyceride results reported here, the free fatty acid values for each of the diets increased significantly following the exercise test indicating increased mobilisation of free fatty acids from fat deposits (Hammel *et al.* 1977; Grandjean and Paragon 1993; McClelland *et al.* 1995). The highest

values, both before and after exercise were seen in Diet 2, indicating higher relative proportions of fat were being metabolised for energy when the dogs were fed this diet (high-protein, high-fat, low-carbohydrate) compared to the other two diets. This confers a performance advantage and highlights that this diet is beneficial for these dogs because by utilising free fatty acids for energy, more limited stores of energy, including muscle glycogen are spared and the onset of fatigue is subsequently delayed.

The oxidative stress exercise causes generates free radicals which have the potential to cause muscle damage (Murphy *et al.* 2007; Rovira *et al.* 2007). The marker that is most commonly used to detect damage to muscles following exercise is creatine kinase (CK) (Piercy *et al.* 2000; Chanoit *et al.* 2001). Creatine kinase activity has been shown to increase with exercise in Labrador Retrievers, sled dogs and Cane Corso dogs (Sneddon *et al.* 1989; Burr *et al.* 1997; Matwichuk *et al.* 1999; McKenzie *et al.* 2005; Rovira *et al.* 2007), however during this study no significant increases were seen with exercise, indicating muscle damage doesn't always occur with exercise. However, it has been reported that peak creatine kinase values are actually not seen until 6-8 hours after exercise (Rovira *et al.* 2007) and as the last sample was taken immediately after exercise in this study, it is possible that there was an exercise induced increase, but it was missed by not taking further samples. Another possibility is that because the animals are trained, and perhaps being exercised well within their capability no or minimal muscle damage was occurring.

In the blood, the lymphocyte population is comprised mostly of T-helper cells (CD4<sup>+</sup>), cytotoxic T-cells (CD8<sup>+</sup>) and B cells. Large changes in either T or B cell numbers can lead to a predisposition to certain types of infection or disease and therefore such changes are undesirable. There were no significant changes in the level of expression of the lymphocyte markers CD4, CD8 or B cells for the dogs fed any of the diets in this study. This is advantageous because significant changes in the level of expression of the T cell markers (CD4, CD8) results in the opposing effect in B cells and such changes are undesirable as they can predispose the animal to certain types of infection and disease. There were also no significant changes observed in the level of expression of the monocyte marker CD14. The level of expression of the lymphocyte marker CD4 for the diets (range of 27.8-30.0 %) were similar to that reported for sled dogs before training of 28.9 % (Davis *et al.* 2008), but somewhat lower to that reported for mixed breed dogs of 42 % (Byrne *et al.* 2000). Compared to these same studies, the level of expression of CD8 in the study reported here (range 35.8-



38.8 %) was much higher than that reported for both the sled dogs (22.4 %) and mixed breed (29 %) dogs (Byrne *et al.* 2000; Davis *et al.* 2008). The reason for this difference may relate simply to differences in the genetics and immune systems of these different breeds of dog or to differences in the level of training they received and exercise they performed.

The phagocyte function assay used in this study specifically measures the ability of cells to ingest foreign particles such as bacteria. There were no significant differences in the phagocytic activity of peripheral blood leukocytes between the three diets. However, there was a significant increase in this parameter between week 0 and week 6 when the dogs were fed Diet 2 (high-protein, high-fat, low-carbohydrate) and this increase in phagocytic activity is an indicator of greater ability to fight infection and disease when the dogs were fed this diet. The fact that the Phagocytic activity increased when the dogs were fed Diet 2 but the level of expression of CD14 did not, indicated that the mechanism for the increase in phagocytic activity was not via an increase in the expression of monocytic cells.

Lymphocyte proliferation measures the ability of the cells to respond to mitogenic stimulation, therefore giving a measure of the readiness of the cells to respond to and fight infection and disease. The three mitogens used; Concanavalin A (ConA), Phytohemagglutinin (PHA) and Lipopolysaccharide (LPS) selectively stimulate the activation and proliferation of specific lymphocyte populations. From the results of this study, the proliferative responses to these three mitogens remained unchanged as no significant increases or decreases were observed. The changes in the proportions of the macronutrients were not expected to enhance the responses, the purpose of the analysis was to ensure these changes did not cause any detrimental changes in these responses, which they did not.

The blood lactate concentrations were not different between the diets and only increased significantly from baseline following exercise when the dogs were fed Diet 1. This indicates there was a significant amount of glycolytic activity in the skeletal muscle during exercise when the dogs were fed this diet (Rovira *et al.* 2007) and further supports the idea that the dogs fed Diet 1 were relying more heavily on carbohydrate utilisation during the exercise test. However, if this is the case it is not clear why when the dogs were fed Diet 3, which contained the highest amounts of carbohydrate, the lactate concentrations did not also increase significantly following the exercise test, especially because of the results determined for the RER for this diet.

The use of indirect calorimetry in this study produced some clear and very interesting results. The RER was the most sensitive marker measured and clearly highlighted dietary differences. The respiratory exchange ratios (RER) determined during the exercise test demonstrated that when the dogs were fed Diet 3 (low protein, low fat, high carbohydrate) they were predominantly utilising carbohydrate sources for energy for exercise, as seen by a RER of 1.0. When the dogs were fed Diet 2 (high protein, high fat) on the other hand, it can be seen they initially utilised predominantly carbohydrate (RER close to 1.0) but at 10 minutes the RER had decreased to 0.91 and it continued to decline, reaching 0.74 after 40 minutes of exercise. This illustrates these dogs switched to utilising predominantly fat sources early (Figure 19). Diet 1 was different again to these two diets. With this diet the dogs initially utilised mainly carbohydrates, with a RER of approximately 1.0, but after 15 minutes of exercise the RER decreased below 1.0, eventually falling to 0.8 after 40 minutes of exercise. This therefore indicates these dogs were probably using a mixture of carbohydrates and fats to support exercise. The one disadvantage of using the respiratory exchange ratios to estimate the respiratory quotient is that at times, during intense exercise some accuracy may be lost because of factors including bicarbonate buffering of hydrogen ions, which can affect the levels of carbon dioxide being expelled (Plowman 2008).

This study further highlights the need for investigations to define the normal responses to the many different types of exercise that dogs perform and establish reference values for these exercise types (Matwichuk *et al.* 1999; Rovira *et al.* 2007). This information would allow the evaluation of differences in performance and exercise intolerance in nutritional studies (Rovira *et al.* 2007). It is also important that a standard protocol for treadmill exercise tests be developed so that results from different studies can be compared more easily.

Conducting more studies in the field, when the dogs are undertaking the exercise they normally perform would also be beneficial and would be a true test of the suitability of the diets fed during this study. This would allow the effects of feeding the diets to be established in a practical setting and the subjective opinions of the owner in regards to the diets and dogs performance to be gathered. As the owner is generally the person purchasing the product this would make sense for pet food companies as it would prove to the owners that the diet is beneficial for their dogs, therefore giving them more reason to purchase and feed it. This is particularly important in New Zealand as many working dog owners, particularly farmers and hunt masters are somewhat sceptical about dry diets and their suitability for their dogs. They

tend to still feed predominantly home kill diets to their dogs because it is what they have always used and they are tentative to experiment with a different feed in case it hinders their dogs' performance.

In conclusion this study determined that pre-feeding profoundly affects fuel utilisation in working dogs and the implications of this are that it can be used to improve the efficiency of energy generation during exercise and improve endurance and performance in these animals. From the results of this study it is clear that a low-carbohydrate diet is preferable for these dogs, as both low-carbohydrate diets (Diet 1 and Diet 2) produced advantages over the high-carbohydrate diet (Diet 3), for example Diet 1 displayed the lowest heart rates and Diet 2 the highest digestibility. Overall the high-protein, high-fat, low-carbohydrate Diet 2 formulated for this study probably has the greatest potential for the 'intermediate' working dog, such as the many dogs used on farms throughout New Zealand. Based on the blood glucose, free fatty acid and indirect calorimetry results from this study it appears these dogs are more similar to the sled dog than the greyhound in the fuels they utilise, and the ideal dietary macronutrient profile they require.

The high-protein, high-fat, low-carbohydrate (Diet 2) not only had a higher apparent digestibility, it also appeared to encourage the animals to predominantly utilise fat sources for energy during exercise, thereby sparing the use of more limited muscle glycogen and blood glucose stores (Kronfeld *et al.* 1994; Reynolds *et al.* 1994; Reynolds *et al.* 1995; Reynolds *et al.* 1997; McKenzie *et al.* 2005). Dogs naturally have a greater capacity to transport free fatty acids and the amount of energy from fat oxidation at rest and during exercise is greater than less aerobic species such as the goat and humans (McClelland *et al.* 1994; McClelland *et al.* 1995). In addition to this, dogs can potentially increase their capacity to metabolise fat further in response to high fat diets (McClelland *et al.* 1994; Reynolds *et al.* 1994; McClelland *et al.* 1995). It would be of interest in future studies to examine if any particular free fatty acids are preferentially utilised for energy during exercise in these dogs, to see how changing the source or sources of fat included in the diet affects the diet's performance and to further narrow down the exact 'ideal' proportions of fat, protein and carbohydrate in these dog's diets. A further area of interest would be to feed Diet 2 to working dogs such as those used on farms or for hunting over a long term period to establish that this diet can keep these dogs in optimum body condition (i.e not under or over weight) and health (including their digestive systems, coat and teeth) during both work seasons and non-work seasons.

# **Chapter Eight**

## **General Discussion**

The aim of this thesis was to investigate the effects of dietary macronutrients on animal physiology and metabolism during exercise or at maintenance (rest) in dogs and cats. The diets tested included high-protein, low-carbohydrate diets with various proportions of fat and commercial low-protein, high-carbohydrate diets. The apparent digestibility of the diets was determined and glycaemic and insulinaemic responses assessed after feeding the diets. Work was simulated by running the dogs on a treadmill and a variety of performance indicators such as heart rate, rectal temperature, RER and concentrations of various biochemical markers including free fatty acids and glucose were determined.

For the initial working dog study testing a high-protein, low-carbohydrate diet and a low-protein, high-carbohydrate diet, when the blood glucose concentrations were plotted against time, no significant differences were found in the AUC, and Cmax values determined for the diets, meaning the total amount of glucose released and maximum concentration of glucose reached was the same for the two diets. In contrast however, Tmax was significantly higher for the high-protein, low-carbohydrate diet, indicating the maximum glucose concentration was reached later in the dogs fed this diet, and a slower release of glucose into the bloodstream and better glycaemic control was occurring.

Due to the nature of the results of the initial study involving working dogs, which showed that the high-protein, low-carbohydrate diet produced better glycaemic control and higher apparent digestibility it was decided to also conduct preliminary studies on the implications of such diets in cats. In line with the dog studies, both of the cat studies demonstrated that the high-protein, low-carbohydrate diets had a higher apparent digestibility than the high-carbohydrate diets fed. During these two cat studies, as seen in the initial dog study, no significant differences were found in the AUC, and Cmax values determined for the diets when blood glucose concentrations were plotted against time. There were also no differences found in Tmax values between the diets for the two studies. However, when the glucose concentrations from the cats involved in the two studies were plotted as changes from baseline values, it was clear that the cats fed the high-protein diet exhibited smaller changes in blood glucose levels compared to the commercial high-carbohydrate diet. Interestingly, in the second cat study it was seen that the wet diet fed produced the smallest changes in blood glucose. Compared to other research conducted in the area, our studies agree with those of Mazzaferro *et al.* (2003), Biourge *et al.* (2000) and Bennett *et al.* (2006), that high-protein, low-carbohydrate diets improve glycaemic control in cats. In contrast, it disagrees with the

findings of Hoenig *et al.* (2000) that no differences in post-prandial glycaemic responses were seen when cats were fed high-protein and low-protein diets. This study by Hoenig *et al.* (2000) did however find that post-prandial insulinaemic responses were different between these diets and subsequently concluded that the low-protein diet leads to hyperinsulinemia.

In addition, the results gathered throughout this study demonstrate that high-protein, low-carbohydrate diets have great potential to be beneficial to both dogs and cats with health issues such as diabetes mellitus and obesity. The slower release of glucose into the bloodstream, smaller fluctuations in blood glucose levels and therefore better glycaemic control and a decreased demand for insulin afforded by this diet illustrate the suitability of this diet for diabetic animals. The incidence of diabetes and obesity in companion animals have been increasing worldwide (Sloth 1992; German 2006), therefore it is important diets are formulated which can not only prevent but also control these health issues. With the current trend of humanisation of pets, owners are willing to buy premium products which they perceive to have nutritional and health benefits and give a better quality of life; therefore a market exists for a product such as this which may control diabetes and/or obesity.

Further work still needs to be carried out in this area of diabetes and obesity control however. It would be useful to conduct a long term study feeding these high-protein, low-carbohydrate diets to overweight and obese cats and dogs and monitor their bodyweights and body composition over the duration of the trial period to see if this diet has any beneficial effects on weight loss and the composition of this weight loss; fat versus lean tissue mass. Conducting a similar long term study feeding this diet to diabetic cats and dogs, or cats and dogs with a risk of developing diabetes mellitus would also be of use to determine if there are actually any benefits of such a diet to these animals in regards to their glycaemic control, medications required and the development of the disease. In addition, the effects of high-protein, low-carbohydrate dry diets on general cat and dog health when fed over an extended period of time could be worth investigating to ensure there are no long term issues such as damage to organs or dietary intolerances created from feeding this high-protein diet. Although no issues are expected because cats and dogs evolved on high protein diets, unless the animal has pre-existing conditions, for example kidney disease, it would be worthwhile to further investigate this and ensure this diet has no detrimental effects. If using a diet such as this for controlling obesity it is important to remember that effective treatment relies on the owner using restricted feeding methods for their cats and dogs because the dry form of the diet has a higher

energy density than wet diets. Hence this feeding regime will only work if the owner feeds it correctly and doesn't increase the volume being fed.

High-protein, low-carbohydrate diets have the potential to benefit working dogs, particularly those that can be considered 'intermediate athletes' such as the hunt dogs used in this study and farm dogs in the work they do. This is supported by the fact that this diet, when fed to hunt dogs produced a slower release of glucose into the animal's bloodstream, with the maximum concentration reached over twice as long after feeding, compared to dogs fed the commercial high-carbohydrate diet, thereby providing them with a supply of energy to fuel their work of significantly longer duration. Taking into account recommendations for feeding working dogs, it seems appropriate that this high-protein, low-carbohydrate diet should be fed four hours before work (Guilford 1997; Toll and Reynolds 2000; Wortinger 2007) and within two hours of completing work so as to replenish muscle glycogen stores (Toll and Reynolds 2000; Case 2005; Wortinger 2007) and ensure a relatively constant blood glucose concentration throughout the day and night. From all three exercise studies, it was also shown that this high-protein, low-carbohydrate diet and the high-protein, low-carbohydrate, high-fat diet display a higher digestibility, which is also beneficial for working dogs. This higher digestibility means less diet is required to be fed in order to meet the animal's daily nutrient requirements, something that is particularly important to both the dog and owner as often working dogs are too tired or stressed after work to eat large amounts, it may be more economical to feed for the owner, and when considering feeding before exercise, it means smaller intestinal bolus for the dog to carry when working. In line with this it was demonstrated in the first working dog trial that the high-protein, low-carbohydrate diet resulted in less large intestinal fermentation of carbohydrate, therefore creating fewer problems with soft stools and diarrhoea in these dogs. It would be very interesting to repeat the first trial protocol using the high-protein, low-carbohydrate, high-fat diet used in the final study to see what effects it has on glycaemic control and large intestinal fermentation of carbohydrate, so this could then be compared to the results using the high-protein, low-carbohydrate diet.

Possibly due in part to the original nature of this research in intermediate working dogs, not only at this university but worldwide, no conclusive findings with regard to performance came from the first two exercise trials. However, overall the results of this part of the study agree with other reports that physiological parameters such as heart rate and rectal

temperature are more sensitive and provide clearer changes with exercise than the blood biochemical markers such as glucose and lactate that were used in this study and in previous studies by others (Burr *et al.* 1997; Matwichuk *et al.* 1999; Steiss *et al.* 2004), as both rectal temperatures and heart rates of the dogs involved in all of the studies here increased significantly with exercise (Sneddon *et al.* 1989). Despite this, these two parameters are probably not the ideal markers to use in studies where differences between diets on exercise performance are being sought, because although changes can be seen with exercise, as a whole there were still no clear differences between the diets in this study. Identifying a reliable biochemical marker may be more useful than these physiological parameters and may be more likely to be affected by differences in diet coupled with exercise, however such a marker needs to be determined and the exercise intensity needed for measurable differences to be observed must also be established. One possibility which may be worth exploring is the use of muscle biopsies to determine muscle glycogen and fatty acid synthase levels pre and post exercise, however this is a much more invasive measurement and due to the relatively large size of the sample that would be needed, one may question the effect this sampling would have on the dogs exercise performance. Other possibilities include determination of markers involved in pathways such as the beta oxidation and insulin signaling pathways.

Interestingly, the differences in glucose concentrations measured before and after exercise were different for all three trials; the dogs fed the high-protein, low-carbohydrate diet in the first study exhibited lower concentrations both before and after exercise on nearly all sampling days compared to the high-carbohydrate diet, perhaps indicating a slower steadier release of glucose into the bloodstream. In contrast, the only difference found in blood glucose concentration during the second study was on day 14 where the pre-exercise concentration was lower for the high-protein diet and in the final study, the concentrations of glucose increased significantly following exercise when the dogs were fed the high-protein, low-carbohydrate, low-fat and low-protein, high-carbohydrate, low-fat diets. The lack of differences in blood glucose concentrations determined during the second study may indicate a balance between hepatic mobilisation of glucose and peripheral glucose uptake (Rovira *et al.* 2007). However it is not clear why the results from the first two studies are so different, given they had similar protocols, used dogs from the same genetic pool and although the high-carbohydrate diet was different, the high-protein, low-carbohydrate diet used was the same as the previous trial. These differences may be related to pre-study feeding differences or differences in the amount and or intensity of exercise they received before the start of the two



studies. The increase in glucose concentration with exercise observed in the final study for the two low-fat diets suggests these diets encourage the dogs to rely more heavily on the use of carbohydrate energy sources such as muscle glycogen, hence the reason for the higher circulation of glucose in the blood.

For all three exercise studies, no real differences were seen between the diets in triglyceride concentrations before or after exercise which is in line with reports that serum triglyceride concentrations may not be as an effective measure of fat mobilisation as free fatty acid and intramuscular triglyceride concentrations (Poso *et al.* 1989). In fact the only differences between the diets were determined on day 56 of the first two studies, when the ambient temperatures were much higher during the exercise test. This may indicate that the test conditions (exercise intensity and ambient temperature) on the other days were simply not high enough for differences to be seen or that there simply were no differences.

The free fatty acid concentrations increased significantly following exercise for all of the diets in all three trials, as found in most other published studies (Kronfeld *et al.* 1977; Reynolds *et al.* 1994; McClelland *et al.* 1995). This demonstrates that during exercise free fatty acids are mobilised from adipose tissue into circulation to be used by the muscles for fuel (Hammel *et al.* 1977; Grandjean and Paragon 1993; McClelland *et al.* 1995). This is beneficial for the dog because it spares the use of more limited stores of muscle glycogen and because muscle glycogen depletion is related to fatigue, it delays the onset of fatigue subsequently improving stamina.

During this study no detrimental effects of any of the diets on any facet of the immune system studied were observed. A diet providing adequate nutrition should not result in any detrimental immune effects, unless there was a problem with the animals' health to begin with, or if the dietary changes created problems with nutrient absorption. However there was a significant increase in phagocytic activity when the dogs were fed the high-protein, low-carbohydrate, high-fat diet, indicating a better ability to fight potential infection and disease. This requires further investigation because there is a lack of research in this area in the dog and also a dearth of information in regards to the impacts of exercise on immunity. In contrast more work has been conducted on the effects of exercise on the immune system in humans. Because of the stress exercise puts the body under, it makes sense that it may impact on the immune system in some manner and may lead to an immunocompromised situation,

especially in animals and humans participating in large amounts of exercise. For example in human athletes such as marathon runners, despite their high levels of fitness they frequently contract illnesses, particularly respiratory infections (Murphy *et al.* 1997). Therefore understanding the mechanisms whereby one could use the diet to stimulate the immune system in dogs and humans undergoing significant training and exercise may aid in minimising illness and disease and improving performance.

By pre-feeding diets varying in macronutrient proportions the primary fuel being utilised for exercise was profoundly affected. This was definitively illustrated during the final study by differences in the RER determined when the dogs were fed the two high-protein, low-carbohydrate and low-protein, high-carbohydrate diets and by some of the differences observed in blood markers such as glucose and free fatty acids throughout all three exercise trials in this study. The implications of this finding are that dietary manipulations can be used to improve the efficiency of fuel utilisation during exercise and therefore improve performance and endurance. This calorimetry study was a novel area of research in dogs and because of this the entire design of the trial, equipment used and the actual masks themselves had to be adapted or invented. Despite this, the protocol and equipment designed and utilised during this study proved highly effective and would be suitable to use in future studies of this kind. However it is recommended that RER studies be properly validated for use in companion animals and in particular exercising dogs before definite conclusions can be drawn from such studies.

From the study conducted here the RER appeared to be the most sensitive marker measured throughout this thesis and clearly highlighted dietary differences in aspects of exercise and performance. From these results, it appears this is a very useful research tool for working dogs and it would be interesting and useful to conduct further studies in this area. It would be particularly interesting to directly compare the effects of the home kill meat diets many New Zealand farmers feed their dogs to the high-protein, low-carbohydrate diets fed throughout this study. This may provide evidence of the advantages of such diets to these dogs and encourage farmers to shift their thinking and actually trial this new dry diet.

Many important lessons have been learned from this study, including the need to formulate and manufacture the diets for trials in house so everything in the diet is constant except the macronutrients of interest. This would have allowed the same batches of ingredients to be

used for all the diets and recipes of all of the diets to be known, which may have helped in explaining the results. Therefore in order to produce more meaningful, explainable results, future studies should ensure all diets tested are made by the same company using the same processing technique if possible, and everything, apart from the ingredient or macronutrient being tested should be kept constant. Considering this, it may have been better to also formulate and produce the high-carbohydrate diets specifically for the trials, rather than using products already available, or at least using the same commercial diet for each of the studies, so direct comparisons could be made. In this regard however, the limitations of pet food manufacturing processes must also be acknowledged. It may therefore have been impossible or very difficult to keep everything constant as some of the manufacturing processes require certain ingredients or levels of nutrients in the recipe for the process to work, for example extrusion requires at least some carbohydrate in the recipe in order for the product to be extruded successfully, without the product being too wet and blocking the machine or not forming into pellets properly or crumbling. The level of carbohydrate in the high-protein, low-carbohydrate, high-fat diet (Diet 2) used in the final trial was nearly at the minimum possible for the product to be made and therefore the extrusion process was difficult and very slow. On the other hand, in using an existing commercial product in these trials, it means the results are relevant and can be directly related to the industry and products currently on the market.

This study clearly highlights just how much research is still required in the area of nutrition for working dogs, including ascertaining basic and optimum dietary requirements for intermediate working dogs, particularly for farm and hunt dogs in New Zealand, to improve their health and maximise their performance. However, before the optimum diet for these animals can be formulated and then tested some basic information needs to be gathered and protocols established. The relative proportions of each fuel utilised for each of the many different types of exercise that intermediate working dogs partake in needs to be determined. Additional reliable, sensitive and easily repeatable markers, other than RERs need to be discovered which can be used to indicate differences in performance, because, as can be seen from this study, lack of previous research meant that the successful choice of markers was achieved gradually by trial and error. Furthermore, the normal responses to exercise for dogs within each exercise category such as sprinters, marathon runners, intermediate etc. need to be defined and a set of reference values for each of these established. From this the markers could be used in combination with the normal reference ranges to allow differences in

performance and exercise intolerance to be evaluated. A standard protocol for treadmill exercise tests also needs to be formulated, as if this protocol was accepted and used by all canine researchers, then the results of studies could be easily compared to one another. Based on the exercise protocol utilised in these studies and the subsequent results from them, it is suggested a standard treadmill exercise protocol for endurance or intermediate working dogs, such as farm dogs here in New Zealand, involve a slope of at least that used in the latter studies here ( $6.3^\circ$ ), be longer than an hour in length and be at a faster pace than that used here; perhaps  $4\text{ms}^{-1}$  or greater for both female and male dogs. It is believed such a protocol would be more intensive and may better reflect the situation and level of exercise that working dogs here in New Zealand partake in.

The dogs used throughout this study were hunt dogs and they are therefore accustomed to running for several hours and covering great distances over mixed terrain. In comparison the one hour of exercise they were subjected to during these studies was very limited compared to the work they are used to. If future studies in working dogs are to be conducted at this university it is imperative that the ethics committee be convinced of the fact that these dogs have a huge capacity for exercise and they need to be tested using a more intense exercise regime than that which they have previously allowed us to use. One study clearly highlighting that a very intense exercise regime is needed for performance differences to be determined was that conducted by Downey *et al.* (1980). This study used Beagles which were run till exhaustion (range 103.7-138.6 minutes) on a treadmill at a  $7^\circ$  slope (compared to  $6.3^\circ$  in this study) at a speed of 9 mph (compared to 7.8 mph for the females and 8.3 mph for the males in this study). These authors measured exhaustion time and distances run and also, like this study rectal temperature and heart rates to determine differences between diets. As seen in the current study, these authors found rectal temperature and heart rate increased significantly with exercise. However, the most definitive difference in performance between the diets fed in the study by Downey *et al.* (1980) was the mean exhaustion time which was significantly longer for the dogs fed the highest protein, lowest carbohydrate diet. However it is very unlikely that such a protocol and use of such an indicator of performance would be approved by any ethics committee today.

An alternative option for future studies of farm and hunt dogs is to carry them out in the field. Although it may be difficult to tightly control all aspects of the trial in a scientific manner, it would have the advantage that the animals would be tested doing the type and intensity of

work they normally perform. This would provide direct evidence of the benefits of any diet and would perhaps be more relevant and be better accepted by working dog owners compared to data collected under laboratory conditions. In addition the opinion of the working dog owners involved in the study could be gathered also. This is important as the owner is generally the person buying the diet and word of mouth on the best diets to feed is an important way to advertise a product. Convincing working dog owners that feeding such a diet could be of benefit to them by enhancing an animal's performance is particularly important in New Zealand where as previously mentioned a large number of farm and hunt dog owners still feed home kill meat diets.

The intermediate working dog, such as the hunt and farm dog appears to be more similar to sled dogs in the exercise they do, primarily partaking in endurance exercise with periods of sprinting and the required proportion of macronutrients in their diet. This study clearly highlights the benefits of high-protein, low-carbohydrate and high-protein, low-carbohydrate, high fat diets for hunt and farm dogs. If a recommendation was to be made for the most suitable proportions of dietary macronutrients for these dogs, it would be for a high-protein, low-carbohydrate, high-fat diet. This contrasts vastly to the ideal diet for human athletes and illustrates the large differences between metabolism and physiology in different species. Humans in comparison to dogs commonly use carbohydrate loading before and during events, and high-carbohydrate diets appear more successful for maximizing performance than other dietary strategies such as the high-protein, high-fat diet suggested here for dogs (Christensen and Hansen 1939; Bergstrom *et al.* 1967; Laurie Rauch *et al.* 1998).

The next step in this research would be to trial the high-protein, high-fat, low-carbohydrate diet from the final study in a field study, using activity monitors, heart rate monitors and perhaps some of the blood parameters trialled during this study to see if any differences can be determined in the field when the dogs are actually performing the work they are accustomed to. During such a study it would be important to ensure the diets are fed to the dogs individually in the correct quantities, and if tested against the home-kill meat diets usually fed, the meat would need to be minced to ensure all dogs were receiving the same parts of the carcass and offal. The daily exercise of each dog would also need to be controlled to ensure all dogs were exercised the same. This would allow the diet to be trialled in the actual setting it would normally be used and for a longer period of time so it could be established if this diet was able to maintain the dogs in optimum body condition and health

during both work seasons and non-work seasons. Supporting this reasoning is data obtained by Reynolds *et al.* (1999) that during training, feeding a diet containing 18 % of energy as protein was insufficient for these sled dogs and resulted in significantly more injuries compared to when higher protein (23 %, 29 % and 35% of energy), lower carbohydrate diets were fed. As mentioned above, the results of such a trial are more relevant and more likely to be accepted by farmers, therefore increasing the likelihood of such a product being purchased and fed to farm dogs if the owners could see benefits such as improved stamina first hand.

In conclusion, this work provides new and valuable information on the energy utilisation for intermediate working dogs such as farm dogs and provides a major starting point for determining the exact nutrient requirements of these types of dogs. In addition it provides important information that can be used for designing exercise trials, and a platform from which to build future trial work, particularly in relation to the best markers to measure and required exercise intensity. The calorimetry work in the final trial is the first of its kind performed at this university and was extremely successful, thus forming a strong base and standard protocol to work from in the future for studies of this kind both at this university and around the world.

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## **APPENDIX ONE:**

### **Publication of Chapter 2**