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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
the degree of**

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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

ω -3	Omega 3 fatty acid
ω -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 ₂	Volume of carbon dioxide produced
VO ₂	Volume of oxygen consumed
RQ	Respiratory quotient
RER	Respiratory exchange ratio
NEFA	Non-esterified fatty acid
HCO ₃ ⁻	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 ₂	Titanium dioxide
rpm	Revolutions per minute
mmol	Millimoles
μ U	Microunits
AUC	Area under the curve
C _{max}	Maximum concentration
T _{max}	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 ⁻¹	Micrograms per millilitre
Uml ⁻¹	Units per milliliter
mM	Millimolar
mM-L ⁻¹	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.

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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