Accelerometry as a tool for evaluating the efficacy of treatment with a green-lipped mussel extract in dogs with joint disease

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Abstract

Accelerometry is a useful objective measure of a dog's daily activity, which is most commonly expressed simply as the raw activity count of a defined epoch such as a minute, hour, or day. The accelerometry data has potential as a tool to categorise a dog's gait or estimate its speed of movement. Thus, accelerometry is a tool that can be used to monitor diseases that affect a dog's activity, such as osteoarthritis. Osteoarthritis (OA) is the most frequently identified musculoskeletal disorder in dogs, with clinical signs including lameness, swelling, and pain. Although incurable, there are a variety of treatments that can reduce the clinical signs of OA, including green-lipped mussel (*Perna canaliculus*) extract (GLME), which has evidence of anti-inflammatory activity. GLME can alleviate clinical signs such as swelling and pain, when given to dogs with OA. New Zealand farm dogs are highly active dogs with a high frequency of joint disease within the population. The level of management of joint disease in the population is low, thus they are a novel and potentially suitable population for trialling treatments of OA. Accelerometry is an obvious means of detecting an effect of treatment in these working dogs.

Therefore, the principle aim of this thesis was to determine if New Zealand working farm dogs are a suitable study population, and if accelerometry is able to detect an effect of GLME nutraceuticals in this population of dogs with mild joint disease. In order to achieve that aim, it was decided to first determine if the chosen accelerometry system could estimate the speed, and characterise the gait of dogs.

In the first trial, dogs (n=8) were exercised on a treadmill that was held at speeds that were comfortable for them to walk, trot, and run. Their gait was visually annotated using a motion capture system, and speed was determined from the treadmill. The association between the basic accelerometry output "delta-G", and the dog's speed and gait in 10 second intervals was tested. It was concluded that there was a delta-G threshold above which, the dogs would reliably be gaiting faster than a walk. However, the linear association between delta-G and speed was poor, and decreased with increasing speed. Thus, it was not possible to accurately predict speed using the accelerometry system.

In the second trial, dogs (n=27) were treated with two dose strengths of a GLME nutraceutical and a placebo for 8 weeks each in a randomised, cross-over, double-blinded study. Linear mixed models were created to estimate the effect of treatment on delta-G, which was collected in 10 second epochs. Accelerometry was able to detect small, but significant effects in this population. In addition, it was concluded that treatment with GLME increased peak activity in working farm

dogs with signs of joint disease, and increased night-time activity slightly. While joint disease is highly prevalent in NZ working farm dogs, there were significant problems and limitations that question the suitability of this population for further similar studies. Nonetheless, the research presented in this thesis suggests that farm dogs with signs of joint disease might benefit from treatment with the GLME used here, even when they are mildly affected.

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Introduction

Osteoarthritis is an incurable, degenerative joint disease. It is the most common musculoskeletal disease reported in dogs and is estimated to affect upwards of 2.5% of dogs (Anderson et al., 2018; O'Neill et al., 2014). Osteoarthritis progression results in increasing debilitation of a dog, particularly impacting a dog's ability to move freely.

Triaxial accelerometers can continuously record acceleration across three orthogonal planes in space that can be quantified to characterise movement in dogs. Simple measurements derived from accelerometers have been used with some success to identify treatment efficacy in dogs with a variety of diseases, most notably in osteoarthritis (Brown, Boston, & Farrar, 2010; Knazovicky, Tomas, Motsinger-Reif, & Lascelles, 2015). However, accelerometry data is often presented in terms and units that are not intuitively easy to understand and are difficult to communicate to audiences unfamiliar with accelerometry.

To address this, accelerometry could be used to produce measurements beyond the simple accelerometer measurements into parameters that are more widely understood, such as speed and gait. This is of interest for two reasons, firstly for reporting purposes, accelerometer measurements are meaningless to people without accelerometry experience, and secondly accelerometer measurements are not intuitive in their application to dog activity, where it is unknown what activity a dog is doing at any given accelerometer value. To date, accelerometry has not been utilised to measure specific gaits nor to predict speed in any study designed to test a treatment's efficacy for osteoarthritis.

Green-lipped mussel extract is a nutraceutical with some evidence of effectiveness in the treatment of osteoarthritis in dogs. However, there is a distinct lack of objective data supporting this. There is a need to obtain objective evidence of green-lipped mussel extract efficacy. Accelerometry could be a useful tool for achieving this, either with the simpler accelerometry measurements or by using accelerometry to estimate the speed and gait of a dog for a time period.

Typically, dogs selected for participation in treatment efficacy trials are sourced from the pet dog population. As an alternative in New Zealand, is to use the working farm dog population, which is a large unutilised population of highly active dogs. New Zealand farm dogs are made up of a few key breeds, principally the Huntaway and the Heading dog. As a consequence of genetics and the frequency of injury in New Zealand farm dogs, joint disease is very common (Cave, Bridges, Cogger, & Farman, 2009). The large number of dogs with joint disease in this

population suggests it may be useful for treatment efficacy studies, particularly because the level of osteoarthritis management in this population is low, and many dogs remain active despite the presence of joint disease.

This thesis consists of a literature review, two experimental chapters and a general discussion chapter. Chapter one is a literature review, split into three sections. The first section is a brief review of the causes, clinical signs, and pathophysiology of osteoarthritis in dogs. The second section reviews literature regarding green-lipped mussel extract, a nutraceutical treatment, as a treatment for the alleviation of clinical signs of osteoarthritis. The third section outlines how treatment efficacy could be evaluated using accelerometry.

Chapter two is the first experimental chapter, which details the assessment of a novel, collar-mounted accelerometer for estimating a range of dog speeds and gaits on a treadmill. The correlation between the delta-G values from the accelerometer and the dogs' speed and gait was tested, to evaluate the potential of the accelerometer to predict gait and speed.

Chapter three is the second experimental chapter, which describes a pilot study that aimed to determine if accelerometry is a feasible tool to study the efficacy of a green-lipped mussel nutraceutical for the treatment of joint disease. It also aimed to determine if New Zealand working dogs are a useful population for evaluating treatment effects. Two green-lipped mussel extract nutraceuticals and a placebo were evaluated in an 8 ½ month cross-over study via the comparison of delta-G values from a continuously recording, collar-attached accelerometer.

The fourth and final chapter consists of an overall discussion of the thesis, including the key findings and suggestions for future research.

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1 A review of literature relevant to evaluating the efficacy of GLME for the reduction of clinical signs of OA with accelerometry

Osteoarthritis (OA) is one of the most common musculoskeletal diseases reported in dogs, effecting their synovial joints (Mele, 2007; O'Neill, Church, McGreevy, Thomson, & Brodbelt, 2014). A major characterisation of OA is the progressive breakdown of articular cartilage covering the articulating bones within the joint (Hutton, 1989). The architecture of healthy articular cartilage enables synovial joints to withstand the stress that normal movement exerts on the joint without damage to the joint or subchondral bone (Arokoski, Jurvelin, Väätäinen, & Helminen, 2000). Once a synovial joint is damaged and the articular cartilage is compromised, irreversible changes to the structure of the joint can occur which inhibit joint function. Altering the joint structure results in the common clinical signs of OA, including a reduced range of motion in the affected joint, stiffness and pain (Cooper, Javaid, & Arden, 2014).

Nutraceuticals are one method of OA management in dogs. Green-lipped mussels (*Perna canaliculus*) are a bivalve marine mollusc endemic to New Zealand with increasing popularity as a nutraceutical. The lipid fraction of green-lipped mussels is comprised of the key fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), novel omega-3 polyunsaturated fatty acids and furan fatty acids. These fatty acids have been strongly linked to inflammation reduction and resolution, and subjectively associated with a reduction in clinical signs of OA in dogs (Halpern, 2000; Mehler, May, King, Harris, & Shah, 2016; Treschow et al., 2007; Ulbricht et al., 2009; Wakimoto et al., 2011). However, objective evidence of GLME efficacy in dogs is scarce.

Joint disease causes dogs to modify their natural movement to compensate, due to pain or structural changes in the joint resulting in altered patterns of movement and abnormal gait characteristics in comparison to their healthy counterparts (Knazovicky, Tomas, Motsinger-Reif, & Lascelles, 2015; Tashman, Anderst, Kolowich, Havstad, & Arnoczky, 2004). Triaxial accelerometers can continuously record acceleration across three orthogonal planes in space. These planes can be orientated to the three planes of a dog's body (sagittal, coronal, and transverse) to model the acceleration experienced by a dog during movement. By quantifying the acceleration across these planes, accelerometry can be used to characterise movement and gait in dogs. This technology could be useful for the evaluation of OA treatment efficacy through the objective quantification of activity in dogs on and off treatment.

In this review, I will justify the use of accelerometry to evaluate the efficacy of a green lipped mussel extract nutraceutical for the treatment of clinical signs of OA in dogs. The first section will describe OA in dogs, including possible causes, clinical signs and prevalence estimations. It will then describe the components of a healthy synovial joint and articular cartilage, and the pathway of degradation, inflammation, and the morphological changes in synovial joints affected by OA. The second section will explore the mechanisms of action of GLME on clinical signs of OA, and review all clinical studies evaluating the efficacy of GLME for the treatment of OA in dogs to date. The final section will explain what accelerometry is and how the data obtained can be used and interpreted. It will also review previous studies that have used triaxial accelerometry to monitor OA and common methods of accelerometer attachment in dogs.

1.1 Osteoarthritis in dogs and the response of articular cartilage to damage

Osteoarthritis (OA) is a degenerative joint disease that develops as a result of damage to, or irregularities in articular cartilage. Not all injuries to a joint result in the onset of OA; in many cases the joint will heal through normal repair mechanisms. It is unknown what determines this outcome, however, severity of injury, the stress applied to the joint whilst it is healing, age and genetics are all likely to play a part in determining the switch from healing to OA pathogenesis. In dogs, OA tends to be caused by an identifiable disease or trauma, such as hip dysplasia, patellar luxation, or traumatic injury (Clements, Carter, Innes, & Ollier, 2006; Johnston, 1997; Mele, 2007). This differs from humans where OA onset often has no identifiable cause and is attributed to the summative effect of various mechanical and biological damage to a joint over a lifetime. Two of the most common causes of trauma in pet dogs are motor vehicle accidents and interactions with other animals (Kolata, 1980; Simpson, Syring, & Otto, 2009). If the trauma causes severe damage to a joint, OA onset is likely.

Hip dysplasia is a common heritable disorder in dogs characterised by abnormal development of the hip joint resulting in hip laxity, possibly due to laxity/underdevelopment of muscles and connective tissue in and around the hip joint. Hip laxity causes irregular movement within the joint that over time can damage the articular cartilage. OA develops if the damage causes an imbalance towards degradation over repair within the articular cartilage. Unlike trauma, the likelihood of OA onset due to a genetic predisposition such as hip dysplasia varies with breed, with different breeds having a very different prevalence of hip dysplasia. To highlight this breed effect, two independent studies based in the US and France estimated breed prevalence and both identified the Cane Corso and Siberian Husky as a breed with a very high prevalence of 44.5-60% and a very low prevalence of 2.1-3.9% respectively (Genevois et al., 2008; Loder & Todhunter, 2017). Hip dysplasia is just one of many diseases that can lead to OA but demonstrates that in the absence of injury the likelihood of any given dog developing OA is extremely varied, with genetic predisposition and environmental effects having a big part to play.

OA is the most frequently identified musculoskeletal disorder in dogs (O'Neill et al., 2014). There are two prevalence estimations of OA in the domestic dog population cited in the literature. The first is a prevalence of 20% in dogs over one year of age and the second is a prevalence of 80% in dogs over eight years of age (e.g. Rialland et al., 2012; Servet, Biourge, & Marniquet, 2006).

Both estimates are credited back to a single publication that is without mention of an 80% estimate, and the 20% estimate was accredited to an unpublished survey more than 20 years ago (Johnston, 1997). Therefore, despite the widely cited nature of these estimates, the inability to investigate an original source and the estimate being based on data at least 20 years old have deterred their use for this review.

VetCompass™ is a database of veterinary clinical records from participating clinics across the UK. Whilst it must be acknowledged that prevalence estimates of OA in dogs from this database only includes dogs that have visited a participating clinic and have clinical signs of OA, VetCompass™ provides reliable, current data on health estimates. Two published studies have used the VetCompass™ database to produce prevalence estimates of OA in dogs in the UK. One study estimated 2.5% of veterinary visits were due to OA while another estimated 6.6% (Anderson et al., 2018; O'Neill et al., 2014). Neither study confirmed all cases of OA with a radiograph, instead using clinical signs and animal history as acceptable evidence of OA which may have resulted in incorrect estimation of prevalence. Further, both studies only included dogs that presented at a veterinary clinic with OA, and therefore may have underestimated the prevalence of OA as there are dogs that would have had OA during a veterinary visit and not have been diagnosed. Additionally, there may be a number of dogs with OA that have not visited a clinic at all.

Although there is no actual prevalence estimate of OA in the domestic dog population, its high frequency is widely acknowledged. As the most common musculoskeletal disease presented to veterinary clinics, its widespread effects leave many dogs with a decreased quality of life that diminishes further as the disease progresses. As OA is currently incurable it will continue to be a leading cause of pain and discomfort in dogs worldwide.

Clinical signs of OA include stiffness after inactivity, a reluctance to exercise, prolonged lameness, and pain on manipulation, which may be obvious, or only manifested as aggression (Cooper et al., 2014; Pettitt & German, 2015). Night-time pain in dogs can also be reported by owners through observed restlessness during the night (Jones & Doherty, 2005; Knazovicky et al., 2015). Clinical signs apparent on examination of the joint include thickening of the joint, atrophy of surrounding muscle, joint effusion, a reduced range of motion and crepitus (Pettitt & German, 2015). Fibrosis and osteophyte formation are responsible for chronic joint swelling, although in acute exacerbations there may be significant joint effusions as well. The loss of the protective articular cartilage causes crepitus between the articulating bones, and pain due to

stimulation of the nerves inside the chondral bone. Osteophytosis and fibrosis also impairs normal joint movement, resulting in a reduced range of motion.

A standard method for OA diagnosis is to identify osseous changes in and around the affected joint using radiography, requiring sedation or general anaesthesia of the dog, making it an expensive procedure in comparison to a physical exam. As an alternative to radiography, a diagnosis can be confidently made using a history aligning with OA onset paired with the presence of clear clinical signs during a physical exam. This is because the presence of a sufficient number of the clinical signs are specific to osteoarthritis, with very few diseases aside from OA presenting in a similar way. These symptoms, when paired with a history of prolonged lameness, or stiffness after periods of inactivity, are indicative of OA. However, a portion of dogs diagnosed will have other disease or injury such as soft tissue damage or bursitis.

Healthy Synovial Joint Composition and Function

Synovial joints are characterised by the synovial fluid that fills the joint cavity (Mele, 2007). The outer layer of the joint cavity is formed by fibrous connective tissue called the articular or joint capsule. The joint capsule attaches to the articulating bones to form the border of the joint cavity and is lined with the synovial membrane. The majority of the synovial membrane is made up of a thin layer of cells called synoviocytes, which secrete the synovial fluid, and a few other cell types including macrophages and helper T cells that secrete mediators (Iwanaga, Shikichi, Kitamura, Yanase, & Nozawa-inoue, 2000; Revell, Mayston, Lalor, & Mapp, 1988). The synovial fluid that fills the joint capsule is a viscous, largely acellular fluid that reduces friction between the articulating bones and facilitates the transfer of nutrients and waste between blood vessels outside the synovial joint and the articular cartilage within the joint. At the point of articulation, the bones are covered in hyaline articular cartilage allowing smooth, low friction movement of the joint (Iwanaga et al., 2000).

Ligaments are bands of fibrous connective tissue that connect articulating bones together to stabilise the joint by preventing bone separation or abnormal movement. Tendons attach the articulating bones to the muscles acting across the joint to facilitate joint movement during muscle contraction. Fat pads and bursae work by keeping moving parts of the joint apart to reduce friction and prevent damage to the various tissues (Riegger-Krugh, Millis, & Weigel, 2014). Articular discs or menisci are also found in some synovial joints, which serve a range of functions including joint stability and shock absorption (Makris, Hadidi, & Athanasiou, 2011).

Articular cartilage

Articular cartilage is an unusual tissue because it is aneural, avascular and lacks lymphatics. It is a form of hyaline cartilage made up of a dense extracellular matrix (ECM), with its wet weight consisting of 65-80% water, 10-20% collagen and 10-20% proteoglycans (Bhosale & Richardson, 2008). Within the ECM is a sparse collection of chondrocytes that make up only between 1 and 5% of the wet weight (Bhosale & Richardson, 2008). The water, along with its electrolytes, is known as the fluid phase of articular cartilage, while the collagen and proteoglycans components are known as the solid phase. There are also a number of other molecules found in low concentrations in articular cartilage including non-collagenous proteins and glycoproteins (Fox, Bedi, & Rodeo, 2009).

Chondrocytes

Chondrocytes are the specialised resident cells within the ECM responsible for the production, degradation, and replacement of the ECM. The cells are found sparsely throughout the ECM, varying in number and form by the zone they are found in, with the greatest numbers being found in the superficial zone (Fox et al., 2009). To survive in the avascular environment, the chondrocytes exchange nutrients and waste with the synovial fluid and subchondral bone. They are only responsible for the maintenance and synthesis of immediately surrounding ECM via the production of a range of enzymes, such as metalloproteinases (see below) as they are unable to migrate within the complex ECM. Their inability to migrate makes cell to cell contact and communication rare, however the cells are very sensitive to their surrounding ECM and will respond to a variety of stimuli including changes in hydrostatic and osmotic pressure, growth factors and cytokines including PGE_2 to change gene expression and protein synthesis (Goldring, Otero, Tsuchimochi, Ijiri, & Li, 2008; Li et al., 2009).

Collagen

There are a few forms of stress applied to a joint, including compressive, shear and tensile stress. Compressive stress is a pushing inward force on a joint, shear stress is stress parallel to the surface of the joint, while tensile stress is the pulling or stretching stress on a joint. Type-2 collagen is the predominant form of collagen fibres in articular cartilage and function to resist these stresses. The orientation of collagen fibres depends on the zone they feature in within articular cartilage, which can be divided into three zones; superficial, transitional and deep. The changing orientation and interactions between the collagen fibres and other molecules allow cartilage to keep its form by providing shear and tensile force resistance (Arokoski et al., 2000;

Fox et al., 2009; Fratzl et al., 1998). Within the thin superficial zone, collagen is densely packed and orientated so that the fibres run parallel to the cartilage surface to protect the underlying layers from shear stress (Fox et al., 2009).The collagen within the transitional zone is orientated in a slanted direction. The deep zone is the layer closest to the articulating bone, and the collagen fibres in this layer are orientated at 90 degrees to the endochondral bone, providing compressive stress resistance.

Proteoglycans

Proteoglycans are protein monomers that have been glycosylated with one or more chain. The monomers can exist alone or in aggregates. Non-aggregating proteoglycans are thought to regulate collagen fibrillogenesis (Kuijer, van de Stadt, de Koning, Jos van Kampen, & van der Korst, 1988). Aggregating proteoglycans, the most common being aggrecan (chondroitin sulfate proteoglycan 1), fill the space between the collagen fibres and provide compression resistance by maintaining a high osmotic pressure in the cartilage. The negatively charged proteoglycan side chains function in two ways, they bind cations and attract water into the cartilage, and their charges cause repulsion from each other to push the proteoglycan molecules apart (Newman, 1998).

Water

Water makes up to 80% of the volume of articular cartilage. It makes up the fluid phase with a collection of electrolytes and aids in the distribution of nutrients to the chondrocytes along with being largely responsible for the resistant properties of articular cartilage (Fox et al., 2009; Newman, 1998). As water is attracted into the cartilage by the proteoglycan chains, the electrostatic interactions between the electrolytes of the fluid phase and proteoglycans in the ECM repel each other, pushing the molecules apart. The fixed collagen structure of the ECM works to constrain the repelling molecules, limiting the cartilage swelling. This constraint produces a constant pressure within the cartilage that provides compressive stiffness (Arokoski et al., 2000; Newman, 1998). When a load is initially applied to a joint, the fluid and solid phases share the load. As the cartilage covering the articulating bones is compressed the fluid phase moves through the permeable solid phase of the ECM. The solid phase, whilst permeable, has a high frictional resistance to the water movement that increases with increasing pressure on the cartilage, decreasing the permeability of the ECM. Consequently, a high hydrodynamic pressure is required to move the interstitial water. It is this pressurisation of the fluid phase that provides up to 75% of the load support (Newman, 1998). Once all the water has been expelled through

the solid phase, the solid phase supports the entire load. On removal of the load, the water flows back into the ECM.

Metabolism and Repair of Articular Cartilage

Normal metabolism of articular cartilage

In healthy articular cartilage there is a balance between ECM degradation and regeneration over a lifetime. The turnover of proteoglycans and collagen is slow, a large proteoglycan aggrecan can take up to 25 years to turnover while collagen fibres can last a lifetime in the absence of damage (Maroudas, Bayliss, Uchitel-Kaushansky, Schneiderman, & Gilav, 1998; Verzijl et al., 2000). While collagen and proteoglycans are synthesised by the chondrocytes directly, degradation is indirectly controlled via the production of a group of enzymes released into the ECM (Fox et al., 2009). This dynamic process conducted by the chondrocytes is regulated by a number of stimuli, both chemical and mechanical, in order to keep a healthy balance (Karsdal et al., 2008). Disturbances in this process can lead to irreparable damage of articular cartilage.

The key enzyme families responsible for ECM degradation are matrix metalloproteinases (MMPs), cathepsins, and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS). Proteoglycans are degraded by ADAMTS and cathepsin B and D. MMPs are produced in an inactive form (zymogen) that requires the removal of a zinc atom in the catalytic site in order to become active. This activation is predominantly done by the protein-activating enzyme Furin (Van Wart & Birkedal-Hansen, 1990). There are many MMP enzymes including collagenases (MMP1, 8, 13), gelatinases (MMP2, 9), and stromelysins (MMP3, 10, 11), each with a slightly different role, that function to breakdown collagen in addition to a number of other proteins (Fox et al., 2009; Karsdal et al., 2008).

Pathogenesis of OA

When a synovial joint is damaged, helper T cells and macrophages in the synovial membrane and chondrocytes release proinflammatory cytokines (Abramson, 2008; Goldring & Otero, 2011; Sandell & Aigner, 2001). The main proinflammatory cytokines identified in osteoarthritic joints are interleukin-1 (IL-1) and tumour necrosis factor−α (TNF- α) which along with insulin-like growth factor 1 (IGF-1) cause flow on effects that incite OA pathogenesis. Effects include increased synthesis and activation of ADAMTS and the suppression of ECM synthesis (Kapoor, Martel-Pelletier, Lajeunesse, Pelletier, & Fahmi, 2011; Sandell & Aigner, 2001). They also stimulate the production of MMP-1, MMP-13 and MMP-3. MMP-3, once activated by Furin, can

activate other MMPs aiding in the upregulated degradation of the collagen structures within the ECM (Goldring, 2000; Kapoor et al., 2011; Westgarth & Ladha, 2017).

IL-1 and TNF- α initiate further proinflammatory cytokines and chemokines that bind to their respective cell surface receptors on chondrocytes. This induces the synthesis of cyclooxygenase 2 (COX-2) and nitric oxide synthetase (iNOS). Nitric oxide, the product of iNOS, inhibits the synthesis of collagen and proteoglycans, activates MMP's and initiates the apoptosis of chondrocytes (Abramson, 2008; Amin & Abramson, 1998; Goldring, 2000; Taskiran, Stefanovicracic, Georgescu, & Evans, 1994).

Cyclooxygenase exists in two forms, COX-1 and COX-2, both of which produce prostaglandin H_2. COX-1 is constitutively expressed in many cells throughout the body, to produce prostaglandins required for normal body functions. One of these normal body functions is maintaining the integrity of the intestinal mucosa, through inhibiting the secretion of gastric acid, increasing mucosal blood flow, increasing the secretion of mucus and bicarbonate, and promoting epithelial cellular turnover (Toki, Aoki, Katsumi, & Takahashi, 2007). Inhibition of prostaglandin synthesis by the inhibition of COX-1 can result in ulceration and bleeding in the gastrointestinal tract, which manifests as loss of appetite, nausea, vomiting, diarrhoea, and in severe cases, haematemesis and melaena (Brinton, 1857; Wallace, 2000). COX-2 is referred to as the inducible form and is responsible for producing proinflammatory prostaglandins in response to joint injury. COX-2 synthesises prostaglandin-H2 from arachidonic acid. The enzyme Prostaglandin E synthase then produces prostaglandin E2 (PGE_2) from PGH_2.

PGE_2 has a diverse role in the body, it is involved in pain sensation and inflammation (Funk, 2001; Kawahara, Hohjoh, Inazumi, Tsuchiya, & Sugimoto, 2015). Its role in pain sensation is one of increased perception of pain (hyperalgesia). When a nerve is damaged in the subchondral bone or surrounding tissues due to cartilage loss and joint damage, PGE2 enhances the pain signalling pathways by decreasing the response threshold of nociceptive cells (Kawabata, 2011). PGE2 is also responsible for increased vascular permeability and vasodilation, causing redness and swelling – cardinal signs of inflammation (Ley, Laudanna, Cybulsky, & Nourshargh, 2007; Omori, Kida, Hori, Ozaki, & Murata, 2014)

Structural changes to synovial joints caused by OA

Normal turnover becomes pathogenic when there is an imbalance between degradation of ECM and the ability of chondrocytes to repair the ECM. Water moving in and out of the ECM with joint loading causes a change in osmotic pressure which can alter chondrocyte behaviour. Under

physiologically normal osmotic conditions, chondrocytes are able to maintain regular metabolic activities and cell morphology via altering gene expression effecting both the ICM and ECM synthesis, where increased osmotic pressure results in increased proteoglycan production to attract and retain water (Johnson, Shapiro, & Risbud, 2014). However, the osmotic pressure can move outside of physiologically normal osmotic conditions, it can be too high due to prolonged stress, or too low due to water swelling the tissue because of the decreasing collagen content. In these instances, chondrocyte function is compromised. This can result in the irreversible suppression of matrix synthesis and the transcription of genes required to maintain homeostasis, with proteoglycan synthesis unable to revert to the levels of synthesis possible at ideal osmotic pressures (Borghetti et al., 1995; Urban, Hall, & Gehl, 1993). Damage to chondrocytes can also result in cell death, which is a major problem for articular cartilage repair as chondrocytes do not readily proliferate. Therefore, unprogrammed cell death as a result of direct injury or secondary to joint degeneration leaves a region of ECM without a mechanism to repair or maintain itself (Fox et al., 2009; Newman, 1998).

Cartilage cannot be repaired when collagen degradation far exceeds collagen synthesis. (Van Meurs et al., 1999). The increase in catabolic activity in the ECM of articular cartilage causes irreversible degradation of the articular cartilage as the anabolic processes, while still active, are unable to replace ECM at the rate of catabolism. The repair process also fails to replicate the specialised ECM architecture. Therefore, the regenerated ECM does not have the same functional capabilities as healthy articular cartilage. The resultant replacement tissue is unable to withstand the loading of the joint through normal movement, stimulating further degeneration of the remaining articular cartilage and thus resulting in irreversible damage (Arokoski et al., 2000).

Alongside cartilage degradation and erosion, changes to the subchondral bone, synovial membrane and surrounding tissues also occur. Cells in the periosteum form bony projections at the margin of the joint at sites of joint loading, and the subchondral bone thickens, possibly in an attempt to maintain joint stability (Goldring & R. Goldring, 2010). Increased cell infiltration and fluid within the capsule causes the synovial membrane to inflame and thicken (Man & Mologhianu, 2014). These structural changes to the joint all work to cause the clinical signs characteristic of OA including a reduced range of motion, joint thickening and crepitus (Pettitt & German, 2015).

1.2 Identification of the efficacious components of green-lipped mussel extract and its use in the treatment of osteoarthritis in dogs

Introduction to the green-lipped mussel

The New Zealand green-lipped mussel (*Perna canaliculus*) industry is extensive, valued at $202 million in 2009 (Alfaro, Jeffs, Gardner, Bollard Breen, & Wilkin, 2011). A major market contributing to the success of this industry is nutraceuticals, particularly the use of green-lipped mussels for the treatment of OA (Paul, 2012). Green-lipped mussels are a bivalve marine mollusc endemic to New Zealand, distinguishable from other species by a green lip and green posterior strip around the ventral margin of their shell. A tolerance for a variety of salinities and temperatures enable green-lipped mussels to occupy much of New Zealand's coastline, although there is a preference for the warmer climates in New Zealand's northern regions (Alfaro et al., 2011). The green-lipped mussel stock for farming is maintained almost entirely by wild spat collections outside of established farms (Alfaro et al., 2011). This reliance on wild spat scavenging threatens the stability of the green-lipped mussel industry, as transport mechanisms for dispersing larvae are largely unknown, making larvae settlement hard to predict, intercept or manipulate (Alfaro et al., 2011; Alfaro & Jeffs, 2003).

Green-lipped mussels have historically been an integral component of the coastal New Zealand diet since Maori settlement approximately 600 years ago, and have since remained a staple in their diet, with the long-standing belief of coastal tribes that the consumption of mussels aid in good health (Cobb & Ernst, 2006; Paul, 2012; Smith, 2013; Ulbricht et al., 2009). Evidence of a reduced incidence of arthritis in coastal Maori populations compared to that of inland Maori populations promoted numerous extensive studies regarding the health benefits of green-lipped mussel extract (GLME) dating back to the 1960s (Brien, Prescott, Coghlan, Bashir, & Lewith, 2008; Cobb & Ernst, 2006; Grienke, Silke, & Tasdemir, 2014; Halpern, 2000; Paul, 2012; Ulbricht et al., 2009).

The efficacy of orally administered GLME has been studied for both osteoarthritis (OA) and rheumatoid arthritis (RA) in humans, prior to any other animal (Paul, 2012). Initial research into GLME opposes more recent studies, which observed either no effect or an exacerbation of clinical signs of arthritis with GLME treatment. The earliest studies found after 4 weeks of treatment, anti-inflammatory activity was equal between placebo and treatment groups (Highton & McArthur, 1975; Huskisson, Scott, & Bryans, 1981). A study in 1985 treated patients with Seatone®, a well-known GLME, and registered more patients reporting a deterioration of clinical signs over the six month period than that of the placebo group (Larkin, Capell, & Sturrock,

1985). Evidence that GLME improved signs of OA was not recorded until a series of studies that reported improved function, reduced morning stiffness and reduced tenderness of affected joints in OA patients after 3 months of treatment (Gibson, Gibson, Conway, & Chappell, 1980; S. Gibson & Gibson, 1998). Subsequent studies also reported reduced joint pain and stiffness of OA patients with GLME treatment (Cho et al., 2003; Coulson, Butt, Vecchio, Gramotnev, & Vitetta, 2013; Coulson, Vecchio, Gramotnev, & Vitetta, 2012; Lau et al., 2004).

There are several possibilities as to why initial trials failed to prove GLME reduced clinical signs of OA. Criticisms within these papers identify early trials as having inadequate participants and treatment length for GLME to have a measurable effect. A further cause could be the lack of quality products used in the trials; GLME was initially an unstable product until development of a stabilisation process in the 1980s (S. Gibson & Gibson, 1998). Lastly, it could be a case of publication bias - as the popularity of GLME increased, so did the motivation for publications to support efficacy.

Mechanism of action

The functional component of GLME is believed to be in the lipid fraction. However, definitive identification of the potently efficacious component within this fraction has yet to occur as many of the constituent fatty acids have anti-inflammatory properties. Omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) are common dietary lipids. Green-lipped mussels are enriched with long chain omega-3 PUFAs that are synthesised by the algae they consume. Arachidonic acid (AA, C20:4 n-6), docosahexaenoic acid (DHA, C22:6 n−3) and eicosapentaenoic acid (EPA, C20:5 n-3), are located within the phospholipids of cell membranes in eukaryotic cells. As a vertebrate's dietary intake ratio of EPA to AA increases, more EPA than AA is incorporated into the membranes.

Following cell injury, 20 carbon PUFA's are liberated from the cell membrane into the cytosol by the action of phospholipase A_2s (PLA_2s), which has a specificity for PUFA at the sn-2 position. The ratio of AA:EPA in the cell membrane determines which PUFA is predominantly liberated, hence the available substrate for metabolism by the key inflammatory cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) pathways (Singh et al., 2008). In these pathways, AA is metabolised into metabolites including leukotriene B_4 (LTB_4), 5-HETE and prostaglandin E_2 (PGE_2), which are pro-inflammatory. PGE_2 causes vasodilation and increased vascular permeability, and heightens pain sensation, while LTB_4 is a potent chemotactin, and activates neutrophils leading to

degranulation and the generation of reactive oxygen species (Caughey, Pouliot, Cleland, & James, 1997; Salmon & Higgs, 1987; Whitehouse et al., 1997).

EPA is a competitive substrate in both the 5-LOX and COX pathways, resulting in a reduced inflammatory response. Firstly, EPA metabolism reduces the amount of AA metabolised, consequently the amount of highly inflammatory prostaglandins such as PGE_2. Secondly, EPA is metabolised slower than AA as it is not the preferred substrate for COX, further reducing prostaglandin production. Thirdly, the metabolism of EPA produces prostaglandins that are less inflammatory than AA, reducing the inflammatory response (Corey, Shih, & Cashman, 1983; Gil, 2002; McPhee et al., 2007). EPA is metabolised by 5-LOX to produce leukotriene B_5 which is up to 30 times less potent at initiating an inflammatory response than leukotriene B_4 (Strasser, Fischer, & Weber, 1985). Examples of other metabolites produced by EPA metabolism include PGE_3, which is synthesised in place of PGE_2 and induces lowered concentrations of inflammatory cytokine synthesis and lowered COX-2 expression (Bagga, Wang, Farias-Eisner, Glaspy, & Reddy, 2003). Thromboxane A_3 is another metabolite of EPA, which is unable to stimulate platelet aggregation unlike its AA derived counterpart TXA_2 (Needleman, Raz, Minkes, Ferrendelli, & Sprecher, 1979).

Derived from EPA and DHA, resolvins and protectins are another group of mediators which promote resolution of inflammation. While the pathway of action remains unclear, chronic inflammation is known to result in the absence of these pro-resolving lipid mediators, reviewed in Serhan and Petasis (2011). However, there are a few known outcomes of these pro-resolving mediators for the resolution of inflammation, including the reduction of leukocyte infiltration and cytokine production, and anti-hyperalgesia properties (Hong, Gronert, Devchand, Moussignac, & Serhan, 2003; Lima-Garcia et al., 2011; Xu et al., 2010).

EPA and DHA have many mechanisms to incite anti-inflammatory effects, however the extent of the effect induced by GLME treatment cannot be justified by the concentrations of these PUFA's found in GLME. Fish oil tablets with greater concentrations of these fatty acids than GLME had a diminished effect on inflammation (Tenikoff, Murphy, Le, Howe, & Howarth, 2005). There must be other efficacious constituents in the lipid fraction for the reduction of inflammation. Therefore, the sizeable effect of GLME could be due to the anti-inflammatory properties of either the furan fatty acids or the novel omega-3 PUFAs, or another, currently unidentified lipid component entirely.

Furan fatty acids are another lipid group found in green-lipped mussels. Using a rat model of adjuvant-induced arthritis, isolated furan fatty acids produced a far greater reduction of swelling than EPA at 10 mg/kg doses (Wakimoto et al., 2011). Whilst the mechanism of action is still undetermined, authors believe these fatty acids work as antioxidants by trapping free radicals (Okada, Kaneko, & Okajima, 1996; Wakimoto et al., 2011). However, given the extremely low concentrations present in green-lipped mussels, it seems unlikely the furan fatty acids can build up in high enough concentrations within the effected joint for radical scavenging to be their primary effect.

There are also several novel omega-3 PUFAs in GLME with anti-inflammatory activity (Halpern, 2000; Singh et al., 2008; Treschow et al., 2007; Whitehouse et al., 1997). Isolation of lipids that inhibited the 5-LOX pathway implicated four uncommon PUFA's, C21:5, C20:4, C19:4 and C18:4 in inflammation reduction (Treschow et al., 2007). The addition of these PUFA's to EPA produced greater inhibition of the LOX pathway than EPA alone (Treschow et al., 2007; Wakimoto et al., 2011).

Gastroprotective effects of GLME

In addition to anti-inflammatory properties, GLME has gastroprotective effects. Initially, the gastroprotective effects were thought to be due to the selective inhibition of COX-2 over COX-1. This was because the side effects associated with the use of non-selective NSAIDs were absent when given in conjunction with GLME, including changes in platelet aggregation, stomach lesions and gastrointestinal upset (Halpern, 2000; Rainsford & Whitehouse, 1980; Whitehouse et al., 1997). However, it has been proven that both COX-1 and COX-2 are inhibited by GLME, indicating the gastroprotective effects have an alternate origin (McPhee et al., 2007). The primary gastroprotective property of GLME is the prevention of stomach lesions. Treatment of pigs with NSAID's resulted in severe stomach lesions, but when administered in conjunction with GLME, there was a significant reduction in both the number of lesions and of their severity (Rainsford & Whitehouse, 1980).

Quality and safety of GLME products

Green-lipped mussel are commonly sold as a whole-mussel, freeze-dried powder or as a lipid extract, whereby the method of preparation may influence product potency. One author estimated the lipid extract was around 125 times more potent than a freeze-dried alternative (Ulbricht et al., 2009; Whitehouse et al., 1997). However, it was not determined whether this was due to an increased concentration of the effective lipid component, or the stability of the

product. The green-lipped mussel is then packaged as an oil, powder, tablet, or as part of a complete diet. The lipid fraction is extracted from mussel meat via supercritical liquid extraction with carbon dioxide or enzymatic digestion (Macrides & Kalafatis, 2002; Whitehouse et al., 1997). The stability of GLME is maintained by the addition of a chemical preservative, which acts as an antioxidant and a metal chelator, preventing lipid peroxidation and aiding in absorption (Kosuge & Sugiyama, 1989; Whitehouse et al., 1997). Currently, there is no industry standard for GLME quality, therefore inefficacious by-products of the mussel production process without lipid fraction can be marketed and sold as freeze-dried GLME protein powder (Halliday, 2008). Also, reliable comparisons of efficacy between products is impeded by the variation in preparation of GLME products in terms of lipid composition and quantity (Ulbricht et al., 2009).

To date there have been no cases of GLME toxicity reported in dogs. Testing for GLME toxicity at concentrations found in a commercially available product for dogs returned haematological, serum biochemical and urinalysis analyses all within normal ranges (Pollard, Guilford, Ankenbauer-Perkins, & Hedderley, 2006). When GLME was administered at a dose that Aspirin inhibits platelet aggregation and cause gastric haemorrhaging in rats there were no observed side effects in rats (Whitehouse et al., 1997). Evaluation of the maximum safe dosage of the closely related species *Perna viridis* in rats had no adverse effects at 2000mg/kg in haematological, serum biochemistry and histopathological analyses and wasn't lethal at 5000mg/kg, both extremely high doses (Chakraborty, Joseph, & Chakkalakal, 2014).

The incidence of GLME adverse effects reported in humans is extremely low. The few stated effects have been nausea, unspecified oedema and epigastric discomfort (Coulson et al., 2012; S. Gibson & Gibson, 1998; Lau et al., 2004). There have also been reports of hepatitis as a possible result of GLME consumption. Elevated transaminases (indicative of liver damage and rectified once the patient stopped taking GLME) and a liver biopsy comparable with drug-induced hepatitis was reported in one patient taking GLME, and another patient developed granulomatous hepatitis (Abdulazim, Hädrich, Montani, & Semmo, 2012; Ahern, Milazzo, & Dymock, 1980). Although these adverse effects are serious, they are nearly isolated cases and not commonplace side effects of taking GLME.

As described previously, rigorous testing of the inhibitory effects of GLME on COX and 5-LOX pathways suggest components of GLME reduce inflammation and pain. Evidence of efficacy in dogs is sufficient to argue the addition of GLME to a diet may improve alleviation of OA clinical signs in dogs, with mild to moderate osteoarthritis. In more severe forms of OA where NSAIDs are required to manage the pain and inflammation, the addition of GLME may reduce the side

effects of NSAIDs in two ways: reducing the amount NSAIDs required, and acting to protect the gastrointestinal tract from the effects of NSAIDs (Rainsford & Whitehouse, 1980; Singh et al., 2008).

Clinical use of green-lipped mussels in the treatment of OA in dogs

To date there have been 5 studies investigating GLME use for OA in dogs (Bui & Bierer, 2001; Dobenecker, Beetz, & Kienzle, 2002; Pollard et al., 2006; Rialland et al., 2012; Servet et al., 2006). Three studies were single treatment, placebo-controlled trials, the remaining 2 studies treated all dogs with GLME and compared outcome variables pre- and post-treatment (Bui & Bierer, 2001; Dobenecker et al., 2002; Pollard et al., 2006; Rialland et al., 2012; Servet et al., 2006). The sample size of dogs varied considerably across trials. In placebo-controlled trials, sample size ranged from 14 to 43 dogs per treatment group, while studies without a control group had up to 84 dogs (Bui & Bierer, 2001; Pollard et al., 2006; Servet et al., 2006). Dogs were selected from patients at participating clinics, or from a shelter in one study (Bui & Bierer, 2001). This resulted in the inclusion of a broad mix of breeds and ages, and a balance between sexes, however, there was a tendency towards larger breeds such as Labradors and, not surprisingly for dogs with OA, older dogs. Whilst radiographic confirmation of OA was required for inclusion in several trials, in others OA was diagnosed with owner-reported clinical signs and veterinary examination (Pollard et al., 2006; Rialland et al., 2012; Servet et al., 2006). In the latter trials, the inclusion criteria included continued or chronic lameness, a reduced range of motion, pain and crepitus (Bui & Bierer, 2001; Pollard et al., 2006; Rialland et al., 2012; Servet et al., 2006).

Using subjective measures of treatment efficacy, the effect of GLME on OA in dogs was unclear until approximately six weeks of treatment, with severity evaluation of dogs at 4 weeks unable to support GLME efficacy (Pollard et al., 2006). In general, study length ranged from 6 to 16 weeks, with continuous monitoring of outcome variables or repeated measurements of outcome variables at set time points over the course of the study (Bui & Bierer, 2001; Pollard et al., 2006; Rialland et al., 2012).

The outcome variables used to evaluate the effect of GLME on OA consisted mostly of subjective measures, including questionnaires for both owners and veterinarians, and physical examinations carried out by a veterinarian. The client-specific outcome measures (CSOMs) questionnaire was a popular subjective measure in studies (Dobenecker et al., 2002; Pollard et al., 2006). This included questions about an owner's perception of their dog's level of activity, attitude, lameness, and signs of pain, along with an overall impression of improvement during

the trial. Veterinary physical examination was used to evaluate changes in the physical characteristics and discomfort of effected joints over the course of the trial. Examinations were used to rank OA severity on an ordinal scale for different parameters including lameness, joint mobility and signs of pain (Bui & Bierer, 2001; Pollard et al., 2006; Servet et al., 2006). Objective measures of OA severity were only used in one study (Rialland et al., 2012). In this study, accelerometry and force plate analysis were used to investigate changes in peak vertical force and total activity intensity with increases in either proposed to indicate improved OA.

Force plate analysis was the only objective measure that identified an effect of GLME on alleviating clinical signs of OA, detecting a significant increase in peak vertical force of affected limbs in dogs after GLME treatment (Rialland et al., 2012). Accelerometry was unable to identify a difference in activity intensity which could be attributed to the addition of GLME alone. The accelerometry output reported activity intensity using activity count. The activity account was converted from Delta-G using proprietary algorithms, further defined in a later section, which was used as a proxy for activity intensity. This activity count was continuously recorded over a 12 week period, with data analysed in two-week blocks including; baseline data collection at 0 to 2 weeks, controlled diet at weeks 3 to 4, and a GLME enriched diet at weeks 5 to 12. When time of day (morning, afternoon and night) and age were accounted for, results identified the baseline activity count was significantly lower than that of the final block of GLME enriched diet at 10 to12 weeks. However, due to the absence of a significant difference in activity intensity between control diet and GLME enriched diet, the increase in activity count could not be attributed to the GLME. There are several reasons for the lack of a difference between control and enriched diet periods. The small sample size of only 7 dogs used in the activity analysis may be responsible for failing to identify an effect within the time frame using accelerometry. There could also be no effect of GLME on activity intensity in dogs with OA, and increased quality of diet (both the control and enriched were high-quality diets) resulted in an increased activity count. Ultimately the proposed relationship between increased activity intensity and improved OA was not explained and may not exist. It was not explained what activity intensity meant in this context, nor how an increase would reflect a reduction of OA clinical signs or OA severity as a whole, which makes conclusions difficult to make from the findings.

The perceived efficacy of GLME is likely to be influenced by the severity of the OA in the dogs selected for the study. Bui and Bierer (2001) identified significant reductions in joint swelling, perceived pain, and total arthritis score (based on the overall condition of the dog and its affected joint) in dogs treated with GLME compared to those treated with a placebo. However,

joint crepitus and range of movement did not improve with treatment. The effects of GLME treatment are thought to be primarily reducing inflammation and pain. This suggests GLME would not influence clinical signs related to permanent changes in joint structure, such as bone remodelling, osteophytosis or the thickening of the joint capsule, which result in crepitus and a reduced range of motion (Bui & Bierer, 2001; Servet et al., 2006). Perhaps, the effectiveness of GLME for OA management should be based on the evaluation of pain, inflammation, and the clinical signs relevant to GLME properties, as opposed to OA as a whole. Certainly, GLME may be more suited for the treatment of dogs with milder forms of OA, and dog selection should reflect that.

Many of these studies used veterinary assessment of clinical signs to evaluate GLME efficacy. Lameness, weight bearing, willingness to bear weight whilst holding up the contra-lateral limb, pain and joint mobility were assessed in a double-blind, placebo-controlled trial by Pollard et al. (2006). The effect of GLME on OA was analysed as a comparative cumulative score of severity judged by rating the severity of the aforementioned clinical signs, and as a comparative holistic view of clinical sign severity (improved/not improved) before and after treatment. At 56 days of treatment, OA had improved in significantly more dogs treated with GLME than the placebo, and the quantitative improvement of these cumulative clinical signs was only significant in the treatment group. In another double-blind, placebo-controlled trial, dogs in both the placebo and treatment group had a perceived reduction of lameness and pain by owners and veterinarians (Dobenecker et al., 2002). The absence of a difference in outcome variables between groups was hypothesised by authors to be due to the low dosage of GLME used in the trial which, as explored later in this section, was far lower than studies that identified a decrease in clinical signs with treatment. Servet et al. (2006) compared clinical signs of dogs with mild to moderate OA before and after a GLME enriched diet in a placebo-less study. A visual score consisting of a dog's effort to climb stairs and the presence of lameness whilst walking and trotting was significantly decreased with a GLME enriched diet. A manipulation score consisting of the degree of swelling, crepitus, pain and a reduced range of motion in affect joints also significantly decreased with a GLME enriched diet. Pain and mobility of joints were the most improved parameters, which, assuming early OA reduction of joint mobility is due to inflammation rather than joint thickening, supports the efficacy of GLME for the treatment of pain and inflammation over later stage structural changes in the joint associated with OA.

The guidelines around dosage of GLME in studies of arthritic dogs appear to be either dose guides from the commercial products used in studies or likely replicating dated literature, such

as reports of reduced paw swelling in adjuvant-arthritic rats with an orally administered dose of 15mg/kg (Whitehouse et al., 1997). The dose size of GLME is an important part of study design. However, there has not been any clear investigation into the minimum effective dose of GLME in dogs. For comparison, GLME doses administered in different studies will be reported as mg of product per kg bodyweight (mg/kg). Due to inconsistencies in reporting, where the dose was provided as a rate of inclusion in diet, rather than a dose per kg of bodyweight, calculations were based on the energy requirements of a 30kg dog (maintenance energy requirements = 1256Kcal). All studies used freeze-dried mussel powder as opposed to lipid GLME for inclusion into diet, with three studies using the same GLME enriched diet (Bui & Bierer, 2001; Rialland et al., 2012; Servet et al., 2006). One study administered GLME as a tablet whereas another added the powder to the normal diet of each dog (Dobenecker et al., 2002; Pollard et al., 2006). In four studies the calculated dose was approximately 33mg/kg, and the other it was 11mg/kg. Of note, a clinical improvement was seen in all studies using the higher dosage (Bui & Bierer, 2001; Pollard et al., 2006; Servet et al., 2006). Only the lowest dosage study failed to discover evidence of GLME efficacy (Dobenecker et al., 2002). On this basis, a dose of 33mg/kg per day appears to be sufficient for an efficacious effect of GLME whole powder to be observed, with a minimum value above 11mg/day.

Alleviated clinical signs in both placebo and treatment groups were noted in two studies, with a reduction in clinical signs in up to 41% of dogs in one of the placebo group (Dobenecker et al., 2002; Pollard et al., 2006). This phenomenon was documented in multiple other trials in dogs with arthritis e.g. (Conzemius & Evans, 2012; Gingerich & Strobel, 2003). Potential explanations for improvements in the placebo groups include coincidence with the onset of warmer weather or a temporary spontaneous reduction of clinical signs. This may be due to a falsely perceived improvement by owners, or true improvement of clinical signs due to study participation prompting owners to take better care of their dogs (Dobenecker et al., 2002; Pollard et al., 2006). Designing future studies with double blinding and a cross-over component should resolve these potential problems, although these observations emphasise the necessity of placebo controls, and maximal blinding.

1.3 Accelerometry and its use to monitor osteoarthritis in dogs

Joint disease causes dogs to modify their natural movement to compensate, due to pain or structural changes in the joint (Knazovicky et al., 2015; Tashman et al., 2004). Therefore, disease progression in dogs could be monitored by measuring movement. One method of measuring movement is with accelerometry. Accelerometry is a relatively new tool for assessing dog health with the advantage of being unobtrusive, lightweight, and able to provide continuous, objective data (Hansen, Lascelles, Keene, Adams, & Thomson, 2007; Helm, McBrearty, Fontaine, Morrison, & Yam, 2016). Triaxial accelerometers are a type of accelerometer capable of continuously recording acceleration across three orthogonal planes in space, these planes can be orientated with the three planes of a dog's body (sagittal, coronal, and transverse). By quantifying the acceleration across these planes, accelerometry can be used to characterise movement and gait in dogs. For simplicity, all references made to accelerometers from here are in reference to triaxial accelerometers. Accelerometer set up and use varies greatly between studies, including how the accelerometer is attached, and how the data is summated and presented from the accelerometer.

What is accelerometry?

Accelerometry is the measure of acceleration. An accelerometer is an electromechanical instrument used to measure the acceleration of an object due to gravity and dynamic forces. Two common detecting components used in accelerometers are piezoelectric crystals and capacitors. A microelectromechanical systems (MEMS) capacitive accelerometer is made up of elements only micrometres in size. The system determines the magnitude of acceleration using a cantilever beam (a beam fixed at one end). A voltage is supplied to the system so that an electrostatic force is produced between the beam and a measuring plate. Capacitance is the ratio of the electric charge on the beam and measuring plate, to the voltage (potential difference) between them. The magnitude of beam deflection determines the capacitance of the system as the smaller the distance between the beam and measuring plate the greater the charge that a capacitor can hold. When a lateral force is applied to the beam it is deflected, changing the distance between the beam and plate and consequently, the capacitance changes. This change in capacitance is used as a proxy for the magnitude of the force applied to the system, and in turn, the acceleration of the object.

A MEMS piezoelectric accelerometer uses a piezoelectric crystal to measure acceleration via the voltage generated from the stress applied to the crystal. Piezoelectric materials generate an electric charge in response to a physical force, converting one type of energy into another. As it

requires a change in force to generate a charge this type of accelerometer is incapable of measuring static forces. A common set up of a MEMS piezoelectric accelerometer system is similar to the capacitive accelerometer. There is a cantilever beam made of the piezoelectric crystal with a constant mass at the unattached end. As the accelerometer accelerates the mass and the acceleration applies a force on the crystal which generates a proportional charge. The voltage produced is then used as a proxy for the acceleration of the object.

There are compromises to using either type of accelerometer. MEMS piezoelectric accelerometers are unable to measure static forces like gravity because they only generate charge in response to a change in force, while MEMS capacitive accelerometers can measure both static and dynamic forces. MEMS piezoelectric accelerometers are also more limited in the frequency of their response (i.e. the rate of which they can measure changes in acceleration) and the temperature in which they can function, compared to MEMS capacitive accelerometers which can have far higher frequency response (Acar & Shkel, 2003; Yazdi, Ayazi, & Najafi, 1998). While MEMS piezoelectric accelerometers can measure a greater G-force than MEMS capacitive accelerometers, the latter are able to measure far smaller amounts of acceleration (Acar & Shkel, 2003; Yazdi et al., 1998). Therefore, the individual needs of the study should dictate the accelerometer of choice. Using accelerometers designed for the specific application of a study ensures the correct parameters are in place. For example, an accelerometer specifically designed for measuring activity in dogs was used in this thesis, it was a micro electro-mechanical (MEMS) capacitive triaxial accelerometer that measures accelerations between +4 and -4 G's in magnitude at a sampling rate of 10 Hz (Heyrex®, Say Systems, Wellington, NZ).

Accelerometry output

Regardless of the type of accelerometer used the output is measured acceleration. However, this is rarely reported. Rather the values are converted to activity counts. Activity counts are effectively the sum of the raw accelerometer values for a given length of time and are determined by the amplitude of the acceleration and the frequency of the change in acceleration after band-pass filtering which removes frequencies of acceleration outside the normal range of animal movement. A limitation of activity counts is the method in which they are generated, activity counts are determined via proprietary algorithms that are known to vary considerably between companies and there are differences in the measured acceleration between the accelerometers themselves (Welk, 2002) Therefore, there can be limitations to the comparability of data obtained by different brands of accelerometers. Delta-G is used as an activity count, it is a unit used to quantify a change in the net acceleration of an object. For

triaxial accelerometers, delta-G is the change in acceleration between two points in time summed across three axes. Increased frequency of changes in acceleration and greater peak accelerations between adjacent points in time increase the change in acceleration and therefore increase delta-G.

While activity intensity is usually defined as the amount of effort required to, or how hard it is to, complete an activity, the measure of acceleration from an accelerometer is limited in its ability to quantify non-ambulatory movements or the intensity specific to an animal (a movement that is low intensity to one animal may be high intensity to another). Therefore, when activity intensity is used in terms of accelerometry output in dogs, it refers to acceleration per unit of time, rather than the true intensity of an activity, therefore any further mention of activity intensity from this point forward is in reference to this definition (Helm et al., 2016; Morrison, Penpraze, Beber, Reilly, & Yam, 2013).

An activity with more frequent changes in acceleration or greater peak acceleration will result in a higher activity count value. Activity intensity is used to identify activity within certain ranges of activity count values. The argument for doing this is that dogs completing more activity in a higher or lower activity intensity category could reflect changes in health. Activity intensity categories are established with guidance from the activity count range associated with activities with different acceleration per unit of time. Walking and resting are examples of activities with a comparatively low acceleration per unit of time compared with jumping, cantering or scratching, which are examples of activities with a comparatively high acceleration per unit of time. Activity count values from activities can overlap so a cut-point within the values must be determined where any values above are classified a higher intensity activity than any values below. Cut-points are defined by researchers and can denote many different classifications, but when applied to studies on dogs, are most often used to classify activity intensity, with categories from sedentary to vigorous activity (Hansen et al., 2007; Helm et al., 2016; Michel & Brown, 2011; Yashari, Duncan, & Duerr, 2015). As the cut-off points for defining the intensity of a dog's activity are decided by individual researcher or companies they vary considerably due to the lack of a standardised method. Consequently, while activity intensity or categories may seem more comparable than activity counts, the variation in their classification makes these outcomes difficult to compare between studies.

Energy expenditure (EE) is the energy used by an animal to carry out an activity. It is very popular in human accelerometry studies as it a biologically meaningful way to present the data. EE can be reported as either activity specific or over a given period of time in a free-living environment

where activity is undefined. In regard to activity specific EE, EE equations may be inadequate for predicting EE across multiple activities. While dog-specific studies are not available, in humans there have been independent evaluations of published EE equations using indirect calorimetry measures, which have surmised that many equations aren't applicable for the estimation of EE outside of the typically small number of activities used to develop the models (Crouter, Churilla, & Bassett, 2006; Lyden, Kozey, Staudenmeyer, & Freedson, 2011). Predicting EE in a free-living environment denotes the estimation of EE with the measurement of undefined activity over extended periods of time. An estimate of total EE of endurance runners, including these periods of undefined activity, from accelerometry was comparable to the estimation of EE obtained from double labelled water (Yoshida et al., 2018). It was concluded that EE estimated using accelerometry from non-training periods, in addition to the EE estimated using the rate of perceived exertion during training periods, was adequate for the estimation of total EE (Yoshida et al., 2018). In another study accelerometry was used to predict EE, as estimated with the double labelled water method. It was determined that the activity count from the accelerometer significantly improved the prediction of EE, with the final model explaining over 75% of the variation in measured total EE (Bonomi, Plasqui, Goris, & Westerterp, 2010). So, while there are many equations available for estimating EE from accelerometry no method is perfect, however that is not to say there will not be an equation created in the future capable of overcoming the current underperformances of existing equations. There are many regression equations. Consequently, there can be huge variability in results, which indicates the need for caution on the selection of the regression equation selected for a given study.

Along with the more general description of dog activity described above, specific gait characteristics can also be determined with accelerometry. Examples of gait characteristics are power, stride regularity, and peak vertical force. In humans, investigation into gait characteristics using accelerometry has already come a long way with reference data for normal gait in humans published, however gait characteristics determined by accelerometry had not been investigated in dogs until 2009 (Auvinet et al., 2002; Barthélémy et al., 2009). Throughout the studies investigating gait characteristics in dogs the variables were calculated consistently, allowing for direct comparison of values between studies unlike activity counts with their proprietary algorithms (Barthélémy et al., 2011; Barthélémy et al., 2009; Clark, Caraguel, Leahey, & Béraud, 2014; Fraysse et al., 2017).

To the author's knowledge, gait characteristics have not been evaluated in dogs with OA using accelerometry, however, gait differences have been identified between dogs with other

muscular-skeletal diseases. Muscular Dystrophy in dogs affects patterns of movement and gait characteristics, with increasing phenotypic severity as the disease progresses, just like OA. Successful identification of differences in gait between dogs with differing levels of phenotypic severity such as speed, total force and regularity of accelerations indicate accelerometry has the potential to be a sensitive tool for disease progression or improvement (Barthélémy et al., 2011; Barthélémy et al., 2009; Fraysse et al., 2017). However, currently gait analysis is a very involved technique, it has required a dog to complete specific movements on a treadmill for set periods of time to obtain results. This limits the usefulness of this technique as many dogs will require treadmill training and the unfamiliar environment may alter the behaviour of the dogs (Sharkey, 2013).

When we are interested in investigating a dog's type of movement rather than the amount of movement, the epoch length is important. When an accelerometry variable, such as intensity or gait parameters, is calculated by the change in acceleration over a period of time, the epoch length can affect the outcome. An animal will only carry out an activity for a limited period of time, irrespective of the epoch length, and if the animal changes activity within a measurement, periods of greater changes in acceleration are summed with periods of smaller changes in acceleration. As this summed acceleration over a measurement is used to estimate activity intensity, the effect is ultimately a value that reflects neither the low intensity nor the high intensity activity observed during the epoch, but rather the mean intensity, ultimately resulting in a misclassification of activity (Chen & Bassett, 2005; Edwardson & Gorely, 2010; Gabriel et al., 2010). This has the greatest effect in an uncontrolled environment where an animal's activity is not dictated and varies rapidly. Michel and Brown (2011) suggested their minute long epoch may have resulted in the loss of vigorous exercise carried out only briefly by dogs during the minute. Ultimately, the longer the epoch length the less accurate the estimate of how a dog is moving (e.g. intensity/gait/stride regularity) so selecting an epoch length should be based on the required specificity of the study.

Accelerometer attachment options

There are many methods of accelerometer attachment to dogs but there are two points of attachment that are more common than others: a harness-based system where the accelerometer sits either on the sternum or along the spine, and a collar-based system where the accelerometer sits on the ventral side of the neck. Out of the three studies that compared these two attachment sites, only one study concluded that attaching the accelerometer to a harness was better than collar attachment for achieving the highest quality data (Hansen et al.,

2007; Preston, Baltzer, & Trost, 2012; Westgarth & Ladha, 2017). It was determined that the harness attached accelerometer output was significantly different between two speeds with 2km/h between them, while the collar attached accelerometer output was not (Preston et al., 2012). However, given that another speed, 2km/h slower, was not significantly different with either attachments, this claim of a harness being a better attachment site seems ill-supported.

A further two studies directly compared the output of accelerometers attached to a collar and accelerometers attached to a harness. One study used the number of activity counts at different levels of activity intensity, and the other compared the activity counts for each one-minute epoch of the study (Hansen et al., 2007; Westgarth & Ladha, 2017). Both studies determined that the output from the two attachment points were highly correlated with an acceptable level of disagreement between counts, and therefore there was no evidence to support either attachment site. The minimal difference between attachment methods means both placement could be considered equally useful. Therefore the choice of attachment location can be decided based on convenience and practicality if the choice made is a consistent one throughout the study (Hansen et al., 2007; Martin, Olsen, Duncan, & Duerr, 2016; Olsen, Evans, & Duerr, 2016).

Variability of collar tightness, height of the collar on the dog's neck and the orientation of the accelerometer was not shown to significantly impact results (Martin et al., 2016; Olsen et al., 2016). However, there was considerable evidence that attaching a lead to the collar with the accelerometer attached influences the output by falsely increasing the activity count (Martin et al., 2016; Preston et al., 2012). To prevent this, a lead should not be attached to the collar used to hold the accelerometer but could be attached to a separate collar, or harness. The overall conclusion of these studies was that researchers should be consistent with the accelerometer attachment method and location throughout the study.

Review of studies monitoring osteoarthritis in dogs

Gait analysis is a commonly used technique to assess dogs with OA using tools such as force plates, owner-based surveys, and pressure mat walkways. Owner-based surveys are convenient but subjective, which can be an issue in evaluating a pet's health/wellbeing where emotion can influence a treatment's perceived effect (Brown, Boston, Coyne, & Farrar, 2007). Objective measures are desirable in this situation, as they avoid personal emotion and perspectives. Both force plate (FP) and pressure walkway (PW) analyses are objective measures of gait but they are limited by several factors that reduces their utility. For example, for gait evaluation the dog is taken out of its home environment into an unfamiliar setting. This may impact the results

obtained as dogs are known to mask/modify their behaviour to pain if they feel uncomfortable (Sharkey, 2013). FP analysis is arguably the most common method of objective gait analysis in dogs with OA, specifically to monitor lameness as a measure of OA severity (Lascelles et al., 2015; Sharkey, 2013). Its usefulness as a technique is reduced by its inability to provide information on multiple limbs on a single pass of the FP, and when multiple limbs are in contact with the force plate with different gaits the FP is unable to adapt to reflect the target limb only (Brown, Boston, & Farrar, 2010; Lascelles et al., 2015). In addition, long term, continuous monitoring is preferable to a single moment as it removes day to day variability of a dog, which FP and PW systems are unable to do. The limitations of the technology currently used in gait analysis of dogs with OA indicate there is a niche here to be filled with a technology that can be used reliably at home, with minimal disruptions to a dog's day to day life, and provide up to date, objective data on a dog's gait.

Unlike FP and PW technology, accelerometers can be used in an environment where activity does not have to be controlled. In terms of investigating OA in dogs, accelerometers have been almost exclusively used for treatment efficacy evaluation. As accelerometers can be used in an environment where a dog can exhibit normal behaviour and activity, they can be employed as an in-home measure of spontaneous activity, and allow comparison of activity levels of dogs (Hansen et al., 2007). In-home measure of activity gives accelerometers an edge over other methods of OA evaluation as the results obtained from dogs at home are more likely to accurately represent their true state of wellbeing compared to attempting to obtain results in a clinic setting (Sharkey, 2013). Many studies successfully have identified an increase in the activity of dogs with OA in response to treatment over a period of time, with all currently published studies using collar mounted accelerometers (Brown et al., 2010; Lascelles et al., 2015; Moreau et al., 2012; Wernham et al., 2011).

Many studies looked for increases in a dog's overall activity count in broad hour groupings to remove the hours where dogs are likely to be sleeping (Brown et al., 2010; Lascelles et al., 2015; Walton, Cowderoy, Lascelles, & Innes, 2013; Wernham et al., 2011). Clinical signs of dogs with OA include a reluctance to exercise and stiffness after exercise. Therefore, increased activity counts during active hours is considered a favourable outcome for treatment efficacy because it is indicative of the dog feeling less pain or more able to move (Cooper et al., 2014). Comparing the change in average activity levels of a dog before and after treatment over an extended period of time reduces the natural variation in activity between dogs and enables comparability

between treatment groups. This variation is present both between dogs and within an individual dog, whose activity can vary greatly day to day (Lascelles et al., 2015).

In humans sleep disturbance is a well-known trait of OA with those affected attributing pain as the causative factor, so increased activity in the hours a dog is most likely to be asleep could be considered indicative of disease progression (Power, Perruccio, & Badley, 2005; Woolhead, Gooberman-Hill, Dieppe, & Hawker, 2010). To the author's knowledge, only one study has focused on the effect of OA treatment on a dog's night-time activity (Knazovicky et al., 2015). Despite the lack of primary evidence that OA caused increased night-time activity in dogs, it was predicted that there would be a decrease in night-time activity in the dogs being treated with an OA medication. Broad hour-long epochs were used to evaluate changes in total activity over night-time, and it was found that there was no difference in activity before or after OA treatment, and therefore no evidence that OA treatment influenced night-time activity (Knazovicky et al., 2015). A clear failing of this study was the lack of primary objective evidence that night-time activity is different between heathy dogs and dogs with OA collected prior to this study. It is very possible there is no measureable effect of OA on night-time activity in dogs.

1.4 Conclusion

OA onset is common in dogs following trauma or other joint disease (Mele, 2007). In the absence of damage, healthy synovial joints have a specialised architecture to withstand the daily pressures applied to the joint during normal movement (Arokoski et al., 2000). This is particularly true for articular cartilage where small changes to the ECM can compromise the resistant properties of healthy cartilage, leading to permanent damage of the joint. Damage to synovial joints can trigger the release of cytokines (Goldring et al., 2008). This elicits enzymatic cartilage degradation and inflammation in addition to further structural changes to the joint. The damage and inflammation presents as clinical signs that are characteristic of OA in dogs such as pain on manipulation, thickening of the joint and crepitus (Pettitt & German, 2015).

There is evidence that lipid portion of green lipped mussels is an efficacious product for the management of inflammation and pain in osteoarthritic dogs when administered orally (Bui & Bierer, 2001). In addition, when GLME is administered alone there is an absence of side effects commonly associated with NSAIDs, and when administered in conjunction with a NSAID there is evidence of gastroprotective properties making GLME an attractive treatment option (Rainsford & Whitehouse, 1980). The efficacious component within the lipid fraction has likely not yet been identified, nor the explicit mechanism of action, due to the sizable effect of GLME on inflammation unexplained by the concentration of known PUFA's EPA and DHA (Tenikoff et al., 2005). Despite some evidence of efficacy, there have also been studies unable to identify an effect, and there has also been a distinct absence of objective evidence (Dobenecker et al., 2002). There is a need here for further evidence of GLME efficacy in dogs with OA using an objective measure.

Accelerometry is an objective tool for quantifying movement in dogs. In studies monitoring OA in dogs there has been clear preference for collar-attached accelerometers, likely due to the convenience of placement for a longer-term study requiring the dogs to keep the accelerometer at all times (Martin et al., 2016). Accelerometry enables the measurement and evaluation of both normal activity and gait characteristics of dogs. While measured gait characteristics can identify small changes in dogs with musculoskeletal disease, gait analysis is a highly technical approach for monitoring disease in dogs, requiring both specialised equipment and training of the dogs (Barthélémy et al., 2009). Activity quantification in dogs is a desirable method for future use because of its simplicity and ability to objectively measure changes in activity for dogs in-home (Hansen et al., 2007). Evaluation of activity in dogs with OA using activity counts and activity classification have been shown to successfully detect treatment efficacy (Brown et al.,

2010). Therefore, gait characteristic investigation may be unnecessarily complex for obtaining desired results in studies investigating OA treatment efficacy.

In conclusion, the aim of this thesis was to evaluate whether accelerometers could be used to detect improvements in dogs with OA that have been treated with a GLME nutraceutical. As the data collated from this study would be extensive, a method for filtering data for specific periods of interest was required. Therefore, a treadmill study was conducted with the aim of developing a model to predict speed and gait of healthy dogs from delta-G for application to the data obtained from the GLME efficacy trial.

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2 Use of a collar-mounted triaxial accelerometer for speed and gait prediction in dogs

2.1 Introduction

Accelerometry is a powerful tool for investigating activity in dogs. Activity counts, a measurement derived from accelerometers, are an arbitrary measure of movement intensity and have been used to identify treatment efficacy in dogs with a variety of diseases, most notably in osteoarthritis (OA). The activity count is the summation of the acceleration across the three planes after company specific data cleaning and amalgamation processes. As a consequence of company specific methods of data manipulation, the activity count can vary slightly in its creation. Delta-G is an example of a company specific activity count, defined as the change in acceleration between adjacent sampling time points summed across three axes. Activity counts have been used to monitor total night-time activity, total daytime activity, and increased intensity of activity, to evaluate the effectiveness of a number of OA treatments such as green-lipped mussel extract (GLME) nutraceuticals (Brown et al., 2010; Knazovicky et al., 2015; Moreau et al., 2012).

However, there is potential to use accelerometry to produce measurements beyond the basic activity count. Triaxial accelerometers work by continuously recording acceleration across three orthogonal planes in space. When these planes are orientated with the sagittal, coronal, and transverse planes of a dog's body, the resultant acceleration measurements can potentially characterise activity and gait in dogs. To date, it does not appear that accelerometry has been used to estimate speed in free-moving dogs. In one study, the authors were able to identify significant differences in the accelerometry data at different speeds (Preston et al., 2012). However, the study only used two different speeds, therefore, the differences in accelerometry output could be due to differences in gait rather than speed.

In contrast to speed, many studies have aimed to measure activity categories with accelerometry. Several studies have used accelerometry to determine if a dog was walking, sleeping, cantering, or in a period of "inactivity" (den Uijl et al., 2017; Gerencsér, Vásárhelyi, Nagy, Vicsek, & Miklósi, 2013; Ladha, Hammerla, Hughes, Olivier, & Ploetz, 2013). For activity classification, the accelerometry data was applied to algorithms, identifying patterns in the acceleration vectors consistent with the activity in question, such as the symmetric gait of a trot, rather than extrapolating from the activity count (den Uijl et al., 2017; Gerencsér et al., 2013;

Ladha et al., 2013). This method has been shown to accurately identify certain activities in dogs, however, classification of activities using the activity count does not appear to have been investigated, probably because the accuracy of classification using this method may be too low. However, using the activity count rather than applying complex algorithms to continuous data would be simpler and easier, especially for long term studies, such as those required to test efficacy of nutraceuticals in the management of OA. Thus, if the activity count is used for classification of a limited number of activities where high accuracy is not paramount, it would be preferable for crude activity classification. To date, accelerometry has not been utilised to measure specific gaits nor to predict speed in any study designed to test a treatment's efficacy for OA.

Characterising locomotion by gait or speed is more complex and requires more data transformation than required to produce the activity count, but there are important benefits to doing so. For reporting purposes, delta-G has little meaning to people without accelerometry experience. Transforming the data into a parameter most people readily understand like gait, or speed, allows for the effective communication of experimental results. Assigning meaning to activity count values also allows for the educated division of periods of continuous activity data into periods of time more likely to contain evidence of disease. It is well recognised that an increasing activity count per unit time reflects an increase in activity intensity, but it does not describe the type of activity in action. Clinical signs of disease such as osteoarthritis, are more likely to be detected during periods of greater activity intensity; for example lameness is more readily detected in dogs while trotting than when walking (Carr & Dycus, 2016).

However, without knowledge of the activity count value at which a dog has transitioned into a faster gait or speed, identifying those periods of interest is speculative. By translating the activity count into a clearly defined variable like speed or gait, activity counts can be divided into more meaningful categories, as opposed to the selection of an arbitrary cut-off point within the activity count data. Thus, for refining the detection of changes in locomotion in response to therapy, the definition of clear gait categories would be useful to select the key activities that may best demonstrate a treatment effect. Therefore, the aims of this study were to measure controlled activity in dogs using accelerometry, and to determine if the activity count "delta-G", could estimate the speed, and characterise the gait of dogs.

2.2 Methods

Animal selection

Eight Huntaway dogs were selected from a population held at Massey University's Canine Colony. All dogs were between three and 10 years of age and deemed healthy by a veterinary physical examination prior to study commencement. Due to the novel nature of this study, sample size was unable to be calculated exactly based on previous studies. However, the most comparable study used 6 dogs to identify optimal accelerometer placement for broad activity categories on a treadmill (Preston et al., 2012). Therefore, it was estimated that eight dogs would provide enough data to validate the accelerometer using our proposed method. This study was approved by the Massey University Animal Ethics Committee (Protocol 18/44).

Accelerometer

A micro electro-mechanical (MEMS) triaxial accelerometer (Heyrex®, Say Systems, Wellington, NZ) weighing 32g and measuring 65x26x18mm, was used for this study (Figure 1). The collar-mounted accelerometer was positioned on the ventral side of the dog's neck. Accelerations between +4 and -4 G's in magnitude were recorded at a sampling rate of 10 Hz. Acceleration was measured across three axes as the change in acceleration between neighbouring samples, reported as delta-G, and summed into one second epochs. When in-range of the specialised receiver, the accelerometer transferred captured acceleration data to proprietary software for cleaning, transformation and summation.

Experimental procedure

The dogs were acclimated to the treadmill and safety harness over a six month period. During the acclimation period the dogs were trained up to three times a week until they could confidently move on the treadmill without excessive interference from handlers. The treadmill was 8.36 metres long and was set without an incline for both the acclimation period and experiment. The safety harness was a vest worn by the dog (Figure 1) and attached by ropes to the frame beside the treadmill. When a dog faltered or accelerated beyond the speed of the treadmill, the ropes supported the dog until someone stopped the treadmill. Otherwise, the ropes were loose enough not to impede the dog's gait.

On the day of data capture, all dogs were fitted with the same adjustable safety harness, an accelerometer was attached to the collar, and adhesive circular markers were placed on the dog (see Video analysis for more detail) as shown in Figure 1. Each dog was led onto the treadmill

and encouraged to move at a slow walk, fast walk, slow trot, fast trot, slow canter and fast canter for 30 to 50 seconds in each gait. For each gait, periods of 10 seconds were recorded using the motion capture software. The recordings were aligned to the measure of delta-G captured by the accelerometer by a precise timestamp with the annotated motion capture. The order that dogs completed each gait differed based on the dog's preference on the day. For example, some dogs were excited and willing to canter when first placed on the treadmill, while others appeared more comfortable walking. For each dog, the speed of the treadmill at each gait was set to a speed that allowed the dog to move comfortably in that gait without changing within the 30 to 50 second interval. In some instances, a dog could not be restricted to a walk, or would not break into a canter, and therefore all gaits were not recorded for all dogs. Periods were discarded from analysis for inconsistent gait, obscuring of the markers, and excessive forwards/backwards movement of the dog that occurred because the dog was moving faster or slower than the set treadmill speed.

Video analysis

On each dog, adhesive circular markers were attached just above the metacarpophalangeal and metatarsophalangeal joints on each foot, on the same side as the cameras (Figure 1). In addition, a 1 centimetre diameter spheroid marker was placed in the dorsal midline between the scapulae on a harness, and another on the dorsal midline at the level of the seventh lumbar vertebra. Six motion capture cameras were set up around the treadmill to provide a 3-dimensional view of the markers on the dog. The motion captured by the marker movement was converted into quantified movement in the vertical, horizontal and transverse axes with a proprietary software system (Qualisys Track Manager v. 2.17, Gothenburg, Sweden). The movement of the feet markers was used for visual gait identification as defined by Nunamaker and Blauner (1985), by following the pattern of feet placement.

Morphometry & estimation of skeletal size

Skeletal measurements were taken from each dog using a flexible tailor's measuring tape. Following the method developed by Leung, Cave, and Hodgson (2018) six morphometric measurements between specific bony locations were taken as described in Table 1. The variable "skeletal size" was calculated using the method described by Leung et al. (2018). Briefly, for each of the six skeletal measurements, the values from the eight dogs were combined using principle component analysis to generate eigenvector values for each measurement (Table 1). The eigenvector values derived from the first principle component for each skeletal measure, were

used to generate a singular variable for each dog that could be considered to account for the majority of variation between the measurements.

Statistics

Statistical analysis was performed using the statistical software R (version 3.5.2, R Development Core Team; R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics and the distribution of variables were investigated. The variables were delta-G, speed, gait, body weight, age, and skeletal size. Pearson's correlation coefficient was used to evaluate the relationship between speed and delta-G, and a comparison of the inter-quartile range of delta-G between the walking and trotting and/or cantering gait categories were compared to evaluate the relationship between gait and delta-G. Delta-G was defined as the change in acceleration between adjacent sampling time points summed across three axes.

Separate multivariable models were constructed to determine if delta-G could be used to predict speed or gait. Speed was expressed in meters per second, and initially gait was an ordinal variable with three levels. However, initial examination of the data could not determine a convincing break between delta-G values for between trotting and cantering, or at different speeds. Consequently, gait was compressed into a two-level binary variable, called "binary gait variable" (BGV), with two levels that were coded 0 if the dog was walking, and 1 if the dog was trotting or cantering.

A mixed effects linear regression model was constructed to predict speed based on delta-G, in addition to body weight, age, and skeletal size. A forward and backward stepwise process was used for selection of fixed effects, which started with a full model and eliminated variables one at a time, and then the eliminated variables were returned into the reduced model to ensure the model was not improved, before removing variables again using the stepwise function in the Mass package in R. The model was then extended to include a random effect for dog, to account for both the repeated measures, and dog-specific gait characteristics unaccounted for in skeletal size. The goodness of fit of the model was assessed by comparing the AIC and log-likelihood between the mixed model and a mixed effects intercept-only model. The model assumption of independence was handled with the inclusion of dog as a random effect, the assumption of normality of residuals was checked visually with a Q–Q plot and equal variance of residuals was assessed with a plot of residuals against fitted values. The assumption of a linear relationship between the outcome variable and the predictor was assessed the addition of a quadratic into the model.

A mixed effects logistic regression model was constructed to predict gait using the two-level categorical variable BGV based on delta-G and any additional explanatory variables that significantly added to the model's predictive ability. A stepwise selection process was utilised to select final fixed effects in the model as described previously in this section. The model was then extended to include a random effect for dog to account for the repeated measures. The goodness of fit of the model was assessed by comparing the log-likelihood ratio statistic and the AIC probability, deviance and AIC of the full mixed model with the mixed effect intercept-only model. Hosmer and Lemeshow's pseudo-R^2 was also calculated for the simple logistic regression (Hosmer Jr, Lemeshow, & Sturdivant, 2013). The model assumption of scale - that is that the relationship between speed and delta-G on the logit scale was linear - was tested with the inclusion of a quadratic of the delta-G term into the model.

2.3 Results

Eight dogs were enrolled in the study. However, prior to analysis one dog was removed from the study due to failure to persist in a singular gait for a 10 second period on the treadmill. Of the seven dogs included in the analysis; six were female and one was male. Body weight ranged from 21kg to 25.8kg. Skeletal size was a variable that accounted for 56% of the variation seen between dogs. The eigenvalue for body length was much smaller than the values for the other measurements, indicating it had less influence on overall skeletal size than the other measurements (Table 1). The relationship between skeletal size and body weight for the seven dogs that contributed data to the study is show in Figure 2.

The seven dogs contributed 345 separate measurements of delta-G over 10 second intervals (delta-G_{10s}). Of those, 34 intervals were removed due to inconsistent gait or speed. The remaining data set included 311 measurements of delta-G_{10s}: 113 were recorded when the dogs were walking, and 198 were recorded when the dogs were either trotting or cantering. Speed ranged from 0.67m/s to 6.87m/s, with a median of 2.69 m/s. The distribution of delta-G_{10s} over all speeds was bimodal (Figure 3). The relationship between speed and delta-G for all the data points is shown in Figure 4, while Figure 5 shows the same relationship for each dog.

There was a strong relationship between speed and delta-G for the whole data set, with a Pearson's correlation coefficient of 0.89. Visual assessment of this relationship indicated that the relationship was linear for each individual dog, though it appeared non-linear as a whole dataset (Figures 4 and 5). Including "dog" in the model as a random effect accounted for this phenomenon, improving the model and negating the need for data transformation.

As stated previously, there was a division in the delta-G-speed curves that corresponded to the change in gait between walking and trotting, but not between trotting and cantering. The interquartile range of delta-G for the walking intervals (min: 14.23, LQ: 23.59, median: 29.5, mean: 33.71, UQ: 42.32, max: 64.42) did not overlap with the trotting/cantering IQR (min: 31.70, LQ: 97.42, median: 122.10, mean: 119.86, UQ: 139.85, max: 205.00). In contrast, there was significant overlap between the delta-G values for dogs trotting and cantering, which led to the binary classification of BGV, rather than a tertiary classification.

The final mixed effects linear regression model to predict speed included delta-G and skeletal size as fixed effects after stepwise selection, and dog as a random effect. Delta-G and skeletal size both significantly predicted speed (Table 2). The F-statistic and effect size of the model without the random effect of dog were both significantly high ($F_{2, 308} = 694.5$, $p < 0.001$; R^2=82%),

and both explanatory variables were significant, indicating the model was a good fit of the data. The model was then checked in its full form, with dog added back in. When compared with the intercept-only mixed model, the mixed model fit better (Table 2). All bar one model assumption was met. The model assumption of equal variance of residuals was shown to be violated, whereby the plot of residuals against fitted values of speed produced a clear funnelling trend (Figure 6).

The final mixed effects logistic regression model constructed to predict BGV contained only delta-G as a fixed effect after stepwise selection of variables, and dog as a random effect. Delta-G significantly predicted BGV, whereby increases in delta-G increased the odds of the dogs trotting or cantering (Table 3). Comparison of the full model with the intercept-only mixed model supported the full model as a better fit of the data, with a significant log-likelihood ratio statistic ($\chi 2_{(1)} = 35403$, $p < 0.001$). The Hosmer and Lemeshow's pseudo-R^2 was high at 87%, indicating the fit of the model improved greatly with inclusion of the delta-G variable. All model assumptions were met.

2.4 Discussion

This study explored whether the change in delta-G measured using a triaxial accelerometer could be used to predict speed and gait of dogs running on a treadmill. Analysis of gait showed that the odds of a dog trotting or cantering increased with delta-G, and the model was a good fit for the prediction of the BGV classification of a dog on the treadmill. The decision to use a binary classification of gait rather than attempt to distinguish between walking, trotting, and cantering, was made because initial exploratory analysis determined that the division of delta-G values between trotting and cantering was not clear, unlike the division between walking and trotting which was clear and within a fairly consistent zone of delta-G values for each dog (Figure 4). While there was clear demarcation between trotting and cantering for some dogs, suggesting that it is possible to use this method for gait classification of individual dogs, the demarcation point varied between dogs considerably. Therefore, it would be required that set points were established for every individual dog – a method that cannot be applied to an undefined population of dogs. Additionally, as the intended use of the resulting model for this study was to isolate periods of activity of interest where a dog was gaiting fast enough to highlight injury, illness, or a therapeutic effect, further gait classification was deemed unnecessary for this study.

None-the-less, the confidence of binary gait classification for reporting purposes is high, which is promising, as like speed, gait is a well understood classification, and the model built in this study has a very good fit. An important function of gait classification beyond reporting is for identifying periods of activity of interest within the delta-G data, so that large data sets can be filtered for easier and more precise analysis. As previously mentioned, clinical signs of disease such as osteoarthritis, are more likely to be detected during periods of greater activity intensity (Carr & Dycus, 2016). Given the fit of the model in this study, the confidence in gait classification for isolating periods of time a dog is trotting (or any faster gait, including cantering and galloping) is high. This crude activity classification is sufficient for identifying the threshold between walking and trotting, but not between trotting and cantering. Other studies that have successfully classified these three or similar activities, have used a more complex method of classification, with algorithms to identify patterns in the accelerometry over and above delta-G (or activity count) (den Uijl et al., 2017; Gerencsér et al., 2013; Ladha et al., 2013). This indicates a more complex method than delta-G analysis may be required for a more detailed classification of gait. The disadvantage of such an approach is the need for more extensive data processing, which is very difficult for long term studies due to the size of the datasets generated.

Analysis of speed showed that a dogs' predicted speed increased by 0.3m/s for each 10 unit increase of delta-G when accounting for skeletal size and the repeated measure of the dog. This study's findings are in agreement with those by Preston et al. (2012), who reported that accelerometer vector magnitude (similar to an activity count as defined in the introduction) can be used to identify a change in speed. The inclusion of skeletal size improved the model's predictive ability significantly, which indicates that in order to determine speed using data from the accelerometer used in this study the dog's size must also be taken into account. The model presented here, capably identifies a change in speed, but the accuracy of the estimation of the absolute speed value is low. Therefore, its use is limited to detecting changes in speed, rather than estimating absolute differences. The desire to estimate speed from the accelerometry data arose because speed is a term understood by most individuals, whereas delta-G is non-intuitive, and quantitatively meaningless without considerable experience, context, and comparative data. Unfortunately, the accuracy of speed estimation decreased with increasing speed, as depicted by the model residuals. It remains to be seen if rough estimates with large confidence intervals are still useful for communication.

For the linear mixed regression model, the assumption of equal variance of residuals was violated. The impact of this violation is on the estimated standard error for the beta coefficients of explanatory variables, where the standard error is possibly underestimated. However, it is not believed that there is a significant impact on the estimation of the coefficient. The extension of modelling in order to address this violation was over and above the scope of this thesis and therefore, while it is acknowledged that there is a violation of the model assumption, the model was used for the remainder of the study.

A major limitation of this study was the controlled environment in which it was carried out. Each interval was recorded on a treadmill at a constant speed and incline for a defined time period, which has implications for the use of both models to predict speed or gait in a free-living environment. When this approach is applied to continuously collected data that is analysed as 10 second epochs, it is unlikely that a dog running free will remain within a consistent gait and uniform speed for a 10 second period that aligns with the epoch defined by the accelerometer. It is far more likely that a 10 second period of consistent activity would be straddling two epochs, or perhaps, particularly for high intensity activity, a dog may not carry out the activity for a complete 10 second period at all. Therefore, inference of results must acknowledge that the averaged activity across the epoch may not reflect the true activity of a dog, and speed may be underestimated, or gait misclassified.

Although not specifically studied in dogs, the effect of a dog gaiting on a treadmill as opposed to the ground is expected to be negligible on the accelerometry output despite the known differences in gait between the two. This is supported by evidence of insignificant differences in activity counts per minute for humans on and off a treadmill while walking and running, supporting the use of models built with treadmill data for use on land data (Hendrick et al., 2010; Vanhelst et al., 2009). Additionally, in horses, the effect of treadmill locomotion on back kinematics in comparison to over-ground locomotion was negligible using motion capture software (Álvarez, Rhodin, Byström, Back, & Van Weeren, 2009). For this reason, it is likely that the accelerometer output between treadmill and ground running would be similar in dogs also.

An important limitation of this study is that neither of the models built have been validated. To validate the use of these models for predicting the speed and gait of dogs on a treadmill, another set of data would need to be obtained and these models applied. Comparison of the predicted values against the true values of this new dataset would reveal the usability of these models. As the small sample of seven dogs used in this study were the same breed, of similar weight and size, and almost all were female, in order to validate these models for application to dogs in general, a far more diverse range of dogs would need to be used, if inference beyond this narrow dog type is desired. Similarly, the validation of these models for predicting the speed and gait of free-moving dogs, would require simultaneous speed and accelerometry measurements in dogs running free. That would be difficult, since the measurement of speed would require the use of a speed radar, time/distance markers, or similar. It would also be difficult to obtain 10 seconds of continuous gait or speed without human intervention.

In conclusion, the results indicate that delta-G can be used to separate a dog's gait into walking or a faster gait (trot, canter or gallop), but estimation of speed above 3m/s was inaccurate. While, the model has not been validated, there is still value in using the model to screen large datasets in the field, to subset those 10-second epochs that involve movement at a gait faster than a walk, such as the dataset in the following chapter. In contrast, the model to predict speed would benefit from further revisions if it was considered necessary to generate accurate standard errors. However, even with this limitation the model may be of use for the identification of changes in speed between epochs rather than absolute speed measures.

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2.6 Tables and figures

Figure 2.1: Picture on the left indicates the placement of markers on each dog for motion capture of the dog as it moved on the treadmill. Picture on the top right is of the accelerometer used in this study. Picture on the bottom right is of the accelerometer on the dog, attached to the collar and positioned on the ventral side of the neck.

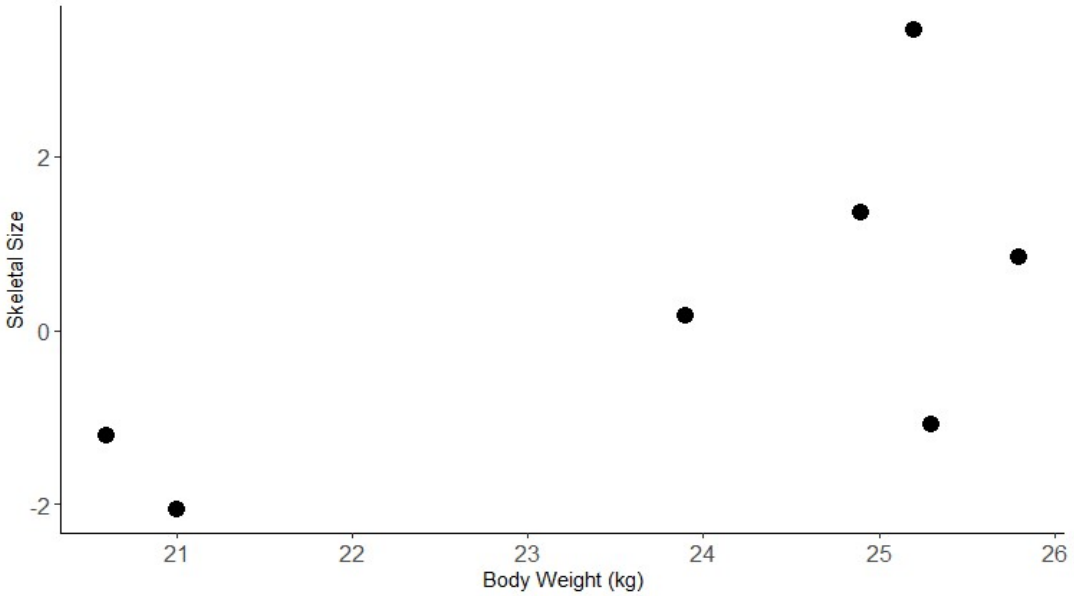

Figure 2.2: Plot of skeletal size variable against the body weight of the seven participating Huntaway dogs. Skeletal size was a variable calculated with the eigenvalues from six morphometric measurements taken of the body.

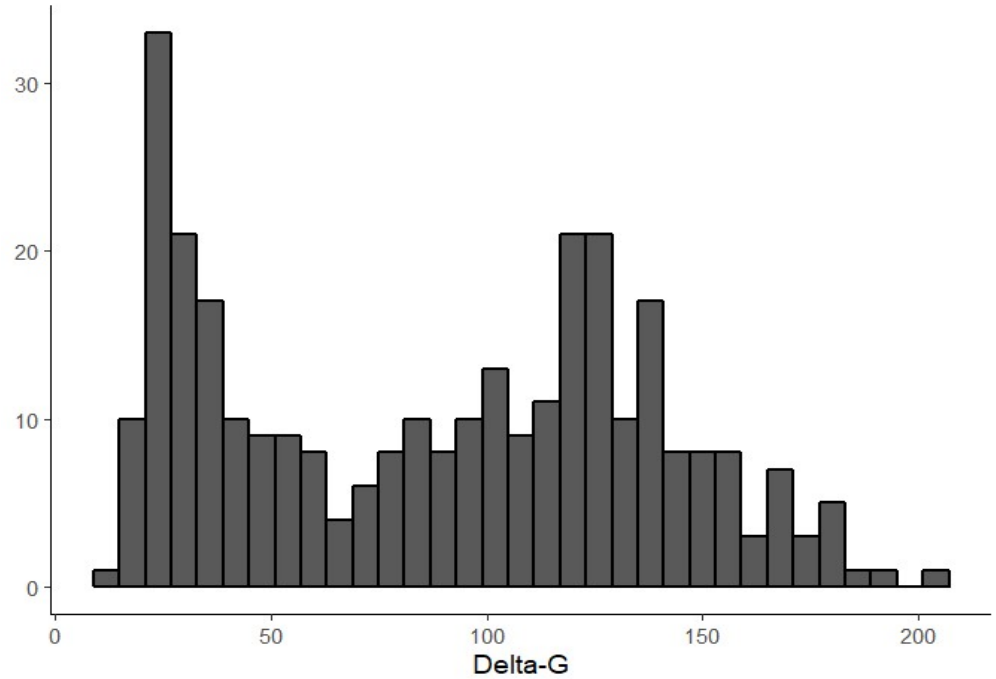

Figure 2.3: Distribution of 311 delta-G values recorded with a collar-mounted accelerometer for 10-second intervals of seven dogs at different speeds on a treadmill (mean: 88.70, median: 94.59, min: 14.23, max: 205.00, range: 190.77, LQ: 37.39, UQ: 126.89, IQR: 89.5).

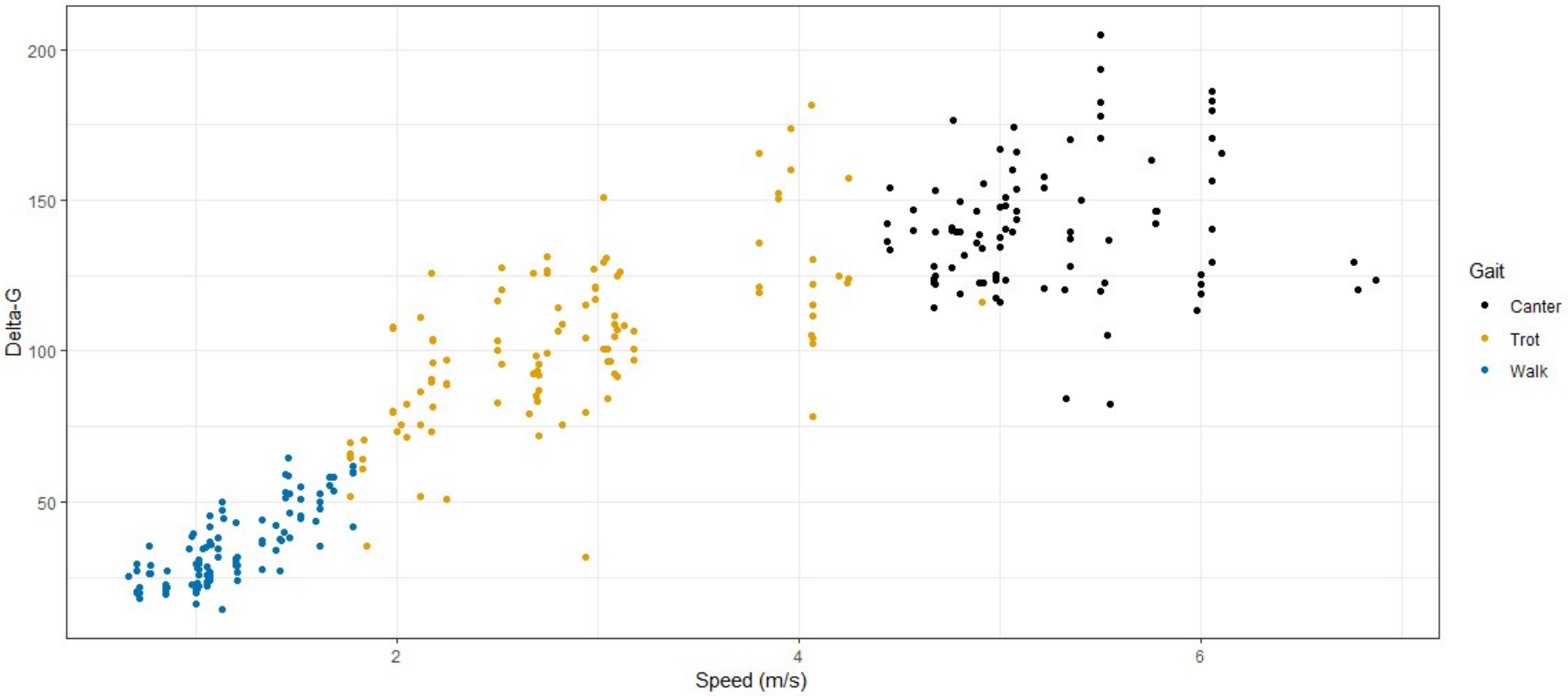

Figure 2.4: Plot of delta-G and speed with the distribution of the three gaits, walk, trot and canter. Data from 311 intervals of seven Huntaway dogs.

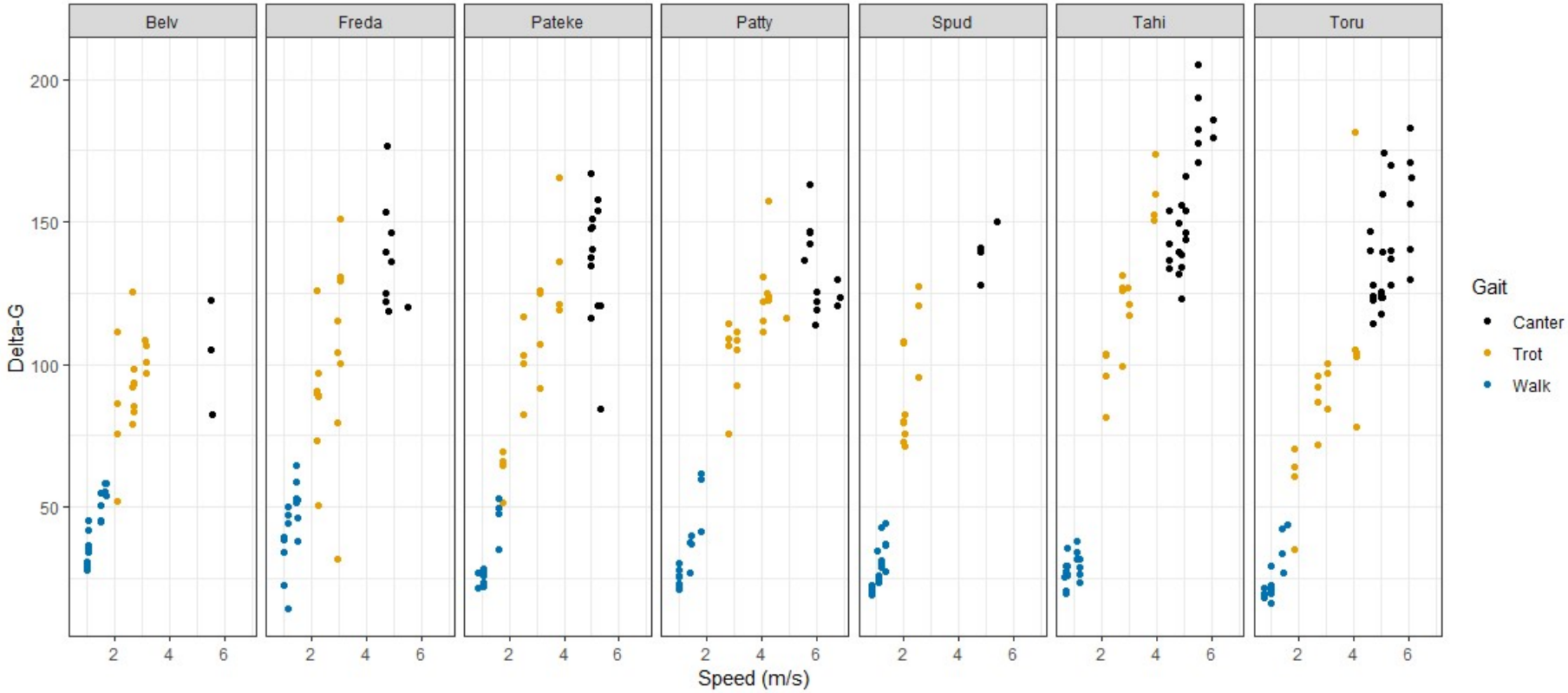

Figure 2.5: Scatterplot of speed and delta-G, and the distribution of gait for seven Huntaway dogs.

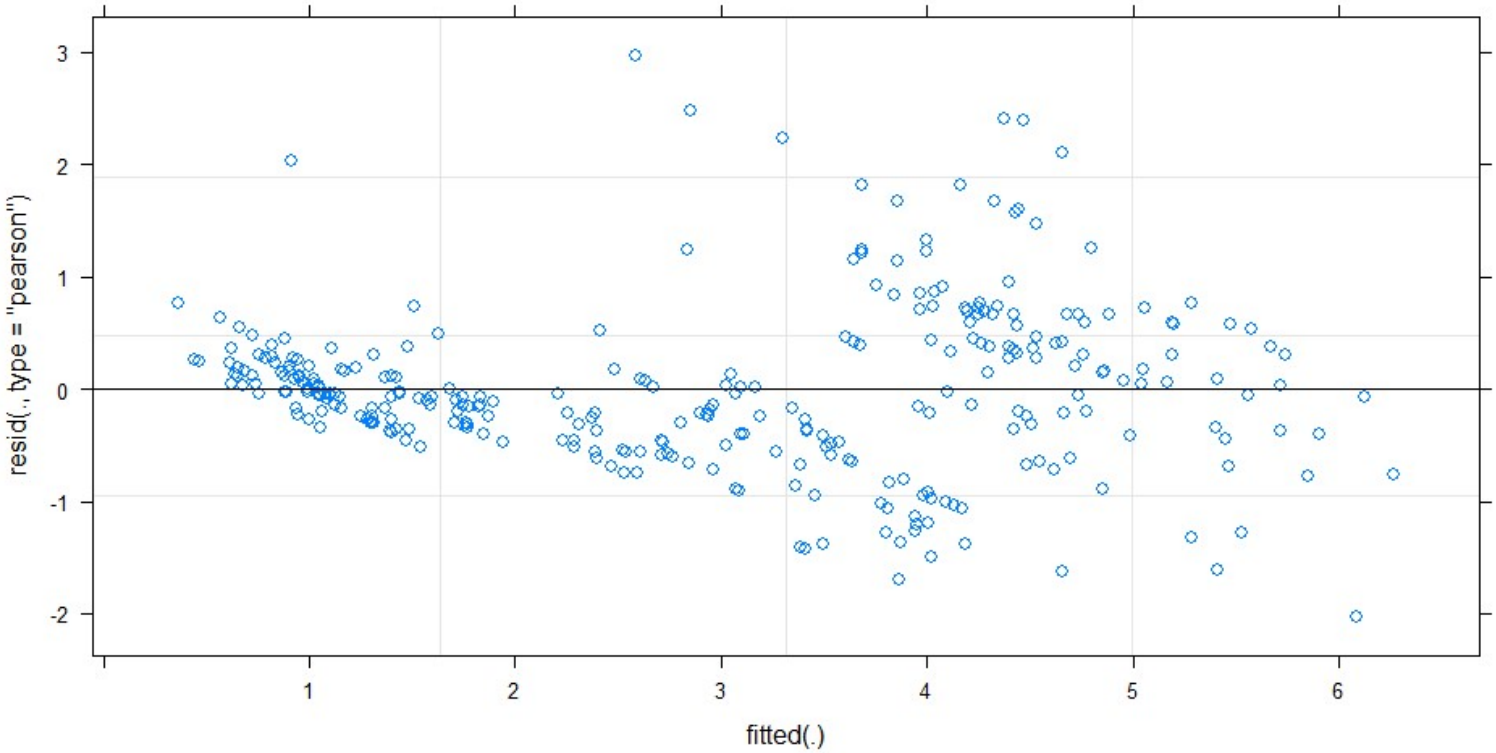

Figure 2.6: Plot of residuals against the fitted speed values for the linear mixed model predicting speed with delta-G and skeletal size, with dog as a random effect.

Table 2.1: Description of the six morphometric measurements (Table taken from Leung et al. (2018)), in addition to the mean morphometric measurements and eigenvalues for the first principal component from the eight participating Huntaway dogs.

Measurement	**Description**	Mean (cm)	1st PC
Head length measurement	Distance from the level of the medial canthi, equidistant between the eyes, to the external occipital protuberance	39.56	0.331
Head circumference	Circumference at the widest point, equidistant between the eyes and ears	13.19	0.387
Foreleg measurement	Distance from the proximal edge of the central foot pad to the olecranon process	70.06	0.481
Hind-leg measurement	Distance from the proximal edge of the central foot pad to the dorsal tip of the calcaneal process with the tarsus in extension	44.62	0.190
Body length	Distance from the dorsal process of thoracic vertebra 1 (T1) to the dorsal process of sacral vertebrae 1 (S1)	27.31	0.477
Thoracic girth	Chest circumference at the level of the xiphoid process	14.25	0.496

Table 2.2: Results from a mixed effects linear regression model predicting speed with repeat measurements in individual dog accounted for with an intercept-only random effect.

	Beta	SE beta	95% CI	P-value
Intercept	0.104	0.099	-0.082, 0.290	
Delta-G	0.031	0.001	0.030, 0.033	< 0.001
Skeletal Size	0.137	0.037	0.067, 0.207	< 0.001
Dog-level random effect				0.25[a]

Deviance of 689 with 5 degrees of freedom. The between dog variance (intercept) was 0.008, and the inter-dog variance (residual) was 0.530.
[a] P-value calculated using the likelihood ratio test

Table 2.3: Results from a mixed effects logistic regression model predicting BGV with delta-G, with repeat measurements in individual dog accounted for with an intercept-only random effect.

	Beta (SE)	P-value	95% CI for odds ratio		
			Lower	Estimated odds ratio	Upper
Intercept	-10.238 (1.849)	< 0.001	9.536e-07	3.577e-05	1.341e-03
Delta-G	0.170 (0.031)	< 0.001	1.115	1.185	1.261
Dog-level random effect		0.550[a]			

Deviance of 53 with 3 degrees of freedom. The between dog variance (intercept) was 0.473.
[a] P-value calculated using the likelihood ratio test

3 A pilot study to evaluate the feasibility of using accelerometry to study the efficacy of a green-lipped mussel nutraceutical for the treatment of joint disease

3.1 Introduction

Osteoarthritis (OA) is the most frequently identified musculoskeletal disorder in dogs, accounting for approximately 7% of veterinary visits (Anderson et al., 2018). Clinical signs of OA include lameness, swelling, pain, a reduced range of motion and joint crepitus (Cooper, Javaid, & Arden, 2014; Pettitt & German, 2015). Although incurable, there are a variety of treatments that may reduce the clinical signs of OA. One such treatment is a nutraceutical product, green-lipped mussel (*Perna canaliculus)* extract (GLME), which has been shown to have anti-inflammatory activity, particularly in rats (Halpern, 2000; Whitehouse et al., 1997).

When used for the treatment of OA in dogs, there is some evidence that GLME is capable of alleviating clinical signs such as swelling and pain (Bui & Bierer, 2001; Servet, Biourge, & Marniquet, 2006). However, the evidence of efficacy of GLME for the treatment of OA in dogs has been inconsistent, with some studies identifying a clear reduction in clinical signs as a result of treatment, while others failed to identify any effect at all (Dobenecker, Beetz, & Kienzle, 2002; Pollard, Guilford, Ankenbauer-Perkins, & Hedderley, 2006). There are several reasons for the inconsistencies in findings related to the efficacy of GLME. Studies have differed significantly in design, with some studies lacking a placebo control, and of those with a placebo there is variation with regard to the blinding of owners and researchers, and trial length (Bui & Bierer, 2001; Dobenecker et al., 2002; Pollard et al., 2006; Rialland et al., 2012; Servet et al., 2006). In general, trials that are not blinded or without a placebo control are more likely to show a treatment effect. Subjective measures of GLME efficacy have been the predominant form of measurement, with owner or veterinary questionnaires being a common form of measurement, whilst objective measures of clinical signs have been underutilised. Force plate analysis is one objective measure that has frequently been used to assess the effect of drugs on lameness, yet only one study of GLME efficacy used force plates (Rialland et al., 2012).

Accelerometry is another objective tool that has been used to evaluate OA treatments in dogs, by investigating trends in a dog's activity typically using a measure known as the activity count (Brown, Boston, & Farrar, 2010; Knazovicky, Tomas, Motsinger-Reif, & Lascelles, 2015). An

activity count is an arbitrary, company-specific measurement used to interpret the measured acceleration. Activity counts are effectively the sum of the raw accelerometer values for a given length of time and are determined by the amplitude of the acceleration and the frequency of the change in acceleration, so movements with greater changes in acceleration, or higher intensity exercise, increase the activity count. The activity count during the day is expected to increase with effective treatment of OA, as mobility is expected to improve in dogs as pain decreases. Conversely, night-time activity has been proposed to be lower in dogs with reduced OA clinical signs, as night-time restlessness is a commonly reported feature of OA (Knazovicky et al., 2015).

New Zealand farm dogs are a unique population of dogs in which accelerometry could be used to monitor progression of joint disease, like OA, and effectiveness of treatments. Farm dogs are made up of a few key breeds, two of which, the Huntaway and the Heading dog, are unique to the New Zealand farming industry, and make up the great majority of farm dogs in New Zealand (Cave, Bridges, Cogger, & Farman, 2009; O'Connell, Scott, Cogger, Jones, & Hill, 2019). These dogs are usually kept outside in kennels all year round, kept very lean and are a highly active group of dogs, with a workload influenced by both season and farm. Further, the dogs are tools for farmers, and therefore, working dogs tend to be treated differently to pet dogs. Owners of pet dogs enrolled in OA treatment studies may change the exercise routine and diet of an affected dog after enrolment, which can confound of the detection of true treatment efficacy. In addition, in placebo-controlled trials, owners may withdraw their animals from trial if they see signs of discomfort returning or a lack of improvement with a particular treatment, impacting the integrity of the study. Consequently, inconsistency of animal treatment and participation throughout the course of a study by pet owners can be a significant source of error. Despite the ability of accelerometry to identify changes in activity over extended periods of time, the behaviour of the owners of pet dogs can have a serious impact on the results. Altered owner behaviour is likely to be less of a contributing factor to erroneous results with working farm dogs.

Another reason for considering New Zealand working dogs as a study population, is that the prevalence of musculoskeletal problems is high. A recent, longitudinal study reported that 43% of working farm dogs had a musculoskeletal abnormality, of which 26% were lame on trot-up (Isaksen et al., in prep). Due to the nature of a farm dog's work, trauma is the most common cause of injury resulting in a veterinary visit, with vehicles and livestock interaction being the two most common causes of injury, and with feet, stifles and tarsi being the most frequent locations of injury (Cave et al., 2009). The most commonly reported musculoskeletal condition

is OA, most frequently in the hip, carpus and elbow (Cave et al., 2009). In addition, the Huntaway is known to have a notably high prevalence of hip dysplasia, disproportionately greater than Heading dogs, and which almost always results in OA (Cave et al., 2009; Hughes, 2001). The large number of dogs with joint disease in the New Zealand working farm dog population suggests it may be useful for treatment efficacy studies. In addition to the prevalence of joint disease in the population, the level of joint disease management in this population is probably minimal, as previous work has shown that once a dog is past puppyhood, nearly 80% of working dog owners indicated they either only occasionally or never visit a veterinary clinic (O'Connell et al., 2019). Despite the presence of joint injuries, they remain active and are highly motivated to work, and therefore they might be a good population of athletic active dogs to study the efficacy of GLME.

Despite the apparent suitability of the working dog population for studying joint disease, there are a number of possible challenges associated with using this population also. In addition, the currently available accelerometry technology may be problematic because working farm dogs spend large amounts of time away from their kennels during the day, with exposure of the accelerometer to potentially damaging conditions such as water, mud, and impact from stock. It remains to be seen if these concerns are sufficient to make them an unsuitable population.

Therefore, a pilot study is needed to determine whether using this population is technically feasible, to describe the variation of activity - both between dogs and within a dog over a long period of time, whether client compliance would be sufficient, and to describe the variation in the response of dogs with mild joint disease to treatment with GLME using accelerometry, in order to design a full study that is properly powered. The aims of this pilot study then, were to determine if New Zealand working farm dogs are a suitable study population, and if accelerometry is able to detect an effect of a GLME nutraceutical in this population of dogs with mild joint disease.

3.2 Methods

Animal selection

Thirty New Zealand working farm dogs with clinical signs of joint disease were recruited for this pilot study. It was predicted that this would be an adequate number of dogs to give sufficient information for the evaluation of aspects of the trial. This number was based on two comparable studies that utilised 23 or 31 dogs to determine the efficacy of GLME in dogs with OA, where one was a placebo-controlled trial, and the other a placebo-controlled, cross over study (Bui & Bierer, 2001; Rialland et al., 2012).

Working farm dogs with joint disease were primarily selected using veterinary examination notes collected prior to this study, from a pre-existing group of South Island farm dogs participating in "TeamMate", a longitudinal study of working dog health. The veterinary examination detailed the presence of physical abnormalities in these dogs, including joint disease. "TeamMate" is described in detail in Isaksen et al. (in prep). disease was defined as the presence of one or more of the following clinical signs on veterinary examination; lameness, crepitus, pain on manipulation, a decreased range of motion, or thickening of joints. Dogs were excluded if clinical signs were indicative of a more severe disease. Additional dogs were sourced from either interested owners volunteering dogs with known joint disease, or a veterinary clinic client base in the North Island. For both groups, the joints of interest were the hip, stifle, carpus, shoulder, elbow, hock and spine. Dogs that exhibited convincing evidence of clinical signs in a joint of interest were considered for inclusion, pending owner consent. This study was approved by the Massey University Animal Ethics Committee (MUAEC 17/103). The study was funded by the manufacturer of the GLME product, Lintbells Ltd, Weston, UK.

Study Design

The study was designed as a double-blinded, cross-over study with three treatments: 180mg full fat green-lipped mussel powder ($GLME_{180}$), a 220mg full fat green-lipped mussel powder ($GLME_{220}$), and a placebo. In order to ensure the researchers and owners were blinded to the treatment, the treatments were packaged and formulated to look nearly identical, and then labelled as A, B, or C (Lintbells Ltd, Weston, UK; Figure 3.1). Each treatment was administered by the farmer for an 8-week period, with a 4-week washout in between treatments. Each dog was allocated to one of 6 treatment orders (e.g. ABC, BCA, CBA, etc.). Dogs with the same owner were allocated to the same treatment group to remove the possibility of the owners mixing up the treatments. Treatment groups were allocated by ordering the owners by number of dogs

and first name, and then allocating them a number from 1 to 6 to produce relatively equal dog numbers in each group while still maintaining randomisation.

Dogs were required to wear a triaxial accelerometer for the entire duration of the study. The collar-mounted accelerometer was attached to a tightly fitted, dedicated leather collar on the dog, in addition to any pre-existing collar, so that it remained unaffected by the attachment of a lead or chain, which occurs commonly in farming practice. The accelerometer was to be removed only for dogs that resided in kennels outside the range of the specialised receiver, and only for the time required for the accelerometer to upload a full memory of data to the receiver, for which overnight once a week was usually sufficient. Owners of those dogs took the accelerometers off their dogs and left them by the receiver overnight, before putting the collar back on in the morning.

Accelerometers and receivers were couriered to the participants prior to trial commencement. Once the accelerometer setup was optimised, and each dog had at least 1 week of data successfully collected, they started their first round of treatment. Treatment required the administration of a single liver flavoured tablet once a day at the owners preferred feeding time, without any other alteration to the dog's regular diet. They either hid the treat in food, fed the treat out of their hand or physically dosed the dog. Activity of the dogs was not controlled. Owners were encouraged to maintain their normal routines.

Surveys were initially sent out at the end of each round to establish any health issues experienced by the dog over the course of the treatment round and to account for any missing doses or issues experienced by the owners. However, the extremely low response rate meant this method was not pursued after the second round. Instead a basic closing questionnaire was administered over the phone at the conclusion of the final round, with questions focussed on owners' perceived effectiveness of any of the three treatments.

Accelerometer

A micro electro-mechanical (MEMS) triaxial accelerometer (Heyrex®, Say Systems, Wellington, NZ) weighing 32g and measuring 65x26x18mm, was used for this study. The collar-mounted accelerometer was positioned on the ventral side of the dog's neck. Accelerations between +4 and -4 G's in magnitude were recorded at a sampling rate of 10 Hz. Acceleration was measured across three axes as the change in acceleration between neighbouring samples, reported as delta-G, and summed into one second epochs. Up to 7 days of data was stored on the collar unit, and when in range of the specialised receiver, which was connected to the internet, the accelerometer transferred captured acceleration data to the manufacturer's servers, where

proprietary software cleaned, transformed, summed, and stored the data. In order to facilitate data transfer from the accelerometer to the receiver, the accelerometer had to be within 30 metres of the receiver, in a line of sight. In cases where the receiver placement did not meet these criteria a number of techniques were enlisted to facilitate data transfer. Where possible, extension cords were utilised to move the receiver in line of sight, and where distance was an issue the receiver was set up with a Wi-Fi extender to a nearer power source, often a farm shed. Where neither were viable options the owners were asked to remove the collar once a week and position the accelerometer by the receiver overnight, before replacing the collar on the dog the next morning.

All accelerometry data were analysed as delta-G in 10 second epochs (delta-G_{10}). Morphometric measurements were also collected for each dog, following the method developed by Leung, Cave, and Hodgson (2018), and described in detail in the previous chapter. Age, weight, breed and sex were also collected from each dog. Body condition score was collected as part of the original data set, however because the time between scoring and the trial was highly variable, and there was a high likelihood it would have changed, and it was decided not to include that in the analysis.

Fatty acid analysis

In order to confirm the composition of the tablets, a sample of each was analysed for their polyunsaturated fatty acid content. One of each of the tablets was crushed and suspended in toluene. Fatty acids were methylated by using methanolic hydrochloride, in culture tubes. Samples were vortexed and heated at 70°C for methylation for 2 hours. After methylation samples were cooled on ice, potassium carbonate and toluene were added. Samples were vortexed and centrifuged at 2500 rpm for 7 minutes at room temperature to separate the solvent layer containing methyl esters and the aqueous layer.

Fatty Acids were detected by using a Shimadzu GC-2010 Plus Gas chromatograph equipped with a flame ionization detector. Fitted with a SupelcoTM-2560 Capillary Column 100mm x 0.25mm x 0.2um film thickness. The oven temperature was programmed to hold at 140^{o}C for 5min then to increase to 240^{o}C at the rate of 4^{o}/min, hold for 38min. Injector temperature was 250^{o}C, Detector temperature 255^{o}C. Standards were purchased from Sigma-Aldrich Co (Auckland, NZ). From the results, it was determined that $GLME_{220}$ was tablet A, $GLME_{180}$ was tablet B and the placebo was tablet C (Table 3.1). The primary researcher and owners were blinded to the results of the analysis until conclusion of the data collection phase.

Data cleaning and filtering

Data was filtered and analysed using the statistical processing software R (Version 3.5.2, R Development Core Team; R Foundation for Statistical Computing, Vienna, Austria). Data from dogs with fewer than two rounds of data were excluded. For the remaining data it was necessary to remove epochs that were invalid or had a high probability of being artefacts. Seven exclusion criteria were applied. The first three criteria involved exclusion of epochs with incorrect timestamps: 1) the epoch was stamped as occurring in 2000, 2) the epoch had a timestamp outside of the start and end date of a round for a dog, and 3) the epoch had a duplicate timestamp. In the case of duplicates both were excluded. The next two criteria removed periods of time when the accelerometer was not on the dog, by identifying a run of 360 consecutive epochs without a change in pitch or roll. The sixth exclusion criteria removed epochs in which no activity was recorded. Finally, epochs were removed if there was rhythmic movement of the accelerometer lasting more than five seconds that the proprietary algorithms classified as "scratching". The reason for this exclusion is that while the proprietary algorithm classified this as "scratching", other intense, rhythmic activities such as running with a collar that is not attached tightly enough, could have caused the movement. In addition, initial surveys of the data revealed periods of "scratching" that occurred during intense running activity, where it was deemed implausible that the dog was completing both activities at the same time. The exclusion criteria were not applied in a step-by-step fashion. Rather new variables were created for each of the seven exclusion criteria. The variables were coded "one" if the epoch meet the exclusion criteria, and "zero" if it did not. A single 10 second epoch could have been marked for exclusion for more than one criteria. An epoch that was marked for exclusion on any of the criteria was then removed from the data set.

Coding the response variables

To investigate the effect of treatment on delta-G_{10}, activity for each day was partitioned into daytime and night-time periods. The night-time period was limited to the hours between 11 pm and 4 am the next day. The decision to limit the night time period was based on a visual inspection of the delta-G_{10} traces, which suggested that was a time period in which the dogs were most settled and likely to be sleeping.

The daytime period was defined as the remaining hours of the day, 4 am to 11 pm on the same day. Within the daytime period, periods of time that a dog was completing lower intensity activity were removed. In order to achieve this, the daytime data were filtered to select epochs with a delta-G_{10} greater than or equal to 60. The cut point of delta-G_{10} 60 was determined based

on the model developed in Chapter 2, in which it was shown that for the dogs used in that study, it was the threshold for the change in gait from walking to trotting (Figure 3.2). Thus, the daytime activity data that was analysed represents all the periods when the dogs were predicted to be gaiting at a pace faster than a walk.

The distributions of delta-G_{10} during night and day periods were then examined separately, and response variables were created that summarised the activity. In both day and night periods the median delta-G_{10} and interquartile ranges for each date, for each dog were determined. Additionally, it was proposed that changes in activity in response to treatment might be seen in the most vigorous or intense activities. Thus, in the daytime dataset the 75^{th} and 90^{th} percentiles for each 24-hour period, for each dog were used to summarise activity.

Statistical analysis

General descriptive statistics of variables were calculated, and initial relationships between the delta-G response variables (median delta-G_{10} per date, 75^{th} and 90^{th} percentiles per date, and the interquartile ranges per date), treatment and the explanatory variables treatment order, season, , sex, breed, weight, age, skeletal size and farm were evaluated with bivariate linear regression models. To describe the variation in activity within and between dogs over the course of the study, a sum of the delta-G measured in the 10 epochs for each month was calculated for each dog and plotted as a boxplot.

Six mixed effects linear regression models were constructed to determine if treatment altered the response variables over the daytime or night-time. The final models included treatment, treatment order, season, and dog as a random effect in order to account for the repeated measures. For the interquartile range model, median was also added as a fixed effect. The p-values for Wald tests for the terms in the model were used to determine if there was an effect of a treatment on the response variables. The manufacturer of the GLME nutraceutical was not party to the results until after the analysis was completed.

3.3 Results

Of the 32 dogs initially recruited for the study, two required immediate replacement after the owner withdrew from the study for personal reasons. A further dog was dropped from the study part way into the first round after it broke its leg. Another owner withdrew their two dogs after the conclusion of their second round because they were unable to commit the time required to meet the study protocol, which on their farm required weekly removal of the accelerometers to place them inside next to the receiver. Two late recruitments were added to replace the first dropouts but had not completed the study at the time of analysis for this thesis, and consequently were not included. Subsequently, a total of 27 dogs were available for analysis.

Eighteen of the 27 dogs included in this study required weekly removal of their accelerometer overnight as their kennels were situated too far away from the nearest possible set-up of the accelerometer receiver. For a further five dogs it was possible to set up a Wi-Fi extender to place the receiver in range of the kennels. Only four dogs did not require extra set-up or weekly collar removal as their kennels were close enough to an existing internet connection point for the receiver.

Over the course of the study all 27 of the dogs had at least one issue with the accelerometer equipment. The most common issue was a breakdown in the uploading between the accelerometer and the receiver, which occurred at least once for all the dogs, irrespective of set-up. The weather, fluctuating quality of internet connection in the rural environment, and damage to the accelerometers were all possible causes of this failure, however none of these could be confirmed. There were also issues with the accelerometers themselves, with six of the dogs needing either replacement of the batteries in their existing accelerometer, or a replacement accelerometer. Of the dogs requiring a replacement accelerometer, two dogs had their accelerometers come off their collar during the working day and the owner was unable to locate it, and one dog appeared to have damaged the accelerometer casing resulting in internal water damage beyond repair.

The 27 dogs were spread across 17 farms: 10 owners contributed one dog, seven owners contributed two dogs, and one owner contributed three dogs to the study. Five dogs were fed other supplements prior to the trial, which were ceased from at least two weeks prior to study commencement until the conclusion of the study. These supplements included two with GLME as a main ingredient, and a flaxseed oil supplement.

The characteristics of the dogs, including the physical description of their joint disease, are presented in Table 3.2. Overall, the dogs ranged from approximately 2 to 11 years old with a

mean approximate age of 5.8 years. Their weight ranged from 16.6kg to 38.6kg with a mean of 26.7kg. As presented in Table 3.2, the most commonly affected joint was the hip, and a reduced range of motion and pain being the two most common clinical signs in the affected joints.

During the study period, the total number of 10 second epochs collected from the 27 dogs was 33,160,430. After removing epochs that met the exclusion criteria, approximately 5.5% of the raw dataset, 31,329,501 epochs remained for analysis, which produced a CSV file 6.8 gigabytes in size (Table 3.3 & 3.4). When categorised according to time, 6,565,232 epochs were available for the night time period, and 24,764,269 epochs were available for the day time period. Of the daytime epochs, there were 554,408 epochs in which the dogs were classified as gaiting at a pace faster than walking, referred to as delta-G_{10} greater than walking for the remainder of this chapter.

Analysis of daytime epochs with a delta-G_{10} greater than walking

Daytime activity data was recorded for 27 dogs for a total of 3,500 days: 1,160 days when 27 dogs were treated with $GLME_{220}$, 1,113 days when 23 dogs were treated with $GLME_{180}$ and 1,227 when 26 dogs were given the placebo. The data from 21 days were excluded as the interquartile range (IQR) in delta-G_{10} was 0. This occurred because there was only one epoch available for the day after excluding errors/artefacts, and epochs with activity less than 60 delta-G_{10}. There were far fewer winter days than during other seasons by design in this study, as it was known *a priori* that New Zealand working dogs are less active during this period as a consequence of a decreased workload on farm. There were 1,218 summer days, 1,189 spring days, 1,088 autumn days and 5 winter days with epochs greater than walking. There were 1,443 days for heading dogs, and 2,057 days for huntaways.

The distribution of the daytime 75th and 90th percentile delta-G_{10}, median and IQR delta-G_{10} are presented in Figures 3.3 to 3.6. Univariate relationships between each of the daytime response variables and each of the explanatory variables are described in Tables 3.5 to 3.8. For each of the daytime response variables there were significant differences between the seasons. The relationships were similar for each of the response variables, so box-plots are only shown for the relationships between 75th percentile and season (Figure 3.7), and between the 90th percentile and breed (Figure 3.8). There was also significant variation in the total activity within the dogs, and between dogs over the course of the study (Figure 3.9).

The results of the mixed effects multivariable linear regression models describing the relationships between each of the response variables and explanatory variables in the daytime dataset, are presented in Tables 3.9 to 3.12. The linear mixed-effects models identified a

relationship between higher intensity activity and GLME treatment. The daytime 90th percentile delta-G_{10} was higher when dogs were treated with the $GLME_{220}$ compared with the placebo, during the spring, and for the treatment order [$GLME_{220}$: $GLME_{180}$: placebo] compared to the treatment order [Placebo: $GLME_{180}$: $GLME_{220}$] (Table 3.9). The daytime 90th percentile delta-G_{10} was not significantly different between when dogs were treated with $GLME_{180}$ and $GLME_{220}$ ($p = 0.74$, not shown in Table 3.9).

The 75th percentile was not significantly associated with GLME treatment (Table 3.10). Neither treatment with $GLME_{220}$ nor $GLME_{180}$ were significantly associated with daytime median delta-G_{10} (Table 3.11). The daytime median delta-G_{10} was highest during the spring. Daytime median delta-G_{10} was not significantly different between periods when dogs were treated with $GLME_{180}$ and $GLME_{220}$ ($p = 0.81$, not shown in Table 3.11). The daytime IQR for delta-G_{10} was larger when dogs were treated with $GLME_{180}$, and during the spring compared with summer (Table 13.2). The daytime interquartile range for delta-G_{10} increased linearly with the median (Figure 3.10). Daytime IQR delta-G_{10} was not significantly different between periods when dogs were treated with $GLME_{220}$ and $GLME_{180}$ ($p = 0.72$, not shown in Table 3.12).

Night-time epoch analysis

A total of 3,780 nights of night time median, and IQR delta-G_{10} were collected from the 27 dogs, of which 1,251 nights were when all 27 dogs were on $GLME_{220}$, 1,177 nights were when 23 dogs were on $GLME_{180}$, and 1,352 were when 26 dogs were given the placebo. The distribution of the night time median delta-G_{10} was skewed, with values ranging from 0 to 22.44 (Figure 3.11). The distribution for the night time interquartile range in delta-G_{10} was skewed, with values ranging from 0 to 64.6 (Figure 3.12).

Univariate relationships between variables and the night time median and IQR for delta-G_{10} are presented in Tables 3.13 and 3.14. Both night time response variables were significantly associated with season, so only a box-plot for the relationship between the median and season has been shown (Figure 3.13). Both night time response variables were significantly associated with treatment order. Specifically, the order [Placebo: $GLME_{180}$: $GLME_{220}$], was significantly associated with a higher median and greater IQR delta-G_{10} than the reverse order [$GLME_{220}$: $GLME_{180}$: placebo] (Figure 3.14).

The results of the mixed effects multivariable linear regression models describing the relationships between each of the response variables and explanatory variables, are presented in Tables 3.15 and 3.16. There was no significant effect of treatment with $GLME_{220}$ and $GLME_{180}$ on the night time median delta-G_{10} (Table 3.15). The night time interquartile range for delta-G_{10}

was greater when dogs were treated with $GLME_{180}$ than when on the placebo (Table 3.16). Overall, the night time interquartile range for delta-G_{10} also increased with increasing median (Figure 3.15). The interquartile range was 0.07 (95% CI = 0.02, 0.12) less when dogs were treated with the $GLME_{220}$, compared to when treated with the $GLME_{180}$ ($p = 0.009$).

3.4 Discussion

This study was primarily designed to determine if accelerometry could be used in New Zealand working farm dogs with signs of joint disease to test the efficacy of a GLME nutraceutical. A secondary aim was to obtain preliminary data on the efficacy of the GLME extract, so that a larger appropriately powered study could be planned. The New Zealand working dog population was selected for this study because it has a substantial number of dogs with joint disease. In particular, dogs were selected from a specific cohort in which dogs had been diagnosed with joint disease, as part of a previous study (Isaksen et al., in prep). The high frequency of joint disease in the working dog population is a consequence of a high risk of injury in their working environment, and because huntaways have a predisposition for hip dysplasia and lumbosacral disease (Cave et al., 2009; Hughes, 2001). This study included 27 dogs, which included dogs with various clinical abnormalities, affecting several different joints.

Although the dogs selected for this study all had convincing signs of joint disease, their signs were probably from a wide range of pathologies. There was likely a large proportion of the dogs with OA, with varying severity. Additionally, there could have also been dogs with joint laxity without OA, or periarticular joint thickening / fibrosis. There may have also been dogs with soft tissue injuries that did not involve the joint. This will undoubtedly increase the variation in any response to GLME, as some conditions may not respond at all, whilst others may have spontaneously improved. An additional factor that may have influenced the response of dogs to the GLME was the variation in diet. As diet was not controlled in this study, the intake of various dietary elements would have a degree of variation between dogs. These additional dietary elements may have the ability to suppress or intensify the response to the GLME.

A limitation of this study is that the diagnosis of joint disease was made as part of another study (Isaksen et al., in prep), and some dogs had been examined up to three years previously. Dogs were selected from the prior study using the pre-existing veterinary notes, which did not include clear descriptions of severity, nor any history of the duration of clinical signs. Consequently, there was no certainty the injury described in the veterinary notes was still present at the time of this study, or that it was of a suitable nature or severity for GLME treatment. In an attempt to negate this, dogs were selected based on the presence clinical signs convincingly consistent with chronic joint disease in joints of interest as described in the methods, which drastically reduced the number of dogs for inclusion from the initial list of candidates. Ideally all candidate dogs would have been evaluated again by a veterinarian, and joint disease known to respond to GLME like OA confirmed radiographically prior to recruitment to ensure they meet the inclusion

criteria at the time of the study, rectifying all of these issues. However, there was insufficient funding to perform that for this pilot study.

The accelerometer system set-up in a rural New Zealand environment was, in many cases, a cumbersome process. The technology was not designed for working farm dog use and consequently there were a number of issues regarding the accelerometry equipment. Approximately 20% of the accelerometers required replacement or maintenance over the course of the study. Setting up the accelerometer system was problematic. The accelerometer was unable to contact the receiver over a range greater than 20 metres, or without a clear line of sight. The location of the dog kennels for many owners did not meet either of these requirements. The use of extension cords and Wi-Fi extenders solved the kennel location issue for some, but not all owners. This meant a number of owners were required to remove the accelerometer from their dog on a weekly basis and set it in range of the receiver overnight.

Ultimately, this accelerometer system was not ideal for this particular population due to aforementioned problems with the accelerometers, the system setup, and the unpredictability of uploading from the accelerometer. There were a number of cases of the accelerometer failing to upload stored data to the receiver later in the study despite there being no change to the dog's location or receiver setup. Due to the remote nature of the farms it was not possible to have study personnel visit them to determine the exact cause of these communication failures. The only feasible solution was to monitor the data being uploaded from the accelerometer to the receiver from the 27 dogs frequently and regularly over the course of the study. Owners were asked to place the accelerometer next to the receiver overnight if there was a gap in the data uploading. Usually, overnight was a sufficient time for an accelerometer with a full memory to upload to the receiver. In some instances, however, an accelerometer would not clear overnight, requiring the owner to take the accelerometer to the receiver multiple times a week. This substantially increased the work required from an owner. Feedback from owners indicated that their willingness to participate in future studies would be low if this technology was used again.

A further limitation of this technology is the absence evidence of repeatability between devices of this particular accelerometer. It is possible that a portion of the variation in data between dogs, or the data from a dog that required a replacement accelerometer, may have been due to inconsistencies between accelerometers.

Aside from the dog selection and accelerometer setup, the final element in this study was the administration of the GLME. This required owners to dose their dogs daily over three 8-week

periods, totalling six months of daily dosing. The study period included the New Zealand summer, the busiest season in the New Zealand sheep and beef farming community in terms of workload, and also included Christmas and the New Year. It is conceivable that dosing was not maintained every day during the study period, particularly during holiday periods. As owners were monitored remotely, the accelerometry system interface made it simple to track owner compliance regarding clearing the accelerometer memory, but it was not possible to know the level of compliance for the everyday dosing of the dogs. An attempt at quantifying the number of doses missed by owners via online questionnaires failed as owner feedback using this media was limited. The lack of feedback was attributed to the owners forgetting to contribute, or the perceived difficulty and time-consuming nature of the questionnaires. While consistent dosing was claimed by all owners that contributed feedback, the feedback came from arguably the more motivated of the owners in the study and it is likely the overall rate of compliance was much lower. The difficulty of establishing estimates of compliance, and the subjective observations of a dog's health over the course of the study, indicated that subjective measures would be unlikely to yield useful information in this population of dogs.

The size of the data in this study posed issues for the statistical analysis. Given the length of the study and the short duration of the epochs, the dataset was large, and required considerable computing power for analysis. For researchers accustomed to smaller datasets, the size of this dataset was challenging, as typical packages used for filtering and manipulating data in the statistical processing software R were unable to process the full dataset on a generic, personal computer, (in this case a 3Ghz processor, with 16 gigabytes of RAM, running a 64-bit operating system). Running mixed models and plotting was also difficult. Sub-setting the dataset negated this issue for this study by decreasing the size of the dataset required to process at one time. Future work with datasets of this size would benefit from forethought regarding how the data could be processed, and the technology that would be required to successfully analyse it.

The activity of the dogs varied greatly between dogs, between days, and across seasons. There was a limited amount of data collected during the winter by design, however, for the other three seasons, spring had consistently higher daytime variables than the summer or autumn. This was unsurprising, as in New Zealand, spring is arguably the busiest season in terms of stock work, which is what the dogs are used for. There was also variation between dog breeds, with heading dogs having consistently higher daytime outcome variables than huntaways. The two breeds are used very differently on farm. Heading dogs are traditionally used for herding stock, often requiring them to run very quickly over extended distances. In contrast, the huntaway is used to drive stock away using its bark and tends to move at a comparably slower pace.

There was a large amount of variation in activity between dogs. This is likely to be a farm effect, as the activity required from a dog can vary greatly between farms, due to differences in farming practices, work load, and topology. Some farmers use their dogs frequently, whilst others refrain from using dogs unless necessary. Smaller, and flatter farms arguably require less work for dogs than larger or steeper farms. Some owners may only have one or two dogs and use them frequently, others may have a larger team of dogs and use each dog less frequently. However, there were not enough dogs per farm to successfully attribute this variation to differences between farms. Therefore, it is not known how much of the variation was a true difference between dogs, let alone if it was due to variation in the severity of the dogs' joint disease.

There was also substantial variation within each dog across the study period. Along with changes in workload with the seasons, the day to day workload of a dog can vary significantly. Some days a dog will spend the majority of its time in a kennel, whilst during other days it may spend more than 12 hours out of its kennel. Huntaways may spend some days doing lower intensity work, such as yard work, which may not have made it above the threshold for inclusion in this analysis (activity greater than walking), which is likely to explain some of the breed differences in activity.

The huge variation in the activity of dogs in this study emphasised the importance of a rigorous study design. This study was randomised, placebo-controlled, with a cross-over, and spanned a two-month period per treatment. Without the cross-over it would have been very difficult to detect an effect of treatment due to the number of potential confounders including season, the differences between farms, the variation in joint disease, and the variation in activity between dogs. In principle, randomisation increases the chance that confounders are evenly distributed amongst treatment groups, but the dynamic nature of these confounders means it cannot account for all the effects.

The GLME treatments were associated with significant changes in delta-G_{10} in New Zealand working farm dogs. Treatment with $GLME_{220}$ increased the daytime 90th percentile delta-G_{10} by 1.72%. Similarly, $GLME_{180}$ increased the daytime 75th delta-G_{10} by approximately 1.5%, and $GLME_{220}$ increased the daytime 75th delta-G_{10} by approximately 1.4%, though neither were statistically significant. This could be inferred to mean that when a farm dog is required to work very hard, GLME treatment may increase the intensity a dog is capable or comfortable working at. Although the difference detected in this study was apparently small, it may be clinically and functionally significant. The linear mixed model showed that $GLME_{220}$ increased the predicted daytime 90th percentile for delta-G_{10} from 151 to 154 when given in spring and when the treatment order was $GLME_{220}$ first and placebo last. When applied to the relationship between

speed and delta-G_{10} for each of the seven dogs used for the treadmill experiment in Chapter 2, this difference equates to an increase in speed of between 0.1 m/s and 0.18 m/s, with an average of 0.12 m/s. Therefore, GLME appears to facilitate an increase in speed, and arguably the performance of working farm dogs. Beyond that though, the biological significance of the effect sizes of GLME on the severity of a dog's joint disease was not explored in this study. However, evidence of an anti-inflammatory effect of GLME, and a reported decrease in swelling and pain in dogs with OA provide probable areas of action for the GLME treatments that resulted in the increased intensity in this study (Bui & Bierer, 2001; Halpern, 2000; McPhee et al., 2007; Servet et al., 2006; Whitehouse et al., 1997).

The median delta-G_{10} was not associated with GLME treatment. The absence of a change in median with treatment was expected as these dogs are working animals, and the majority of their daily activity is dictated by the work they are required to do, whether they experience some degree of joint pain or not. Consequently, a dog with mild to moderate joint disease will complete a certain amount of activity irrespective of the severity of clinical signs and therefore the median activity for a dog was not expected to change. This is in contrast to pet dogs, in which the majority of their activity is likely to be spontaneous, and the severity of their disease will impact the activity they complete to a greater extent that the working dogs in this study. Nonetheless, this emphasises the importance of a more detailed analysis than total or average activity counts for detecting small effects of treatment.

In the multivariable models, daytime 90th percentile and IQR were higher during the treatment order [GLME220: GLME180: Placebo] than the reverse, [Placebo: GLME180: GLME220]. Similarly, the night time median was smaller during the treatment order [GLME220: GLME180: Placebo] than the orders that began with the placebo. During the night time, the treatment orders that began with the placebo round were significantly associated with a lower median than the reference treatment order, [$GLME_{220}$: $GLME_{180}$: placebo] (Table 3.15). This study was designed with a four week washout period that was intended to eliminate any residual effect of the treatments. However, the results suggest that the residual effect period for GLME treatment may have exceeded four weeks. Prior to the study the washout period was considered generous, so the possibility of a residual effect was surprising and requires further investigation to determine if this effect is repeatable. The association with treatment order and outcome variables emphasises the importance of the cross-over design, with dogs going through the treatments in different orders, if, as in this study, the washout period is insufficient.

The IQR for delta-G was included as a measure of variability as it was hypothesised that this may change with treatment as initial exploratory visualisation of the data suggested that there was an effect of treatment on the IQR. The IQR is not an immediately intuitive outcome variable and has not been previously used to describe accelerometer-derived activity measures in dogs. In this study, the IQR was largest during treatment with GLME for both the daytime and night time periods. Although treatment was not associated with an increase in the median delta-G_{10} and treatment, the median was positively correlated with the IQR, as depicted in Figure 3.10. Therefore, it would appear that the increase in IQR with treatment is due to an increase in activity with higher delta-G_{10} values, as shown with the higher 90^{th} and 75^{th} percentiles with treatment.

It was hypothesised that during the night time, treatment with GLME would reduce the response variables, indicative of a reduction in clinical signs of joint disease, particularly pain, which would allow for a more settled night time period. Similar to the daytime, this study did not reveal an effect of GLME treatment on the median delta-G_{10}. However, the interquartile range of delta-G_{10} during the night increased with $GLME_{180}$ treatment, and the IQR increased with increasing median delta-G_{10}, which was opposite to the hypothesis. This was an unexpected result, and it was not possible to determine what caused it with the information collected during this study. One possible reason is that with treatment caused a reduction of joint stiffness and pain experienced during recumbency, dogs are able to move more freely and increase their activity during the night. Other possible explanations for the increase of IQR delta-G_{10} with GLME treatment should be considered. These could include the possibility that GLME impairs sleep, through effects on the brain, or peripheral effects that lead to restlessness. At this time, it is not possible to corroborate either theory using the accelerometry data due to the inability to identify the circumstances around recorded activity with an accelerometer. To do so would require the addition of a visual monitoring system, with which it may have been possible to identify the cause of the change in night time activity Therefore, while there are a number of possible explanations for this trend observed in the night time data, the lack of corroborating evidence from other sources, and the lack of video recording in this study means it is not possible to conclude with an explanation for these results.

An assumption made in this study, was that all dogs were on the same sleep cycle and therefore, the same night time hours could be used for all dogs. It is possible that the use of a set night time period rather than tailoring the period to the dog influenced the trends seen in the data, potentially diluting the effect of GLME on night time activity. Only one other study has investigated the effects of treatment for joint disease on night-time activity of dogs using

accelerometry, in which the authors hypothesised that a decrease in night-time activity in dogs with OA would indicate treatment efficacy, and that study did not identify an effect (Knazovicky et al., 2015). However, the details of how data in Knazovicky et al (2015) was analysed were not explained clearly and as such the absence of an effect in that study may simply have been due to inadequate analysis.

The outcome variables selected for this study were unguided by previous work, with other studies using total activity over a treatment period and looking for differences between a baseline or placebo period (Brown et al., 2010; Knazovicky et al., 2015). Finer measures of delta-G, namely the percentiles, IQR, and median, were used in this study with the ambition of identifying smaller changes in the dogs' activity that would be undetected with the broad summation of data used in previous studies. It is unknown how this treatment may be affecting the pathogenesis of joint disease, whether it is acting as a pain inhibitor, which has implications for potentially facilitating further damage to the joints, or whether the GLME is actually reducing the damage within a joint via its direct anti-inflammatory effect. It is possible that the effect of treatment on the delta-G_{10} response variables does not translate into a sizable enough effect on the severity of a dog's joint disease to justify treatment. However, for a population of dogs that work, it could be argued that treatment is of value for any slight improvement in health that could translate into improved performance.

The delta-G_{10} response variables selected in this study were relatively simple. By investigating working dog activity in greater detail, there may be a more nuanced effect of GLME on activity. One example of a more in-depth evaluation of the activity data would be to subset the activity of dogs during nights that followed days of high intensity or prolonged activity and compare that with nightly activity that followed days of little activity. Another would be to evaluate the effect of treatment on a subset of days with prolonged high intensity activity, since the effect of GLME may be more pronounced on those days. Alternatively, the dogs could be subjected to set activities, such as a standardised fitness tests, which would reduce the variation in activity between dogs, and provide a consistent measure of improvement. However, this could be practically difficult with owners.

In conclusion, the result of this study suggests that treatment with GLME increases peak activity in working farm dogs with signs of joint disease, and may improve their ability to complete the activities required of them as a working dog. As a working dog, these dogs were completing a certain amount of activity regardless of treatment, consequently, changes in response to treatment are subtle. Although accelerometry appears to be an excellent tool to objectively

detect small, but significant effects, the system used was too fraught to recommend for future studies in this population. Joint disease is highly prevalent in the NZ working farm dog population, but the remote location of many dogs in relation to the researchers reduces the suitability of this population. Nonetheless, this study suggests that even mildly affected working farm dogs might benefit from treatment of their joint disease.

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3.6 Tables and figures

Table 3.1: Polyunsaturated fatty acid composition of $GLME_{220}$ and $GLME_{180}$, and the placebo.

FATTY ACIDS	$GLME_{220}$	$GLME_{180}$	Placebo
	g/100g	g/100g	g/100g
C6:0 Caproic	<0.01	<0.01	<0.01
C8:0 Caprylic	ND	ND	ND
C10:0 Capric	0.01	<0.01	ND
C11:0 Undecanoic	<0.01	<0.01	<0.01
C12:0 Lauric	0.01	0.01	<0.01
C13:0 Tridecanoic	<0.01	ND	ND
C14:0 Myristic	0.14	0.07	0.03
C14:1n5 - cis-9-Myristoleic	<0.01	<0.01	<0.01
C15:1n5 - cis-10-Pentadecenoic	ND	ND	ND
C16:0 Palmitic	0.89	0.72	1.50
C16:1n7 - cis-9-Palmitoleic	0.35	0.18	0.01
C17:0 Margaric	0.02	0.02	0.02
C17:1n7 - cis-10-Heptadecenoic	ND	ND	ND
C18:0 Stearic	0.50	0.45	1.32
C18:1n9t Elaidic	0.01	<0.01	<0.01
C18:1n7t Vaccenic	<0.01	<0.01	<0.01
C18:1n9c Oleic	0.32	0.12	0.19
C18:1n7c Vaccenic	0.07	0.03	0.02
C18:2n6t Linolelaidic	ND	ND	ND
C18:2n6c Linoleic	0.17	0.03	0.17
C20:0 Arachidic	0.01	0.01	0.02
C18:3n6 - cis-6,9,12-Gamma linolenic	0.02	ND	<0.01
C20:1n9 - cis-11-Eicosenoic	0.06	0.03	<0.01
C18:3n3 - cis-9,12,15-Alpha linolenic	0.05	0.03	0.01
C21:0 Heneicosanoic	ND	ND	ND
C20:2n6 - cis-11,14-Eicosadienoic	0.03	0.01	0.01
C22:0 Behenic	0.01	<0.01	0.01
C20:3n6 - cis-8,11,14-Eicosatrienoic	0.02	<0.01	0.01
C22:1n9 - cis-13-Erucic	0.01	ND	ND
C20:3n3 - cis-11,14,17-Eicosatrienoic	0.01	<0.01	ND
C20:4n6 - cis-5,8,11,14-Arachidonic	0.14	0.03	0.11
C23:0 Tricosanoic	ND	<0.01	<0.01
C22:2n6 - cis-13,16-Docosadienoic	ND	ND	ND
C24:0 Lignoceric	0.01	0.01	0.01
C20:5n3 - cis-5,8,11,14,17-Epa	1.57	0.23	<0.01
C24:1n9 - cis-15- Nervonic	<0.01	ND	ND
C22:5n3 - cis-7,10,13,16,19-DPA	0.18	0.02	0.01
C22:6n3 - cis-4,7,10,13,16,19-DHA	1.06	0.16	0.01
Total Fatty Acids	5.63	2.18	3.46

Table 3.2: Summary of dog parameters. For joints, there may be more than one effected joint in a dog, and a dog may have more than one clinical sign.

Parameter	Number of dogs	% Dogs
Sex		
Male	19	70
Female	8	30
Breed		
Huntaway	17	63
Heading	10	37
Effected joint		
Hip	17	63
Carpus	12	44
Stifle	6	22
Elbow	3	11
Tarsus	5	19
Shoulder	2	7
Clinical signs of joint disease		
ROM	13	48
Pain	12	44
Lameness	6	22
Crepitus	6	22
"Stiff"	4	15
Joint swelling/thickening	4	15
X-ray confirmed	2	7

Table 3.3: Number and percentage of epochs removed, stratified by exclusion criteria.

Exclusion Criteria	Epoch Count	% Epochs
Duplicate timestamps	989,748	3.2
Scratching > 6	157,243	0.5
Consecutive zero values	565,699	1.8
Data from the 2000's	277,606	0.9
No change in roll	207,634	0.7
No change in pitch	178,531	0.6
Outside of round timeframe	702,937	2.2
Total epochs removed	1,830,929	5.5

Criteria are not exclusive, and epochs may have been excluded for more than one criteria.

Table 3.4: Number and percentage of epochs that were removed stratified by the number of exclusion criteria violated.

Number of Exclusion Criteria Violated	Epoch Count	% Epochs
1	1,012,938	3.05
2	441,257	1.33
3	330,191	1.00
4	39,342	0.12
5	7,201	0.02

Table 3.5: Results from bivariate linear regression models showing the relationship between ***daytime 90th percentile delta-G$_{10}$*** *and possible explanatory variables. Dataset consisted of 3,500 days from 27 dogs.*

Parameter	Beta	SE	p-value
Sex			
Female	REF		
Male	0.38	1.37	0.78
Breed			
Heading	REF		
Huntaway	-16.73	1.22	<0.001
Season			
Autumn	REF		
Spring	5.06	1.53	<0.001
Summer	0.76	1.52	<0.001
Winter	-33.48	16.35	<0.001
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	-10.74	1.96	<0.001
GLME180: GLME220: Placebo	18	2	<0.001
GLME180: Placebo: GLME220	11.06	1.74	<0.001
Placebo: GLME220: GLME180	-14.41	1.98	<0.001
Placebo: GLME180: GLME220	-27.34	1.85	<0.001
Farm			
Farm A	REF		
Farm B	27.67	2.18	<0.001
Farm C	11.24	2.65	<0.001
Farm F	6.17	3.21	0.05
Farm G	43.77	2.21	<0.001
Farm H	-9.62	2.42	<0.001
Farm I	31.8	2.69	<0.001
Farm J	-5.11	2.71	0.06
Farm K	-9.68	2.42	<0.001
Farm L	-26.12	2.13	<0.001
Farm N	29.83	2.23	<0.001
Farm O	19.31	2.19	<0.001
Farm P	-19.02	2.8	<0.001
Farm Q	23.25	3.09	<0.001
Farm R	23.65	3.39	<0.001
Farm S	-6.31	4.78	0.19
Weight	-0.49	0.11	<0.001
Age	-3.22	0.23	<0.001

Table 3.6: Results from bivariate linear regression models showing the relationship between ***daytime 75th percentile in delta-G$_{10}$*** *and possible explanatory variables. Dataset consisted of 3,500 days from 27 dogs.*

Parameter	Beta	SE	p-value
Sex			
Female	REF		
Male	0.004	1.05	0.997
Breed			
Heading	REF		
Huntaway	-10.35	0.94	<0.001
Season			
Autumn	REF		
Spring	4.93	1.17	<0.001
Summer	1.16	1.16	<0.001
Winter	-21.54	12.47	<0.001
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	-3.29	1.54	0.03
GLME180: GLME220: Placebo	11.32	1.57	<0.001
GLME180: Placebo: GLME220	9.21	1.36	<0.001
Placebo: GLME220: GLME180	-7.54	1.55	<0.001
Placebo: GLME180: GLME220	-17.69	1.45	<0.001
Farm			
Farm A	REF		
Farm B	19.69	1.72	<0.001
Farm C	12.63	2.09	<0.001
Farm F	5.81	2.53	0.02
Farm G	31.26	1.74	<0.001
Farm H	-7.92	1.91	<0.001
Farm I	19.84	2.12	<0.001
Farm J	-5.14	2.14	0.02
Farm K	-6	1.91	<0.001
Farm L	-20.03	1.68	<0.001
Farm N	18.31	1.76	<0.001
Farm O	14.29	1.72	<0.001
Farm P	-9.06	2.21	<0.001
Farm Q	16.84	2.44	<0.001
Farm R	20.49	2.67	<0.001
Farm S	-7.84	3.77	0.04
Weight	-0.13	0.08	0.1
Age	-1.76	0.18	<0.001

Table 3.7: Results from bivariate linear regression models showing the relationship between ***daytime median delta-G_{10}*** *and possible explanatory variables. Dataset consisted of 3,500 days from 27 dogs.*

Parameter	Beta	SE	p-value
Sex			
Female	REF		
Male	0.08	0.67	0.9
Breed			
Heading	REF		
Huntaway	-4.04	0.61	<0.001
Season			
Autumn	REF		
Spring	3.27	0.75	<0.001
Summer	1.55	0.74	<0.001
Winter	-11.91	8	<0.001
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	0.84	1.02	0.41
GLME180: GLME220: Placebo	4.47	1.04	<0.001
GLME180: Placebo: GLME220	4.79	0.9	<0.001
Placebo: GLME220: GLME180	-1.8	1.03	0.08
Placebo: GLME180: GLME220	-8.41	0.96	<0.001
Farm			
Farm A	REF		
Farm B	12.11	1.15	<0.001
Farm C	11.63	1.4	<0.001
Farm F	6.49	1.7	<0.001
Farm G	16.52	1.17	<0.001
Farm H	-3.03	1.28	0.02
Farm I	6.4	1.42	<0.001
Farm J	-3.81	1.43	0.01
Farm K	-2.59	1.28	0.04
Farm L	-11.18	1.13	<0.001
Farm N	10.12	1.18	<0.001
Farm O	8.67	1.16	<0.001
Farm P	-0.57	1.48	0.7
Farm Q	11.53	1.63	<0.001
Farm R	17.62	1.79	<0.001
Farm S	-4.12	2.53	0.1
Weight	0.09	0.05	0.08
Age	-0.63	0.12	<0.001

Table 3.8: Results from bivariate linear regression models showing the relationship between ***daytime interquartile range in delta-G_{10}*** *and possible explanatory variables. Dataset consisted of 3,479 days from 27 dogs.*

Parameter	Beta	SE	p-value
Sex			
Female	REF		
Male	-0.45	0.85	0.6
Breed			
Heading	REF		
Huntaway	-9.36	0.76	<0.001
Season			
Autumn	REF		
Spring	3.34	0.94	<0.001
Summer	0.37	0.94	<0.001
Winter	-18.17	10.04	<0.001
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	-4.1	1.23	<0.001
GLME180: GLME220: Placebo	9.57	1.25	<0.001
GLME180: Placebo: GLME220	7.79	1.09	<0.001
Placebo: GLME220: GLME180	-7.43	1.24	<0.001
Placebo: GLME180: GLME220	-15.06	1.16	<0.001
Farm			
Farm A	REF		
Farm B	13.83	1.39	<0.001
Farm C	5.08	1.69	<0.001
Farm F	2.35	2.05	0.25
Farm G	24.84	1.41	<0.001
Farm H	-8.16	1.54	<0.001
Farm I	18.13	1.71	<0.001
Farm J	-3.78	1.73	0.03
Farm K	-5	1.56	<0.001
Farm L	-16.34	1.36	<0.001
Farm N	12.84	1.42	<0.001
Farm O	10.4	1.39	<0.001
Farm P	-11.26	1.78	<0.001
Farm Q	11.08	1.99	<0.001
Farm R	10.36	2.17	<0.001
Farm S	-6.91	3.04	0.02
Weight	-0.22	0.07	<0.001
Age	-1.6	0.14	<0.001

Table 3.9: Results from a mixed effects linear *regression model of the effect of treatment on* ***daytime 90th percentile delta-G$_{10}$*** *greater than walking, with repeat measurements in individual dogs accounted for with an intercept-only random effect.*

Parameter	Beta (SE)	95% CI	p-value
Treatment			
Placebo	REF		
GLME220	2.6 (1.2)	0.253,4.94	0.03
GLME180	2.19 (1.22)	-0.21,4.58	0.073
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	-8.1 (13.17)	-35.48,19.28	0.545
GLME180: GLME220: Placebo	18.99 (14.31)	-10.77,48.74	0.199
GLME180: Placebo: GLME220	13.12 (12.39)	-12.65,38.89	0.302
Placebo: GLME220: GLME180	-11.71 (12.43)	-37.56,14.14	0.357
Placebo: GLME180: GLME220	-26.26 (12.41)	-52.06,-0.45	0.046
Season			
Spring	REF		
Autumn	-5.38 (1.24)	-7.8,-2.95	<0.001
Summer	-3.93 (1.19)	-6.27,-1.59	0.001
Winter	-15.91 (13.02)	-41.45,9.62	0.222

Intercept was 151.16 (SE = 8.85).
Dog added as a random intercept, standard deviation of 19.45 (95% CI: 14.3-26.45) with a residual of 28.29 (95% CI: 27.63-29.0).

Table 3.10: Results from a mixed effects linear *regression model of the effect of treatment on* ***daytime 75th percentile delta-G$_{10}$*** *greater than walking, with repeat measurements in individual dogs accounted for with an intercept-only random effect.*

Parameter	Beta (SE)	95% CI	p-value
Treatment			
Placebo	REF		
GLME220	1.74 (0.95)	-0.115,3.6	0.066
GLME180	1.81 (0.97)	-0.08,3.71	0.061
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	-1.14 (10.1)	-22.14,19.86	0.911
GLME180: GLME220: Placebo	11.97 (10.98)	-10.85,34.8	0.288
GLME180: Placebo: GLME220	10.52 (9.51)	-9.25,30.29	0.281
Placebo: GLME220: GLME180	-6 (9.54)	-25.84,13.83	0.536
Placebo: GLME180: GLME220	-17.34 (9.52)	-37.13,2.46	0.083
Season			
Spring	REF		
Autumn	-4.93 (0.98)	-6.85,-3.01	<0.001
Summer	-3.47 (0.94)	-5.32,-1.62	<0.001
Winter	-6.16 (10.31)	-26.37,14.06	0.55

Intercept was 121.41 (SE = 6.79).
Dog added as a random intercept, standard deviation of 14.91 (95% CI: 11.0 - 20.3) with a residual of 22.39 (95% CI: 21.87-22.93).

Table 3.11: Results from a mixed effects linear *regression model of the effect of treatment on* ***daytime median delta-G_{10}*** *greater than walking, with repeat measurements in individual dogs accounted for with an intercept-only random effect.*

Parameter	Beta (SE)	95% CI	p-value
Treatment			
Placebo	REF		
GLME220	0.64 (0.64)	-0.614,1.9	0.316
GLME180	0.49 (0.65)	-0.8,1.77	0.456
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	2.06 (6.37)	-11.18,15.3	0.75
GLME180: GLME220: Placebo	4.77 (6.92)	-9.61,19.15	0.498
GLME180: Placebo: GLME220	5.22 (5.99)	-7.24,17.67	0.394
Placebo: GLME220: GLME180	-1.34 (6.01)	-13.85,11.16	0.826
Placebo: GLME180: GLME220	-8.69 (6)	-21.17,3.78	0.162
Season			
Spring	REF		
Autumn	-3.42 (0.66)	-4.72,-2.12	<0.001
Summer	-1.79 (0.64)	-3.04,-0.54	0.005
Winter	-2.87 (6.98)	-16.55,10.82	0.681

Intercept was 93.83 (SE = 4.29).
Dog added as a random intercept, standard deviation of 9.38 (95% CI: 6.89 - 12.78) with a residual of 15.16 (95% CI: 14.8 -15.52).

Table 3.12: Results from a mixed effects linear *regression model of the effect of treatment on* ***daytime IQR delta-G_{10}*** *greater than walking, with repeat measurements in individual dogs accounted for with an intercept-only random effect.*

Parameter	Beta (SE)	95% CI	p-value
Treatment			
Placebo	REF		
GLME220	1.05 (0.56)	-0.05,2.15	0.063
GLME180	1.25 (0.57)	0.12,2.37	0.03
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	-4.52 (3.36)	-11.51,2.47	0.193
GLME180: GLME220: Placebo	5.82 (3.64)	-1.74,13.39	0.125
GLME180: Placebo: GLME220	4.42 (3.15)	-2.14,10.97	0.176
Placebo: GLME220: GLME180	-5.53 (3.18)	-12.15,1.1	0.097
Placebo: GLME180: GLME220	-7.39 (3.17)	-13.98,-0.8	0.03
Season			
Spring	REF		
Autumn	-0.6 (0.58)	-1.75,0.55	0.304
Summer	-1.43 (0.56)	-2.53,-0.33	0.011
Winter	-4.34 (6.12)	-16.34,7.67	0.479
Median	0.84 (0.01)	0.81,0.87	<0.001

Intercept was -32.13 (SE = 2.69).
Dog added as a random intercept, standard deviation of 4.86 (95% CI: 3.52-6.69) with a residual of 13.31 (95% CI: 13.0-13.62).

Table 3.13: Results from bivariate linear regression models showing the relationship between ***night time median delta-G_{10}*** *and possible explanatory variables. Dataset consisted of 3,780 nights from 27 dogs*.

Parameter	Beta	SE	p-value
Sex			
Female	REF		
Male	-0.01	0.01	0.69
Breed			
Heading	REF		
Huntaway	-0.02	0.01	<0.001
Season			
Autumn	REF		
Spring	-0.05	0.02	<0.001
Summer	-0.03	0.02	<0.001
Winter	-0.07	0.17	<0.001
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	0.06	0.02	0.01
GLME180: GLME220: Placebo	0.02	0.02	0.45
GLME180: Placebo: GLME220	0.03	0.02	0.12
Placebo: GLME220: GLME180	0.09	0.02	<0.001
Placebo: GLME180: GLME220	0.12	0.02	<0.001
Farm			
Farm A	REF		
Farm B	-0.03	0.03	0.38
Farm C	-0.02	0.03	0.6
Farm F	-0.05	0.04	0.21
Farm G	-0.01	0.03	0.73
Farm H	0.04	0.03	0.27
Farm I	0.02	0.04	0.62
Farm J	-0.07	0.04	0.06
Farm K	0.03	0.03	0.31
Farm L	0.08	0.03	<0.001
Farm N	-0.05	0.03	0.1
Farm O	0.02	0.03	0.44
Farm P	0.02	0.04	0.63
Farm Q	0.12	0.04	<0.001
Farm R	0.11	0.04	0.02
Farm S	-0.05	0.05	0.35
Weight	-0.003	0.001	0.03
Age	-0.003	0.003	<0.001

Table 3.14: Results from bivariate linear regression models showing the relationship between ***night time IQR delta-G_{10}*** *and possible explanatory variables. Dataset consisted of 3,780 nights from 27 dogs.*

Parameter	Beta	SE	p-value
Sex			
Female	REF		
Male	-0.15	0.05	<0.001
Breed			
Heading	REF		
Huntaway	-0.05	0.04	<0.001
Season			
Autumn	REF		
Spring	-0.12	0.05	<0.001
Summer	0.02	0.05	<0.001
Winter	-0.045	0.53	<0.001
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	0.03	0.07	0.69
GLME180: GLME220: Placebo	0.13	0.08	0.09
GLME180: Placebo: GLME220	0.04	0.07	0.53
Placebo: GLME220: GLME180	0.07	0.07	0.35
Placebo: GLME180: GLME220	0.22	0.07	<0.001
Farm			
Farm A	REF		
Farm B	-0.07	0.09	0.45
Farm C	-0.028	0.11	0.79
Farm F	-0.01	0.14	0.93
Farm G	0.08	0.09	0.4
Farm H	0.13	0.1	0.22
Farm I	0.45	0.11	<0.001
Farm J	-0.11	0.11	0.33
Farm K	0.03	0.1	0.75
Farm L	0.13	0.09	0.13
Farm N	-0.07	0.09	0.44
Farm O	0.05	0.09	0.58
Farm P	0.12	0.12	0.32
Farm Q	0.48	0.12	<0.001
Farm R	0.14	0.14	0.32
Farm S	0.05	0.16	0.74
Weight	-0.011	0.004	<0.001
Age	-0.004	0.008	<0.001

Table 3.15: Results from a mixed effects linear *regression model of the effect of treatment on* ***median night time delta-G_{10},*** *with repeat measurements in individual dogs accounted for with an intercept-only random effect.*

Parameter	Beta (SE)	95% CI	p-value
Treatment			
Placebo	REF		
GLME220	0.03 (0.02)	-0.003,0.06	0.075
GLME180	0.02 (0.02)	-0.01,0.05	0.231
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	0.07 (0.04)	-0.01,0.15	0.094
GLME180: GLME220: Placebo	0.02 (0.04)	-0.06,0.11	0.574
GLME180: Placebo: GLME220	0.04 (0.04)	-0.03,0.12	0.263
Placebo: GLME220: GLME180	0.1 (0.04)	0.02,0.18	0.019
Placebo: GLME180: GLME220	0.13 (0.04)	0.05,0.21	0.002
Season			
Spring	REF		
Autumn	0.06 (0.02)	0.02,0.09	0.001
Summer	0.03 (0.02)	-0.002,0.06	0.069
Winter	0.06 (0.17)	-0.27,0.38	0.741

Intercept was -0.002 (SE = 0.03).
Dog added as a random intercept, standard deviation of 0.05 (95% CI: 0.03-0.08) with a residual of 0.4 (95% CI: 0.39-0.41).

Table 3.16: Results from a mixed effects linear *regression model of the effect of treatment on* ***IQR of night time delta-G_{10},*** *with repeat measurements in individual dogs accounted for with an intercept-only random effect.*

Parameter	Beta (SE)	95% CI	p-value
Treatment			
Placebo	REF		
GLME220	0.04 (0.03)	-0.01,0.09	0.115
GLME180	0.11 (0.03)	0.06,0.16	<0.001
Order of treatment			
GLME220: GLME180: Placebo	REF		
GLME220: Placebo: GLME180	-0.13 (0.11)	-0.35,0.1	0.247
GLME180: GLME220: Placebo	0.07 (0.12)	-0.17,0.31	0.565
GLME180: Placebo: GLME220	-0.05 (0.1)	-0.26,0.15	0.594
Placebo: GLME220: GLME180	-0.17 (0.1)	-0.38,0.05	0.118
Placebo: GLME180: GLME220	-0.12 (0.1)	-0.33,0.09	0.237
Season			
Spring	REF		
Autumn	-0.03 (0.03)	-0.09,0.02	0.196
Summer	0.06 (0.03)	0.01,0.11	0.012
Winter	-0.03 (0.26)	-0.55,0.48	0.9
Median	2.8 (0.03)	2.75,2.85	<0.001

Intercept was 0.05 (SE = 0.08).
Dog added as a random intercept, standard deviation of 0.15 (95% CI: 0.11-0.21) with a residual of 0.62 (95% CI: 0.61-0.64).

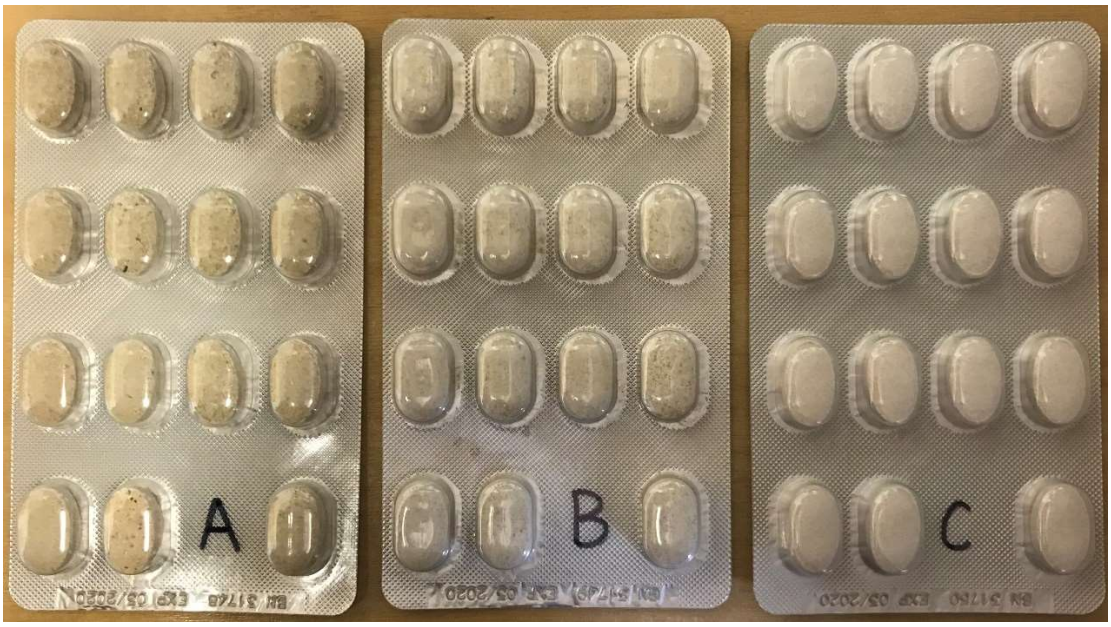

Figure 3.1: Tablets labelled as A, B and C in the form they were distributed to the owners of the New Zealand working farm dogs. Tablet A was the treatment $GLME_{220}$, tablet B was the treatment $GLME_{180}$ and tablet C was the placebo.

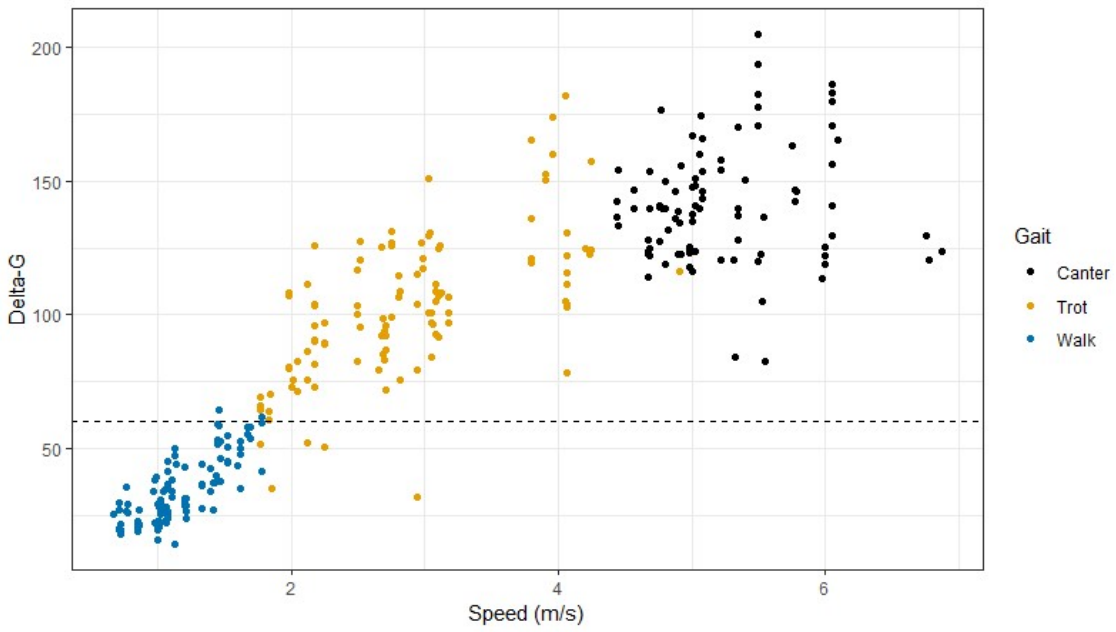

Figure 3.2: Plot of delta-G and speed with the distribution of the 3 gaits, walk, trot and canter. Data from 311 intervals of 7 Huntaway dogs. The dotted line depicts the cut-point of 60 delta-G_{10} 60, which was chosen as giving high confidence that values of delta-G_{10} greater than that, a dog could be confidently classified as having a gait faster than a walk.

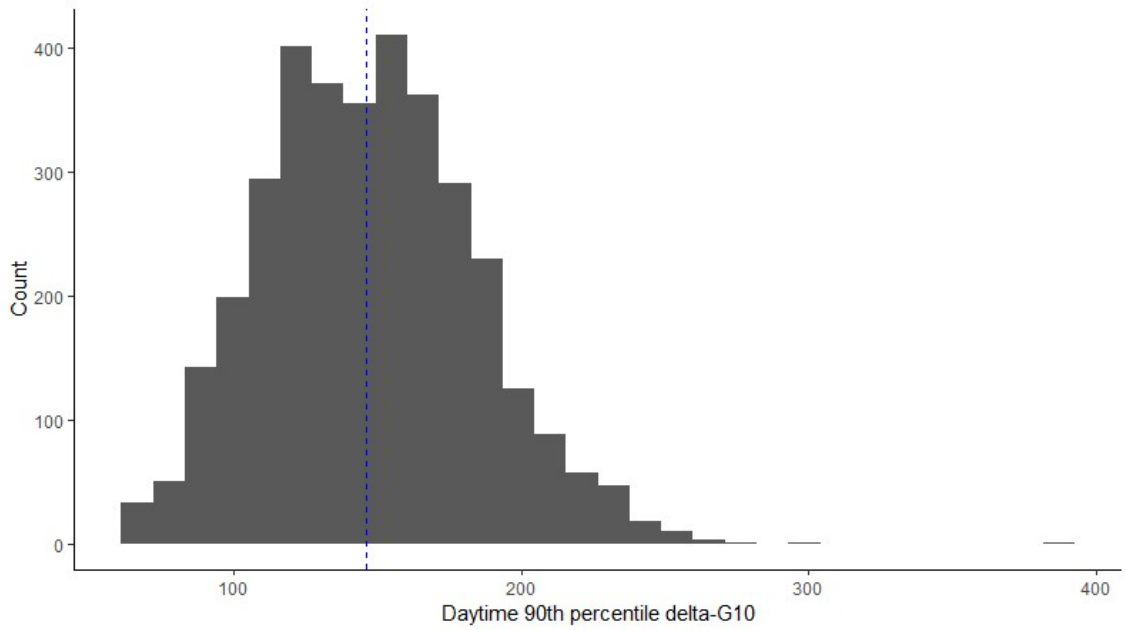

*Figure 3.3: Histogram of **daytime 90th percentile delta-G$_{10}$** for epochs greater than walking, with median marked in a blue dashed line (min= 61.5, max=382.3, mean=147.3, median=146.2, LQ=120.6, UQ=171.4).*

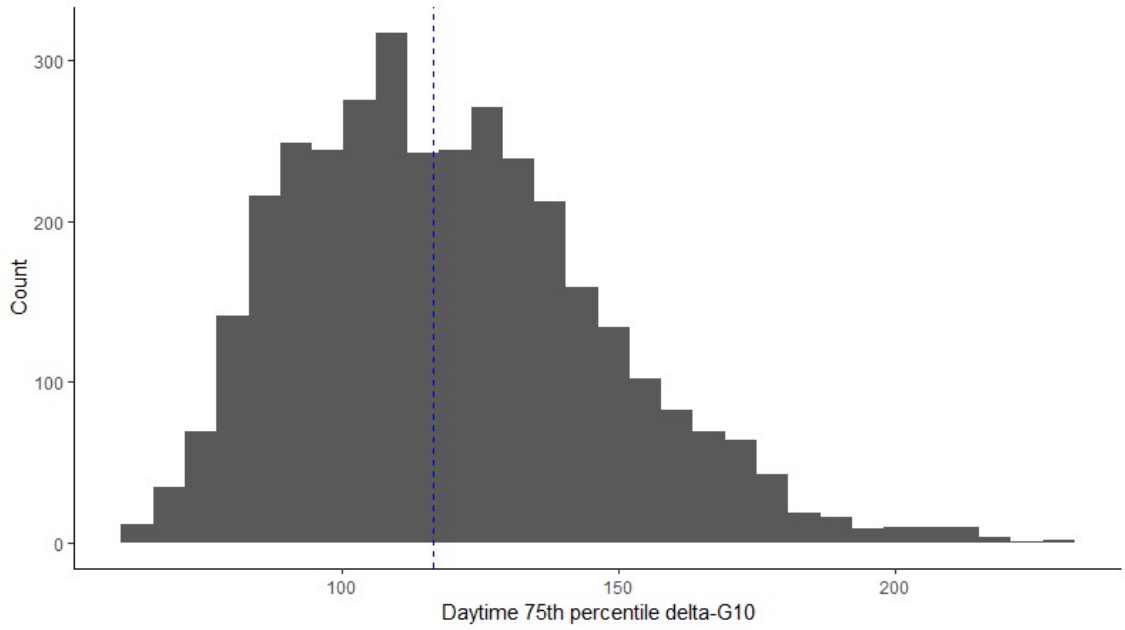

*Figure 3.4: Histogram of **daytime 75th percentile delta-G$_{10}$** for epochs greater than walking, with median marked in a blue dashed line (min= 61.4, max=227.8, mean=119.4, median=116.6, LQ=98.2, UQ=136.7).*

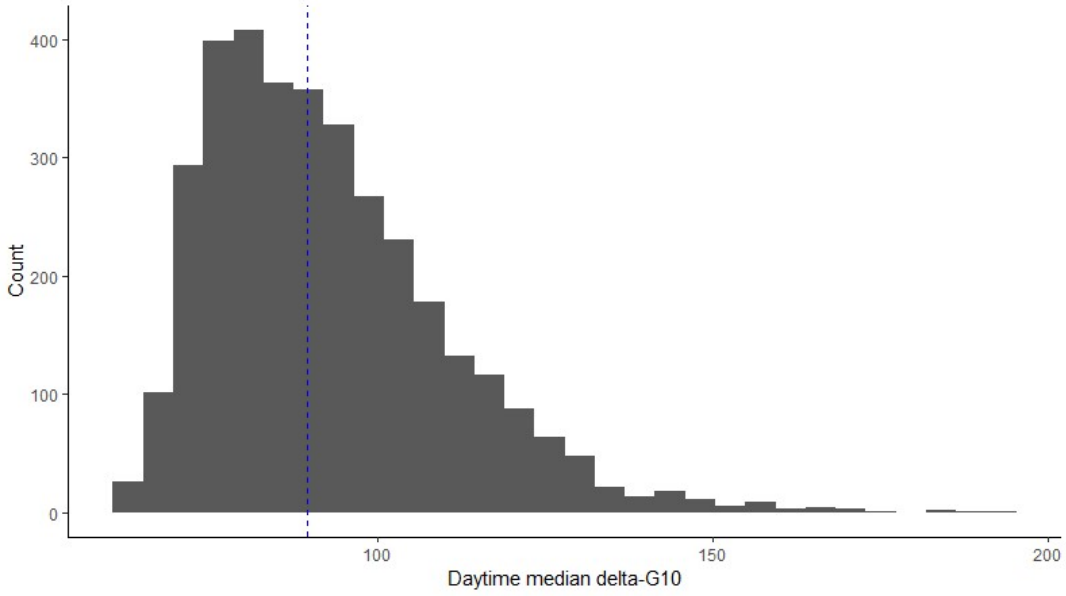

*Figure 3.5: Histogram of **daytime median delta-G_{10}** for epochs greater than 60 delta-G, with median marked* in a blue dashed line *(min= 61.13, max=191.31, mean=92.67, median=89.51, LQ=79.07, UQ=102.42).*

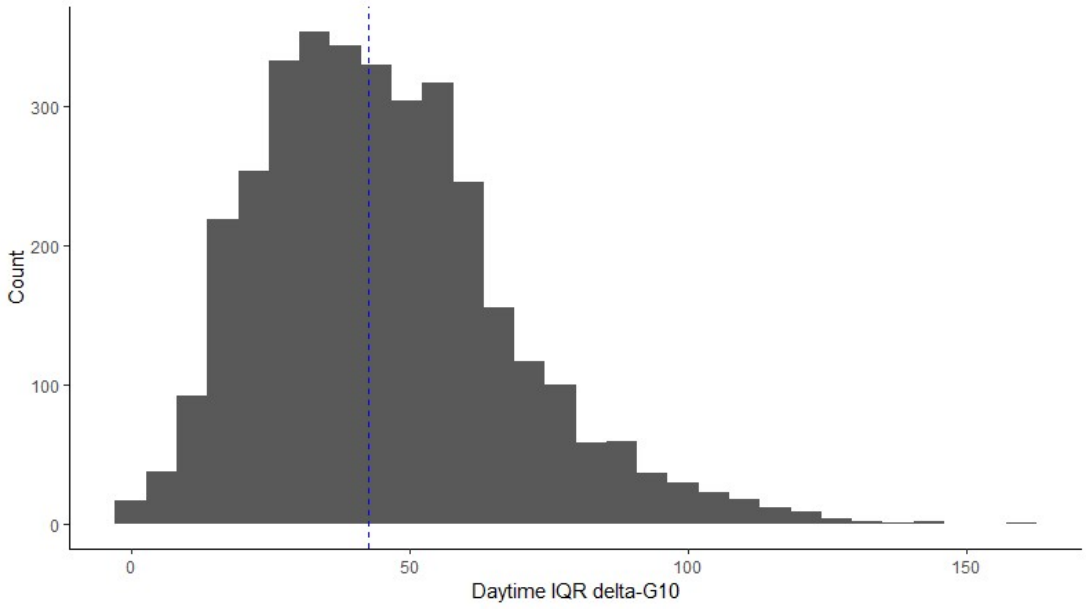

*Figure 3.6: Histogram of **daytime IQR in delta-G_{10}** for epochs greater than walking, with median marked* in a blue dashed line *(min= 0.02, max=159.89, mean=45.39, median=42.58, LQ=29.10, UQ=58.07).*

*Figure 3.7: Box-plot of **daytime 75th percentile delta-G$_{10}$** each day, by season. Based on 3500 days from 27 dogs (p-value < 0.001).*

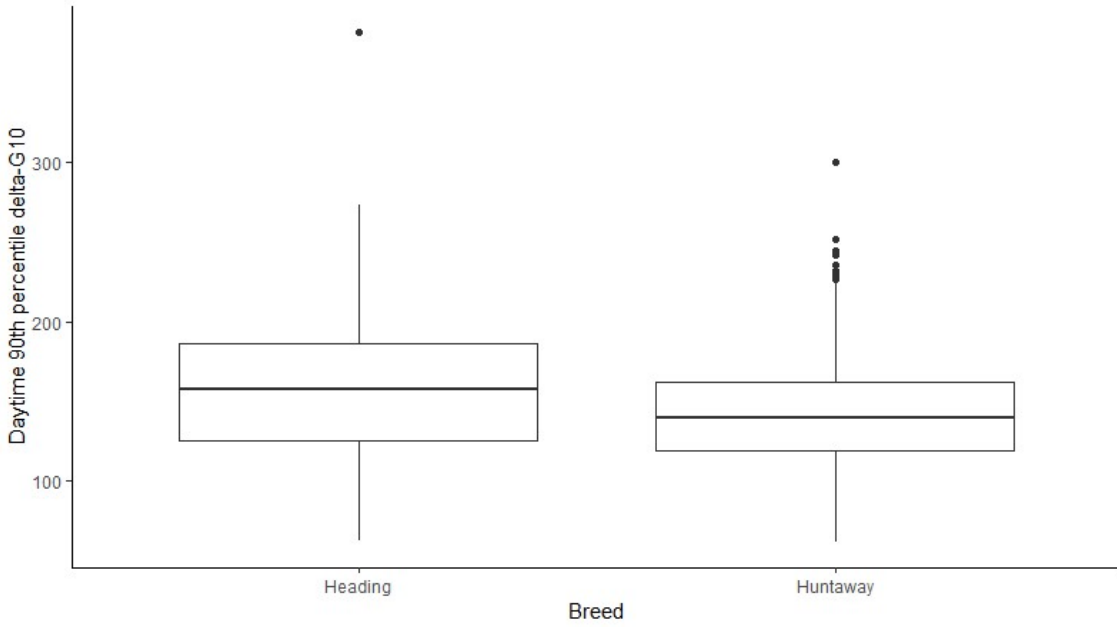

*Figure 3.8: Box-plot of **daytime 90th percentile delta-G$_{10}$** each day, by breed. Based on 3500 days from 27 dogs (p-value <0.001).*

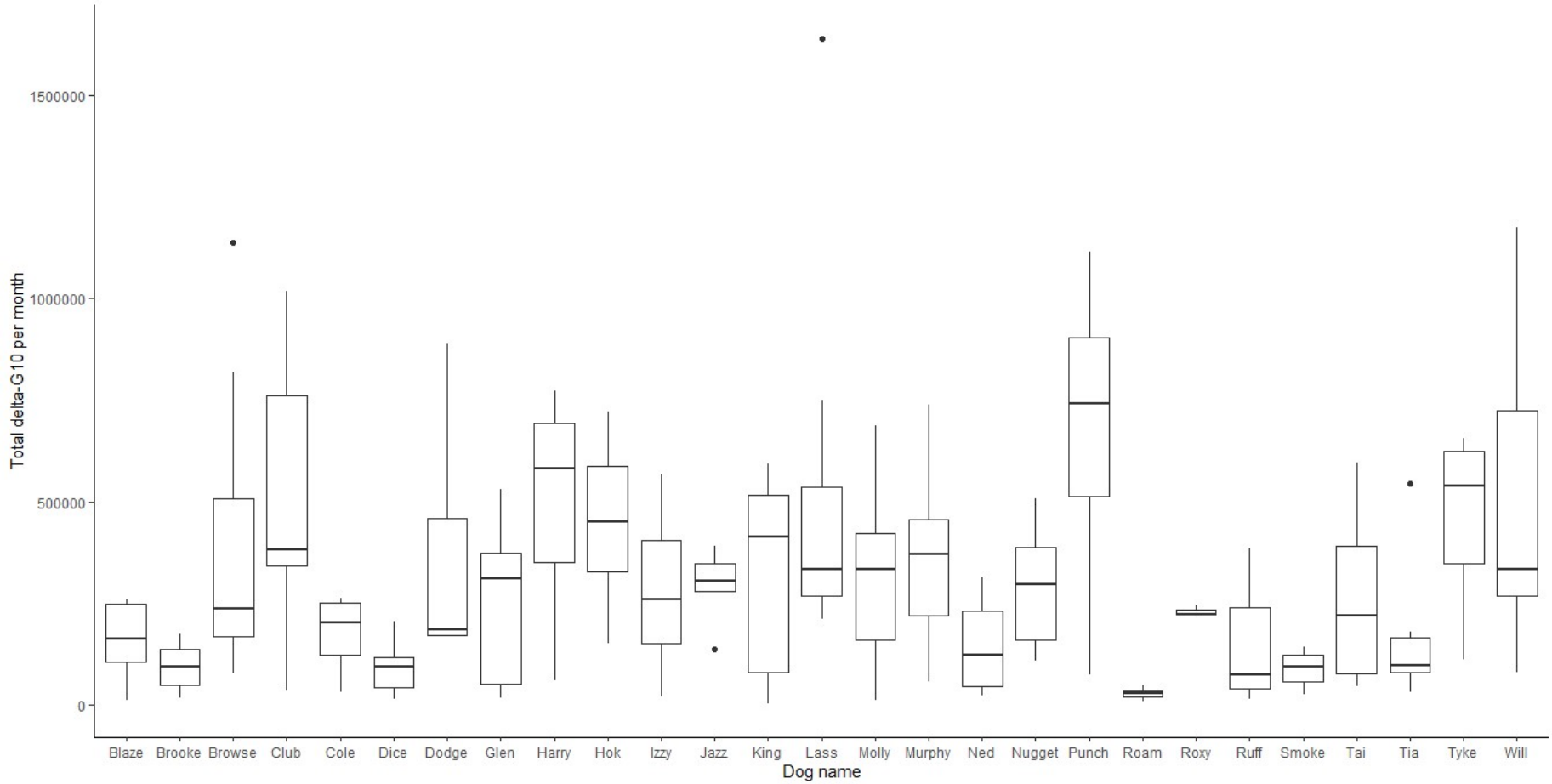

Figure 3.9: Box-plot of the monthly sum of delta-G10 for each of the 27 dogs over the course of the study. This includes the months on GLME treatment and placebo.

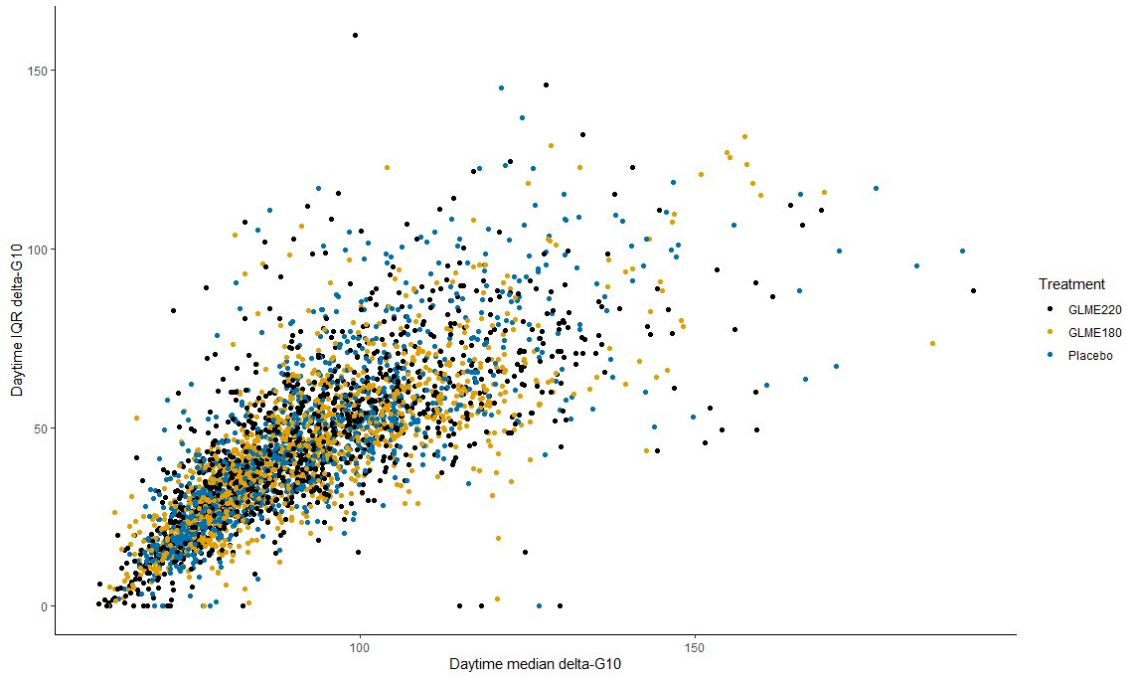

Figure 3.10: Scatterplot of daytime median delta-G_{10} and daytime IQR delta-G_{10}, coloured coded by treatment. There are 3500 days from 27 dogs.

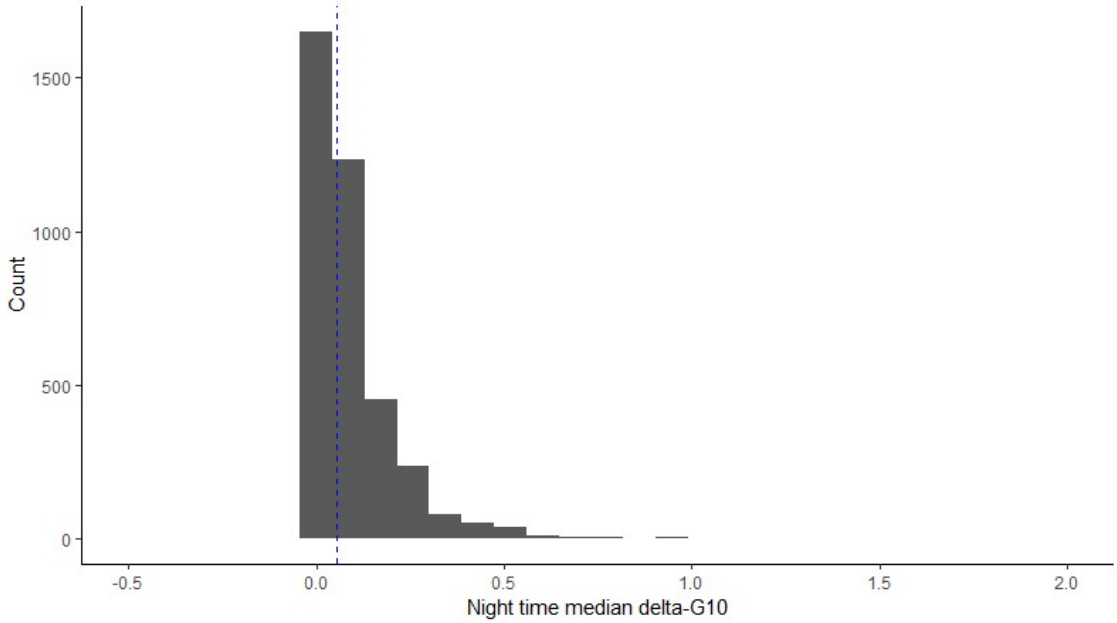

*Figure 3.11: Histograms of **night time median for delta-G_{10}** for all treatments, with the median marked in a blue dashed line (min=0, max=22.44, median=0.06, mean=0.10, LQ=0.01, UQ=0.13). There were 11 outlier median values removed from this graph.*

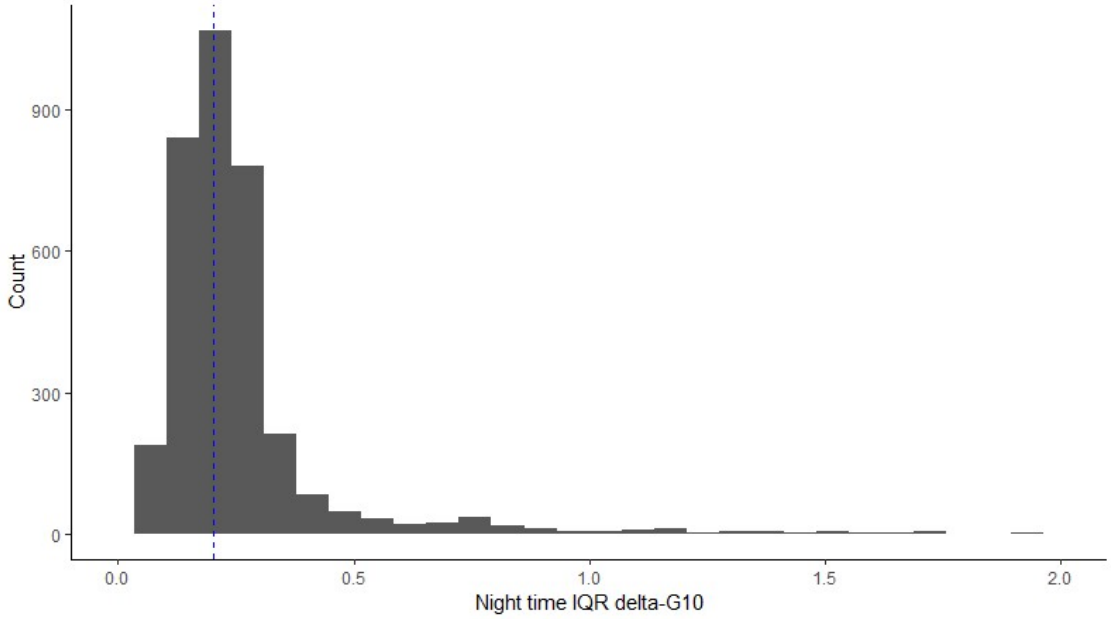

*Figure 3.12: Histogram of **night time IQR for delta-G_{10}** for all treatments with median marked in a blue dashed line (min=0, max=64.6, median=0.2, mean =0.33, LQ=0.16, UQ=0.27). There were 54 outlier IQR values removed from this graph.*

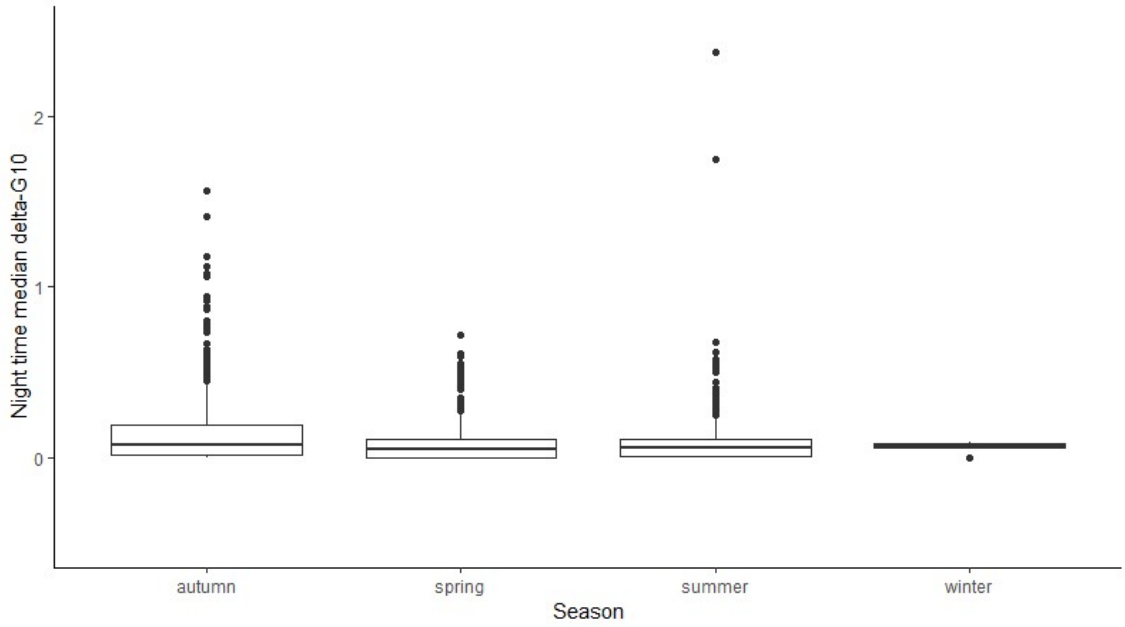

*Figure 3.13: Box-plot of n**ight time median delta-G$_{10}$** each day, by season. Based on 3,780 nights from 27 dogs. There have been four outliers removed for this graph (p-value = 0.042).*

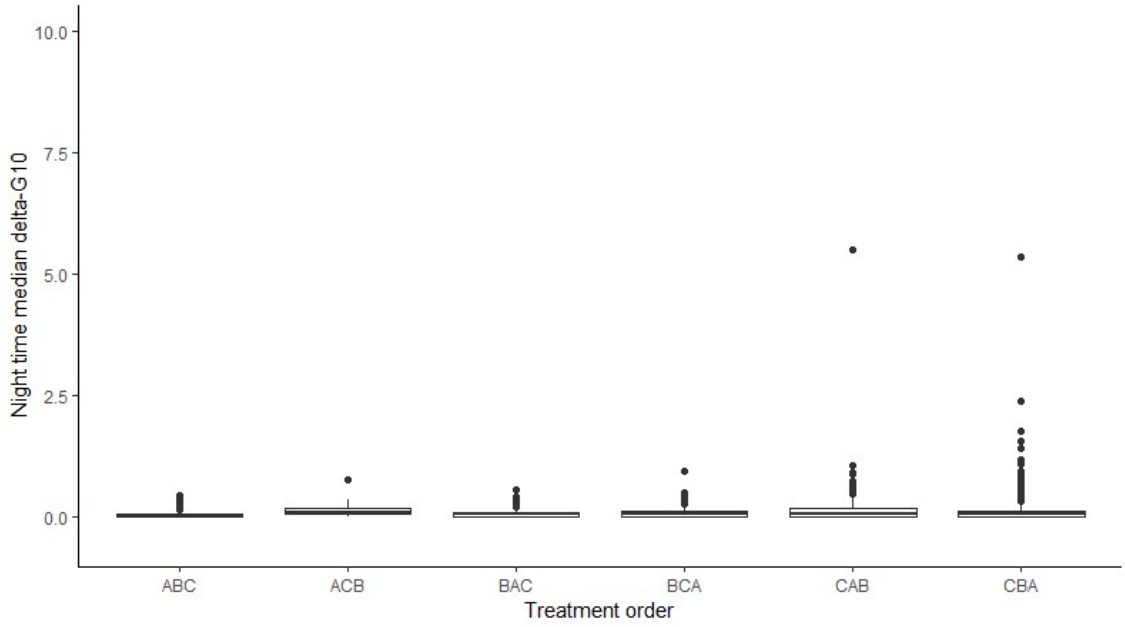

*Figure 3.14: Box-plot of n**ight time median delta-G$_{10}$** each day, by treatment order. Based on 3,780 nights from 27 dogs. There has been one outlier removed from this graph (p-value <0.001).*

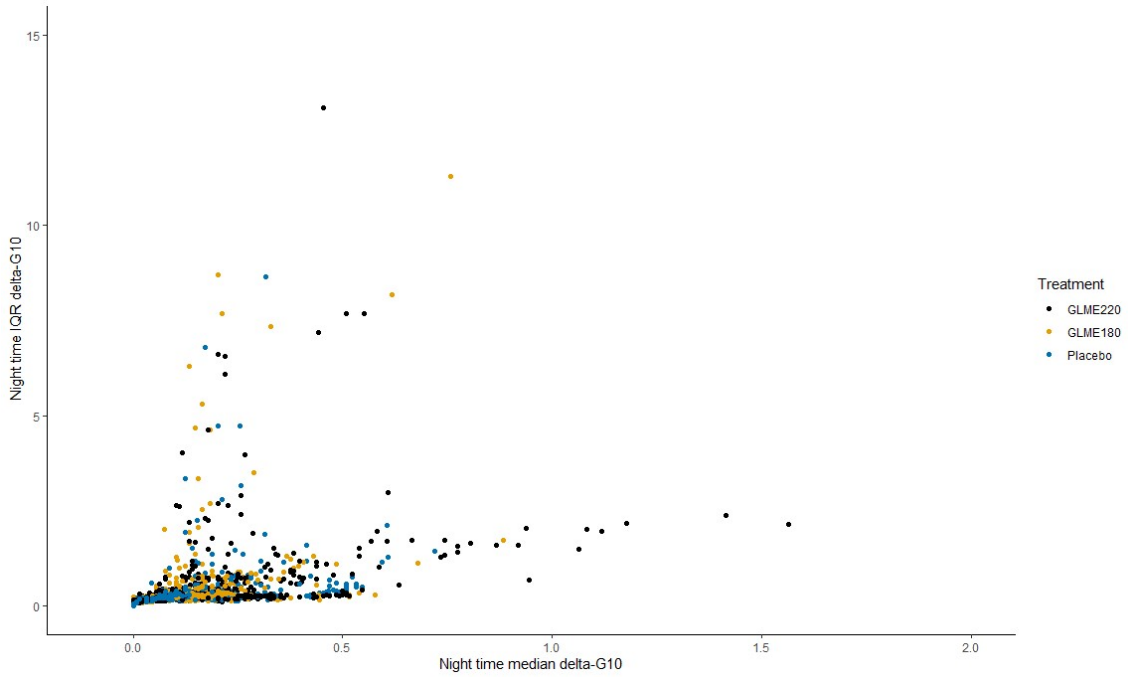

Figure 3.15: Scatterplot of night time median delta-G_{10} and night time IQR delta-G_{10}, coloured coded by treatment. There are 3,780 days from 27 dogs. There have been 5 outliers removed from this graph.

4 General discussion

4.1 Summary

Osteoarthritis (OA) is one of the most common musculoskeletal diseases reported in dogs, estimated to account for between 2.5% to 6.6% of veterinary visits for pet dogs (Anderson et al., 2018; O'Neill, Church, McGreevy, Thomson, & Brodbelt, 2014). A major feature of OA is the progressive breakdown of articular cartilage covering the articulating bones within a joint (Hutton, 1989). Once a joint is damaged and the articular cartilage is compromised, irreversible changes to the structure of the joint can occur which results in the common clinical signs of OA, including a reduced range of motion in the affected joint, stiffness and pain (Cooper, Javaid, & Arden, 2014). Joint disease causes dogs to modify their natural movement to compensate, due to pain or structural changes in the joint (Knazovicky, Tomas, Motsinger-Reif, & Lascelles, 2015; Tashman, Anderst, Kolowich, Havstad, & Arnoczky, 2004). Triaxial accelerometers can be used to model the acceleration experienced by a dog during movement. By quantifying the acceleration of a dog, accelerometry can be used to characterise movement and gait. This technology could be useful for the evaluation of joint disease treatment efficacy through the objective quantification of activity in dogs on and off treatment.

Green-lipped mussels are a popular nutraceutical for the management of OA in dogs. The lipid fraction of green-lipped mussels is comprised of the key fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), along with novel omega-3 polyunsaturated fatty acids, and furan fatty acids. These fatty acids have been strongly linked to inflammation reduction and resolution, and subjectively associated with a reduction in clinical signs of OA in dogs (Halpern, 2000; Mehler, May, King, Harris, & Shah, 2016; Treschow et al., 2007; Ulbricht et al., 2009; Wakimoto et al., 2011). The aim of this thesis was to evaluate the application of accelerometry technology for detecting an effect of a GLME on the activity of dogs with joint disease.

Chapter Two describes the attempt to characterise the gait of dogs in a controlled environment, on a treadmill with set speeds for each 10 second epoch. The results of that study showed that there was a delta-G threshold above which dogs would be reliably characterised as gaiting faster than a walk on a treadmill. While it was possible to identify a change in speed, it was not possible to accurately predict speed using the accelerometry system. Chapter Three describes a randomised, placebo-controlled trial of a green-lipped mussel extract in free-living New Zealand working farm dogs with joint disease. By applying the delta-G threshold established in Chapter Two, in addition to novel outcome variables obtained from the accelerometry data,

accelerometry was able to detect small, but significant effects of treatment. However, the technology was challenging to utilise in that population. Nonetheless, it was possible to conclude that treatment with GLME increased peak activity in working farm dogs with signs of joint disease. This may be a result of the GLME decreasing the clinical signs of joint disease, so the dogs are able to move at a greater intensity when carrying out high intensity activity. There was also a slight increase in night-time activity, which was not intuitive and opposite to the hypothesis of the study. This increase in night-time activity remains unexplained, however it could be due to an improvement in clinical signs of joint disease enabling dogs to move more freely during the night. Therefore, GLME could enhance the performance of dogs with mild joint disease. As a population, New Zealand working farm dogs have a high prevalence of joint disease but there were significant problems and limitations that question the suitability of this population for further similar studies. Overall, the research presented in this thesis suggests that farm dogs with signs of joint disease might benefit from treatment with the GLME used here, even when they are mildly affected.

4.2 Future directions

The data obtained in the first study predicting the gait and speed of dogs on the treadmill, did not produce a clear cut-point in the delta-G that enabled reliable differentiation between trotting and cantering. However, for three of the seven dogs used in this study, there was some distinction between the delta-G values for their trotting and cantering trials that may have been clear enough to develop an acceptably accurate delta-G cut-point between a walk, trot and canter on an individual basis. The inability to identify a trotting-cantering cut-point in this study was compounded by the low number of trials at faster speeds and gaits. Any future attempt would have to include more trials that included the point at which the dog transitioned from trotting to cantering transition, to increase the likelihood of establishing a useful delta-G cut-off. Such research could possibly increase the accuracy of speed prediction, which was poor at the higher speeds. Additionally, the accelerometer 10 second epochs are set with a coordinated universal time system, as opposed to a start-stop system that could be easily matched the time of a trial. Therefore, the trials should be timed to match the accelerometer epochs more closely than what was achieved during this study to ensure each trial delta-G is in encompassed within a single epoch.

During the treadmill study, the trial data from all seven dogs was collated. Both the cut-point for gait transition, and speed estimation were completed with this pooled dataset, as opposed to on an individual dog basis. When looking into the data from each dog it was clear that there

were dog-specific trends in terms of the delta-G value that a dog transitioned between gaits or moved at a certain speed. Future studies would benefit from the use of individual cut-points for dogs, which would improve the accuracy of predictions for both speed and gait.

The second study evaluated the efficacy of a GLME treatment for New Zealand working dogs with joint disease, using accelerometry as the outcome measure. The general New Zealand working dog population is ideal for obtaining dogs with untreated joint disease, as demonstrated by only 5 of the 27 dogs being regularly treated for their joint disease prior to participation in this study. There were concerns that as these dogs are working dogs, there would not be an identifiable effect of treatment on activity as they would be carrying out the activity required of them - even in discomfort - at their owner's command. However, the results of this study indicate that this is not the case. While a working dog remains active, as shown by the lack of a difference in median activity, the upper 75th and 90th percentiles of activity were higher during treatment with GLME, indicating the dog may be able to move at greater intensity or speed. Overall then, it appeared that GLME improved the activity of working dogs with joint disease.

Despite the suitability of the dogs for this study, there were a number of issues with the accelerometry system used. Future studies would benefit greatly from careful consideration of the brand and set-up design used. The technology used in this study had a number of benefits over other brands, including a long battery life, seven-day memory storage, and local support from the company for researchers. However, its ability to upload stored data from the accelerometer to the receiver was repeatedly compromised over the course of the study without clear causes. Other accelerometers without a memory would be very unsuited in this environment as they would need to be within range of a receiver at all times, which is impractical for working dog use. However, a continually uploading system would have the benefit of avoiding corruptions of epochs through incorrect timestamping, and duplicates, which were issues in this study. Other accelerometers have a much shorter battery life compared with the Heyrex® sensor, and usually require charging overnight or weekly. Initially this seems to be impractical for two main reasons, these dog owners are very busy, and this requirement would likely become tedious over a long term, 8-month study such as this, and this would remove the possibility for the collection of night time data. However, as a number of owners in this study were required to take their accelerometers in to the receiver overnight once a week anyway, this may not be an entirely impractical option. Additionally, a more stringent owner selection would have been of benefit in this study in terms of the proximity of the kennels to the receiver, and the quality of the connection to the internet. This would have reduced the input required

from the owners dramatically and would have definitely improved the quality and quantity of the data collected.

4.3 Review of study design

For the study that evaluated the efficacy of a GLME treatment for New Zealand working dogs with joint disease using accelerometry, the study design was robust, lending a high confidence in the results. The study was double-blinded, with both the owners and the primary researcher being blinded to the treatments for the entire duration of the data collection phase. Each dog was trialled on both the GLME treatments, and the placebo, and there were six different orders of treatment. This allowed them to act as their own control, which was important as there was significant between-dog variation, and comparison of activity between dogs on different treatments would have required a much larger number of dogs participating to identify a treatment effect. The different treatment orders minimised confounding of the results due the variation in activity of dogs between seasons, which was known to vary significantly *a priori*, and was confirmed in this study, with season consistently being significantly associated with the delta-G_{10} response variables for both the daytime and night time.

The likelihood of the association between treatment and the delta-G_{10} response variables being by chance for the IQR variables is very low, as their associated p-values were less than 0.001. For the 90^{th} percentile, with a p-value of 0.03 there is a 3% chance that the association was a random chance finding. Admittedly, the understanding around the significance of an association between the IQR and treatment is limited and therefore the interpretation of what the effect size of the treatment on the IQR means in respect to a change in activity of a dog is difficult. However, the effect size of the treatment $GLME_{220}$ on the 90^{th} percentile of daytime delta-G_{10} greater than walking, when applied to the data collected from the study in chapter two, was consistent with a small but notable increase in speed. This effect could be said to be performance enhancing in dogs with joint disease.

The dose of GLME trialled in this study was low compared to other studies of the effectiveness of GLME for the treatment of joint disease in dogs (Bui & Bierer, 2001; Dobenecker, Beetz, & Kienzle, 2002; Pollard, Guilford, Ankenbauer-Perkins, & Hedderley, 2006; Rialland et al., 2012; Servet, Biourge, & Marniquet, 2006). Although most studies investigating the effects of GLME on the joint disease of dogs found a reduction in clinical signs, the dose closest to that used in this study was unable to find an effect of treatment on joint disease (Dobenecker et al., 2002). However, there has not been any dedicated study into the minimum dose required for a GLME to be efficacious. It has also been noted that not all GLME products are equal, with differences

in the quality of the mussels, the quantity of the lipid fraction, and the content of the efficacious lipids themselves (Halliday, 2008; Ulbricht et al., 2009). The quality of this product could be high compared to other products, resulting in its efficacy at a comparably low dose. In this study, the weight of dogs ranged from 18kg to 38.6kg. This meant the at the higher dose, $GLME_{220}$, the dogs received a minimum dose of 5.7mg/kg and a maximum dose of 12.2mg/kg. At the lower dose, $GLME_{180}$, they received a minimum dose of 4.7mg/kg and a maximum dose of 10mg/kg. Even at these low doses, GLME appears to improve the quality of life of dogs with joint disease.

4.4 References

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