Use of triaxial accelerometers and machine learning algorithms for behavioural identification to assess the effectiveness of a joint supplement in old domestic cats (*Felis catus*).

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Synopsis

Osteoarthrosis has a 90% prevalence rate in older cats and adversely affects quality of life. Long-term administration of non-steroidal anti-inflammatory drugs is usually required, but can have adverse effects such as vomiting, diarrhoea and kidney failure. Nutraceuticals have been shown to relieve joint pain in cats without such side effects, however, due to differences in dose rates and formulations there is no consensus within the academic community as to their efficacy. Most studies have utilised subjective measures such as veterinary or owner-assessed changes in activity, which are not very sensitive. Accelerometers can provide objective data on cat activity and have recently been shown to identify specific behaviours (Smit et al., 2023).

In the present study, triaxial accelerometers (ActiGraph®, Pensacola, FL, USA) were fitted to the collars of the cats for 20 weeks to assess activity and behaviour. Two groups (n=8 per group) of old cats (aged 11-16 years; Massey University, Palmerston North, NZ) were fed the same diet (Chef: Kraft Heinz Wattie's Ltd., Hastings, NZ) for four weeks to collect baseline acceleration data. The 16 cats selected for the main study were then divided into two groups balanced for physical activity. The test group received a joint supplement added to the baseline diet while the control group were fed the diet unsupplemented for 16 weeks. The commercial joint supplement contained glucosamine, chondroitin sulphate, curcumin and green-lipped mussel (Tandem, Wellington, NZ). The aim was to assess the effectiveness of the joint supplement in maintaining or promoting activity and specific behaviours in the cats.

Overall physical activity was not different between groups at baseline but was higher over the study in the test group compared to the control animals ($P<0.03$). Grooming, as identified by the algorithm, showed a tendency ($P=0.051$) to be higher in the test group than in the control. These results suggest that the supplement had a positive effect on both overall activity and more specifically on grooming behaviour in old cats.

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1.1 Introduction

Degenerative joint disease (DJD) is a blanket term used to describe a number of diseases, such as osteoarthritis (OA) and spondylosis deformans (SD), which cause chronic inflammation of the joint due to either progressive degeneration of articular cartilage (OA) or bony growths (SD) (Kimura et al., 2020). In cats over 12 years of age, a 90% prevalence of DJD has been reported, and in cats over six years of age, 61% have OA in at least one joint (Bennett et al., 2012a). Both OA and SD cause joint pain, which generally reduces the physical activity level of cats and adversely affects their welfare (Bennett et al., 2012a, 2012b).

The most common treatments for DJD are non-steroidal anti-inflammatory drugs (NSAIDs), but these can have adverse effects such as vomiting, diarrhoea and kidney failure. Dietary joint supplements are becomingly increasingly common to improve the mobility and quality of life of cats with DJD (Corbee et al., 2013; Khan & McLean, 2012; Rosenbaum et al., 2010). The effectiveness and content of various active ingredients in joint supplements have not yet been standardized, and the active ingredients in various joint supplements are still being researched (Johnson et al., 2020). Another challenge is the methods used to assess the efficacy of such supplements, with researchers commonly using subjective-based measures of physical activity levels and mobility. In the past, most assessment tools have been presented in the form of questionnaires, which has led to results that are heavily influenced by subjectivity and often associated with owner-based biases (Monteiro & Steagall, 2019). Technologies such as accelerometry are increasingly being used to monitor overall physical activity (Lascelles et al., 2007; Monteiro & Steagall, 2019; Rosenbaum et al., 2010), and more recently, behaviour of animals (Smit et al., 2023). However, there are many important considerations when implementing such technologies to assess the effectiveness of dietary supplements for DJD.

This review focuses on the common dietary supplements currently marketed for cats with DJD, including fish oil and chondroitin sulphate, and discusses the need for a combination of ingredients. In addition, the review will evaluate the tools available for monitoring the efficacy of joint supplements for use in companion animals with DJD.

1.2 Prevalence of degenerative joint disease (DJD)

The incidence of DJD is high in cats 11 years of age or older (Dowgray & Comerford, 2020). The prevalence in appendicular joints alone can be as high as 90% (Kerwin, 2010). In two prospective cross-sectional studies in cats, the prevalence of DJD reported by Slingerland et al. (2011) was 61% of 100 cats, whereas Kimura et al. (2020) reported 74% of 101 cats. It is worth noting that many studies report that it is a reduction in the mobility or physical activity as observed by the owner, not lameness, that leads to veterinary check and resulting diagnosis of DJD (Bennett et al., 2012a; Bonecka et al., 2023; Clarke et al., 2005; Kimura et al., 2020). It has also been shown that radiographic findings in cats with DJD do not match orthopaedic findings, with 34% of orthopaedic procedures in joints thought to be painful during manipulation not having any radiographic signs of DJD (Lascelles et al., 2012). This means that early onset DJD is often undiagnosed in cats. Moreover, the true prevalence of DJD is likely to be underestimated in cats as recognising pain in cats is difficult (Bennett et al., 2012a), and a wide range of joints can be affected by the disease. Many cats aged 10 years or older have been found to have DJD in multiple joints, with the shoulder, hip, elbow, knee, and ankle joints most commonly affected (Deabold et al., 2023). The lumbosacral spine is also a common site of DJD in cats, with reports of between 60-90% of cats over the age of 10 years showing evidence of DJD most commonly in the thoracic, lumbar, or sacral regions of the spine (Lascelles et al., 2020).

In cats, the prevalence of DJD is also correlated with breed, with hip abnormalities shown to be higher in breeds, such as Maine Coon, Persian and Siamese (Bonecka et al., 2023). Abyssinian and Devon Rex cats are more prone to patellar dislocation and thus secondary DJD, and in Scottish fold cats, severe arthritis is likely to affect multiple joints due to abnormal cartilage development in the breed (Bonecka et al., 2023; Clarke et al., 2005).

1.3 Pathology of DJD

Degenerative joint disease is a complex multifactorial disease resulting from damage to the musculoskeletal system that includes OA and SD (Kimura et al., 2020). Osteoarthritis is a pathognomonic change of synovial type II joint lesions characterized by degeneration of articular cartilage, bone spur formation, bone remodelling, changes in adjacent tissues, and varying degrees of low-grade, nonsuppurative inflammation (Clarke et al., 2005). When this inflammation involves the cartilaginous joints of the spine, it becomes deformational spondylosis. In metaplastic spondylosis, degeneration is usually due to disc degeneration resulting in narrowing of the intervertebral space, endplate sclerosis, and bone spur formation (Clarke et al., 2005). While degenerative joint disease and OA are often used interchangeably, in truth OA is a type of DJD, albeit the most common type in companion animals (Clarke et al., 2005).

The inflammation and joint changes associated with DJD often result in chronic pain, which affects the quality of life of the cat (Benito et al., 2012). The major factors contributing to destructive and pathological conditions include cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE_2), interleukin-1β (IL-1β), matrix metalloproteinase-13 (MMP-13), inducible nitric oxide synthase (iNOS), tumour necrosis factor (TNF-α) and nuclear factor kappaB (NF-kB) (Fox, 2016). Of these, IL-1β and TNF-α are thought to be the main inflammatory cytokines mediating the pathogenesis of DJD (Gruen et al., 2017). Abnormally high levels of IL-1β and TNF-α and IL-6, three pro-inflammatory cytokines metabolised in articular cartilage (as shown in Figure 1-1), are frequently detected in synovial fluid, synovium and cartilage tissues of DJD patients. Although the exact mechanism is not clear, elevated levels of these markers are thought to be an important factor in cartilage loss in subjects (Wang & He, 2018).

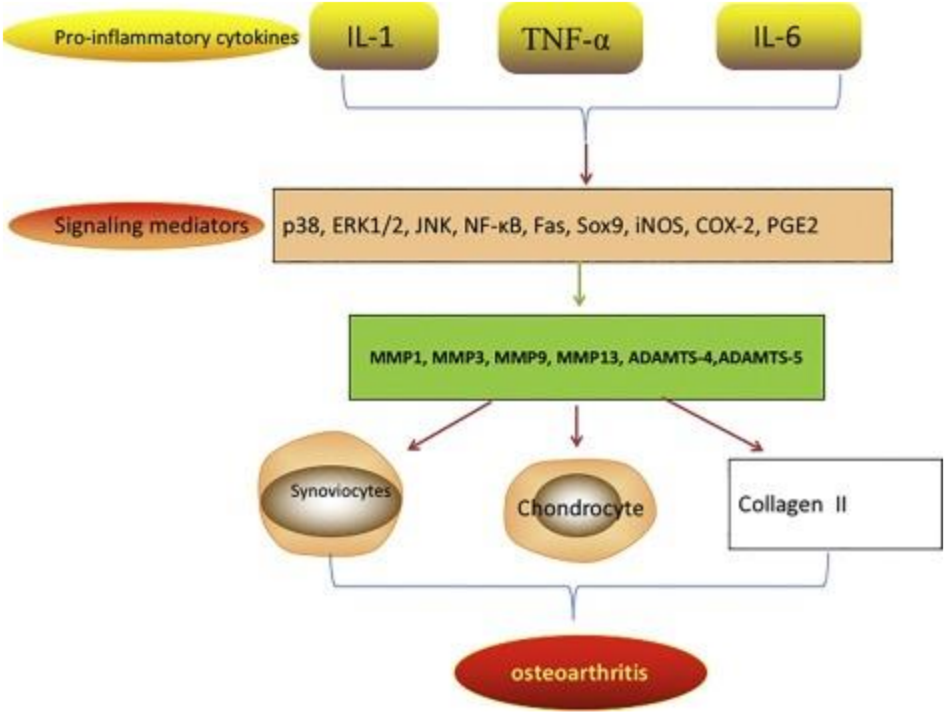


Figure 1-1 The effects of IL-1, TNF-α, and IL-6 on articular cartilage during OA (DJD). (Reproduced from Wang & He, 2018).

In addition, chronic inflammatory states of the bone and joint can also lead to an increase in matrix metalloproteinases (MMPs), which are commonly found in almost all inflammatory states. MMPs are a family of extracellular proteases responsible for the degradation of collagen articular cartilage in DJD. Matrix metalloproteinases, particularly MMP-1 and MMP-13, cause irreversible damage to the cartilage matrix by digesting type 2 collagen and subsequently releasing matrix proteoglycans from the cartilage (Hansen et al., 2008). The destroyed cartilage matrix leads to further release of MMPs, and ultimately initiates a cycle of permanent cartilage destruction and inflammation (Ansari et al., 2020; Gruen et al., 2017; Lepetsos & Papavassiliou, 2016). Therefore, reducing the chronic inflammatory state not only helps to alleviate the chronic pain associated with DJD, but can also reduce the further progression of DJD by breaking this cycle.

In cats, the cause of DJD is not clear and likely multifactorial, and it is often associated with age (Slingerland et al., 2011), breed (Clarke et al., 2005), and live weight (Bonecka et al., 2023). There is a positive correlation between the onset of DJD and age in cats (Bonecka et al., 2023;

Lascelles, 2010). As cats age, the cartilage that supports the joint degenerates and is worn away, leading to persistent damage within and around the joint resulting in an increase in inflammatory substances and arthritis (Slingerland et al., 2011).

Obesity also contributes to both the symptoms and progression of DJD, and numerous studies have shown that cats and dogs with a body condition score (BCS) $\geq 7/9$ are more likely to develop inflammation and DJD than non-obese cats and dogs (Johnson et al., 2020). The reason for this is twofold, firstly weight gain leads to increased load on the joints, thus increasing mechanical damage to the joints (Thijssen et al., 2015). Secondly, obesity leads to the development of chronic inflammation, which increases reactive oxygen species, thereby triggering oxidative stress and thus potentially irreversible cellular damage (Martins et al., 2023). Lipocalins in adipose tissue are thought to affect joint health, and cartilage degeneration occurs when functional lipocalin receptors are expressed in human and mouse chondrocytes (Francisco et al., 2020). In addition, adipocytes also produce leptin, a peptide hormone with circulatory concentrations that directly reflect the amount of adipose tissue present in the body (Wang & He, 2018). Studies in a mouse model have shown that leptin may exert a catabolic effect on normal cartilage *in vivo*, implying that the increase in leptin associated with obesity and excess bodyweight has a detrimental effect on joint heath (Bao et al., 2010). Therefore, weight loss is probably the most common non-pharmacological approach for managing and slowing the development of DJD in companion animals.

1.4 Management and prevention of DJD in cats

Since DJD is difficult to cure, prevention and pain management are the focus of DJD treatment (Monteiro, 2020). As previously mentioned, obesity is a high risk factor for DJD, so weight loss is one of the most important modalities for the prevention and management of DJD (McLaughlin, 2000). As for the pain caused by DJD, NSAIDs are widely used, although the long-term use of such drugs may adversely affect liver, kidney, and gastrointestinal tract function (McLaughlin, 2000). Another promising avenue is the use of dietary supplements (i.e., nutraceuticals), which are better suited for long-term use and safer when compared to NSAIDs.

A number of ingredients that have been shown to improve DJD, but the effectiveness of many of these ingredients is still being studied for cats (Johnson et al., 2020). This section will evaluate these treatment approaches in turn.

1.4.1 Weight loss

Obesity has been shown to be a risk factor for DJD in cats (Johnson et al., 2020). Controlling the body weight of animals, therefore, can be slow the progression of DJD. In dogs, Impellizeri et al. (2000) reported that weight loss of approximately 11-18% reduced lameness in dogs with painful DJD with concurrent improvement in mobility. In cats, Lascelles et al. (2010) suggested weight loss may be associated with improved activity counts using accelerometers, however there are no specific data to support this, and further research is needed. Weight loss is thought to reduce the load on the joint, thereby reducing pain and promoting increased activity (Monteiro, 2020). Overall, several reviews have concluded that weight management is important for cats with DJD, but the relationship between body weight and DJD in cats needs to be further investigated (Kerwin, 2010; Monteiro, 2020; Monteiro & Steagall, 2019).

1.4.2 Anti-inflammatory drugs: Non-steroidal anti-inflammatory and analgesic drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are the medication of choice for use in veterinary medicine for the treatment of DJD (White & Morrow, 2020). Clinically, commonly used NSAIDs used for the treatment of DJD include meloxicam and ropecoxib (Rychel, 2010). These drugs are non-selective COX inhibitors and prevent inflammation by inhibiting the production of PGE_2, a key hormone that stimulates the production of proinflammatory cytokines (Wang & He, 2018). Long-term use of NSAIDs, however, can cause gastrointestinal ulcers and both liver and kidney damage (McLaughlin, 2000). In particular, older cats often have some degree of underlying renal insufficiency even before receiving NSAID treatment (Brown et al., 2016), so close monitoring of the animal's tolerance to the drug is required when using NSAIDs (Rychel, 2010).

Analgesics are a common option for animals that cannot tolerate NSAID administration, or that have severe liver, kidney, or gastrointestinal disorders (McLaughlin, 2000). Gabapentin and tramadol are used clinically to relieve pain caused by DJD, respectively, and both drugs have been shown to be effective (Rychel, 2010). However, gabapentin causes side effects such as sedation, ataxia, drowsiness, weakness, and muscle tremor (Guedes et al., 2018). Tramadol is a synthetic opioid with multiple mechanisms of action that works by interacting with opioid, serotonin and adrenergic receptors. In addition to some hepatotoxicity in cats, side effects such as sedation, pupil dilation and euphoria have been observed in cats with DJD receiving tramadol (Guedes et al., 2018; Monteiro et al., 2017). As a result, non-pharmacological treatments are increasingly becoming the preferred treatment for DJD in companion animals (Kerwin, 2010; McLaughlin, 2000; Monteiro, 2020; Rychel, 2010).

1.4.3 Dietary supplementation

The use of dietary joint supplements to alleviate and prevent pain due to DJD in animals has become a common method for veterinarians to use in the treatment of osteoarthropathies (Rychel, 2010). Several ingredients included in dietary supplements (e.g., green-lipped mussel (GLM), fish oils, glucosamine sulphate (Glu), and chondroitin sulphate (CS)) have been shown to be effective in ameliorating the symptoms of DJD (McLaughlin, 2000; Monteiro, 2020). A number of other ingredients such as curcumin (e.g., from turmeric), blackcurrant leaf extract, and green tea polyphenols have also been used to alleviate joint pain in companion animals (Corbee, 2022).

1.4.3.1 Fatty acid supplementation

Fatty acids are one of the most widely used supplements to support animals with DJD (Johnson et al., 2020). The omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are active ingredients in many joint support products (Lenox & Bauer, 2013). The two most common ingredients rich in these substances are GLM extract and fish oil (Beale, 2004).

Servet et al. (2006) studied 85 dogs with joint disease which were offered their original diet supplemented with 0.3% powdered freeze dried GLM. After 50 days of supplementation, the dogs' visual mobility impairment scores, joint manipulation impairment scores and summed total arthritis score (TAS) were reduced by between 33 and 36%, with most dog owners and vets regarding this supplement as 'efficient' or 'efficacious'. Rialland et al. (2013) found that the provision of a diet supplemented with GLM led to an increase in plasma omega-3 fatty acid concentrations and an improvement in peak vertical force during walking in dogs, suggesting that the GLM diet has a beneficial effect on gait function. In cats, there are few studies of GLM with only one mixed dog and cat study. Corbee (2022) showed that adult cats with mild or moderate osteoarthritis and who were otherwise healthy showed improvements in grooming, physical activity, play, and stair climbing with GLM supplementation. In their study, however, the supplement contained of a combination of GLM, curcumin (*Curcuma longa*) and black currant (*Ribes nigrum*) leaf extract, and due to a lack of data on the individual components used, it is difficult to determine whether the effect was due to the GLM or the combined effect the substances (Corbee, 2022). Interestingly, despite the limited experimental data on cats, GLM has been suggested as a source of omega-3 supplementation in various treatments and pain management for DJD (Kerwin, 2010; McLaughlin, 2000; Monteiro, 2020; Monteiro & Steagall, 2019; Rychel, 2010). However, it is important to note that this recommendation may have been made with insufficient evidence to fully support its efficacy. In the future, more trials should focus on the effects of GLM on arthritis in cats.

In comparison to GLM, fish oil is more widely used and therefore has more supporting data. Studies have shown that omega-3 fatty acids can alter the fatty acid composition of cell membranes (Harris & Bulchandani, 2006; Stillwell et al., 2006). This alteration leads to competition between EPA and arachidonic acid (AA) for cyclooxygenase. EPA promotes anti-inflammatory mediators (i.e., leukotriene B_5, prostaglandin E_3, and thromboxane 3 series), while AA promotes pro-inflammatory mediators (i.e., leukotriene B_4, prostaglandin E_2, and thromboxane 2 series) (Corbee et al., 2013). In mice, supplementation with 0.14%-0.7% dietary fish oil has been shown to increase anti-inflammatory mediators, decrease the production of matrix metalloproteinases (MMPs) and increase the production of tissue inhibitors of MMPs (Kavazos et al., 2015). These data suggest that dietary fish oils have the potential to decrease inflammation in companion animals, indeed, the provision of dietary fish oils has been shown to decrease markers of DJD (e.g., pro-MMP-9) in both cats and dogs (Corbee et al., 2013; Hansen et al., 2007).

Corbee et al. (2013) also showed that treating cats with oral liquid fish oil for up to 10 weeks at a dose of 200µl of fish oil per kg of bodyweight was linked to improvement in various behaviours using an owner-based questionnaire. The results showed that compared to the untreated control group, cats consuming fish oil showed significant improvements in gait stiffness and jumping levels (p=0.03), as well as a trend towards improvements in activity levels, walking up and down stairs, and interaction with their owners (p=0.07). It is likely that increased activity is associated with reduced pain in arthritic cats, although more research is needed to confirm this.

Studies have demonstrated that dogs, when provided with a fish oil supplement containing 90 mg of combined EPA and DHA per kg of bodyweight per day, show a decrease in synovial pro-MMP-9 expression (Hansen et al., 2008; Knott et al., 2011). Notably, pro-MMP-9, which upon cleavage, gives rise to MMPs, which have been established as key enzymes mediating the degradation of the extracellular matrix in connective tissues. The breakdown of joint cartilage involves endogenous enzymes that degrade the components of the connective tissue matrix. Therefore, a reduction in the expression of pro-MMP-9 leads to a decrease in MMPs, subsequently slowing down the degradation of joint cartilage and contributing to an improvement in overall joint health in dogs (Hansen et al., 2008). Similarly, Hielm-Björkman et al. (2012) found that dogs given a diet supplemented with fish oil for 16 weeks showed improvements in several key variables (e.g., peak vertical force (PVF), Helsinki Chronic Pain Index (HCPI) and the use of rescue NSAIDs) relative to those receiving placebo (corn oil). In the study, the fish oil group exhibited significant improvements in PVF and impulse on force plates compared to the placebo group. Peak Vertical Force refers to the maximum vertical force exerted when a dog's paw touches the ground while walking, running or jumping, and when the PVC produces a positive change, it means that joint pain has improved in these dogs. Furthermore, the fish oil group significantly decreased their use of rescue NSAIDs, indicating improved joint health. Quality of life, as measured by QOL-VAS (Quality of life visual analog scale), also significantly improved in the fish oil group. Overall, the fish oil group demonstrated significant enhancements in exercise, daily situations, and skin and hair aspects compared to baseline. These combined results underscore the potential for fish oil supplementation to positively impact joint health and quality of life. Similarly, it has been shown that supplementation of 0.2 ml/kg of fish oil in dogs with DJD significantly reduces malondialdehyde (MDA) levels in the blood, suggesting a reduction in reactive oxygen species concentrations (Barrouin-Melo et al., 2016). In DJD-affected tissues, lipid metabolism involves multiple peroxidative reactions associated with inflammation, oxidative stress, and cartilage/bone tissue damage, and a reduction in MDA signals an improvement in DJD (Barrouin-Melo et al., 2016).

Omega-3 fatty acids, however, can potentially cause adverse side-effects in dogs and cats. The most common side effects that have reported are an altered platelet function and blood clotting problems, gastrointestinal adverse effects, deleterious effects on wound healing, lipid peroxidation, the potential for nutrient overload, toxin exposure, weight gain, altered immune function, sensitization to the effects of glycaemic control and insulin, and nutrient-drug interactions (Lenox & Bauer 2013). Therefore, it is important when introducing these types of ingredients into joint supplements that they stay within safe dose ranges and reference doses should be established based on past studies where possible (Lenox & Bauer, 2013).

Doses of omega 3 fatty acids included in supplements in past studies and given in guidelines for the treatment of OA in dogs and cats are generally based on the active ingredients, i.e., EPA and DHA, with a recommended dosage of 230 mg EPA and DHA per kg $BW^{0.75}$, up to a safe upper limit of 370 mg EPA and DHA per kg $BW^{0.75}$ (Bauer, 2011).

1.4.3.2 Glucosamine & chondroitin-sulphate

Glucosamine (Glu) and chondroitin-sulphate (CS) are substances produced by cells within and near cartilage (Bhathal et al., 2017). Most Glu supplements contain either glucosamine hydrochloride or glucosamine sulphate, which can help rebuild and repair cartilage damaged by DJD (Johnson et al., 2020). Many of these supplements also contain CS, a compound that is obtained from animal cartilage and increases the elasticity of cartilage and prevents cartilage breakdown (Barbeau-Grégoire et al., 2022). Dietary supplements of Glu and CS are widely used to treat degenerative joint disease in dogs, cats and horses (Barbeau-Grégoire et al., 2022).

In a number of studies in which dogs with DJD were given a dietary supplements containing Glu and CS, authors reported improved pain scores, increased weight bearing, and greater play behaviour when compared to their pre-treatment scores (d'Altilio et al., 2007; Eleotério et al., 2015; Gupta et al., 2012; Martello et al., 2022; McCarthy et al., 2007). Notably, one study compared the effectiveness of meloxicam and Glu-CS supplementation in the treatment of feline DJD (Sul et al., 2014). After 14 and 42 days of supplementation, both the meloxicam and Glu-CS groups showed significant improvements in owner activity scores compared to pre-treatment, however, when the animals were switched to placebo, the majority of animals in the meloxicam group showed a deterioration in all owner assessment scores from day 70 to day 89, whereas scores in the Glu-CS group stayed more stable than in the meloxicam group. This suggests that Glu-CS can be used as an adjunct in long-term applications compared to meloxicam.

Chondroitin-sulphate is rarely provided on its own, but is commonly given in conjunction with Glu (Bennett et al., 2012b). While there is a wealth of data supporting the use of CS in dogs, there is relatively little data on Glu-CS in cats (Bhathal et al., 2017). This indicates that more research is needed to fully understand the potential benefits of Glu-CS supplementation in cats. Therefore, studies on cats should be conducted to determine optimal dosage, given there is relatively little data in this area.

1.4.3.3 Polyphenols

Polyphenols are compounds found in fruits, vegetables and plants that have been widely recognized for their anti-inflammatory and antioxidant activity (Mével et al., 2014). Several studies have demonstrated the potential inhibitory effects of polyphenols on DJD (see review by Gambari et al. 2022). In patients with DJD, levels of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1 are significantly elevated. These pro-inflammatory cytokines further trigger the production of other inflammatory molecules, including cytokines, matrix metalloproteinases (MMPs) and prostaglandins. In addition, they inhibit the synthesis of anti-inflammatory cytokines such as proteoglycans and type II collagen. As a result, pro-inflammatory cytokines increase while anti-inflammatory cytokines decrease. Thus, they play a key role in cartilage matrix degradation and bone resorption in DJD (Wang & He, 2018). In contrast, polyphenols have antioxidant properties that inhibit pro-oxidant genes and increase the expression of antioxidant genes such as catalase (Ansari et al., 2020). Inhibition of pro-inflammatory signalling pathways (e.g., MAPK, AP1, and NFκB), reduce the production of inflammatory cytokines such as TNF-α, IL-1, and IL-6, and lead to the alleviation of DJD (Wang & He, 2018). Therefore, polyphenols are effective against DJD.

Blackcurrant leaf extract contains several phytochemicals, such as proanthocyanidins (PAC), which inhibit the inflammatory response and have a protective effect on cartilage in patients with DJD (Garbacki et al., 2002). PAC also contains anthocyanins, which have been shown to inhibit the expression of COX-2, thereby moderating the inflammatory state of DJD (Pomilio et al., 2022).

Curcumin is a polyphenolic compound which has been shown to inhibit the production and breakdown of pro-inflammatory cytokines such as IL-1β and TNF-α (Shakibaei et al., 2007). A study in dogs demonstrated that curcumin inhibited macrophage proliferation and was associated with a potent reduction in the regulation of TNFα and activation of fibrinolysis (Colitti et al., 2012). This implies that curcumin can inhibit the expression of inflammatory factors in canine DJD, thereby reducing the symptoms of this condition.

Green tea phenols are derived from extracts of green tea (Ansari et al., 2020). A study by Comblain et al. (2017) showed that dogs given a joint supplement containing a mixture of green tea extracts for three months displayed lower pain severity scores than unsupplemented control animals. Although the supplement in that trial consisted of a variety of polyphenols, and the improvement in pain could have been the result of a combination of substances, it provides some support for the idea that green tea phenolics can help alleviate DJD symptoms.

Although less research has been done on cats, there are still studies that show that citrus polyphenols can significantly improve the inflammatory state of obese cats (Jeusette et al., 2010; Leray et al., 2011). Jeusette et al. (2010) demonstrated that, compared to cats fed saturated fat alone, cats supplemented with citrus flavanones in a saturated fat diet had higher plasma lipid levels and urinary F2-iso concentrations were lower. Plasma lipids and obesity are correlated in cats, whereas urinary F2-isoprostane is one of the indicators of oxidative stress, so citrus polyphenols can improve the inflammatory state caused by obesity. Given obesity is one of the important influences on DJD (Thijssen et al., 2015), citrus polyphenols could be beneficial in improving DJD.

There is some concern that the safety of some anti-inflammatory products which contain polyphenols have not been established. In a study of dogs, high intake of green tea polyphenols led to multiple deaths in beagles resulting in the study being discontinued, although the mechanism by which green tea polyphenols caused the deaths was unclear (Khan & McLean, 2012). This case illustrates the need for research to determine the safety range of adding polyphenols to joint supplements. However, polyphenols have been shown to be effective against oxidative stress in dogs (McMichael, 2007), and there are several dog-related experiments to illustrate the improvement in joint health following polyphenol supplementation (Comblain et al., 2016), so polyphenols should still be chosen for the design of joint supplements. However, due to the complexity of the types and sources of polyphenols, there are few relevant therapeutic guidelines that provide a safe range for polyphenols intake for cats or dogs. Therefore, several past studies on polyphenol intake in cats were taken into consideration when designing polyphenol intake levels in the current work (Corbee, 2022; Leray et al., 2011; Thomas et al., 2024).

1.5 Evaluation of degenerative joint disease

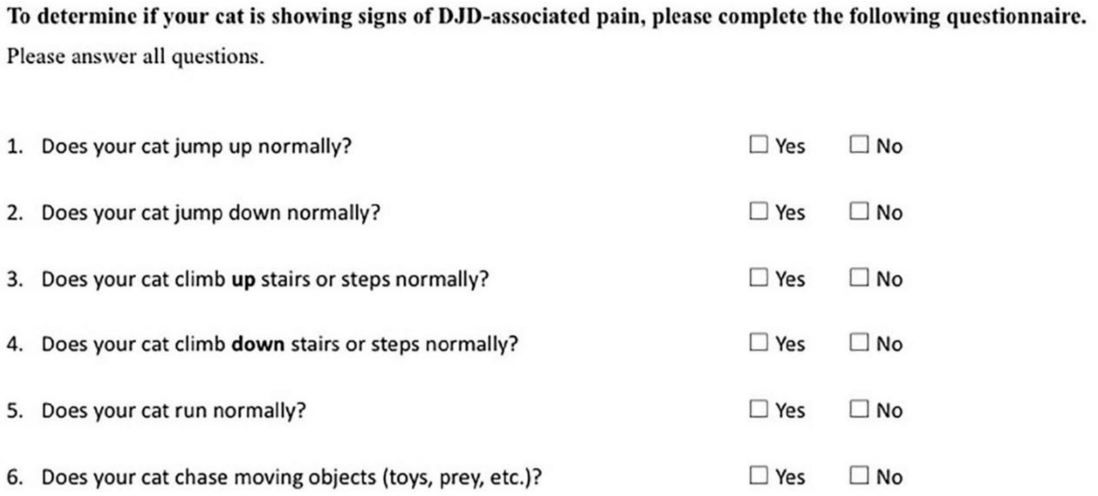
Degenerative joint disease cannot be cured, therefore, pain management is a key aspect of its treatment (Kerwin, 2010; Lascelles, 2010). DJD-related pain indicators have been used as a criterion for assessing whether DJD has improved. The following section describes the strengths and weaknesses of commonly used pain assessment tools currently used in studies of DJD pain in cats. These tools include owner and veterinarian assessment of animal pain, movement and behaviour and blood markers of inflammation. In addition, the use of accelerometers as an objective assessment tool for evaluating improvement in chronic musculoskeletal pain in cats will be discussed.

1.5.1 Common clinical tools used to evaluate of DJD

Currently, common questionnaire-based methods for assessing chronic pain status in feline DJD include: Feline Musculoskeletal Pain Index (FMPI) (Benito et al., 2013), Musculoskeletal Pain Screening Checklist (MiPSC) (Enomoto et al., 2020), Montreal Arthritis Test for Cats - Caregiver's Instrument (MICAT-C) (Klinck et al., 2015), Client-Specific Outcome Measure (CSOM) (Lascelles et al., 2007) and the Quality of Life (QoL) (Yeowell et al., 2021). The majority of these questionnaires consist of a variety of cat behavioural scores (Monteiro & Steagall, 2019).

The FMPI contains questions on the ability of the animal to do various activities such as walking, running, jumping and playing. These questions are used to monitor changes in the cat's mobility. The questionnaire categorises lying, sitting, eating, and using the litter box as "activity". Benito et al. (2013), however, reported no difference in scores for lying or sitting, eating and using the litter box in cats with DJD compared to normal cats when they tested the reliability of the FMPI. The researchers argued that this may have been due to p-values being corrected for multiple comparisons (using Bonferroni correction), or the limitations of the FMPI itself. Regardless of either of these two explanations the data suggest that these behaviours do not fit into the definition of activity in DJD cats.

The MiPSC questionnaire was developed by Enomoto et al. (2020) to assess DJD in cats. The questionnaire consists of 6 questions that relate to cat behaviour (Fig.1-2). The authors noted that the checklist had an accuracy of 71% and suggested that it should be used as a screening tool in conjunction with clinical palpation.



To determine if your cat is showing signs of DJD-associated pain, please complete the following questionnaire.
Please answer all questions.

1. Does your cat jump up normally? ☐ Yes ☐ No
2. Does your cat jump down normally? ☐ Yes ☐ No
3. Does your cat climb **up** stairs or steps normally? ☐ Yes ☐ No
4. Does your cat climb **down** stairs or steps normally? ☐ Yes ☐ No
5. Does your cat run normally? ☐ Yes ☐ No
6. Does your cat chase moving objects (toys, prey, etc.)? ☐ Yes ☐ No

Figure 1-2. Feline Musculoskeletal Pain Screening Checklist (Feline MiPSC). Reproduced from Enomoto et al., 2020.

The MICAT-C questionnaire was developed by Klinck et al. (2015) to assess the clinical signs of feline DJD by veterinarians. It contains 38 assessment criteria which include agility, social, play, and exploratory behaviours, self-maintenance, and body condition. The results of the study, however, showed that no scale items reached statistically significant DJD detection. Some negative correlations for gait and body posture scores were identified but were highly variable and had poorly reliability.

Lascelles et al. (2007) reported that the Client Specific Outcome Measure (CSOM) scale could detect behaviours associated with pain relief in arthritic cats. The CSOM is a pain assessment tool based on the owner's assessment of the impact of chronic pain on the cat's ability to perform specific animal-specific activities. It has no set scoring items and usually requires the owner to select three activities observed in the home environment to assess with the help of a veterinarian or technician. This assessment is therefore very time consuming and relies on a clear understanding by the owner (Monteiro & Steagall, 2019). In this scale assessment, however, objective activity data were not used to validate the subjective assessment system. In a clinical setting CSOM should therefore only be used as an auxiliary tool not as a gold standard for assessing the progression of DJD.

The Health-Related Quality of Life (HRQoL) model, is defined as "a subjective assessment of a situation that includes changes in health status and related interventions", and is an assessment tool for chronic pain. In cats with DJD, the HRQoL is categorised into seven domains: mobility, appearance, energy and vitality, mood, pain performance, socialisation and physical and mental health (Figure 1-3)(Yeowell et al., 2021). There is a lack of consensus in the field to develop a uniform and valid assessment form, however, the three domains of pain expression, mobility, and physical and mental health have been identified as the focus for evaluating the quality of life of cats with DJD (Monteiro & Steagall, 2019). These assessment models, however, still rely on the subjective judgment of the owner and the veterinarian (Yeowell et al., 2021).

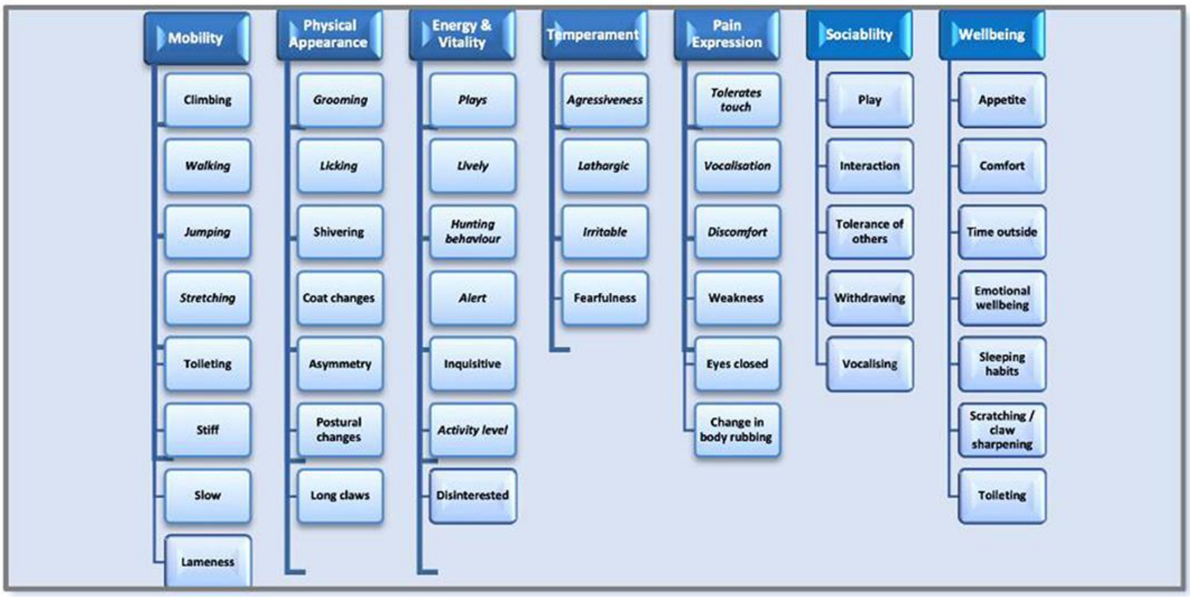


Figure 1-3. Quality-of-life domains. Reproduced from (Yeowell et al., 2021).

Overall, scoring cat activity and joint health relies on the subjective observations of the observer, which lacks objective, quantifiable and validated tools for the assessment of DJD-induced pain. Therefore, objective and quantifiable methods are needed in the development of future assessment tools.

1.5.2 Objective tools for assessing changes in cat activity.

1.5.2.1 Blood Inflammation Indicators

Blood concentrations of inflammatory cytokines such as interleukin-1β and TNF-a have been shown to be elevated in cats with DJD (Barrouin-Melo et al., 2016). The measurement of blood concentrations of these cytokines can be used to assess the efficacy of treatments to alleviate inflammation due to DJD (Gruen et al., 2017). A decrease in blood levels of inflammatory factors, however, does not necessarily correlate with an increase in behaviour or a decrease in pain (Gruen et al., 2017). This approach, therefore, should only be used as an aid to diagnosis and not as a method of assessing pain.

1.5.2.2 Kinetic gait analysis

Kinetic gait analysis is an important tool to assess the impact of DJD on cat movement (Guillot et al., 2012). When an animal walks, the limbs are subjected to reaction forces from the ground. By walking a cat over a force plate the reaction forces generated can be recorded to determine if the movement of the animal is abnormal (Schnabl & Bockstahler, 2015). Motion analysis techniques include the measurement of ground reaction forces (GRFs), joint and spine kinematics, and electromyography (EMG) (Schnabl & Bockstahler, 2015). These data can then be characterised as either normal or abnormal (Corbee et al., 2014). A drawback of this technique, however, is that the cat needs to be trained to walk on the plate before use, and even after training, some cats are unable to be measured successfully (Lascelles et al., 2007). Currently this technique is only used for scientific research and is usually not available for clinical use (Monteiro & Steagall, 2019).

1.5.2.3 *Accelerometers*

Accelerometers are small, non-invasive devices that record the acceleration associated with an animal's activity, and calculations of these accelerations can yield the intensity, frequency, duration, and activity patterns of the animal's movements (Sántha and Hermann, 2013). Accelerometers have been applied and proven to be effective in assessing activity in domestic cats (Andrews et al., 2015; Andrews et al., 2022; Lascelles et al., 2008; Smit et al., 2023). For domestic cats, accelerometers are used to detect physical activity and metrics such as motor activity (MA) and overall dynamic body acceleration (ODBA) are calculated from the acceleration data. In Guillot's (2013) study, accelerometers were set to create a count value every 2 minutes. The magnitude of each count was then converted to a numerical value (from 0 to infinity) which referred to the intensity of locomotor activity, and the median of the activity counts collected by the sensors for each time period were processed to obtain MA, which reflects the variation in the animal's locomotion, with higher values indicating greater locomotion. Overall dynamic body acceleration is a variable that is used to measure the level of activity of an animal, and is calculated using the formula shown in Figure 1-4 (Smit et al., 2023).

$$DBA = SUM_{axis} - moving\ average$$

$$ODBA = \sum_{i=1}^{N} |DBA_X| + |DBA_Y| + |DBA_Z|$$

Figure 1-4 The ODBA formula. Reproduced from Smit et al., (2023).

The formula combines the dynamic elements of acceleration recorded in all three dimensions in order to measure acceleration and thus energy expenditure caused by body movement, therefore, ODBA can be used to detect changes in animal movement (Guillot et al. 2013; Martin Lopez et al. 2022; Wilson et al. 2006).

Accelerometers detect the acceleration of an object through the deformation of piezoelectric elements which generates electricity in response to mechanical stress (Plitnik, 2022). Accelerometers use between one and three plasma sensors, which consist of a plasma element and an electromagnetic block. The sensors are deformed by gravitational acceleration and inertial acceleration from motion and during deformation generate a waveform voltage signal proportional to the acceleration applied. In a triaxial accelerometer, three sensors in each of the X, Y and Z axes are orthogonally aligned (Figure 1-5) and each accelerometer measures acceleration in a single plane or dimension (Sántha & Hermann, 2013).

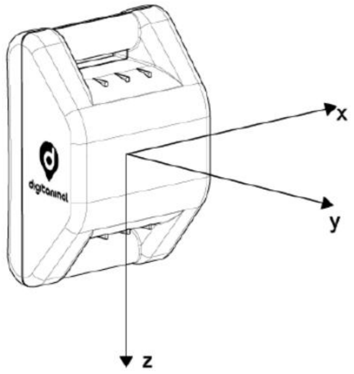


Figure 1-5 Diagram of an accelerator showing the X, Y and Z axes Reproduced from (Santha & Hermann, 2013)

While the accelerometer voltage output is constant when the animal is at rest, any whole-body movement produces fluctuating acceleration waveforms with different cycles that can be used to infer body posture and body movement for a particular behaviour (Plitnik, 2022). There is high variance between measurements of each axis with different movements which allow different movements to be distinguished. As shown in Figure 1-6, Eerdekens et al. (2022) differentiated the electrical signals produced by dogs when lying, sitting, standing, walking, running, sprinting, eating, and drinking.

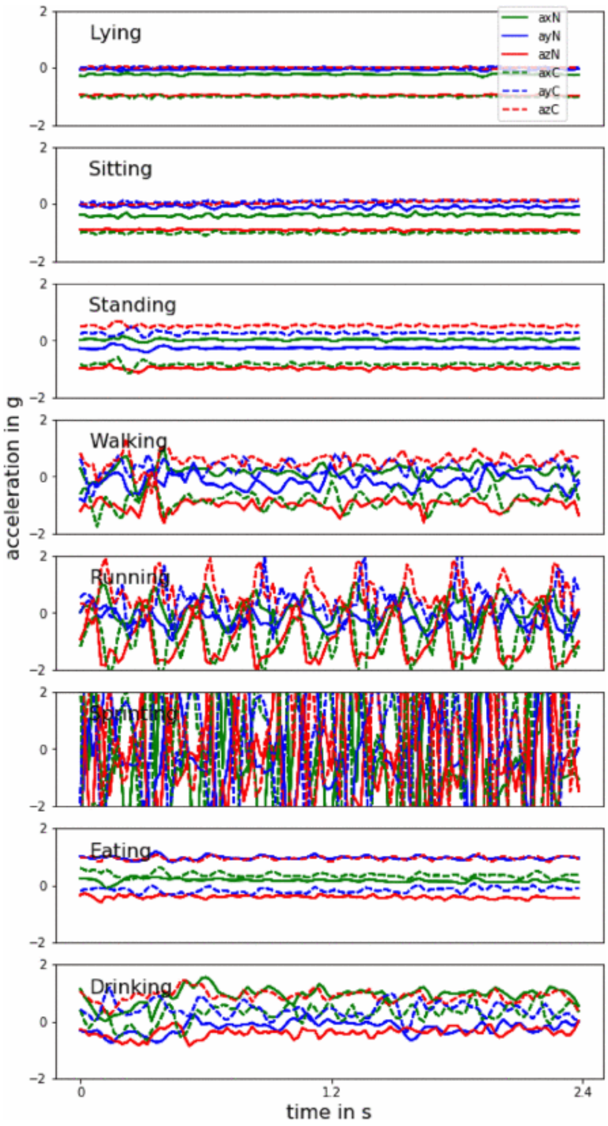


Figure 1-6 Typical accelerometer traces showing lying, sitting, standing, walking, running, sprinting, eating and drinking for a 2.4 second window. The dashed green, blue and red lines indicate the X, Y and Z axes signals from a chest mounted accelerometer, respectively. The solid red, green and blue lines indicate the X, Y and Z axis signals from a neck mounted accelerometer, respectively. Reproduced from Eerdekens et al., 2022.

Overall dynamic body acceleration (ODBA) is an important metric calculated from accelerometer data. It is determined by measuring the three-dimensional acceleration of the body using accelerometers and then integrating these values to derive a comprehensive indicator of overall movement intensity. Specifically, ODBA is computed by obtaining acceleration data along the x, y, and z axes, calculating the total acceleration, and subsequently smoothing the values to yield a representative measure of the dynamic activity level (Gleiss et al., 2011). Wilson et al. (2006) found that ODBA was positively correlated with the rate of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) in cormorants (Figure 1-7). This suggests that ODBA can used to estimate an animal's energy expenditure in addition to the intensity of their movement. In recent years, ODBA has been used to assess energy expenditure in large and small aquatic animals (Martin Lopez et al., 2022) and cormorants (Laich et al. (2011).

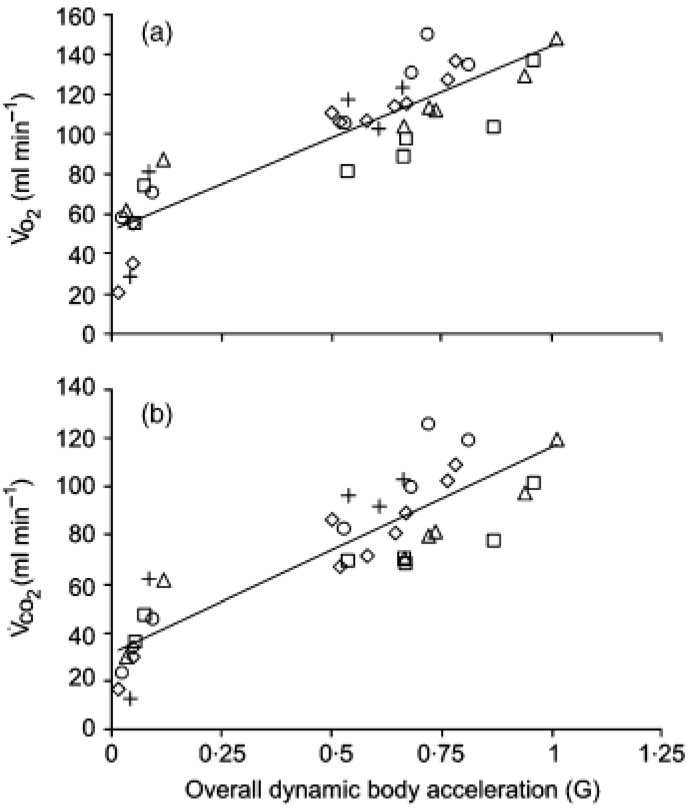


Figure 1-7 Relationship between overall dynamic body acceleration (G) and (a) oxygen consumption (VO_2) and (b) carbon dioxide production (VCO_2) for five great cormorants resting and walking at different speeds on a treadmill (Wilson et al., 2006).

The efficacy of meloxicam in cats with DJD was assessed by Guillot et al. (2013) using accelerometers to calculated the motor activity (MA). They reported an increase in MA intensity of +3.7% in the group dosed with 0.025 mg/kg meloxicam and +5.2% in the 0.05

mg/kg meloxicam group compared to the placebo control group. This study provided evidence that MA intensity measured by accelerometery can be used to monitor the effectiveness of feline DJD treatment.

Accelerometers have also been used to monitor changes in the specific behaviours of cats and dogs (Hansen et al., 2007; Lascelles et al., 2008). In dogs, accelerometers were used to monitor behaviours such as eating, drinking, walking, running, sleeping (Kumpulainen et al., 2021), and have been used in the management of chronic pain (Brown et al., 2010; Muller et al., 2018). In cats, accelerometer outputs have been used to investigate the effect of age and physical condition on the activity of domestic cats (Andrews et al., 2015; Lascelles et al., 2008; Smit et al., 2022), oestrus behaviour (Andrews et al., 2022), activity following treatment for DJD disease (Yamazaki et al., 2020), and the quality of life in cats with cardiomyopathy (Coleman et al., 2020).

Accelerometers have been used to determine the effect of treatment of cats with DJD by detecting changes in movement patterns. Gruen et al. (2017) reported that among 85 cats with DJD and 15 cats without, those with DJD had flatter activity and intensity curves detected using the devices (Figure 1-8).

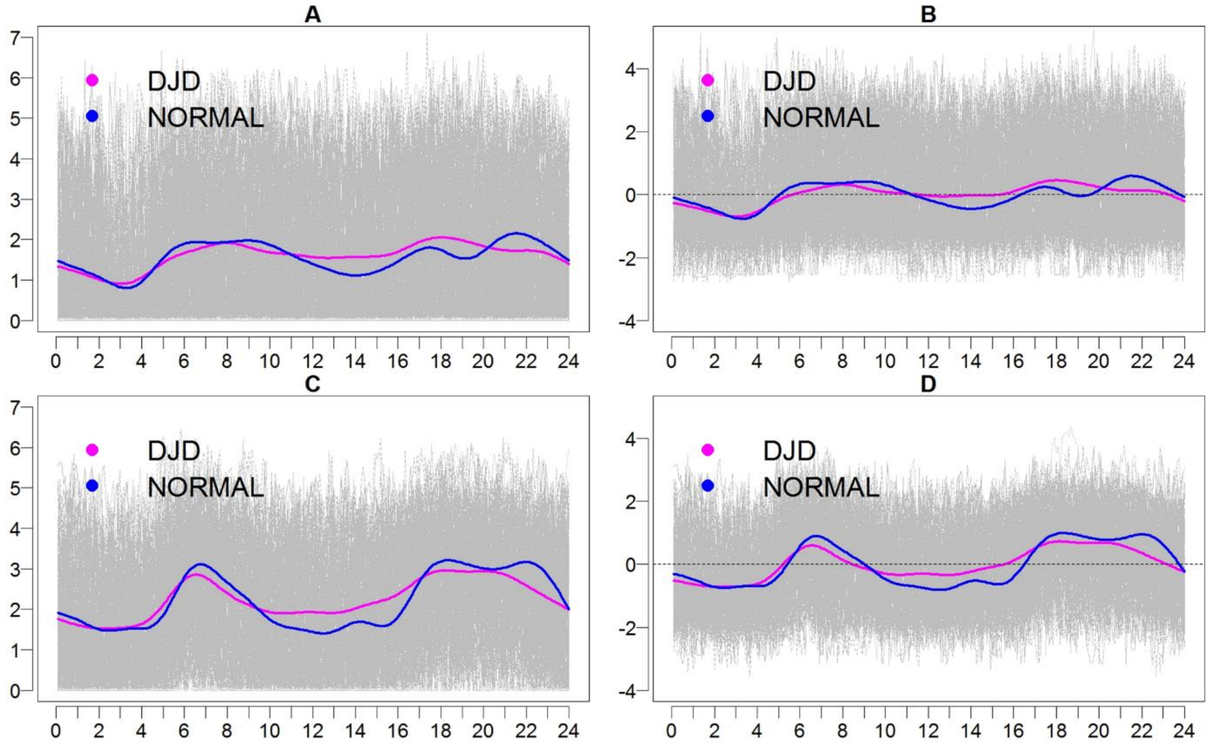


Figure 1-8 Activity (A and C) and intensity (B and D) profiles for cats in the Normal group (blue) and those with DJD (pink). Reproduced from Gruen et al., 2017.

1.6 Thesis aims and objectives:

This study seeks to investigate a novel joint supplement for cats, and using accelerometers, will assess changes in mobility and behaviour of older animals prior to and after administration of the supplement. To date there has been relatively few studies of the effect of joint supplements in cats, and the effectiveness of many supplement ingredients are controversial (e.g., chondroitin sulphate and polyphenols). Currently there is a lack of objective and quantifiable methods to assess changes in movement in DJD cats, and although accelerometers are promising, data generated from them in felines are scarce. This experiment will use triaxial accelerometers to objectively assess the effects of a dietary supplement containing a blend of antioxidants, polyphenols, glucosamine and chondroitin sulphate on the activity and behaviour of older cats. This will provide information on the effectiveness of this novel ingredient combination, and more data on the use of accelerometers in felines.

Chapter 2:

Methods

Chapter 2: Methods

The study was conducted at the Centre for Feline Nutrition, Massey University, Palmerston North, New Zealand (latitude 40°23′ S, longitude 175°36′ E), between 03 May and 22 September 2023. The study design was approved by the Massey University Animal Ethics Committee (MUAEC 22/77).

2.1 Animals

All cats used in the study were part of a colony of domestic shorthair cats housed and managed at the facility. All cats were greater than nine years of age, neutered and weighed between 2845 and 4612 g at the start of the study (Table 2-1). Throughout the study, the cats were housed in two semi-outdoor colony cages, offered *ad libitum* access to a balanced commercial canned food diet (Heinz Wattie's Ltd., Hastings, New Zealand), and provided *ad libitum* access to water.

Table 2-1. Cats enrolled in the study showing their age (years), live weight (g) and sex.

Cat identification	Group	Age (years)	Sex	Bodyweight (g)	Removed	Died
Coby	Test	12.4	Female	3214		
Dalu	Test	12.5	Male	4261		
Katy	Control	12.5	Female	2858		
Raven	Control	16.1	Male	4498		
Red	Control	11.3	Male	4612		
Blur	Test	11.3	Female	2845		
Muse	Test	11.3	Male	3192		
Pocket	Test	11.4	Female	3327		
Vilma	Control	12.2	Female	3226		
Jetty	Test	9.4	Female	2986		
Jetro	Control	10.1	Male	3744		X
Jed	Control	12.1	Female	4364	X	
Token	Test	10.1	Male	3178	X	
Nova	Test	10.2	Female	3255		X
Obi	Control	12.1	Male	4558	X	

Switch	Control	9.4	Female	3891

2.2 Experimental design

The study consisted of two phases: habituation (Phase 1) and data collection (Phase 2). During the habituation phase, 16 neutered domestic shorthair cats (male = 8, female = 8) ranging in age from 9.4 to 16.1 years (mean ± SD, 11.62 ± 1.74 years) were assessed for inclusion in the study. In phase 1, cats were habituated to wearing an ActiGraph wGTX-BT (Figure 2-1) accelerometer attached to their collar over a two-week period as indicated in the Figure 2-2. The acclimatization time was gradually increased in order to allow the cats to adapt to the devices, as shown in Table 2-2. Cats at the Centre for Feline Nutrition (CFN) routinely wear a collar but had not previously been fitted with an ActiGraph device. Three cats did not habituate to wearing the collar with the device and were removed from the study (Table 2-1). In addition, two cats died of a disease not related to the study during the experimental phase, so the data from the remaining 11 animals are analysed and presented.

Figure 2-1 ActiGraph wGTX-BT.

Figure 2-2 Cat wearing ActiGraph wGTX-BT.

The data collection phase consisted of 13 cats (male = 4, female = 7), ranging in age from 9.4 to 16.1 years (mean ± SD, 11.84 ± 1.78 years), all of which had successfully completed the habituation period. The cats were divided into two groups, one receiving oral supplementation and the other (control group) receiving no oral supplementation. In the second phase, each cat wore an accelerometer on a collar for 72 consecutive hours, once a fortnight (Table 2-3). At the very beginning of the data collection phase (baseline week 0), 13 cats were divided equally into two groups according to age and sex and baseline data were collected using accelerometers. At the end of this collection period, the data were analyzed to ensure that there were no significant differences in behavioral activity between the two groups.

Table 2-2 Habituation schedule for a cat to get used to a collar and/or monitor.

	Day					
	1	2	3	4	6	7
Period Devices Worn for (hours)	2	4	6	8	24	Device removed
Supervised	Yes	Yes	No	No	No	

Table 2-3. Timeline of the study showing each study phase and weeks in which supplements were given and accelerometer data was recorded.

Phase	Study week	Supplement given daily	Accelerometer data collection	Blood samples
habituation	Adaptation week 1		X	
habituation	Adaptation week 2		X	
collection	Baseline week 0		X	X (Day 0)
collection	Week 1	X		
collection	Week 2	X	X	
collection	Week 3	X		
collection	Week 4	X	X	
collection	Week 5	X		
collection	Week 6	X	X	
collection	Week 7	X		
collection	Week 8	X	X	
collection	Week 9	X		
collection	Week 10	X	X	
collection	Week 11	X		
collection	Week 12	X	X	
collection	Week 13	X		
collection	Week 14	X	X	
collection	Week 15	X		
collection	Week 16	X	X	X (Day 126)

2.3 Supplement Ingredients

The supplements were provided in the form of oral soft chewable strips (Figure 2-3) at a dose of 2.2 g per cat (Figure 2-4). This dose was calculated on the basis of the dose of each active ingredient required per kilogram of body weight, and since the majority of cats weighed in at around 4.5 kg, the dose of the supplements was calculated on the basis of each active ingredient required per 4.5 kg.

Figure 2-3 Joint supplements made into oral soft chewable strip.

Figure 2-4 Daily dose of supplements per cat.

All supplements were fed in the morning between 9-10am when the cats were eating, with one-to-one feeding of each cat to ensure that all cats received all of the dose. Active ingredients added as supplements included GLM, salmon oil (New Zealand King Salmon Ltd., Nelson, New Zealand), vitamins C and D3, chondroitin, glucosamine sulphate, curcumin, and blackcurrant extract (Table 2-4).

Table 2-4 Active ingredients included in the supplement showing the inclusion rate and unit of measurement.

Ingredients	Per kg of supplement (as fed)	Unit
Green-lipped mussel	40001	mg
Curcumin	10003	mg
Blackcurrant extract	16700	mg
Vitamin C	17000	mg
Vitamin D3	2000	IU
Salmon oil	30700	mg
Glucosamine	187.5	mg
Chondroitin sulphate	150	mg

2.4 Blood sample collection

Blood was drawn from all cats at Day 0 (D0) and D126. The area of skin above each jugular vein was shaved and cleaned with methylated spirits. At least 30 minutes prior to sampling Emla cream (2.5% lignocaine and 2.5% Prilocaine) or 2% Xylocaine cream (Aspen Pharmacare Australia Pty Ltd., Saint Leonards, NSW, Australia) was applied to the blood sampling site to reduce pain. A 2 mL blood sample was taken from the jugular vein using a 3 ml syringe and 25G needle.

Each blood sample was divided into two, half of the samples were placed in purple-topped vacutainer tubes containing Ethylenediaminetetraacetic acid (EDTA) for immediate submission to IDEXX for complete blood count (CBC) and biochemistry analysis, while the other half of the samples were stored in red-topped tubes for 3hr, then spun at 157g for 10 min before the serum was removed and stored in a -80 °C freezer until the second set of blood was removed and then taken together for cytokine analysis.

2.5 Accelerometery

Triaxial accelerometers (ActiGraph wGTX-BT, Pensacola, FL, USA) were fitted to the collar of each cat. The devices weighed 19 g and measured 33 mm × 46 mm × 15 mm and were placed ventrally in a consistent position for all cats (Figure 2-5). The X, Y, and Z axes were oriented laterally, dorsoventrally, and cranio-caudally, respectively. Acceleration data were sampled at 30 Hz (raw acceleration data) and had a dynamic range of ±8 g.

The devices were fitted to each cat for the 2-week of the habituation period, then a further two weeks to collect baseline activity data. Activity data were then collected fortnightly for 72 consecutive hours for a total of 8 times during the remaining 16 weeks of the study (weeks 2, 4, 6, 8, 10, 12, 14 and 16).

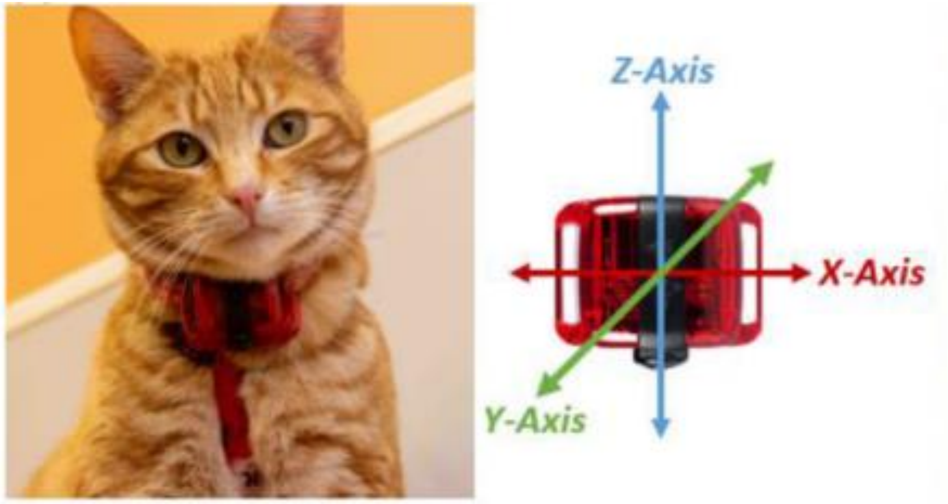


Figure 2-3 ActiGraph wGT3X-BT Accelerometer Placement and Orientation on Collar.

2.6 Statistical analysis

Raw acceleration data for each axis were exported from the devices using ActiLife software (version 6.13.4; ActiGraph, Pensacola, FL, USA). Using RStudio v1.4.1, a total of 32 identifier variables were derived from the raw accelerometer data and summarized into 1 second epochs. The identifier variables included descriptive statistics including mean, sum, minimum, maximum, standard deviation, skewness and kurtosis. In addition, vector magnitude (VM), overall dynamic body acceleration (ODBA) and dynamic body acceleration (DBA) were calculated; see Table 2-5 for detailed description of each identifier variable).

The data generated by the accelerometers were modelled and analysed using a random forest (RF) model. The RF model developed by Smit et al. (2023) was used to assess cat behaviour using activity data. The model classified cat behaviour into Locomotion, Eating, Grooming, Scratching, Littering, Lying, Sitting and Standing. All behaviours were classified as either active or inactive. The active behaviours include Locomotion, Eating, Grooming, Scratching, and Littering, while the inactive behaviours include Lying, Sitting, and Standing. Normality was tested for all activity counts using the Shapiro-Wilk normal distribution test. Differences between treatment and control groups were compared using independent t-tests for ODBA satisfying normal distribution. All activity counts were analysed using generalised linear equations (GEE) as a way to see the effect of week and supplement. The blood sample data were compared for their within-group correlations at week 0 and week 16 using Wilcox analysis and paired analyses, respectively.

Table 2-5 Description of each identifier variable.

Identifier Variable	**Description**
Mean	Mean, calculated for every second using the raw acceleration data (30 measures per second).
Sum	Sum, calculated for every second using the raw acceleration data. $Sum(Axis)=\sum i=1NAxisi$
Minimum (min)	Minimum value of every 30 measures within each second.
Maximum (max)	Maximum value of every 30 measures within each second.
Standard deviation (sd)	Measures the spread of the data.
Skewness	Asymmetry of the distribution.
Kurtosis	Weight of the tails relative to a normal distribution.
Vector magnitude (VM)	$VM=X2+Y2+Z2$
Overall dynamic body acceleration (ODBA)	$ODBA=\sum i=1N\lvert DBAX\rvert+\lvert DBAY\rvert+\lvert DBAZ\rvert$
Dynamic body acceleration (DBA) [1]	$DBA=Sumaxis-moving\ average$

Accelerometer data from each axis were individually smoothed using the moving average over 1 s. [1]The DBA was not included as an identifier variable.

Chapter 3:

Results

Chapter 3: Results

3.1 Bodyweights

Mean body weight of cats in the control and test groups did not differ (P>0.05) at week 0 or 16 (Table 3-1). There was also no effect (P>0.05) of week on the body weight of the cats in either the control or test groups.

Table 3-1 The live weight(g, mean ±SEM) of cats in the control and experimental groups at week 0 and 16.

	Live weight (g)		
Group	Week 0	Week 16	P-value week
Control (n=5)	3817 ± 279	3598 ± 169	0.14
Test (n=6)	3318 ± 214	3552 ± 186	0.17
P-value group	0.22	0.96	

3.2 Activity and Behaviour

The mean daily overall physical activity, as indicated by total daily ODBA, did not differ (P>0.05) between the control and test group animals during the baseline recording period. Between week 2 to 16, the test group had a greater (P=0.03) mean daily ODBA than the control group (Table 3-2).

Table 3-2 Differences in daily mean ODBA between groups (mean ± SEM) during the 14-week experiment.

	ODBA(Δg)	
Week	Baseline (0-1)	2-16
Control (n=5)	2912 ± 71.2	2959 ± 119.0
Test (n=6)	3077 ± 65.5	3211 ± 70.2
P-Value group	0.43	0.03

Throughout the study period, among active behaviours the proportion of time spent grooming each day tended to be greater in the test group of cats than in the control group (P=0.051) (Figure 3-1). There were no differences in the proportion of time spent each day exercising, defecating, eating, or scratching between cats in the control and test groups (P>0.05). As for inactive behaviours (lying, sitting and standing), there were no significant differences in the proportion of time spent each day expressing the behaviours between the cats in the control and experimental groups.

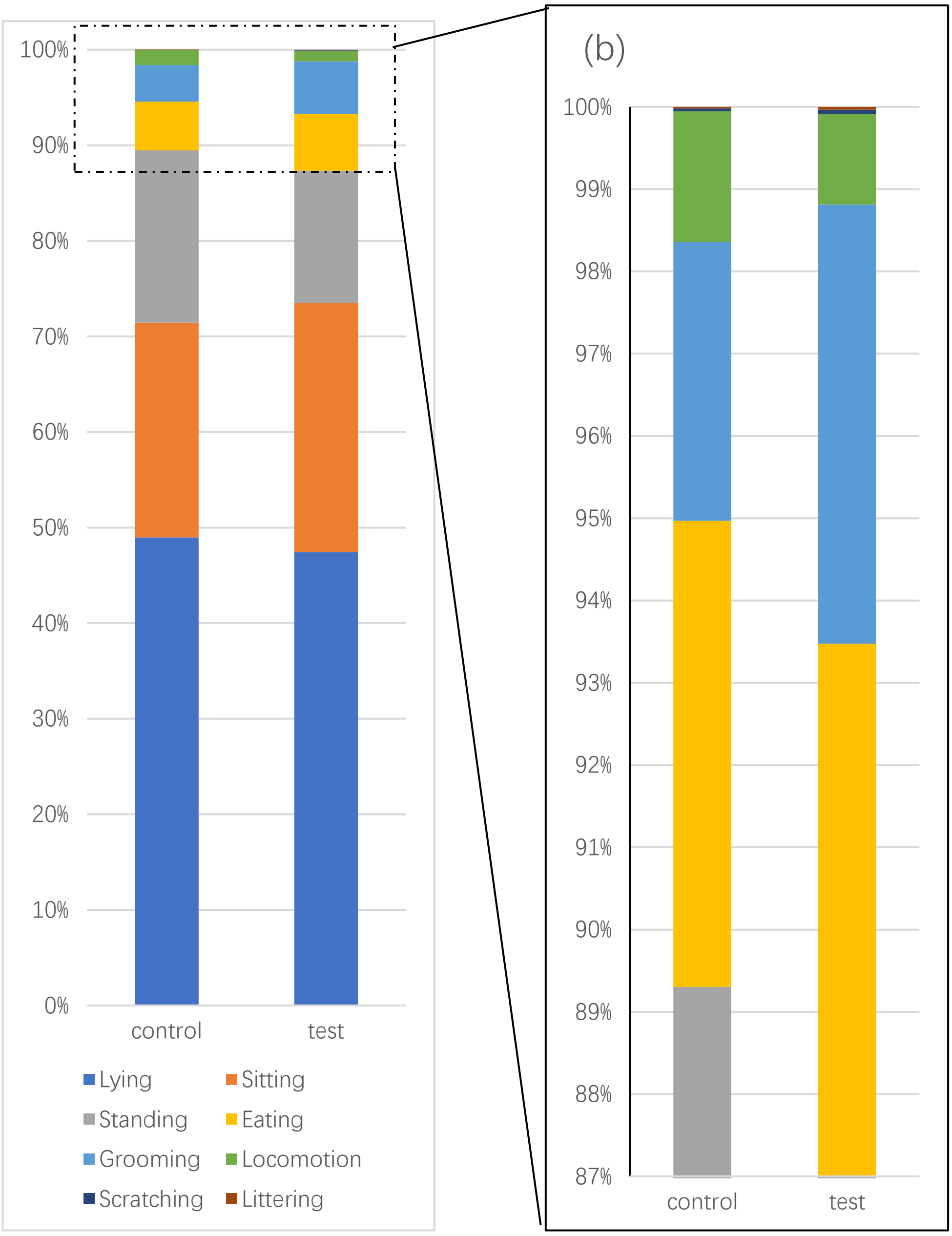


Figure 3-1 (a) Percentage of time spent exhibiting each behaviour per day (%, mean) between the cats in the control and test groups throughout the study period. (b) expanded section showing infrequent behaviours (< 10% of daily behaviour).

Lying behaviour showed a significant interaction between week and treatment (p=0.04; Table 3-3). The change in the percentage of time cats spent on each behaviour before and after taking the oral supplement is shown in Figure 3-2. All other behaviours had a non-significant interaction between the number of weeks and treatment. However, during individual weeks during the study slight differences in behaviours between the two groups were observed. During week 12, the treatment group showed slightly more littering behaviour than the control group (p=0.05). At week 16, the control group showed more locomotion behaviour than the treatment group (p=0.007). While treatment had a significant effect on scratching behaviour in the first (p=0.001) and fourth (p=0.002) weeks, the effect disappeared at other times. Interestingly, there was a repeated effect of treatment on grooming on weeks 2 (p=0.047), 4 (p=0.002), 6 (p=0.004), 8 (p=0.01), and 14 (p=0.004), with the treatment group showing more grooming behaviour than the control group (Table 3-4). However, at weeks 0, 10, 12 and 16, there were no significant differences between the treatment and control groups.

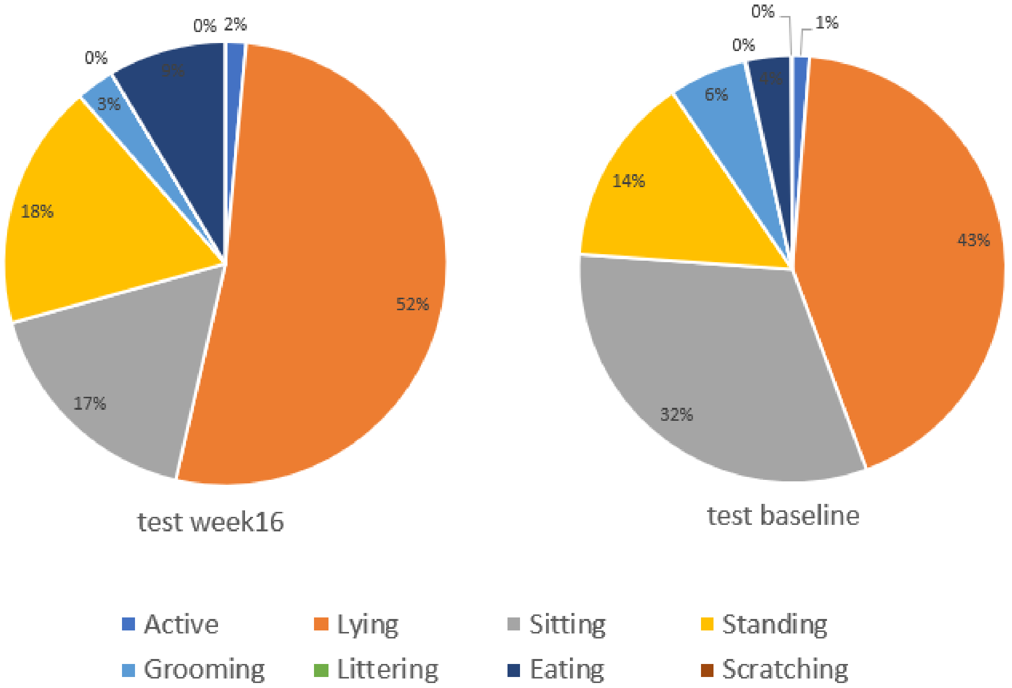


Figure 3-2 Changes in the percentage of time cats in treatment group (n=6) spent on each behaviour before (baseline) and after (16 week) taking the oral supplement.

Table 3-3 Percentage of weekly time spent exhibiting inactive behaviours (lying, sitting, and standing) with corresponding 95% confidence intervals (CI) between cats in the control (n=5) and test groups (n=6) in each observation week.

Behaviour (%)	Week	Control (n= 5)	Test (n = 6)	P-Value
Lying	0	46.58 (39.55-53.62)	40.22 (33.84-46.61)	NS
	2	49.05 (35.52-62.57)	45.18 (36.71-53.64)	NS
	4	46.47 (38.15-54.80)	43.60 (32.99-54.21)	NS
	6	55.23 (43.74-66.71)	41.14 (35.09-47.20)	0.024
	8	46.98 (35.92-58.05)	44.09 (38.64-49.55)	NS
	10	46.92 (36.97-56.88)	44.99 (33.76-56.22)	NS
	12	52.69 (41.77-63.62)	58.30 (52.58-64.01)	0.022
	14	52.85 (44.71-60.99)	60.16 (53.89-66.43)	NS
	16	46.46 (37.24-55.68)	56.31 (48.79-63.83)	NS
	Overall			0.04
Sitting	0	27.63 (23.14-32.12)	35.36 (29.50-41.22)	NS
	2	25.88 (21.36-30.41)	23.38 (19.23-27.52)	NS
	4	23.07 (15.06-31.08)	28.43 (20.82-36.04)	NS
	6	21.85 (14.85-28.85)	27.13 (22.64-31.63)	NS
	8	26.04 (22.83-29.24)	29.39 (22.10-36.68)	NS
	10	27.49 (16.99-37.99)	30.57 (22.79-38.35)	NS
	12	18.74 (13.97-23.50)	15.71 (10.02-21.40)	NS
	14	11.05 (4.08-18.02)	16.52 (11.33-21.71)	NS
	16	15.28 (8.36-22.20)	18.53 (12.02-25.04)	NS
	Overall			0.45
Standing	0	15.90 (10.62-21.19)	13.06 (9.68-16.45)	NS
	2	18.03 (8.78-27.29)	18.78 (12.00-25.55)	NS
	4	15.41 (9.39-21.43)	15.75 (8.96-22.53)	NS
	6	14.81 (9.36-20.26)	19.00 (11.90-26.10)	NS
	8	17.01 (12.39-21.62)	14.25 (7.05-21.45)	NS
	10	14.13 (10.97-17.28)	11.95 (7.91-16.00)	NS
	12	22.00 (14.51-29.49)	11.40 (7.12-15.69)	0.016
	14	21.53 (13.23-29.84)	9.87 (4.06-15.67)	0.024
	16	25.47 (17.38-33.56)	11.64 (6.61-16.68)	0.004
	Overall			0.08

Table 3-4 Percentage of time per day (mean with 95% CI in parentheses) spent exhibiting active behaviours (locomotion, eating, grooming, scratching, and littering) between cats in the control (n=5) and test groups (n=6) in each observation week.

Behaviour (%)	Week	Control	Test	P-Value
Locomotion	0	1.48 (1.17-1.80)	1.11 (0.83-1.41)	NS
	2	1.34 (1.03-1.65)	1.09 (0.85-1.33)	NS
	4	0.98 (0.60-1.36)	1.06 (0.80-1.32)	NS
	6	1.46 (1.05-1.86)	1.20 (0.85-1.55)	NS
	8	2.30 (0.92-3.67)	1.22 (0.66-1.78)	NS
	10	1.59 (1.08-2.10)	1.22 (0.71-1.73)	NS
	12	1.48 (0.59-2.36)	1.13 (0.75-1.49)	NS
	14	1.46 (0.88-2.04)	0.98 (0.74-1.21)	NS
	16	2.13 (1.28-2.98)	0.93 (0.77-1.09)	0.007
	Overall			0.41
Eating	0	2.85 (1.18-4.53)	3.94 (2.53-5.35)	NS
	2	2.95 (1.03-4.86)	4.02 (1.86-6.17)	NS
	4	4.51 (2.47-6.56)	4.08 (2.02-6.14)	NS
	6	5.01 (3.25-6.77)	4.43 (2.14-6.72)	NS
	8	4.35 (3.58-5.12)	4.22 (2.13-6.31)	NS
	10	4.29 (3.67-4.91)	5.98 (4.07-7.90)	NS
	12	8.42 (5.53-11.32)	10.09 (7.50-12.68)	NS
	14	8.54 (6.76-10.32)	9.12 (6.87-11.36)	NS
	16	7.25 (4.77-9.73)	9.81 (7.46-12.16)	NS
	Overall			0.82
Grooming	0	5.51 (3.45-7.57)	6.16 (3.92-8.40)	NS
	2	4.83 (2.87-6.79)	7.46 (5.76-9.15)	0.047
	4	3.29 (1.75-4.83)	7.00 (5.25-8.74)	0.002
	6	3.92 (2.79-5.06)	7.00 (5.24-8.76)	0.004
	8	3.79 (2.36-5.21)	6.70 (5.01-8.40)	0.01
	10	3.37 (2.14-4.61)	5.19 (3.79-6.59)	NS
	12	2.86 (1.54-4.19)	3.32 (2.38-4.27)	NS
	14	2.17 (1.23-3.11)	3.31 (2.77-3.85)	0.04
	16	2.86 (1.48-4.24)	2.75 (1.66-3.84)	NS
	Overall			0.051
Scratching	0	0.04 (0.02-0.05)	0.09 (0.06-0.11)	0.001

	2	0.04 (0.02-0.07)	0.06 (0.04-0.09)	NS
	4	0.03 (0.02-0.04)	0.05 (0.04-0.07)	0.002
	6	0.05 (0.02-0.07)	0.06 (0.04-0.08)	NS
	8	0.05 (0.02-0.07)	0.07 (0.05-0.10)	NS
	10	0.05 (0.03-0.08)	0.06 (0.04-0.08)	NS
	12	0.02 (0.01-0.03)	0.03 (0.01-0.04)	NS
	14	0.02 (0.01-0.03)	0.03 (0.01-0.05)	NS
	16	0.02 (0.01-0.03)	0.02 (0.01-0.02)	NS
	Overall			0.08
Littering	0	0.01 (0.00-0.02)	0.05 (0.00-0.10)	NS
	2	0.01 (0.00-0.02)	0.04 (0.00-0.10)	NS
	4	0.02 (0.00-0.03)	0.03 (0.00-0.07)	NS
	6	0.05 (0.00-0.10)	0.04 (0.00-0.07)	NS
	8	0.01 (0.00-0.04)	0.05 (0.00-0.11)	NS
	10	0.03 (0.00-0.06)	0.04 (0.00-0.08)	NS
	12	0.01 (0.00-0.03)	0.03 (0.02-0.04)	0.05
	14	0.01 (0.00-0.02)	0.02 (0.01-0.03)	NS
	16	0.01 (0.00-0.02)	0.02 (0.00-0.04)	NS
	Overall			0.87

NS = not significant, p>0.05

In week 6 cats in the control group spent more time lying than cats in the test group (P=0.02). In week 12, however, cats in the control group spent less time lying than the test group (P=0.02) (Table 3-5). Cats in the treatment group spent less time standing than the control group on weeks 12 (P=0.016), 14 (P=0.024), and 16 (P=0.004) (Table 3-5). There was no effect of treatment group (P>0.05) on the percentage of the day that cats were identified as sitting.

3.3 Blood assessments

3.3.1 Cytokines

All the cytokines were above their minimum detectable concentrations. There were no differences (P>0.05) in the concentrations of any of the cytokines measured between the control and test groups at week 0 or week 16 (Table 3-6).

Table 3-3 Concentration of cytokines (pg/ml, mean ± SEM) of cats in the control and test groups at week 0 and week 16 along with the minimum detectable concentration.

	Week 0		Week 16		Minimum detectable concentration
Cytokine	Control	Test	Control	Test	
Fas	8.1 ± 1.0	43.6 ± 46.4	6.7 ± 5.1	56.2 ± 63.9	4
Flt-3L	66.5 ± 5.7	72.3 ± 6.8	43.4 ± 5.2	59.4 ± 5.8	4
GM-CSF	6.0 ± 0.0	7.1 ± 2.3	6.0 ± 0.0	9.6 ± 10.9	6
IFN-gamma	43.0 ± 0.0	43.0 ± 0.0	62.3 ± 17.9	303.1 ± 157.1	43
IL-1 beta	47.45 ± 8.37	38.9 ± 29.5	24.3 ± 11.9	55.6 ± 33.0	14
IL-2	50.03 ± 3.39	50.0 ± 60.7	47.2 ± 28.3	174.8 ± 150.7	4
PDGF-BB	2169.5 ± 536.0	1975.5 ± 736.7	965.0 ± 409.8	466.5 ± 300.5	198
IL-12p40	398.5 ± 62.3	424.5 ± 105.6	324.7 ± 70.7	121.7 ± 131.0	9
IL-13	10.92 ± 2.0	15.5 ± 9.7	13.5 ± 8.9	30.8 ± 29.4	7
IL-4	72.57 ± 7.2	65.6 ± 33.4	110.2 ± 111.1	78.1 ± 25.9	30
IL-6	98.47 ± 33.2	101.3 ± 53.7	80.7 ± 45.4	105.4 ± 63.5	25
IL-8	43.2 ± 6.8	28.4 ± 14.0	8.7 ± 7.5	8.7 ± 6.6	7
KC	9.07 ± 2.1	37.3 ± 8.0	84.4 ± 35.9	19.8 ± 18.3	1
SDF-1	343.3 ± 48.7	960.8 ± 572.0	633.3 ± 620.3	673.7 ± 275.8	97
RANTES	17.84 ± 3.56	32.1 ± 22.9	26.9 ± 25.6	63.7 ± 68.3	1
SCF	888.1 ± 190.7	707.1 ± 346.7	874.6 ± 781.5	735.4 ± 365.6	48
MCP-1	23.03 ± 2.92	34.7 ± 14.3	18.8 ± 8.4	19.5 ± 4.7	164
TNFa	477.7 ± 55.9	290.0 ± 101.0	138.3 ± 135.6	214.6 ± 230.8	7
IL-18	53.1 ± 6.2	56.4 ± 50.1	135.1 ± 163.5	157.6 ± 123.8	30

Fas = Tumour Necrosis Factor Receptor Superfamily Member 6; Flt-3L = Fms-like Tyrosine Kinase 3 Ligand; GM-CSF = Granulocyte-Macrophage Colony-Stimulating Factor; IFN-γ = Interferon-gamma; IL-1β =

Interleukin-1β; IL-2 = Interleukin-2; PDGF-BB = Platelet-Derived Growth Factor BB; IL-12p40 = Interleukin-12 p40; IL-13 = Interleukin-13; IL-4 = Interleukin-4; IL-6 = Interleukin-6; IL-8 = Interleukin-8; KC = Keratinocyte-derived Chemokine; SDF-1 = Stromal Cell-Derived Factor 1; RANTES = Regulated on Activation, Normal T Cell Expressed and Secreted; SCF = Stem Cell Factor; MCP-1 = Monocyte Chemoattractant Protein-1; TNFα = Tumour Necrosis Factor alpha; IL-18 = Interleukin-18.

3.3.1 Biochemistry and complete blood count

Blood biochemical showed no differences (P>0.05) between the test and control groups at week 0 and week 16 (Table 3-7). Blood glucose data at week 0 could not be determined due to a problem in analysing the samples.

Table 3-4 Biochemical parameters (mean ± SEM) in control (C) and test (T) cats at week 0 and week 16 with reference ranges.

Parameter	**Ref range**	**Unit**	week0-C	week0-T	week16-C	week16-T
SDMA*	0.0 - 14	ug dL^{-1}	12.8 ± 1.0	12.7 ± 1.3	11.1 ± 4.9	12.7 ± 4.4
Glucose	0.0 - 344	IU L^{-1} at 37ºC	-	-	3.0 ± 0.5	3.0 ± 0.2
CK*	0.0 - 66	IU L^{-1} at 37ºC	169.4 ± 27.1	129.0 ± 20.9	145.0 ± 42.3	175.5 ± 23.8
AST*	0.0 - 85	IU L^{-1} at 37ºC	35.6 ± 5.9	46.0 ± 5.1	32.0 ± 8.6	32.7 ± 3.1
ALP*	0.0 - 100	IU L^{-1} at 37ºC	38.6 ± 4.5	36.8 ± 4.8	46.0 ± 15.4	37.2 ± 5.8
ALT*	0.0 - 5	ug dL^{-1}	86.0 ± 15.9	56.5 ± 2.7	55.0 ± 17.6	58.3 ± 10.9
Bilirubin	63 - 83	µmol L^{-1}	2.8 ± 0.2	2.1 ± 0.2	3.2 ± 0.8	3.0 ± 0.4
Total Protein	26 - 40	g L^{-1}	75.6 ± 1.9	78.8 ± 1.6	76.4 ± 2.3	75.2 ± 1.6
Albumin	24 - 49	g L^{-1}	32.2 ± 1.3	29.8 ± 0.6	30.8 ± 3.6	27.7 ± 0.5
Globulin	0.6 - 1.6	ratio	43.4 ± 2.3	49.0 ± 1.0	45.6 ± 3.3	47.5 ± 2.1
A.G. Ratio*	5.7 - 12.9	mmol L^{-1}	0.7 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.6 ± 0.0
Urea	70 - 159	µmol L^{-1}	12.5 ± 1.8	10.8 ± 1.4	10.4 ± 2.4	12.9 ± 1.4
Creatinine	1.3 - 5.8	mmol L^{-1}	14.1 ± 1.5	10.5 ± 1.2	9.8 ± 1.5	14.4 ± 1.7
Phosphate	1.8 - 2.7	mmol L^{-1}	124.0 ± 14.4	109.3 ± 7.5	104.6 ± 8.6	158.2 ± 19.6
Calcium	1.5 - 6.0	mmol L^{-1}	1.5 ± 0.1	2.2 ± 0.1	1.6 ± 0.1	1.7 ± 0.2
Cholesterol	147 - 156	mmol L^{-1}	2.3 ± 0.2	2.7 ± 0.3	2.3 ± 0.7	2.3 ± 0.3
Sodium	3.5 - 5.0	mmol L^{-1}	148.8 ± 2.7	146.8 ± 1.8	3.5 ± 2.1	149.3 ± 1.5
Potassium	108 - 128	mmol L^{-1}	4.1 ± 0.3	4.9 ± 0.1	4.7 ± 0.2	4.6 ± 0.2
Chloride	108 - 128	mmol L^{-1}	116.6 ± 2.6	114.8 ± 1.9	117.2 ± 1.9	118.7 ± 1.5

*SDMA= Symmetric Dimethylarginine; CK=Creatine Kinase; AST=Aspartate Aminotransferase; ALP= Alkaline Phosphatase; ALT= Alanine Aminotransferase; A.G. Ratio=Albumin to Globulin Ratio.

Complete blood count is shown in Table 3-8. There were no differences (P>0.05) between the control and test groups at week 0 and week 16.

Table 3-5 Complete blood cell counts (CBC) (mean ± SEM) in control (C) and test (T) cats at weeks 0 and 16 with reference ranges.

Parameter *	**Ref range**	**Unit**	week0-C	week0-T	week16-C	week16-T
Retic. Hb*	13.2-20.8	pg	16.2 ± 4.0	16.4 ± 0.9	17.7 ± 1.1	16.0 ± 0.2
RBC*	5.0-10.0	*10^{12}/L	7.2 ± 0.2	7.7 ± 0.5	6.8 ± 0.3	7.4 ± 0.2
Haemoglobin	80-150	g/L	107.4 ± 8.5	112.0 ± 5.9	105.4 ± 2.1	110.0 ± 2.7
HCT*	0.24-0.45	L/L	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
MCV*	39-55	fL	43.0 ± 1.4	43.5 ± 2.4	44.2 ± 1.4	45.3 ± 0.6
MCH*	13-18	pg	14.4 ± 0.9	14.8 ± 0.6	15.8 ± 0.5	15.2 ± 0.2
MCHC8	290-360	g/L	330.0 ± 7.9	311.0 ± 6.7	103.6 ± 0.9	104.5 ± 1.3
Platelets	N/A	*10^9/L				
Absolute Retic	19-107	*10^9/L	686.0 ± 0.0	279.2 ± 47.6	215.1 ± 29.8	156.4 ± 24.3
WBC*	5.5-19.5	*10^9/L	14.1 ± 5.5	11.6 ± 3.6	11.7 ± 1.5	17.9 ± 1.2
Seg. neutrophil	N/A	% WBC	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.7 ± 0.0
	2.4 - 12.5	X 10^9 L^{-1}	14.0 ± 3.8	15.5 ± 2.6	10.6 ± 1.8	14.9 ± 1.3
Lymphocytes	N/A	% WBC	0.7 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.7 ± 0.1
	1.5 - 7.0	X 10^9 L^{-1}	9.1 ± 2.7	7.8 ± 1.2	11.7 ± 2.2	11.6 ± 1.0
Monocytes	N/A	% WBC	0.1 ± 0.1	0.2 ± 0.0	0.5 ± 0.1	0.6 ± 0.1
	0.0 - 0.9	X 10^9 L^{-1}	2.6 ± 0.8	3.1 ± 0.5	6.3 ± 1.7	5.6 ± 1.0
Eosinophils	N/A	% WBC	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	0.0 - 1.5	X 10^9 L^{-1}	0.7 ± 0.1	0.5 ± 0.1	0.0 ± 0.0	0.2 ± 0.1
Basophils	N/A	% WBC				
	0.0 - 0.1	X 10^9 L^{-1}				

* Retic. Hb = Reticulocyte Haemoglobin; RBC = Red Blood Cell; HCT = Haematocrit; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC= Mean Corpuscular Haemoglobin Concentration; WBC = White Blood Cell.

Chapter 4:

Discussion

Chapter 4: Discussion

This study assessed the effects of an oral joint supplement on the behaviour and physiology of a group of old cats (age > 9 years, n=11) over a 16-week period. The results of the study showed that in terms of activity levels, cats taking the oral supplement had greater daily activity compared to cats not taking the supplement (p=0.03). Whereas, in terms of behavioural changes, an increase in grooming behaviour was observed during weeks 2 (p=0.047), 4 (p=0.002), 6 (p=0.004), 8 (p=0.01), and 14 (p=0.004), and an overall trend for a treatment effect on grooming activity (P=0.051). This finding suggests that the increased activity observed may have been associated with changes in grooming behaviour. The results of the complete blood counts, biochemistry and cytokines were not significantly different, and showed no significant negative effects on cat health.

Overall dynamic body acceleration is the sum of the overall dynamic acceleration of organisms, and therefore higher ODBA suggests greater activity levels and therefore greater energy expenditure (Gleiss et al., 2011). Thus, the cats in the current study that received the supplement were more active than those that were not supplemented. This result is consistent with previous findings that joint supplements containing similar classes of compounds could improve the activity of cats with DJD (Coleman et al., 2020; Corbee et al., 2013; Gruen, et al., 2017; Guillot et al., 2013; Lascelles et al., 2010; McCarthy et al., 2007). Polyphenols, glucosamine, chondroitin sulphate and omega-3 fatty acids have been reported to improve activity levels in cats with DJD (Sul et al., 2014; Corbee et al., 2013). Since the supplements used in the current study contained multiple active ingredients, I was unable to determine whether it was the effect of one ingredient or the effect of multiple ingredients working together that produced the effects I observed. Additionally, Parker et al. (2022) reported that cats are less active during winter months compared to summer months, and our study was conducted from May to September, which overlaps with the New Zealand winter season (June-August). This implies that cat activity may have been reduced by environmental factors during the treatment period. However, I observed higher activity levels in cats receiving the supplement despite the winter season, implying that the improvement of activity levels in older cats with this oral supplement was not affected by seasonal factors. In addition, aging has been shown to result in reduced voluntary physical activity (PA) in cats (Smit et al., 2022). All cats used in this experiment were elderly, aged >9, but the control group did not experience significant changes in ODBA before or after the treatment, suggesting that age did not affect cat activity levels in this experiment.

The results of the current study showed that supplemented cats spent 50% more time grooming than unsupplemented cats particularly in the first 8 weeks of the study. It has been reported that cats (both long-haired and short-haired) spend 25-30% of their time grooming (Kim et al., 2019) , but grooming behaviours are more difficult to measure than behaviours such as running and jumping, and in the present experiment cats spent around 3% of their time grooming, which may be related to the inability of accelerometers to accurately identify grooming. However, Monteiro & Steagall (2019) reported that grooming behaviour decreased when cats were in chronic pain resulting in matted fur. Therefore, the increase in grooming behaviour observed in the current study may reflect a reduction in pain associated with DJD. In addition, Smit et al. (2024) found that a reduction in physical activity in cats were likely to be caused by reductions in both active and grooming behaviours, implying that when cats' physical activity increased, grooming behaviours were likely to increase as well. This is supported by the increase in grooming behaviour in the present study.

Locomotion behaviour in the current study showed no differences until the final week of the study when supplemented cats showed less time exhibiting locomotion than control cats (P=0.007). In previous studies of cats, accelerometers have been used to determine overall energy expenditure and while changes in overall locomotor activity have been reported they did not specifically identify locomotion behaviours (Corbee et al., 2013; Lascelles et al., 2008). This result was markedly different to studies that used questionnaires to categorise cat behaviour. In such surveys, changes usually occurred in movement-related behaviours such as going up and down stairs, running, jumping. These types of questionnaires, however, are highly subjective and lack objective data-based measures. In contrast to previous studies, there were no significant changes in the overall locomotor behaviour of the cats in the present study. Previous studies have shown that joint supplements are effective in improving locomotor levels in DJD cats (Benito et al., 2013; Enomoto et al., 2020; Guillot et al., 2013; Klinck et al., 2015;

Lascelles et al., 2007; Monteiro & Steagall, 2019). One possible explanation for this is that despite the large number of past studies of the effect of joint supplementation on behaviour in cats with DJD (Coleman et al., 2020; Corbee et al., 2013; Gruen et al., 2017; Guillot et al., 2013; Lascelles et al., 2010; McCarthy et al., 2007), these studies were assessed differently compared to the current study, with most using force plates (Corbee et al., 2013) or questionnaires (Cunningham et al., 2022; Sul et al., 2014), and the few studies that used accelerometers (Gruen et al., 2017; Guillot et al., 2013; Lascelles et al., 2007), there was little breakdown of the cats' behavioural patterns, with only differences in overall exercise being identified. Thus, in past studies using accelerometers, many other behaviours were attributed to movement, which may account for the discrepancy in results between these studies and mine. In future studies, it may be possible to measure more behavioural changes that are not readily observable if the behavioural patterns recorded by accelerometers can be more finely classified.

None of the cats that received the supplements in the current study showed changes in the concentration of a range of inflammatory cytokines. Pain mechanisms in DJD cats are dominated by inflammatory cytokines (Fox, 2016), and pain scores in DJD cats have been positively correlated with the blood cytokines IL-8, IL-2, and TNF-α (Gruen et al., 2017). The dietary supplement used in this study contained omega-3 polyunsaturated fatty acids (PUFA) and polyphenols with anti-inflammatory and antioxidant properties. Previous studies have shown that omega-3 fatty acids (n-3 PUFA), especially EPA and DHA, reduce the production of thromboxane A2 and leukotriene B4, which inhibit pro-inflammatory mediators such as interleukin 1, interleukin 2, and tumour necrosis factor in cartilage, and that supplementation with n-3 PUFA produces antioxidant effects, which can improve the clinical symptoms of DJD patients (Corbee et al., 2013; Johnson et al., 2020). In the case of polyphenolic compounds, they have been shown to have potent antioxidant, anti-inflammatory, antimicrobial, antirheumatic, neuroprotective, anticarcinogenic, hepatoprotective, and cardioprotective properties, even though their mechanism(s) of action are not fully known (Sirše, 2022). In a study on rats, curcumin which was also used in this study significantly reduced the levels of TNF-α, IL-1β, IL-6, COMP and CRP in rat synovial tissue (Corbee, 2022). Interestingly, in past studies, samples used in the cytokine assays have typically come from synovial fluid rather than systemically, so while the present study saw no differences in circulating proinflammatory cytokine concentrations within the blood of cats following 16 weeks of dietary supplementation, there may have been local changes within the synovial joint (Corbee, 2022). However, the lack of change in cytokine markers following four months of administration did indicate that there were no negative effects of supplementation, which suggests that the supplement is safe for cats.

The control and test groups in the current study did not show any differences in routine blood and biochemical tests either before or after the experiment. This is not unexpected given that DJD rarely results in changes in routine blood and biochemical indices, diagnosis usually relies on subtle changes in synovial fluid composition (Lemetayer & Taylor, 2014).

Chapter 5:

Conclusions and future research

Chapter 5 Conclusion and future research

The joint supplements used in this study were a unique combination of active ingredients commonly found in commercially available joint supplements (antioxidants, polyphenols, and chondroitin sulphate) included at levels proven to be effective in peer-reviewed studies. Their effectiveness was assessed using accelerometers and blood analyses, which is an objective and quantifiable method of assessing effectiveness. To my knowledge, the current study is the first to use accelerometers to compare specific behaviours in cats with degenerative joint disease (DJD). The results of this study suggest that the oral supplement was effective in restoring activity in older cats with DJD. Secondly, this study found a trend for an improvement in grooming behaviour following oral administration of the supplement, which further supports the effectiveness of the supplement and also suggests that grooming may be one of the most significant behaviours that change when effective joint supplements are administered. In addition, blood analyses did not show significant differences, which may be caused by the fact that the oral supplement acted locally, whereas the samples for cytokine detection came from the systemic circulation. Therefore, testing synovial fluid rather than venous blood should be considered in future studies. Moreover, in the future research on accelerometers, more studies are needed to categorise the accelerometer data into activities, so that the accelerometers can perform more accurate detection of specific cat behaviours and identify health abnormalities in cats according to the changes in each behaviour.

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