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A pilot study to detect the effects of a green-lipped mussel (*Perna canaliculus*) nutraceutical on working farm dogs with musculoskeletal abnormalities using accelerometry

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ABSTRACT

Aims: To obtain preliminary data on changes in gait from the use of a green-lipped mussel (*Perna canaliculus*) extract product in working farm dogs with musculoskeletal abnormalities using accelerometry.

Methods: New Zealand working farm dogs (n = 32) with signs of musculoskeletal abnormalities were enrolled in a double-blinded, placebo-controlled cross-over study. Each dog was allocated to one of six groups to receive three trial substances (180 mg full fat green-lipped mussel extract ($GLME_{180}$); 220 mg full fat green-lipped mussel extract ($GLME_{220}$); placebo) in one of the six possible different orders. Each trial substance was administered orally once a day for an 8-week period, with a 4-week washout in between each. Dogs wore a collar-mounted triaxial accelerometer for the study duration. Diet and activity were not controlled. Accelerations were recorded continuously and analysed (n = 27) in 10-second activity epochs partitioned into daytime and night-time periods. Analysis of activity during the daytime period was limited to epochs when dogs were gaiting faster than a walk. The median and IQR of activity were determined for the daytime and night-time. Additionally, the 75th and 90th percentiles of daytime activity for each 24-hour period were determined. Mixed effects linear regression models were constructed to determine if each trial substance altered the response variables.

Results: During the daytime, the 90th percentile was higher when dogs were given $GLME_{220}$ compared with the placebo (β coefficient 2.6; 95% CI = 0.25–4.94; p = 0.03). Dogs that started the trial with the GLME products had a higher 90th percentile activity compared with dogs that began with the placebo (β coefficient 26.26; 95% CI = 0.45–52.06; p = 0.046). The 75th percentile for activity was not affected by the GLME product. The daytime IQR was larger when dogs were given the $GLME_{180}$ product compared with the placebo (β coefficient 1.25; 95% CI = 0.12–2.37; p = 0.03). Night-time median activity and the IQR was greater in dogs that started the trial with the GLME products than in dogs that began with the placebo. The night-time IQR for activity was greater for $GLME_{180}$ than for the placebo.

Conclusions: Administration of a low dose of the GLME-containing product increased peak activity in working farm dogs with signs of musculoskeletal abnormalities and may improve their performance.

Clinical relevance: Even mildly affected working farm dogs might benefit from support of their musculoskeletal abnormalities, and this particular GLME-based product shows promise as an adjunct to other management strategies.

Abbreviations: GLME: Green-lipped mussel extract; NSAID: Non-steroidal anti-inflammatory drugs; OA: Osteoarthritis; PGE_2: Prostaglandin E2

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Introduction

Musculoskeletal problems are common reasons for presentation to veterinarians, accounting for 8.6% of disorders recorded in dogs under primary veterinary care in the UK (O'Neill *et al.* 2021). There are various clinical signs of joint disease, including lameness, joint swelling, pain on manipulation, a reduced range of motion, and joint crepitus (Cooper *et al.* 2014; Pettitt and German 2015). Several clinical signs are improved with non-steroidal anti-inflammatory drugs (NSAID), which inhibit the production of prostaglandin E2 (PGE_2). In addition to NSAID, some extracts from the green-lipped mussel (*Perna canaliculus*), have been shown to inhibit PGE_2 production, and have a clinically significant effect in animals and humans with osteoarthritis (OA) (Whitehouse *et al.* 1997; Halpern 2000).

Similar to other species, when administered to dogs with osteoarthritis, there is evidence that some green-

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lipped mussel extracts (GLME) are capable of alleviating clinical signs such as swelling and pain (Bui and Bierer 2003; Servet *et al.* 2006). However, despite the consistency of the *in vitro* effect of efficacious products, the evidence of efficacy of GLME for the treatment of OA in dogs has been inconsistent; some studies identified a clear reduction in clinical signs as a result of treatment, while others failed to identify any effect at all (Dobenecker *et al.* 2002; Pollard *et al.* 2006). There are several potential explanations for the inconsistencies in findings related to the efficacy of GLME. Firstly, the extract is a complex biological product that has no standard definition, and it can vary between seasons, and greatly between producers (Juliano *et al.* 2016; Miller and Tian 2018). Given that the precise efficacious components have yet to be defined, it is not surprising that there is significant variation between studies using different products.

Studies of the efficacy in dogs have also differed 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, blinding of researchers, and trial length (Dobenecker *et al.* 2002; Bui and Bierer 2003; Pollard *et al.* 2006; Servet *et al.* 2006; Rialland *et al.* 2012). In general, those trials that do not include blinding or a placebo control are more likely to show a treatment effect. GLME efficacy has predominantly been measured subjectively, commonly using questionnaires administered to owners or veterinarians. Objective measures of clinical signs, such as force plate analysis, have been less frequently used, with only one study of GLME efficacy that used force plates, showing an increase in peak vertical force when dogs were fed a GLME-supplemented diet (Rialland *et al.* 2012).

Accelerometry is another objective tool that has been used to evaluate management of musculoskeletal abnormalities in dogs. Typically, trends in a dog's activity have been expressed in terms of the activity count (Brown *et al.* 2010; Knazovicky *et al.* 2015). 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. Thus, movements with greater changes in acceleration (i.e. higher intensity exercise) increase the activity count. However, different manufacturers of accelerometers convert these measurements into an activity count in differing ways. Effective management of musculoskeletal problems is expected to increase the activity count during the day as inflammation and pain decrease. Conversely, night-time activity has been proposed to be lower in dogs with reduced clinical signs of OA, as night-time restlessness is a commonly reported feature of OA (Knazovicky *et al.* 2015).

New Zealand farm dogs are a population in which the effectiveness of treatments could be measured using accelerometry. They are comprised almost entirely of two breeds, the Huntaway and the Heading Dog, which are unique to the New Zealand farming industry (Cave *et al.* 2009; O'Connell *et al.* 2019). The dogs have a high athletic requirement, a high incidence of injury, and high prevalence of musculoskeletal problems. A recent longitudinal study reported that 43% of working farm dogs had a musculoskeletal abnormality on physical examination, of which 26% were lame (Isaksen *et al.* 2020), and OA is the most commonly reported musculoskeletal condition in dogs presented for veterinary examination (Cave *et al.* 2009). However, despite the prevalence of joint injuries and OA, the frequency of treatment is low, and yet many dogs remain active and are highly motivated to work, and therefore they are a good population of athletic, active dogs in which to study the efficacy of GLME.

Further, unlike owners of pet dogs, farm dog owners are less likely to change the exercise routine and diet of working dogs after enrolment on a trial, which is a significant component of the placebo effect that confounds 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. These concerns are less, or perhaps absent, in working farm dogs.

Despite the apparent suitability of the working dog population for studying musculoskeletal abnormalities, there are a number of possible challenges associated with using this population. Working dog activity can vary greatly between farms and by season, and this high variation may complicate the identification of a trial substance effect with accelerometry (Hunt *et al.* 2018). In addition, the 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 study population.

We describe here a pilot study that was designed to obtain preliminary data on the use of accelerometery to assess changes in gait from the use of a GLME-based nutraceutical in working farm dogs with mild musculoskeletal abnormalities. Specifically, we aimed 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 musculoskeletal abnormalities to administration of a GLME-based product using accelerometry, in order to design a full study that is properly powered.

Materials and methods

Animal selection

Thirty-two New Zealand working farm dogs with clinical signs of musculoskeletal abnormalities were recruited for this pilot study. It was predicted that this would be enough dogs to provide an estimate of suitability of both working dogs as a population for evaluation, and accelerometry as a tool for identifying the efficacy of a trial substance in the working dog population. This number was based on two comparable studies that used 23 and 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 and Bierer 2003; Rialland *et al.* 2012).

Working farm dogs with musculoskeletal abnormalities were primarily selected from a pre-existing group of farm dogs participating in a longitudinal study of working dog health (Isaksen *et al.* 2020). Additional dogs were sourced from either interested owners volunteering dogs with known musculoskeletal abnormalities, or a veterinary clinic client base in the North Island. A veterinarian examined each participating dog, and all limb joints were palpated, the range of motion and the presence of lameness was assessed by observation while the dog trotted. Musculoskeletal abnormalities were 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. To be included, dogs had to have one or more of the following joints affected: hip, stifle, carpus, shoulder, elbow, or hock. Dogs with appropriate clinical signs in a relevant joint were considered for inclusion, pending owner consent. Any supplements were discontinued prior to commencement, and no dog was being treated with NSAID or corticosteroids immediately prior to or during the trial. It was known *a priori* that New Zealand working dogs are less active during winter than during other months due to the seasonal nature of their farm work, therefore the study was conducted from August 2018 to September 2019. The study was approved by the Massey University Animal Ethics Committee (MUAEC 17/103) and funded by the manufacturer of the GLME-based product, Lintbells Ltd. (Weston, UK).

Study design

The study was designed as a double-blinded, cross-over study with three trial substances: a nutraceutical containing 180 mg full fat green-lipped mussel extract ($GLME_{180}$), a nutraceutical containing 220 mg full fat green-lipped mussel extract ($GLME_{220}$), and a placebo (all Lintbells Ltd.). All tablets weighed approximately 1 g. To ensure that the researchers and owners were blinded to the trial substances, they were packaged and formulated to look nearly identical, and then labelled as A, B, or C. Each trial substance was administered by the farmer for an 8-week period, with a 4-week washout in between each substance. Each dog was allocated to one of six groups to receive the trial substances in one of the six possible different orders (e.g. ABC, BCA, CBA, etc.). Dogs with the same owner were allocated to the same group to remove the possibility of the owners mixing up the trial substances. Groups were allocated by ordering the owners by number of dogs and first name, and then allocating them a number from 1–6 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. Once the accelerometer setup was optimised, and each dog had at least 1 week of data successfully collected, the first round of trial substance administration was started. This required the administration of a single liver-flavoured tablet once a day at the owners preferred feeding time. The owner either hid the tablet in food, fed the tablet out of their hand, or physically dosed the dog. Diet and activity of the dogs was not controlled. Owners were encouraged to maintain their normal routines. Morphometric measurements were also collected for each dog, following the method developed by Leung *et al.* (2018). Age, weight, breed, and sex of each dog were also recorded.

Accelerometer

A micro electro-mechanical triaxial accelerometer (Heyrex; Say Systems, Wellington, NZ) weighing 32 g and measuring $65 \times 26 \times 18$ mm was used for this 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, and was positioned on the ventral side of the dog's neck. The accelerometer uses the deformation of a piezoelectric crystal to generate a continuous voltage output that is proportional to acceleration in the direction of deformation. The voltage output is expressed in units of standard gravity (G), and the change as delta-G. Accelerations between +4 and −4 G in magnitude were recorded at a rate of 10 samples per second. Accelerations were measured simultaneously across three orthogonal axes as the change in acceleration between neighbouring samples. The raw data was collected as the delta-G over each 1 second then it was summed into periods (epochs) of 10 seconds for analysis (delta-G_{10}). This data compression was performed to smooth out the large second by second variations, and to reduce the data set to a more manageable size for statistical analysis.

Up to 7 days of data were 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. To facilitate data transfer from the accelerometer to the receiver, the accelerometer had to be within 30 m 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 used to move the receiver into line of sight, and where distance was an issue the receiver was set up with a Wi-Fi extender to a nearer power source. Where neither were viable options, the owners were asked to remove the collar once a week and position the accelerometer by the receiver overnight to upload a full memory of data, before replacing the collar on the dog the next morning.

Fatty acid analysis

To confirm the composition of the tablets, a sample of each type was analysed for its polyunsaturated fatty acid content. Each tablet was crushed and suspended in toluene. To methylate fatty acids methanolic hydrochloride was added. Samples were mixed with a vortex mixer, heated at 70°C for 2 hours and cooled on ice. Potassium carbonate and toluene were added and samples were mixed again and centrifuged at 2,500 rpm for 7 minutes at room temperature to separate the solvent layer containing methyl esters from the aqueous layer.

Fatty acids were detected by using a Shimadzu GC-2010 Plus gas chromatograph equipped with a flame ionisation detector (Shimadzu, Kyoto, Japan) and a 0.2-µm film thickness biscyanopropyl GC column (SupelcoTM-2560; Supelco, Bellefonte, PA, USA). The oven temperature was programmed to hold at 140°C for 5 minutes, increase to 240°C at the rate of 4°/minute, then hold for 38 minutes. Injector temperature was 250°C and detector temperature 255°C. Standards were purchased from Sigma-Aldrich (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. The fatty acids indicative of the GLME content are the n-3 polyunsaturated fatty acids. The proportion of fat composed of n-3 polyunsaturated fatty acids in tablets A, B, and C were 50.9%, 20.2%, 0.9% respectively. 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 were filtered and analysed prior to unblinding using the statistical processing software R (Version 3.5.2, R Development Core Team; R Foundation for Statistical Computing, Vienna, Austria). Data from dogs that had completed fewer than two of the treatment periods were excluded. For the remaining data epochs that were invalid or artefactual were removed on the basis of the following exclusion criteria: (1) incorrect time-stamps; (2) periods of time when the accelerometer was not on the dog, defined as a run of 360 consecutive epochs without a change in pitch or roll; (3) epochs in which no activity was recorded; (4) epochs containing rhythmic movement of the accelerometer lasting more than 5 seconds, which is caused by either scratching, or other intense, rhythmic activities such as running with a collar that was not attached tightly enough. The exclusion criteria were not applied in a step-by-step fashion; rather, new variables were created for each of the exclusion criteria.

Coding the response variables

To investigate the effect of each trial substance on delta-G_{10}, activity 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 the period in which the dogs were most settled and likely to be sleeping.

The daytime period was defined as 4 am to 11 pm on the same day. Within the daytime period, periods of low intensity activity, such as lying down, were removed. In order to achieve this, the daytime data were filtered to select epochs with a delta-$G_{10} \geq 60$, which we had previously shown was the threshold for the change in gait from walking to trotting (Bolton *et al.* 2021). Thus, the activity data that were analysed from the daytime periods represent all the epochs when the dogs were inferred 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 IQR for each date of the trial were determined. Additionally, it was hypothesised that changes in activity in response to the trial substances would be seen in the most vigorous or intense activities, thus the 75th and 90th percentiles for each date were used to summarise activity in the daytime data set only.

Statistical analysis

General descriptive statistics of variables were calculated, and initial relationships between the delta-G response variables (median delta-G_{10} per date, 75th and 90th percentiles per date, and the IQR per date)

and single explanatory variables were evaluated with unadjusted linear regression models. In addition to "treatment," the other individual explanatory variables were "days into treatment" (i.e. how many days since commencing the treatment phase of the trial for each dog), "treatment order," season, sex, breed, weight, age, skeletal size, and farm. To describe the variation in activity within and between dogs over the course of the study, the sum of the delta-G_{10} for each month was calculated for each dog and the monthly summed delta-G_{10} values plotted as a boxplot for each dog.

Six mixed effects linear regression models with multiple predictor variables were constructed to determine if each trial substance, referred to in statistical models as "treatment," altered the response variables over the daytime or night-time. The final models included "treatment," "treatment order," season, with dog as a random effect in order to account for the repeated measures. To check model assumptions, standardised residuals were checked for normality, a zero mean, and a constant SD. For the model predicting IQR, median was also added as a fixed effect. Initially, the term "days into treatment" was added into the models, with an interaction term with "treatment order." However, when it was apparent that there was a "treatment order" effect, "days into treatment" was deemed unnecessary as "treatment order" accounted for the effect of "days into treatment," and it was removed from the final models. Given the size and complexity of the dataset, we assumed the correlation between measurements taken on the same dog would be constant, regardless of how far apart in time the measurements were. The p-values for Wald tests for the terms in the model were used to determine if there was an effect of a predictor variable on the response variables. Variables were excluded from the models in a stepwise manner if $p \geq 0.05$. The manufacturer of the GLME nutraceutical was not party to the results until after the analysis was completed.

Results

Of the 32 dogs recruited for the study, five were excluded from the analysis. Four were withdrawn from the trial for personal reasons or time constraints, and one due to breaking its leg. This left 27 dogs for analysis. The 27 dogs were spread across 16 farms, with two farms having more than one dog owner working on them. Ten 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 until the conclusion of the study.

The characteristics of the dogs, including the physical description of their musculoskeletal abnormalities, are presented in Supplementary Table 1. Dogs' ages were estimated by farmers with a mean age of 5.8 (min 2, max 11) years. Mean bodyweight was 26.7 (min 16.6, max 38.6) kg. As presented in Supplementary Table 1, the most commonly affected joint was the hip, and a reduced range of motion and pain on manipulation were 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. A total of 1,830,929 epochs (5.5% of the total dataset) met the exclusion criteria and were deleted prior to analysis (Supplementary Tables 2 and 3). When categorised according to time, 6,565,232 epochs were available for the night-time period. For the daytime period, to retain an epoch the dogs needed to be classified as gaiting at a pace faster than walking, which resulted in a total of 554,408 epochs for analysis. These remaining daytime epochs are referred to as "delta-G_{10} greater than walking."

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

Daytime activity data were recorded for 27 dogs for a total of 3,500 days: 1,160 days when 27 of the dogs were given $GLME_{220}$, 1,113 days when 23 of the dogs were given $GLME_{180}$, and 1,227 when 26 of the dogs were given the placebo. On 21 of the 3,500 days, the delta-G_{10} IQR was 0, because after excluding errors/artefacts and epochs with activity < 60 delta-G_{10}, there was only one 10-second epoch available for the day. Therefore, the data were excluded. There were 1,218 summer days, 1,189 spring days, 1,088 autumn days, and 5 winter days with epochs greater than walking. Of those, 1,443 days were for Heading Dogs, and 2,057 days were for Huntaways.

The distribution of the daytime 75th and 90th percentile delta-G_{10}, median and IQR delta-G_{10} are presented in Supplementary Figures 1–4. The relationships between each of the daytime response variables and each of the explanatory variables are described in Supplementary Tables 4–7. For each of the daytime response variables there were significant differences between autumn as the reference category, and spring, summer, and winter. The relationships were similar for each of the response variables, so boxplots are only shown for the relationships between 75th percentile and season (Supplementary Figure 5), and between the 90th percentile and breed (Supplementary Figure 6). There was also notable variation in the total activity within and between the dogs over the course of the study (Supplementary Figure 7).

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 1–4. The main

treatment effect in each table is presented as the beta-coefficient compared to the placebo, which is the average increase in the response variable (e.g. 90th percentile delta-G_{10}) when dogs received each of the GLME products, with the 95% CI. Similarly, the effect of treatment order, and the effect of the season during which the treatment was given are also included.

The daytime 90th percentile delta-G_{10} was significantly higher when dogs were given the $GLME_{220}$ compared with the placebo, significantly higher during the spring compared to the autumn and the summer, and significantly higher for the order [$GLME_{220}$: $GLME_{180}$: placebo] compared to the order [placebo: $GLME_{180}$: $GLME_{220}$] (Table 1). The daytime 90th percentile delta-G_{10} was not significantly different between periods when dogs were given $GLME_{180}$ and $GLME_{220}$ ($p = 0.74$).

There was no evidence of a change in the 75th percentile with GLME administration (Table 2). The 75th percentile was highest during the spring. The daytime 75th percentile delta-G_{10} was not significantly different between periods when dogs were given the $GLME_{180}$ and $GLME_{220}$ ($p = 0.94$).

There was no evidence of an effect of GLME220 nor GLME180 administration on daytime median delta-G_{10} (Table 3) when compared with placebo. The daytime median delta-G_{10} was highest during the spring. There was also no evidence of a difference in daytime median delta-G_{10} when dogs were given GLME180 or GLME220 ($p = 0.81$).

The daytime IQR for delta-G_{10} was larger when dogs were treated with $GLME_{180}$ compared with the placebo, and during the spring compared with summer (Table 4). The daytime IQR for delta-G_{10} increased linearly with the median. Daytime IQR delta-G_{10} was not significantly different between periods when dogs were given $GLME_{220}$ and $GLME_{180}$ ($p = 0.72$).

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 were nights when all 27 dogs were on $GLME_{220}$, 1,177 when 23 of the dogs were on $GLME_{180}$, and 1,352 when 26 of the dogs were given the placebo. The distributions of the night-time median delta-G_{10} (median = 0.12; min 0, max 22.44) and delta-G_{10} IQR (median = 0.80; min 0, max 64.6) were both skewed.

The unadjusted relationships between individual predictor variables and the night-time median and IQR for delta-G_{10} are presented in Supplementary Tables 8 and 9. Both night-time response variables were significantly associated with season, so only a box-plot for the relationship between the median

Table 1. Results of a mixed effects linear regression model[a] of the effect of oral treatment for 8 weeks each with 180 or 220 mg green-lipped mussel (*Perna canaliculus*) powder (GLME) per day or a placebo on the 90th percentile delta-G_{10}[b] recorded during daytime (4:00–23:00) from individual New Zealand working farm dogs (n = 27) fitted with collar-mounted accelerometers.

Parameter	Beta coefficient (SE)	95% CI	P-value[c]
Treatment			
Placebo	REF		
$GLME_{220}$	2.6 (1.2)	0.253–4.94	0.03
$GLME_{180}$	2.19 (1.22)	−0.21–4.58	0.073
Order of treatment			
$GLME_{220}$: $GLME_{180}$: Placebo	REF		
$GLME_{220}$: Placebo: $GLME_{180}$	−8.1 (13.17)	−35.48–19.28	0.545
$GLME_{180}$: $GLME_{220}$: Placebo	18.99 (14.31)	−10.77–48.74	0.199
$GLME_{180}$: Placebo: $GLME_{220}$	13.12 (12.39)	−12.65–38.89	0.302
Placebo: $GLME_{220}$: $GLME_{180}$	−11.71 (12.43)	−37.56–14.14	0.357
Placebo: $GLME_{180}$: $GLME_{220}$	−26.26 (12.41)	−52.06 to −0.45	0.046
Season			
Spring	REF		
Autumn	−5.38 (1.24)	−7.8 to −2.95	< 0.001
Summer	−3.93 (1.19)	−6.27 to −1.59	0.001
Winter	−15.91 (13.02)	−41.45–9.62	0.222

[a]Overall intercept was 151.16 (SE 8.85). Dog was included with a random intercept (SD 19.45 (95% CI = 14.3–26.45)) with a residual of 28.29 (95% CI = 27.63–29.0).
[b]Delta-G_{10} = change in acceleration in three orthogonal axes, summed over a 10 second epoch filtered to only include epochs with delta G_{10} greater than walking.
[c]Significance of the coefficient in the model. Variables were only included in the model if the global p-value from a Wald test for the inclusion of the variable had an associated p-value > 0.05.
REF = reference category.

Table 2. Results of a mixed effects linear regression model[a] of the effect of oral treatment for 8 weeks each with 180 or 220 mg green-lipped mussel (*Perna canaliculus*) powder (GLME) per day or a placebo on the 75th percentile delta-G_{10}[b] recorded during daytime (4:00–23:00) from individual New Zealand working farm dogs (n = 27) fitted with collar-mounted accelerometers.

Parameter	Beta coefficient (SE)	95% CI	P-value[c]
Treatment			
Placebo	REF		
$GLME_{220}$	1.74 (0.95)	−0.115–3.6	0.066
$GLME_{180}$	1.81 (0.97)	−0.08–3.71	0.061
Order of treatment			
$GLME_{220}$: $GLME_{180}$: Placebo	REF		
$GLME_{220}$: Placebo: $GLME_{180}$	−1.14 (10.1)	−22.14–19.86	0.911
$GLME_{180}$: $GLME_{220}$: Placebo	11.97 (10.98)	−10.85–34.8	0.288
$GLME_{180}$: Placebo: $GLME_{220}$	10.52 (9.51)	−9.25–30.29	0.281
Placebo: $GLME_{220}$: $GLME_{180}$	−6 (9.54)	−25.84–13.83	0.536
Placebo: $GLME_{180}$: $GLME_{220}$	−17.34 (9.52)	−37.13–2.46	0.083
Season			
Spring	REF		
Autumn	−4.93 (0.98)	−6.85 to −3.01	<0.001
Summer	−3.47 (0.94)	−5.32 to −1.62	<0.001
Winter	−6.16 (10.31)	−26.37–14.06	0.55

[a]Overall intercept was 121.41 (SE 6.79). Dog was included with a random intercept, (SD 14.91 (95% CI = 11.0–20.3)) with a residual of 22.39 (95% CI = 21.87–22.93).
[b]Delta-G10 = change in acceleration in three orthogonal axes, summed over a 10 second epoch filtered to only include epochs with delta G10 greater than walking.
[c]Significance of the coefficient in the model. Variables were only included in the model if the global p-value from a Wald test for the inclusion of the variable had an associated p-value > 0.05.
REF = reference category.

Table 3. Results of a mixed effects linear regression model[a] of the effect of oral treatment for 8 weeks each with 180 or 220 mg green-lipped mussel (*Perna canaliculus*) powder (GLME) per day or a placebo on the median delta-G_{10}[b] recorded during daytime (4:00–23:00) from individual New Zealand working farm dogs (n = 27) fitted with collar-mounted accelerometers.

Parameter	Beta coefficient (SE)	95% CI	P-value[c]
Treatment			
Placebo	REF		
$GLME_{220}$	0.64 (0.64)	−0.614–1.9	0.316
$GLME_{180}$	0.49 (0.65)	−0.8–1.77	0.456
Order of treatment			
$GLME_{220}$: $GLME_{180}$: Placebo	REF		
$GLME_{220}$: Placebo: $GLME_{180}$	2.06 (6.37)	−11.18–15.3	0.75
$GLME_{180}$: $GLME_{220}$: Placebo	4.77 (6.92)	−9.61–19.15	0.498
$GLME_{180}$: Placebo: $GLME_{220}$	5.22 (5.99)	−7.24–17.67	0.394
Placebo: $GLME_{220}$: $GLME_{180}$	−1.34 (6.01)	−13.85–11.16	0.826
Placebo: $GLME_{180}$: $GLME_{220}$	−8.69 (6)	−21.17–3.78	0.162
Season			
Spring	REF		
Autumn	−3.42 (0.66)	−4.72 to −2.12	<0.001
Summer	−1.79 (0.64)	−3.04 to −0.54	0.005
Winter	−2.87 (6.98)	−16.55–10.82	0.681

[a]Overall intercept was 93.83 (SE 4.29). Dog was included with a random intercept, (SD 9.38 (95% CI = 6.89–12.78)) with a residual of 15.16 (95% CI = 14.8–15.52).
[b]Delta-G_{10} = change in acceleration in three orthogonal axes, summed over a 10 second epoch filtered to only include epochs with delta G_{10} greater than walking.
[c]Significance of the coefficient in the model. Variables were only included in the model if the global p-value from a Wald test for the inclusion of the variable had an associated p-value > 0.05.
REF = reference category.

Table 4. Results of a mixed effects linear regression model[a] of the effect of oral treatment for 8 weeks each with 180 or 220 mg green-lipped mussel (*Perna canaliculus*) powder (GLME) per day or a placebo on the IQR of delta-G_{10}[b] recorded during daytime (4:00–23:00) from individual New Zealand working farm dogs (n = 27) fitted with collar-mounted accelerometers.

Parameter	Beta coefficient (SE)	95% CI	P-value[c]
Treatment			
Placebo	REF		
$GLME_{220}$	1.05 (0.56)	−0.05–2.15	0.063
$GLME_{180}$	1.25 (0.57)	0.12–2.37	0.03
Order of treatment			
$GLME_{220}$: $GLME_{180}$: Placebo	REF		
$GLME_{220}$: Placebo: $GLME_{180}$	−4.52 (3.36)	−11.51–2.47	0.193
$GLME_{180}$: $GLME_{220}$: Placebo	5.82 (3.64)	−1.74–13.39	0.125
$GLME_{180}$: Placebo: $GLME_{220}$	4.42 (3.15)	−2.14–10.97	0.176
Placebo: $GLME_{220}$: $GLME_{180}$	−5.53 (3.18)	−12.15–1.1	0.097
Placebo: $GLME_{180}$: $GLME_{220}$	−7.39 (3.17)	−13.98 to −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 to −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

[a]Overall intercept was −32.13 (SE 2.69). Dog was included with a random intercept, (SD 4.86 (95% CI = 3.52–6.69)) with a residual of 13.31 (95% CI = 13.0–13.62).
[b]Delta-G_{10} = change in acceleration in three orthogonal axes, summed over a 10 second epoch filtered to only include epochs with delta G_{10} greater than walking.
[c]Significance of the coefficient in the model. Variables were only included in the model if the global p-value from a Wald test for the inclusion of the variable had an associated p-value > 0.05.
REF = reference category.

and season has been shown (Supplementary Figure 8). Both night-time response variables were significantly associated with trial substance 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] (Supplementary Figure 9).

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 5 and 6. There was no evidence of an effect of administering $GLME_{220}$ or $GLME_{180}$ on the night-time median delta-G_{10} compared to placebo (Table 5).

Treatment orders beginning with the placebo, [placebo: $GLME_{220}$: $GLME_{180}$] and [placebo: $GLME_{180}$: $GLME_{220}$], were associated with a higher night-time median delta-G_{10} than the reference order [$GLME_{220}$: $GLME_{180}$: placebo], as were autumn and summer compared with spring. There was no evidence of a difference in night-time median delta-G_{10} between periods when dogs were given $GLME_{180}$ and $GLME_{220}$ (p = 0.59).

The night-time IQR for delta-G_{10} was greater when dogs were given $GLME_{180}$ than when given the placebo (Table 6). Overall, the night-time IQR for delta-G_{10} also increased with increasing median. The IQR was 0.07 (95% CI = 0.02–0.12) less when dogs were given $GLME_{220}$ compared to $GLME_{180}$.

Table 5. Results of a mixed effects linear regression model[a] of the effect of oral treatment for 8 weeks each with 180 or 220 mg green-lipped mussel (*Perna canaliculus*) powder (GLME) per day or a placebo on median delta-G_{10}[b], recorded during night-time (23:00–4:00) from individual New Zealand working farm dogs (n = 27) fitted with collar-mounted accelerometers.

Parameter	Beta coefficient (SE)	95% CI	P-value[c]
Treatment			
Placebo	REF		
$GLME_{220}$	0.03 (0.02)	−0.003–0.06	0.075
$GLME_{180}$	0.02 (0.02)	−0.01–0.05	0.231
Order of treatment			
$GLME_{220}$: $GLME_{180}$: Placebo	REF		
$GLME_{220}$: Placebo: $GLME_{180}$	0.07 (0.04)	−0.01–0.15	0.094
$GLME_{180}$: $GLME_{220}$: Placebo	0.02 (0.04)	−0.06–0.11	0.574
$GLME_{180}$: Placebo: $GLME_{220}$	0.04 (0.04)	−0.03–0.12	0.263
Placebo: $GLME_{220}$: $GLME_{180}$	0.1 (0.04)	0.02–0.18	0.019
Placebo: $GLME_{180}$: $GLME_{220}$	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

[a]Overall intercept was −0.002 (SE 0.03). Dog was included with a random intercept, (SD of 0.05 (95% CI = 0.03–0.08)) with a residual of 0.4 (95% CI = 0.39–0.41).
[b]Delta-G_{10} = change in acceleration in three orthogonal axes, summed over a 10 second epoch filtered to only include epochs with delta G_{10} greater than walking.
[c]Significance of the coefficient in the model. Variables were only included in the model if the global p-value from a Wald test for the inclusion of the variable had an associated p-value > 0.05.
REF = reference category.

Table 6. Results of a mixed effects linear regression model[a] of the effect of oral treatment for 8 weeks each with 180 or 220 mg green-lipped mussel (*Perna canaliculus*) powder (GLME) per day or a placebo on the IQR of delta-G_{10}[b], recorded during night-time (23:00–4:00) from individual New Zealand working farm dogs (n = 27) fitted with collar-mounted accelerometers.

Parameter	Beta coefficient (SE)	95% CI	P-value[c]
Treatment			
Placebo	REF		
$GLME_{220}$	0.04 (0.03)	−0.01–0.09	0.115
$GLME_{180}$	0.11 (0.03)	0.06–0.16	<0.001
Order of treatment			
$GLME_{220}$: $GLME_{180}$: Placebo	REF		
$GLME_{220}$: Placebo: $GLME_{180}$	−0.13 (0.11)	−0.35–0.1	0.247
$GLME_{180}$: $GLME_{220}$: Placebo	0.07 (0.12)	−0.17–0.31	0.565
$GLME_{180}$: Placebo: $GLME_{220}$	−0.05 (0.1)	−0.26–0.15	0.594
Placebo: $GLME_{220}$: $GLME_{180}$	−0.17 (0.1)	−0.38–0.05	0.118
Placebo: $GLME_{180}$: $GLME_{220}$	−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

[a]Overall intercept was 0.05 (SE 0.08). Dog was included with a random intercept, (SD 0.15 (95% CI = 0.11–0.21)) with a residual of 0.62 (95% CI = 0.61–0.64).

[b]Delta-G_{10} = change in acceleration in three orthogonal axes, summed over a 10 second epoch filtered to only include epochs with delta G_{10} greater than walking.

[c]Significance of the coefficient in the model. Variables were only included in the model if the global p-value from a Wald test for the inclusion of the variable had an associated p-value > 0.05.

REF = reference category.

Discussion

This study was designed to obtain preliminary data on the use of accelerometery to assess changes in gait from the use of a GLME-based nutraceutical in working farm dogs with mild musculoskeletal abnormalities, with a view to testing the efficacy in a larger, appropriately powered study. The New Zealand working dog population was selected for this study because it has a substantial number of active dogs with musculoskeletal abnormalities.

Administration of the $GLME_{220}$ trial substance increased the daytime 90th percentile delta-G_{10} by 1.72 (95% CI = 0.95–3.1)%. For the daytime 75th percentile, although the median increase for both $GLME_{180}$ and GLME200 was 1.5 and 1.4% respectively, the data are consistent with both a small increase and a small decrease (95% CI = −0.2–2.87 and −0.3–2.07% respectively) and there is no statistical confirmation of these differences. This could be inferred to mean that when a farm dog is required to work very hard, supplementation with the nutraceutical containing GLME increases the intensity that a dog is capable of, or comfortable working at. Although the difference detected in this study was apparently small, it may be clinically and functionally significant. In addition, the small effect size may be because a single tablet was given to each dog once a day in this study, for simplicity of administration. However, the manufacturer's recommended daily amount for the bodyweight of the dogs included in the study is four tablets per day during a loading period of 4–6 weeks followed by two tablets per day for the maintenance period. Therefore, the effect size may have been greater if a loading period for each trial substance had been incorporated into the study design or if twice the number of tablets had been given to each dog per day. The linear mixed model showed that giving $GLME_{220}$ increased the 90th percentile value of delta-G_{10} from 151 to 154 on average, compared with the placebo (Table 1). When applied to the relationship between speed and delta-G_{10} developed by the authors previously, this difference equates to an increase in speed of between 0.1 and 0.18 m/s, with an average of 0.12 m/s (Bolton *et al.* 2021). Therefore, supplementation with the nutraceutical containing GLME appears to facilitate an increase in speed, and arguably the performance of working farm dogs with signs of musculoskeletal abnormalities.

Beyond these observations, the mechanism of effect of the GLME-based products on the dogs was not explored in this study. However, GLME extracts have been shown to competitively inhibit both cyclooxygenase and lipoxygenase enzymes and can decrease swelling and pain in dogs with OA (Bui and Bierer 2003; McPhee *et al.* 2007; Treschow *et al.* 2007). Further study is needed to determine if the effect of the GLME-based product, allowing a higher intensity of activity, is dependent on the competitive inhibition of those enzymes.

The median delta-G_{10} was not associated with supplementation with GLME, which was not surprising. The dogs in the trial were working animals, and the majority of their daily activity was dictated by the commands of their owners, whether they experienced some degree of joint pain or not. As a consequence, a dog with mild to moderate impairment 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 voluntary, so the severity of their impairment would be expected to impact the activity they complete to a greater extent than 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, the daytime 90th percentile and IQR were higher during the trial substance order ($GLME_{220}$: $GLME_{180}$: placebo) than the reverse (placebo: $GLME_{180}$: $GLME_{220}$). The night-time median was smaller during the order ($GLME_{220}$: $GLME_{180}$: placebo) than the orders that began with the placebo. This difference suggests the presence of a

long duration of action of the GLME-based products, which carried over into the placebo phase. This study was designed with a 4-week washout period that was intended to eliminate any carry-over effect. Despite the washout period, it is very plausible that there was a longer duration of effect of GLME administration prior to the placebo round. Consequently, placebo rounds that followed administration of GLME would show an artefactual effect during the round due to the residual GLME effect, while giving the placebo first would lack this hangover effect. Whilst efficacy of some GLME products has been demonstrated in dogs before, we are not aware of a previous description of evidence for a prolonged duration of effect.

The outcome variables selected for this study were unguided by previous work, with other studies using total activity over a trial 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, which would be undetected with the broad summation of data used in previous studies.

The IQR is not an immediately intuitive outcome variable and has not been previously used to describe accelerometer-derived activity measures in dogs. However, initial exploratory visualisation of the data suggested that there was an effect of trial substance on the IQR, thus it was included in the analysis. The larger IQR meant that there was greater variation in the delta-G_{10} values for the day or night periods with the GLME trial substances. Although trial substance was not associated with an increase in the median delta-G_{10}, the median was positively correlated with the IQR. This likely means that the increase in IQR is due to an increase in activity with higher delta-G_{10} values, as evident in 90th and 75th percentiles.

It was hypothesised that during the night-time, supplementation with GLME would reduce the response variables, indicative of a reduction in clinical signs, particularly pain, which would allow for a more settled night-time period. However, the IQR of delta-G_{10} during the night increased with $GLME_{180}$, and the IQR increased with increasing median delta-G_{10}, which was opposite to the hypothesis. It is feasible that with the reduction of joint stiffness and pain experienced during recumbency, dogs can move more freely and increase their activity during the night. However, alternative explanations for the increase of IQR delta-G_{10} with GLME treatment are considered. These include the possibility that GLME impairs sleep through effects on the brain, or from peripheral effects that lead to restlessness. NSAID have been shown to disturb sleep patterns in humans, leading to an increase in awakenings and the time spent awake, and it is possible that altering the products of cyclooxygenase or lipoxygenase by the GLME may have had a similar effect (Murphy *et al.* 1994). Only one study has investigated the effects of treatment of dogs with OA with a NSAID on night-time activity (Knazovicky *et al.* 2015). In that study, the authors similarly hypothesised that a decrease in night-time activity would indicate treatment efficacy, but the study did not identify a decrease with treatment. Further research of the effect of GLME or NSAID on sleep patterns in dogs is required to better understand this observation.

The apparent dose-dependent effect between the two GLME products was inconsistent across all the different analyses. This is likely due to a combination of low sample size, and the small dose leading to a small biological difference. We suggest that the accumulation of evidence across the different analyses is what gives confidence of a meaningful and statistically valid effect, when reliance on a single measure might not.

There are several important limitations of this study. Firstly, the identification of musculoskeletal abnormalities was made as part of another concurrent study (Isaksen *et al.* 2020). Dogs were selected using pre-existing veterinary notes, which did not include standardised descriptions of severity, nor any history of the duration of clinical signs. In addition, although the dogs selected all had convincing musculoskeletal abnormalities, they were probably from a range of pathologies. It is likely that a large proportion of the dogs had OA, but of 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.

Ideally all candidate dogs would have been selected on the basis of radiographically confirmed OA prior to recruitment. Nonetheless, many dogs are treated with NSAID and nutraceuticals such as GLME products based on clinical and historical findings without definitive diagnoses, and it is of some use to know the efficacy in a less well-defined population.

The accelerometer system used has the potential to produce continuous, remotely monitored activity data that can define activity intensity, and has some capacity to describe gait and speed of dogs (Bolton *et al.* 2021). However, the system was not ideal for this particular population due to difficulties with the system setup, unpredictability of uploading from the accelerometer, and inadequacy of the radio transmission distance that required several owners to remove the dogs' collars regularly for data upload. Feedback from owners indicated that their willingness

to participate in future studies would be low if this technology was used again.

Compliance with administration of the trial substances, like in many field studies of this type, could not be guaranteed. The tablets were administered by the owners, who were required to give the tablets to their dogs daily over three 8-week periods. This study was active over the summer period, which encompasses both the holiday period and the season with the highest workload for New Zealand sheep and beef farmers. Therefore, it is conceivable that compliance with daily tableting was incomplete, despite regular contact by the researchers.

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 2-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 musculoskeletal abnormalities, and the variation in activity between dogs. In principle, randomisation increases the chance that confounders are evenly distributed amongst trial groups, but the dynamic nature of these confounders means it cannot account for all the effects.

In conclusion, this study suggests that administration of this particular GLME-containing product increases peak activity in working farm dogs with musculoskeletal abnormalities and may improve their ability to complete the activities required of them as a working dog. Due to their working roles, these dogs were completing a certain amount of activity regardless of the product they were receiving, consequently, changes in response to the products were subtle. Musculoskeletal abnormalities are highly prevalent in the New Zealand 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 musculoskeletal abnormalities, and this particular GLME-based product shows promise as an adjunct to other management strategies.

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References

Bolton S, Cave N, Cogger N, Colborne G. Use of a collar-mounted triaxial accelerometer to predict speed and gait in dogs. *Animals* 11, 1262, 2021. https://doi.org/10.3390/ani11051262

Brown DC, Boston RC, Farrar JT. Use of an activity monitor to detect response to treatment in dogs with osteoarthritis. *Journal of the American Veterinary Medical Association* 237, 66–70, 2010. https://doi.org/10.2460/javma.237.1.66

Bui LM, Bierer TL. Influence of green lipped mussels (*Perna canaliculus*) in alleviating signs of arthritis in dogs. *Veterinary Therapeutics: Research in Applied Veterinary Medicine* 4, 397–407, 2003

Cave N, Bridges J, Cogger N, Farman R. A survey of diseases of working farm dogs in New Zealand. *New Zealand Veterinary Journal* 57, 305–12, 2009. https://doi.org/10.1080/00480169.2009.60926

*__Cooper C, Javaid MK, Arden N.__ Epidemiology of osteoarthritis. In: Arden N, Blanco FJ, Cooper C, Guermazi A, Hayashi D, Hunter D, Javaid MK, Rannou F, Reginster J-Y, Roemer FW (eds). *Atlas of Osteoarthritis*. Pp 21–36. Springer, London, UK, 2014. https://doi.org/10.1007/978-1-910315-16-3_2

Dobenecker B, Beetz Y, Kienzle E. A placebo-controlled double-blind study on the effect of nutraceuticals (chondroitin sulfate and mussel extract) in dogs with joint diseases as perceived by their owners. *The Journal of Nutrition* 132, 1690S–1691S, 2002. https://doi.org/10.1093/jn/132.6.1690S

Halpern GM. Anti-inflammatory effects of a stabilized lipid extract of *Perna canaliculus* (Lyprinol). *Allergie et Immunologie* 32, 272–8, 2000

Hunt H, Cave N, Bridges J, Gedye K, Hill K. Plasma NT-pro BNP and cell-free DNA concentrations after prolonged strenuous exercise in working farm dogs. *Journal of Veterinary Internal Medicine* 32, 135–41, 2018. https://doi.org/10.1111/jvim.14835

Isaksen KE, Linney L, Williamson H, Cave NJ, Norman EJ, Cogger N. TeamMate: a longitudinal study of New Zealand working farm dogs. II. Occurrence of musculoskeletal abnormalities. *Frontiers in Veterinary Science* 7, 624, 2020. https://doi.org/10.3389/fvets.2020.00624

Juliano C, Manconi P, Cossu M. Characterisation of commercial *Perna canaliculus* samples and development of extemporaneous oral veterinary paste formulations containing perna. *Natural Product Research* 30, 2041–8, 2016. https://doi.org/10.1080/14786419.2015.1107557

Knazovicky D, Tomas A, Motsinger-Reif A, Lascelles BDX. Initial evaluation of nighttime restlessness in a naturally occurring canine model of osteoarthritis pain. *PeerJ* 3, e772, 2015. https://doi.org/10.7717/peerj.772

Leung YM, Cave NJ, Hodgson B. Creation of a predictive equation to estimate fat-free mass and the ratio of fat-free mass to skeletal size using morphometry in lean working farm dogs. *New Zealand Veterinary Journal* 66, 248–56, 2018. https://doi.org/10.1080/00480169.2018.1492984

McPhee S, Hodges L, Wright P, Wynne P, Kalafatis N, Harney D, Macrides T. Anti-cyclooxygenase effects of lipid extracts from the New Zealand green-lipped mussel, *Perna canaliculus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 146, 346–56, 2007. https://doi.org/10.1016/j.cbpb.2006.11.001

Miller MR, Tian H. Changes in proximate composition, lipid class and fatty acid profile in Greenshell mussels (*Perna*

canaliculus) over an annual cycle. *Aquaculture Research* 49, 1153–65, 2018. https://doi.org/10.1111/are.13565

Murphy PJ, Badia P, Myers BL, Boecker MR, Wright KP. Nonsteroidal anti-inflammatory drugs affect normal sleep patterns in humans. *Physiology & Behavior* 55, 1063–6, 1994. https://doi.org/10.1016/0031-9384(94)90388-3

O'Connell A, Scott I, Cogger N, Jones BR, Hill KE. Parasitic nematode and protozoa status of working sheepdogs on the North Island of New Zealand. *Animals* 9, 94, 2019. https://doi.org/10.3390/ani9030094

O'Neill DG, James H, Brodbelt DC, Church DB, Pegram C. Prevalence of commonly diagnosed disorders in UK dogs under primary veterinary care: results and applications. *BMC Veterinary Research* 17, 1–14, 2021. https://doi.org/10.1186/s12917-021-02775-3

Pettitt RA, German AJ. Investigation and management of canine osteoarthritis. *In Practice* 37, 1–8, 2015. https://doi.org/10.1136/inp.h5763

Pollard B, Guilford W, Ankenbauer-Perkins K, Hedderley D. Clinical efficacy and tolerance of an extract of green-lipped mussel (*Perna canaliculus*) in dogs presumptively diagnosed with degenerative joint disease. *New Zealand Veterinary Journal* 54, 114–8, 2006. https://doi.org/10.1080/00480169.2006.36622

Rialland P, Bichot S, Moreau M, Guillot M, Lussier B, Gauvin D, Martel-Pelletier J, Pelletier J-P, Troncy E. Clinical validity of outcome pain measures in naturally occurring canine osteoarthritis. *BMC Veterinary Research* 8, 1–12, 2012. https://doi.org/10.1186/1746-6148-8-162

Servet E, Biourge V, Marniquet P. Dietary intervention can improve clinical signs in osteoarthritic dogs. *The Journal of Nutrition* 136, 1995S–1997S, 2006. https://doi.org/10.1093/jn/136.7.1995S

Treschow A, Hodges L, Wright P, Wynne P, Kalafatis N, Macrides T. Novel anti-inflammatory ω-3 PUFAs from the New Zealand green-lipped mussel, *Perna canaliculus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 147, 645–56, 2007. https://doi.org/10.1016/j.cbpb.2007.04.004

Whitehouse M, Macrides T, Kalafatis N, Betts W, Haynes D, Broadbent J. Anti-inflammatory activity of a lipid fraction (Lyprinol) from the NZ green-lipped mussel. *Inflammopharmacology* 5, 237–46, 1997. https://doi.org/10.1007/s10787-997-0002-0

*Non-peer-reviewed