

EFFECTS OF A HYPERIMMUNIZED EGG PRODUCT ON VOLUNTARY PHYSICAL
ACTIVITY LEVELS, SERUM INFLAMMATORY MARKERS, AND OWNER
PERCEPTION OF JOINT PAIN OF DOGS WITH OSTEOARTHRITIS

BY

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THESIS

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ABSTRACT

Osteoarthritis (OA) affects about 20% of adult dogs in North America, resulting in reduced range of motion, difficulty climbing and jumping, reduced physical activity, and lower quality of life. A double-blind, placebo-controlled study was conducted to evaluate the effects of short-term supplementation of hyperimmunized egg (HIE) on voluntary activity levels, serum chemistry and inflammatory markers, and owner perception of joint pain in dogs diagnosed with OA. Eighty-two client-owned dogs with clinical signs and veterinary diagnosis of OA were enrolled and sixty-nine dogs (mean age = 8.0 ± 3.4 yr; mean BW = 32.3 ± 11.3 kg) completed a 49-d study composed of a 7-d baseline period followed by a 42-d treatment period. The Institutional Animal Care and Use Committee at the University of Illinois approved all procedures, and owner consent was received prior to experimentation. Enrolled dogs were randomly assigned to one of three treatment groups: treatment 1 (placebo; 0 g HIE/chew; n = 22), treatment 2 (2 g HIE/chew; n = 24), and treatment 3 (3 g HIE/chew; n = 23). Assigned treatments were given in the form of soft-chew treats, with dogs receiving one treat for every 9.07 kg BW daily. A physical examination, radiographs, and blood sample collection were performed at the time of enrollment (d -7) and dogs were sent home with the assigned treatment and a HeyRex (Wellington, New Zealand) activity monitor to wear continuously for 49 d. In addition, owners were required to complete canine brief pain inventory (CBPI) and Liverpool osteoarthritis in dogs (LOAD) survey questionnaires at baseline (d -7), and on d 14, 28, and 42 of the study. On the last day of the study (d 42), another blood sample was collected. Blood samples were used for serum chemistry and inflammatory marker measurements. Statistical analysis was conducted using SAS® (version 9.3; SAS Institute, Inc., Cary, NC) using the Mixed Models procedure with a repeated measures design. Data were reported as means \pm SEM with statistical

significance set at $P < 0.05$ with $P < 0.10$ considered a trend. Results of CBPI survey data showed some significant time effects, with average pain; pain as of right now; interference with the ability to rise from a lying position; interference with the ability to walk; interference with the ability to climb; pain severity score; and pain interference score decreasing ($P < 0.05$) over time. CBPI scores for pain at its least and interference with general activity tended to decrease ($P < 0.10$) over time. Blood C-reactive protein concentrations tended to be greater ($P < 0.10$) in dogs fed the low-dose HIE compared to those fed the placebo or high-dose HIE. Voluntary physical activity was different due to treatment and time. Weekly, weekday, and weekend activity data were greater ($P < 0.05$) in dogs fed the low-dose HIE than for dogs fed placebo or high-dose HIE. Baseline activity data were highest in this group, however, so these differences did not appear to be due to HIE treatment. Weekly, weekday, and weekend activity decreased ($P < 0.05$) over time. In conclusion, results of the current study did not show significant changes in physical activity levels, blood markers, or owner perception of joint pain after consuming a HIE product. Although HIE products of a different source, of higher quality, or of higher dosage may be of interest for future research, the results of the current study do not support the use of HIE for alleviating clinical signs associated with canine OA.

Thank you, God.

*This achievement could not have been accomplished without
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CHAPTER 1

INTRODUCTION

According to the American Pet Products Association (APPA), there are currently about 89.7 million dogs living in the United States (APPA, 2018). In 2016, about 59% of pet owners classified their pet as a family member or child. This common view of pets in the household has led to owners taking greater interest in their health and well-being for a better quality of life (QOL) of the animals. Although the improved nutritional and veterinary care have greatly contributed to extending the life expectancy of pets in recent years, with it has come new challenges. Chronic progressive diseases like osteoarthritis (OA), for instance, are more prevalent with aging and reduce overall QOL of an animal.

Osteoarthritis is a debilitating, irreversible condition that affects the joint structure of an animal. It has been reported that over 20% of dogs over one year of age and 80% of dogs over eight years of age suffer from OA (Johnston, 1997). Also known as a “wear and tear” disease, OA has a much higher prevalence in older dogs due to the mechanical and chemical stress of joints with age. Reported clinical signs include lameness, reduced activity level, pain, and stiffness. Most commonly reported sites of OA in dogs are the hips, elbows, and stifles. Currently, there are many different treatment options for OA, the most common being surgery, pharmacological agents, physical rehabilitation, weight management, and nutraceutical supplementation. Treatment options are carefully selected by veterinarians according to the severity and progression of the disease and oftentimes a multimodal approach using a combination of treatments is used to not only alleviate pain but also to slow down disease progression. Non-steroidal anti-inflammatory drugs (NSAID) are the most commonly used pharmacological agents to help with pain, but long-term use of this medication has been

associated with adverse reactions to the gastrointestinal tract (64%), renal system (21%), and liver (14%), which is concerning for pet owners and veterinarians (Hampshire et al., 2004).

In recent years, there has been increasing interest in using nutraceuticals as an alternative to improve OA signs and improve the overall QOL of animals. Glucosamine, chondroitin, and omega-3 fatty acids are nutraceuticals commonly used to reduce disease signs in OA patients. The published literature is equivocal for most nutraceutical products tested. Although there are some studies reporting improvements in OA symptoms, others have not shown positive changes, leading to inconclusive results. As novel nutraceuticals emerge in the human OA market, there has been great interest in applying and further testing the safety and potential beneficial effects of these products on pets with OA.

The objective of this study was to evaluate the effects of short-term supplementation of hyperimmunized egg (HIE) on voluntary activity levels, serum chemistry and inflammatory markers, and owner perception of joint pain of dogs diagnosed with OA. We hypothesized that HIE would increase voluntary physical activity levels, decrease serum inflammatory markers, and decrease owner's perception of joint pain of dogs diagnosed with OA.

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CHAPTER 2

LITERATURE REVIEW

DOG POPULATION AND INCIDENCE OF OSTEOARTHRITIS

According to a survey by the American Pet Products Association (APPA), there are 60.2 million households in the United States that own dogs, resulting in a total of about 89.7 million dogs (APPA, 2018). In 2016, about 59% of pet owners reported that they consider their pets as a family member or even as a child (AVMA, 2012). The common view of pets as family members is correlated with an increased willingness to spend money on their pets in an attempt to provide a better quality of life (QOL) and increase longevity. Though improved veterinary care and nutrition have helped to increase the life span of companion animals in recent years, with it has come an increased incidence of diseases associated with aging such as osteoarthritis (OA), which often occurs due to prolonged wear and tear of the articular joint due to biochemical stress exerted on the joints. An increase in the obese dog population over the years also has likely contributed to the development and progression of joint disease.

Osteoarthritis, also known as degenerative joint disease (DJD), is a chronic condition commonly associated with joint dysfunction, pain, and stiffness due to joint deterioration. It is a debilitating disease that leads to exercise intolerance and an unwillingness or inability to climb or jump due to pain, lowering the overall QOL for the animal. It is characterized by articular cartilage deterioration; osteophyte formation; bone remodeling; changes in the periarticular tissue; and a low-grade non-purulent inflammation of various degrees due to disruption of normal cartilage structure and homeostasis (Johnston, 1997).

There is often a misconception that OA is a disease that only affects the articular cartilage. In fact, it affects the entire structure of the joint, which includes the hyaline cartilage, synovial membrane and fluid, subchondral bone and the surrounding supporting structures. Also known as “wear and tear” arthritis, OA is known to originate due to biomechanical alterations and the aging process, but studies have shown that inflammatory processes play a key role in pathogenesis of OA (Yuan et al., 2003).

Prevalence and Risk Factors

There are several risk factors associated with OA development, such as breed, sex, age, obesity, and genetics. Some large breed dogs like Golden Retrievers, German Shepherds and Labrador Retrievers have higher OA incidence than average (Smith et al., 2001). Interestingly, a study that surveyed 148,741 dogs from 93 clinics across England reported that purebred dogs had higher ($P < 0.05$) prevalence of DJD compared to crossbred dogs, possibly due to inherited genetic defects in particular breeds (Dan et al., 2014). Studies have indicated that Labrador Retrievers, in particular, have a 3.4-fold increased risk for hip dysplasia, 20.5-fold increased risk for elbow dysplasia, and 5.5-fold increased risk for cranial cruciate ligament rupture compared to mixed breed dogs (LaFond et al., 2002; Duval et al., 1999). More research is needed to evaluate particular genes that may predispose animals to OA.

Age is one of the strongest risk factors for OA, affecting nearly 27 million adult humans in the United States (Lawrence et al., 2008). The data in dogs is similar. In North America, for instance, it is estimated that the prevalence of OA is about 20% of dogs over one year of age and 80% of dogs over eight years of age based on clinically diagnosed cases (Johnston, 1997). While OA can develop at any age, its higher prevalence in older dogs indicates that it is a disease of

aging. It is often a result of long-term wear and tear of the articular joint, with higher prevalence and diagnosis in older dogs when mobility is significantly affected with obvious clinical signs due to OA. Although the exact mechanisms on how age affects OA is unknown, it is likely related to a combination of changes in the capacity of joint tissues to adapt to biomechanical damage (Neogi and Zhang, 2013).

Obesity is a major risk factor of OA in dogs, with an increased incidence of orthopedic disease reported in overweight dogs although exact numbers were not reported (German, 2006). Long-term stress exerted on the joint due to excess BW has been associated with cartilage loss (Cooper et al., 2000; Impellizeri et al., 2000). Although the exact mechanism is unknown, it has been suggested that adiposity, which is associated with abnormal hormone and growth factor concentrations, greater bone mineral density, and other metabolic intermediates, plays a role in increasing the risk of OA (Nevitt and Lane, 1999). Incidence of obesity in pets has been increasing in recent years. A recent clinical survey reported that about 56% of dogs in the United States were considered to be overweight [body condition score (BCS) of 6-7 on 9-point score] or obese (BCS of 8-9) by veterinarians. Given the United States dog population, this translates to approximately 50.2 million dogs above the healthy body weight range (APPA, 2018) and at increased risk for OA.

The sex of the animal also has been associated with increased risk for OA, with male dogs having a greater risk of developing hip OA (Hays et al., 2007). Sex hormones modulate the joint tissues during tissue development and throughout the life cycle. Although there is little evidence on how sex hormones modulate the joint tissue of dogs, in humans it has been shown that chondrocytes have sex-specific responses to sex steroids due to differing actions of the hormone and receptor numbers (Boyan et al., 2013).

Commonly reported sites of OA in dogs include the stifles, hips, and elbows (Pettitt and German, 2015). Osteoarthritis can occur as a primary or secondary form, although the latter is more common. It often occurs as a secondary reaction to an injury such as cruciate ligament or articular rupture, or as a secondary form of inherited genetic conditions such as hip or elbow dysplasia.

JOINT STRUCTURE AND OSTEOARTHRITIS

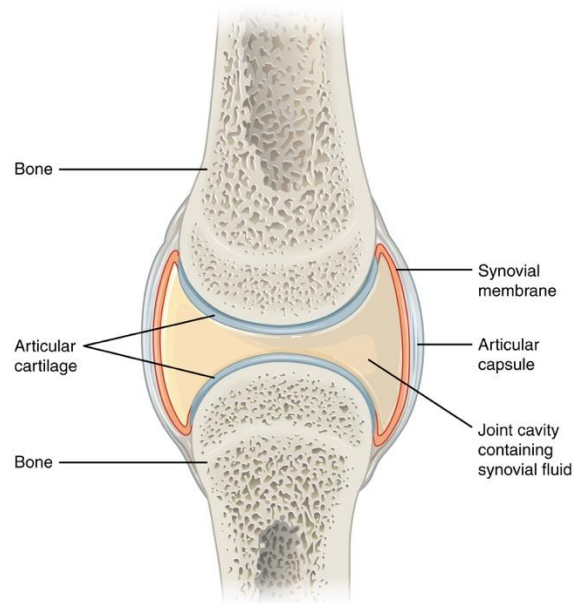


Figure 2.1. Synovial joints (Cohen, 2013)

Articular Cartilage

Articular cartilage is the white, smooth tissue covering the ends of bones where it connects to form joints (Figure 2.1). Its primary function is to provide a smooth, lubricated surface of articulation and to facilitate the transmission of loads with low friction (Fox et al., 2009). Although articular cartilages are only a few millimeters thick, it is designed to endure compression and distribute loads to allow pain-free movement with great durability throughout the majority of a lifespan (Buckwalter and Mankin, 1997).

Articular cartilage is composed of a dense extracellular matrix (ECM) and chondrocytes. In normal articular cartilage, ECM represents about 65 to 80% of the total weight and it mainly provides the mechanical properties needed to help distribute the load stress. Extracellular matrix is composed of water and cartilaginous macromolecules such as collagens, proteoglycans, and other non-collagenous proteins.

Chondrocytes are highly specialized and metabolically active cells that play an essential role in the development, maintenance, and repair of the ECM. Depending on the anatomical site of the cartilage, chondrocytes differ in number, shape, and size. These cells are in charge of the turnover of the ECM in its surrounding and have the ability to sense changes in the matrix composition to synthesize an adequate amount and type of macromolecules needed. However, the lack of blood vessels, nerves, or lymphatics in the articular cartilage limits the ability of chondrocytes to replicate, making it difficult to recover from injury or damage on its own. Therefore, maintaining an optimal chemical and mechanical environment is needed for chondrocyte survival.

Subchondral Bone

Subchondral bone is the thin layer of bone that is present beneath the cartilage. Its ability to deform under pressure allows it to function as a shock absorber and load distribution in weight-bearing joints. Development of OA has been associated with the thickening and stiffening of subchondral bone due to the loss of cushioning ability of cartilage matrix from degradation. However, because the timespan of these occurrences is unclear, it has not been confirmed if bone thickening precedes OA or if it occurs as a consequence of OA.

The involvement of subchondral bone in OA has long been thought as being secondary to cartilage alterations and considered a late-stage sign of disease. Recent clinical findings using magnetic resonance imaging (MRI) in humans, however, have shown that changes in bone occurs in the early stages of the disease even before changes in the cartilage occurs (Funck-Brentano and Cohen-Solal, 2015). In dogs, the biggest changes in both the subchondral bone and cartilage are typically found around the major weight-bearing areas of the femoral head, suggesting that mechanical stress exerted on the joints can play an important role in initiating OA (Johnston, 1997).

Osteophytes

Osteophytes, also called bone spurs, are bony outgrowths that are found at the joint margin of OA patients. It is a common feature of OA that can be detected by a radiograph and is often used to determine the presence and progression of disease (Felson et al., 2005). Osteophytes have been recognized to develop in the presence of inflammation, suggesting that synovial membrane inflammation may play a role (Johnston, 1997). Osteophyte formation is known to take weeks in the presence of chronic inflammation; however, in experimentally induced OA, it has been shown to take only 3 to 7 days to observe osteophyte formation. It has been speculated that osteophyte formation does not have a direct relationship with OA initiation, but rather with the risk of disease progression. Osteophytes often develop at sites adjacent to cartilage loss causing joint malalignment. This can become a source of pain and result in a loss of function due to nerve compression, limitation in joint mobility, and tissue obstruction.

Joint Capsule and Synovial Fluid

The joint capsule or articular capsule is an envelope surrounding the synovial joint that can be divided into three different layers: synovial membrane (intima), subsynovial layer, and joint capsule. The synovial membrane or the intima is the inner most layer. It is a thin layer where two types of cells reside: type A synoviocytes, which have a macrophage-like function and type B synoviocytes that produce hyaluronan and degradative enzymes. The function of the synovial membrane is to produce synovial fluid and provide a low-friction lining to the joint (Johnston, 1997). The subsynovial layer is vascular and contains free nerve endings. Located in between the other layers, it allows movement of the synovial membrane from the joint capsule. The outermost layer is the joint capsule, which is a fibrous layer that is vascular and innervated and functions to provide joint stability. In the presence of OA, the joint capsule becomes thickened with increased vascularization. Synoviocytes are an important source of cytokines and leukotrienes that attract inflammatory cells and release prostaglandins and other inflammatory mediators. This contributes to OA disease progression, resulting in decreased range of motion, pain, and stiffness.

PATHOPHYSIOLOGY OF OSTEOARTHRITIS

For a long time, it was thought that OA was a single disease that was primarily cartilage driven, with matrix metalloproteinases (MMP) being recognized as the primary proteases responsible for the initiation of cartilage degradation because subjects with OA had elevated MMP in the bloodstream. The breakdown of the cartilage matrix leads to the development of fibrillation (softening of articular cartilage) and fissures, eroding the surface of the joint and accompanied with changes to bone with osteophyte formation and thickening of the subchondral

plate. Moreover, it was thought that inflammation was a change observed only in the advanced clinical stages of the disease, with pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), IL-1B, IL-17, and leukemia inhibitory factor (LIF) being produced by the synovial membrane and diffused into the cartilage through the synovial fluid. These pro-inflammatory cytokines, especially IL-1B and TNF- α , are major catabolic signals influencing articular tissue degradation (Martel-Pelletier, 1999).

Recent evidence has shown that OA is not a single disease, but rather a failure of the joint, with bone and synovial tissue playing an important role in disease development and progression. Metabolic changes in subchondral bone tissue have been identified as an active component of OA, especially the expression of pro-inflammatory cytokines in the synovial membrane and subchondral bone that cause structural changes and lead to disease progression of the joint (Kapoor et al., 2011). Inflammation seems to occur during the early onset of the disease; however, the exact pathological sequence of OA is still unclear.

DIAGNOSIS OF OSTEOARTHRITIS

Diagnosis of OA requires a complete patient assessment that usually includes a physical examination, orthopedic and neurologic examination, and diagnostic imaging. Additional measures include gait analysis, blood biomarker analysis, synovial fluid analysis, and others that are less commonly used in a clinical setting. The challenge of OA diagnosis is detecting it at an early stage before overt clinical symptoms are manifested. The earlier OA can be detected, the faster an animal can be treated to delay disease progression, hopefully ensuring a better QOL.

The use of blood biomarkers such as C-reactive protein (CRP) or MMP-3 could be a more sensitive diagnostic method to allow early detection of OA. However, these methods have not

been fully validated for application in canine OA and more research is needed before it can be used in a clinical setting. Other methods such as gait analysis are sensitive methods for detecting abnormal gait in an animal, and although this method has been validated for use in dogs, the need for specialized personnel and equipment makes it difficult to become a common diagnostic method. Synovial fluid analysis, also called arthrocentesis, is a validated method of diagnosing OA. The physical and biochemical characteristics of the fluid can provide detailed information regarding the disease progression. However, it is an invasive procedure that is more expensive than the routine examination methods, so it is often only used when further diagnostic testing is needed.

Physical Examination and Pain Assessment

Pain is the most common clinical sign associated with OA and often is accompanied with lameness. OA may cause pain for several reasons, such as bone structural change at the joint margins and beneath areas of damaged cartilage (Fox, 2016). Lameness may be identified by the owner or by a veterinarian during routine examination. It is important to determine if the animal is actually unable to use the limb or is just unwilling to do so when being assessed. While an inability to use a limb may be attributed to changes in musculoskeletal structure that require physical rehabilitation, unwillingness to use a joint is often a behavior to avoid pain. Pain assessment and quantification is an important aspect of OA diagnosis. Unlike other diseases, physiological parameters such as heart rate, respiratory rate, blood pressure, and body temperature might not be a good indicator of pain. There are several tools developed for this purpose, such as the Glasgow Pain Scale, Canine Brief Pain Inventory (CBPI), and Liverpool Osteoarthritis in Dogs (LOAD). They are multidimensional questionnaires aimed at gathering

information about the intensity, sensory, and affective qualities of pain by asking owners qualitative questions about pain severity. Although it aims to provide an objective measure of pain, these surveys are completed by pet owners, so the responses are subject to each owner's perception of pain.

Orthopedic examination in conjunction with a neurologic examination should be conducted to identify potential neurologic causes for pain or lameness. While a consistent examination pattern is recommended, the procedure will vary depending on the veterinary specialist. Palpation should be performed on all bones for any abnormalities and signs of pain. Leaving the most painful limb for last avoids early elicitation of pain in the animal. During this time, range of motion should be measured to see if normal flexion, extension, abduction, adduction, and rotation are possible. It has been reported by Cook et al. (2005) that instable shoulder joints had larger ($P < 0.001$) abduction angles compared to unaffected shoulders when measured with a goniometer in sedated dogs ($n=33$). A goniometer, which is a non-invasive and simple method to quantify the range of motion of a joint, can be used to assess the angle of the joint. This method has been successfully validated in dogs (Jaegger et al., 2002). In that study, 16 Labrador retrievers were assessed, with the results of goniometric measurements between sedated dogs and awake dogs being compared. In that study, radiographic measurements of joint angle did not significantly differ, making goniometric measurements in awake dogs a reliable measure. Gait analysis is another method of measuring normal or abnormal gait by identifying specific features that might be abnormal such as lameness. This is usually measured by allowing the animal to walk on a force plate. A force plate is a platform that contains sensing elements able to record the magnitude of force. The forces exerted on the plate are converted into electric signals that are amplified and recorded by a computer (McLaughlin, 2001; DeCamp, 1997). Gait

analysis requires specialized equipment and staff but provides objective and quantifiable information about a dog's gait that can be practical in detecting subtle lameness that might not be otherwise observable with the human eye.

Diagnostic Imaging

Once a dog has been physically assessed by a veterinarian, diagnostic imaging is commonly used to confirm the presence of OA, locate the exact site of lesion, and identify the disease stage of the animal. Imaging methods are non-invasive and help the veterinarian determine the degree of OA severity and help decide on a treatment strategy. Radiography (X-ray) is the most widely used and validated method to diagnose OA due to its accuracy and ability to provide spatial resolution at a reasonable cost. However, there are many other types of imaging methods available, each with its own advantages and disadvantages. Additional imaging diagnostic methods are used based on the information sought about the affected site.

Ultrasonography is a real-time noninvasive method that does not use ionizing radiation like X-rays. However, it provides limited access to the joint region, so it is not ideal when more detailed information regarding the specific affected joint area is needed. Nuclear medicine (scintigraphy) has a high sensitivity, allowing early detection and enabling one to track disease progression, but it lacks resolution quality compared to radiography and is expensive due to the special equipment and trained personnel needed. Computed tomography (CT) is advantageous because it is able to record thousands of separate opacities compared to conventional X-rays, but requires animals to be deeply sedated for imaging. Magnetic resonance imaging is excellent in capturing tissue contrast, which could help detect early tissue damage, but is a costly procedure requiring a specialized technician.

Biomarkers

Current diagnosis of OA is based on radiographic changes of the affected joint area, but those changes are observed at a relatively late stage of the disease. Therefore, there has been increased interest in using biochemical markers for early detection of OA as well as determining severity and monitoring response to different therapies. Biomarkers can be classified in different groups: synovial fluid biomarkers, enzymes and inhibitors, and extracellular products that degenerate the cartilage (de Bakker et al., 2017). Synovial fluid biomarkers are the pro-inflammatory mediators produced at an early stage of tissue damage and include IL-1 β , -6, and -8, TNF- α , CRP, and reactive oxygen species (ROS). Enzyme and inhibitor biomarkers play an important role in tissue destruction such as MMP-1, -2, -3, -9, tissue inhibitor of metalloproteinase (TIMP)-1, -2, and tartrate resistant acid phosphatase (TRAP) (Fujita et al., 2005, Boland et al., 2014). The third type of biomarkers that are extracellular include proteoglycans (PG), cartilage oligomeric matrix protein (COMP), tenascin-C (TN-C), and fibronectin (FN). Although different biomarkers have been identified and studied in animal models, more research is needed in order to validate the use of a specific biomarker for diagnosing OA.

CURRENT TREATMENTS FOR CANINE OSTEOARTHRITIS

Because OA is a chronic condition that affects the overall QOL and currently has no biological cure, the treatment goals are to alleviate pain or discomfort, delay disease progression, restore near-normal function of the joints, and minimize joint instabilities. There are many potential treatment and management options, with many being used in combination to relieve the clinical symptoms and ensure a better QOL for an animal.

Weight Loss

Obesity is one of the risk factors of OA and the stress exerted on the joint due to excess BW has been shown to lead to articular cartilage breakdown (Joshua, 1970). There are a number of studies showing the association between obesity and OA development, but the study by Kealy et al. (2000) has some of the strongest data. That study evaluated the radiographic evidence of OA in 48 Labrador Retrievers undergoing non-restricted or restricted (25% less food) feeding from 8 weeks to 8 years of age. Those researchers reported that the incidence and severity of shoulder, hip, and elbow OA were lower in dogs in the restricted dietary group, which had a much lower BW than non-restricted dogs (Kealy et al., 2000). Because that was a prospective study and controlled food intake over a long period of time, it indicates that caloric restriction and/or weight control are effective treatments for preventing incidence and reducing severity of OA.

Another clinical trial evaluated the effects of weight loss on lameness in dogs with OA and found that after losing 11 to 18% of their initial BW over a 10- to 19-week weight loss period, the animals showed a significant improvement in joint health as indicated by lower lameness scores (Impellizeri et al., 2000). This evidence suggests that weight loss through a calorie-restricted diet should be practiced in conjunction with exercise. Maintaining an optimal BW also may help mitigate pain and reduce lameness by reducing the biomechanical stress exerted on the joints.

Medicinal Management

There are two broad categories of pharmacological agents used to manage OA: symptom-modifying drugs and structure-modifying drugs (Altman et al., 1996; Sanderson et al., 2009).

Structure-modifying drugs require strict criteria to validate their use in dogs so they are less available and will not be discussed in this review. Symptom-modifying drugs are more commonly used, with non-steroidal anti-inflammatory drugs (NSAID) being the current gold standard pharmaceutical treatment for dogs with OA due to their anti-inflammatory, analgesic, and anti-pyretic properties. Non-steroidal anti-inflammatory drugs have been shown to be effective, with studies reporting reduction ($P < 0.05$) in clinical signs of OA following 4 weeks of meloxicam therapy, with minimal side effects reported and the drugs being accepted by the animal with no problems (Doig et al., 2000). Non-steroidal anti-inflammatory drugs help relieve clinical signs of pain by suppressing prostaglandins (PG), mainly PGE₂ that is produced from the substrate arachidonic acid within the prostanoid cascade. PGE₂ has several important roles in OA: 1) lowering the threshold of nociceptor activation; 2) promoting synovitis in the joint lining; 3) enhancing the formation of degradative metalloproteinases; and 4) depressing cartilage matrix synthesis by chondrocytes.

Even though inflammation is commonly the primary focus, PG also have positive metabolic roles on the body (platelet aggregation to prevent excessive bleeding, maintaining GI tract integrity and help with renal function) so balance is the key. The goal of NSAID management is to inhibit formation of PG that contribute to clinical signs and pathways of OA, while sparing PG production associated with beneficial functions. There are currently six NSAID that are approved by the United States Food and Drug Administration (FDA) for use in dogs: carprofen, deracoxib, firocoxib, grapiprant, meloxicam, and robenacoxib, with the last drug being restricted for uses longer than three days in dogs with OA. These drugs are given orally or by injection. The recommended mode of delivery found in the literature is greatly variable, with some recommending intermittent use and others a continuous use of these drugs. Benefits of

continuous NSAID use include better pain management, improved mobility, and slowing of OA progression (Innes et al., 2010). However, long-term use of these drugs could lead to intolerance, and NSAID have been reported to result in adverse reactions to the gastrointestinal tract (64%), renal system (21%), and liver (14%). These complications are common due to overdosing of NSAID and often related to concurrent use with corticosteroids. Therefore, it is important that NSAID administration be given at a minimally effective dose to help maintain an optimal balance of PG production in the body over a long time.

Surgery

Surgical intervention may be necessary for some patients in order to replace the whole joint and restore near normal joint function in severe cases of OA. Surgery often involves the removal of inciting cause, or stabilization of the affected joint when an animal is or becomes nonresponsive to other forms of treatment. Indication for surgical intervention include cruciate ligament tear, severe to end-stage hip, elbow and stifle disease, chronic shoulder luxation, among other conditions (Fox, 2016).

Physical Rehabilitation

Physical rehabilitation often is performed in conjunction with pharmaceutical management of OA to relieve or improve joint dysfunction, pain, and stiffness that often leads to reduced range of motion, flexibility, and overall activity level. There are many different methods of physical rehabilitation, from advanced techniques that require a specialist to simple ones that can be performed at home by pet owners. The main goal of physical rehabilitation is to restore, maintain, and promote optimal joint function and support better QOL for an animal. Simple

methods, such as passive stretching, have proven to be effective rehabilitation methods. Crook et al. (2007) tested passive stretching using 10 Labrador retrievers with OA and with reduced range of motion on an affected joint. Dog owners were asked to stretch the affected joint for 10 seconds, 10 times, repeating the routine twice daily over a 21-day period. Goniometric measurement results showed that dogs had increased ($P < 0.0005$) their range of motion on the affected joints after they had completed the stretching program, with an overall increase in the range of motion of 7 to 23% (Crook et al., 2007).

Other methods such as aquatic therapy might require the assistance of a specialist who can evaluate if swimming could improve hip joint function in dogs with OA. In a study conducted by Nganvongpanit et al. (2014), 55 dogs were assigned to one of three groups: 1) OA with swimming ($n=22$); 2) healthy controls with swimming ($n=18$); and 3) healthy controls with no swimming ($n=15$). Dogs were allowed to swim for a total of 8 weeks (twice a week, three cycles of 20-minute intervals with 5-minutes rest). Blood was collected every two weeks to measure OA biomarkers, including chondroitin sulfate epitope WF6 (CS-WF6) and hyaluronan. Results at the end of the study showed that dogs with OA that swam had decreased ($P<0.05$) circulating CS-WF6 concentrations and decreased ($P<0.05$) pain on palpation compared to pre-exercise. Overall, the effects of swimming twice a week over a period of 8 weeks showed significant improvement of OA joint function.

NUTRACEUTICALS

Nutraceuticals have been the fastest growing group of healthcare products, both in humans and animals, as a nutritional means of preventing and managing diseases such as OA. The term ‘nutraceutical’ is defined as a non-drug substance produced in a purified or extracted form and

administered orally to provide agents required for normal body structure and function with the intent of improving health and wellbeing (Fox, 2016). Unlike pharmacological agents, nutraceuticals are not as closely regulated by FDA and cannot claim to treat or cure a disease. Due to the many side effects reported with NSAID usage, there has been great interest in nutritionally-based healthcare products for managing OA in both humans and animals. Although these products are not subject to the same regulations as pharmacological agents, it is still important to prove their efficacy by conducting appropriate research experiments. There are many different products on the market, but more clinical trials are needed to test those available so that pet care providers and owners can be confident that they are suitable treatment options for their animals.

Glucosamine and Chondroitin

Glucosamine and chondroitin sulfate (CS) are two commonly used nutraceuticals that have been used to treat OA in several species. Glucosamine is a component of proteoglycans, which is the building block of articular cartilage (Bassleer et al., 1992). Chondroitin sulfate, which is a polymer of repeating disaccharide units, is a predominant component of articular cartilage and other body tissues, including tendons, bones, and vertebral discs (Paroli et al., 1991). The use of glucosamine and CS in combination was reported to reduce OA symptoms in dogs (McCarthy et al., 2007). These researchers conducted a randomized, double-blind, positive-controlled clinical study in 35 dogs. Dogs were treated with either carprofen (positive control; n=19) or a glucosamine (475 mg/g)/CS (350 mg/g) (n=16) combination for 70 days, with veterinarian assessment on days 14, 42, 70, and 98. Dogs in the glucosamine/CS group showed improvement ($P<0.001$) in disease scores for pain, weight-bearing, and overall condition at day 70 compared

to baseline. However, lameness and joint mobility scores did not significantly improve compared to baseline. Not surprisingly, dogs in the Carprofen group showed significant improvement in all outcomes measured. Results of this study show that the onset of improvement of OA symptoms in glucosamine/CS-treated dogs was delayed compared to the Carprofen-treated dogs, meaning that this nutraceutical is a viable treatment option but longer supplementation would likely be necessary for owners to observe improvements (McCarthy et al., 2007).

Another study compared the effects of a glucosamine/CS combination with a glucosamine/CS combination plus undenatured type II collagen (UC-II) on activity level of dogs. Twenty dogs were recruited and divided into four groups and orally treated for 120 days: placebo group (n=5), UC-II (10 mg) group (n=5), glucosamine (2000 mg) / CS (1600 mg) group (n=5) and glucosamine (2000 mg) /CS (1600 mg) /UC-II (10 mg) group (n=5). This study showed that dogs given the glucosamine/CS/UC-II treatment had decreased overall pain (57%), pain upon limp manipulation (53%), and exercise-associated lameness (53%) when assessed through owner questionnaire. However, once the animals were withdrawn from these treatments, they experienced pain relapse (D'Altilio et al., 2007). Although some studies have reported positive effects of supplementing glucosamine and/or CS, some studies have shown no significant improvements based on orthopedic surgeon or owner assessment (Moreau et al., 2003; Altinel et al., 2007; Gupta et al., 2012). This may be due to the dosage tested, the mode of administration (orally or intramuscular injection), length of administration of treatment and many other different factors. No adverse effects have been reported for a glucosamine/CS mix usage with dogs.

Omega-3 Fatty Acids

Omega-3 fatty acids are those with a double bond located at the third carbon from the methyl (i.e., omega) end. Omega-3 fatty acids are mainly found in fish oil, flaxseed, and other vegetable oils. Many omega-3 fatty acids are commonly present in pet foods, including α -linolenic acid, which is required in the diet of both dogs and cats (NRC, 2006). Long-chain polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are rich in marine products, may influence inflammatory cell responses by being incorporated into cell membrane phospholipids. Increased omega-3 fatty acid intake has been reported to increase the amount of EPA and DHA present in cell membranes and have an anti-inflammatory effect in both humans and animal models (Darlington and Stone, 2001). There have been several studies conducted in dogs, with results showing that a diet enriched in omega-3 fatty acids (3.5% fish oil) led to significant improvement in overall OA condition, degree of lameness, ability to rise from a resting position, and ability to walk when compared to dogs fed a conventional diet (Roush et al., 2010).

Avocado-Soybean Unsaponifiables

Avocado-soybean unsaponifiables (ASU) are plant extracts from unsaponifiable residues and soybean oil mixed at a one-third to two-third ratio (Gabay et al., 2008; Henrotin, 2008; Henrotin et al., 2011). Major components of ASU are phytosterols β -sitosterol, campesterol, and stigmasterol (Lippiello et al., 2008). In vitro studies (chondrocytes) have shown ASU to possess anti-inflammatory and anti-catabolic effects. An in vivo study using 24 dogs with OA showed that supplementation of ASU (4 mg/kg) either every day or every 3 days led to an increase in TGF- β 1 and TGF- β 2 concentrations in synovial fluid. TGF- β 1 and β 2 are growth factors that

stimulate the synthesis of matrix components by chondrocytes (Altinel et al., 2007). In that study, ASU-treated dogs (n=8) had an increase ($P<0.05$) in TGF- β 1 and TGF- β 2 concentrations compared to control dogs (n=8). However, the direct benefit of the observed change on the articular cartilage was not known (Altinel et al., 2007).

Another study evaluated the effects of ASU in an OA-induced canine model by anterior cruciate ligament transection (Boileau et al., 2009). Dogs were treated with 10 mg/kg of ASU per day for 8 weeks following transection. Microscopic lesion of the cartilage and loss of subchondral bone were decreased ($P=0.04$) in dogs treated with ASU compared to the placebo group. Cartilage analysis showed that ASU-treated dogs had a decrease in MMP-13 ($P=0.01$) and nitric oxide synthase ($P<0.05$), which play an important role in collagen type II degradation in OA cartilage (Boileau et al., 2009). However, the short treatment period and the experimentally induced OA model make it difficult to determine the exact mode of action and if ASU would result in improvements in a progressive, natural course of OA. More research is needed for ASU supplementation in dogs with OA to determine the exact mode of action and assess physical improvements in terms of pain and lameness.

Hyperimmunized Egg

Hyperimmunized egg (HIE) product is produced by repeated vaccination of one or more antigens to a hen, which induces an immune response in the hen and develops antibodies. The antibodies produced are transferred to eggs, which are then pasteurized and spray-dried to be utilized as an oral supplement. The concentrated egg protein extract is the final product that contains the specific antibody to provide passive immunity (Dean, 2000). The use of HIE product as an alternative to antibiotics has been proposed in farm animals. A meta-analysis on 61

studies reported that HIE administration reduced the risk of gastrointestinal infection in mice, poultry, piglets and calves (Diraviyam et al., 2014). However, there are few reports on this nutraceutical in dogs. One study evaluated the effect of HIE supplementation in early weaned Alaskan husky puppies (n=36), with results showing improved fecal quality and increased fecal IgA after supplementation (Reynolds and Knorr, 2006). Another study supplemented beagles (2 mo old) with 2 g of HIE containing *Canine Parvovirus 2* (CPV2) specific antibodies three times a day (n=3) and showed evidence of a protective effect upon CPV2 challenge. The control puppies (n=4) in the study lost weight, and had vomiting and diarrhea (Van Nguyen et al., 2006). However, a limitation of this study is the low sampling population number. Overall results of HIE seem to show a potential as a nutraceutical to target against specific canine pathogens when administered to puppies before gut closure occurs (Mila et al., 2017).

The use of HIE for dogs with joint disease has not been studied. For this condition, there is evidence from only an open-label study that was conducted in human patients with arthritis (n=13). This study explored the effect of HIE in the disease condition by providing 4.5 g of HIE supplement daily. Results showed significant improvement ($P<0.05$) of mean global assessment score in these patients and 40% of patients reported some degree of benefit during the experimental period (Greenblatt et al., 1998). However, because the study was an open-label study, a low number of patients were tested, and reports of improvement from patients might be due to a placebo effect, more evidence is needed. In addition, the subjects evaluated had different types of arthritis, including OA, rheumatoid arthritis and psoriatic arthritis. Although they are all arthritis, the pathophysiology for each specific type might differ and HIE might have a different mechanism for targeting the disease. There are many different anecdotal accounts of the efficacy

of HIE in OA, however, there are no clinical trials or literature that provide evidence of HIE being a suitable nutraceutical for managing OA.

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CHAPTER 3

EFFECTS OF A HYPERIMMUNIZED EGG PRODUCT ON VOLUNTARY PHYSICAL ACTIVITY LEVELS, SERUM INFLAMMATORY MARKERS, AND OWNER PERCEPTION OF JOINT PAIN OF DOGS WITH OSTEOARTHRITIS

ABSTRACT

Osteoarthritis (OA) affects about 20% of adult dogs in North America, resulting in reduced range of motion, difficulty climbing and jumping, reduced physical activity, and lower quality of life. A double-blind, placebo-controlled study was conducted to evaluate the effects of short-term supplementation of hyperimmunized egg (HIE) on voluntary activity levels, serum chemistry and inflammatory markers, and owner perception of joint pain in dogs diagnosed with OA. Eighty-two client-owned dogs with clinical signs and veterinary diagnosis of OA were enrolled and sixty-nine dogs (mean age = 8.0 ± 3.4 yr; mean BW = 32.3 ± 11.3 kg) completed a 49-d study composed of a 7-d baseline period followed by a 42-d treatment period. The Institutional Animal Care and Use Committee at the University of Illinois approved all procedures, and owner consent was received prior to experimentation. Enrolled dogs were randomly assigned to one of three treatment groups: treatment 1 (placebo; 0 g HIE/chew; n = 22), treatment 2 (2 g HIE/chew; n = 24), and treatment 3 (3 g HIE/chew; n = 23). Assigned treatments were given in the form of soft-chew treats, with dogs receiving one treat for every 9.07 kg BW daily. A physical examination, radiographs, and blood sample collection were performed at the time of enrollment (d -7) and dogs were sent home with the assigned treatment and a HeyRex (Wellington, New Zealand) activity monitor to wear continuously for 49 d. In addition, owners were required to complete canine brief pain inventory (CBPI) and Liverpool

osteoarthritis in dogs (LOAD) survey questionnaires at baseline (d -7), and on d 14, 28, and 42 of the study. On the last day of the study (d 42), another blood sample was collected. Blood samples were used for serum chemistry and inflammatory marker measurements. Statistical analysis was conducted using SAS® (version 9.3; SAS Institute, Inc., Cary, NC) using the Mixed Models procedure with a repeated measures design. Data were reported as means \pm SEM with statistical significance set at $P < 0.05$ with $P < 0.10$ considered a trend. Results of CBPI survey data showed some significant time effects, with average pain; pain as of right now; interference with the ability to rise from a lying position; interference with the ability to walk; interference with the ability to climb; pain severity score; and pain interference score decreasing ($P < 0.05$) over time. CBPI scores for pain at its least and interference with general activity tended to decrease ($P < 0.10$) over time. Blood C-reactive protein concentrations tended to be greater ($P < 0.10$) in dogs fed the low-dose HIE compared to those fed the placebo or high-dose HIE. Voluntary physical activity was different due to treatment and time. Weekly, weekday, and weekend activity data were greater ($P < 0.05$) in dogs fed the low-dose HIE than for dogs fed placebo or high-dose HIE. Baseline activity data were highest in this group, however, so these differences did not appear to be due to HIE treatment. Weekly, weekday, and weekend activity decreased ($P < 0.05$) over time. In conclusion, results of the current study did not show significant changes in physical activity levels, blood markers, or owner perception of joint pain after consuming a HIE product. Although HIE products of a different source, of higher quality, or of higher dosage may be of interest for future research, the results of the current study do not support the use of HIE for alleviating clinical signs associated with canine OA.

INTRODUCTION

According to the American Pet Products Association (APPA), there are currently about 89.7 million dogs living in the United States (APPA, 2018). In 2016, about 59% of pet owners classified their pet as a family member or child (APPA, 2018). This common view of pets in the household has led to owners taking greater interest in the health and well-being for a better quality of life (QOL) of the animals. Although improved nutritional and veterinary care have greatly contributed to extending the life expectancy of pets in recent years (Inoue et al., 2018), with it has come new challenges. Chronic progressive diseases like osteoarthritis (OA), for instance, are more prevalent with aging and reduce QOL.

Osteoarthritis is a debilitating, irreversible condition that affects the joint structure of animals. It has been reported that over 20% of dogs over one year of age and 80% of dogs over eight years of age suffer from OA (Johnston, 1997). Also known as a “wear and tear” disease, OA has a much higher prevalence in older dogs due to the mechanical and chemical stress of joints with age. Reported clinical signs include lameness, reduced activity level, pain, and stiffness. Most commonly reported sites of OA in dogs are the hips, elbows, and stifles. Currently, there are many different treatment options for OA, the most common being surgery, pharmacological agents, physical rehabilitation, weight management, and nutraceutical supplementation. Treatment options are carefully selected by veterinarians according to the severity and progression of the disease and oftentimes a multimodal approach using a combination of treatments is used to not only alleviate pain but also to slow down disease progression (Fox, 2016). Non-steroidal anti-inflammatory drugs (NSAID) are the most commonly used pharmacological agents to help with pain, but long-term use of this medication has been associated with adverse reactions to the gastrointestinal tract (64%), renal system

(21%), and liver (14%), which is concerning for pet owners and veterinarians (Hampshire et al., 2004).

In recent years, there has been increasing interest in using nutraceuticals as an alternative to improve OA signs and improve the overall QOL of animals. Glucosamine, chondroitin, and omega-3 fatty acids are nutraceuticals commonly used to reduce disease signs in OA patients. The published literature is equivocal for most nutraceutical products tested. Although there are some studies reporting improvements in OA symptoms, others have not shown positive changes, leading to inconclusive results. As novel nutraceuticals emerge in the human OA market, there has been great interest in applying and further testing the safety and potential beneficial effects of these products on pets with OA.

Hyperimmunized egg (HIE) product is produced by repeated vaccination of one or more antigens to a hen, which induces an immune response in the hen and develops antibodies. The antibodies produced are transferred to eggs, which are then pasteurized and spray-dried to be utilized as an oral supplement. The concentrated egg protein extract is the final product that contains the specific antibody to provide passive immunity (Dean, 2000). The use of HIE product as an alternative to antibiotics has been proposed in farm animals and been shown to reduce the risk of gastrointestinal infection in mice, poultry, piglets and calves (Diraviyam et al., 2014). Few reports exist in dogs, but it has been shown to positively affect fecal scores, fecal IgA concentrations, and response to viral infection in growing puppies (Reynolds and Knorr, 2006; Van Nguyen et al., 2006).

The effects of HIE on joint health in dogs were unknown, but of interest. Therefore, the objective of this study was to evaluate the effects of short-term supplementation of HIE on voluntary activity levels, serum chemistry and inflammatory markers, and owner perception of

joint pain in dogs diagnosed with OA. We hypothesized that HIE would increase voluntary physical activity levels, decrease serum inflammatory markers, and decrease owner's perception of joint pain in dogs diagnosed with OA.

MATERIALS AND METHODS

Animals and Study Design

A total of eighty-two adult dogs of various breeds were recruited and sixty-nine dogs (mean age = 8.0 ± 3.4 yr.; mean BW = 32.3 ± 11.3 kg) completed a double-blind, placebo-controlled study in a completely randomized design. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee and dog owners signed an informed consent form prior to starting the study. Dogs were selected based on clinical signs, patient history, owner assessment of pain and OA diagnosis by a board-certified small animal orthopedic surgeon. A 14-d washout period prior to enrollment was required for animals being treated with interfering medications, including corticosteroids, non-steroidal anti-inflammatory drugs (NSAID), and nutraceuticals. Prior to enrollment in the study, dogs underwent physical, neurological and orthopedic examination followed by radiographs to confirm OA diagnosis at the University of Illinois Veterinary Teaching Hospital. The total duration of the study was 49 d and consisted of a baseline period of 7 d followed by a 42-d treatment period. The study was conducted at the University of Illinois Veterinary Teaching Hospital. Dogs enrolled in the study were required to visit the clinic on d -7 and d 42. In addition, dog owners were required to complete and submit canine brief pain inventory (CBPI) and Liverpool osteoarthritis in dogs (LOAD) surveys in person or electronically on d 14 and d 28 of the study.

Exclusion Criteria

Dogs that were unable to remain off of interfering medications during the washout period, as well as during the 49-d study period, were excluded from the study. Dogs that were under 18.2 kg BW; had evidence of neurological disease affecting gait, neoplasia or acute instability of the joint; and female dogs that were pregnant or planned on being bred during the study also were excluded.

Treatments

All treatments were provided by Trouw Nutrition USA (Highland, IL) in the form of soft-chew treats. There were three different treatments, all containing the same base ingredients and only differing in HIE inclusion concentration as follows:

- Treatment 1 (placebo): 0.0 g HIE/soft-chew
- Treatment 2: 2.0 g HIE/soft-chew
- Treatment 3: 3.0 g HIE/soft-chew

Dogs were randomly allotted to each treatment through a continuous enrollment over a period of 12 mo so that an even number of dogs were assigned to each treatment. Treatment dosage was calculated by rounding the BW of each dog to the nearest 4.54 kg increment and assigned one treat for every 9.07 kg BW. On d -7, dog owners were sent home with a 2-wk supply of the assigned treatment. Owners were instructed to begin supplementing the appropriate treatment and dosage starting on d 0 and consequently returning to the Veterinary Teaching Hospital on d 14 and d 28 to receive a refill of the treatment.

Blood Collection and Serum Chemistry and Inflammatory Marker Analyses

On d -7 and 42, up to 20 mL of blood was collected via jugular venipuncture after overnight fasting by University of Illinois small animal veterinary staff. On d -7, blood was collected while the animal was under sedation for radiographic assessment. Blood was aliquoted into two 10 mL BD Vacutainer® SST™ tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) and allowed to clot at room temperature. One of the 10 mL Vacutainer tubes was sent to the University of Illinois Veterinary Diagnostic Laboratory (Urbana, IL) for serum chemistry analysis. The second 10 mL Vacutainer tube was centrifuged at 1,210 x g for 15 min at 4°C. Isolated serum was pipetted into cryogenic vials and stored at -80°C until serum inflammatory marker analysis. The following inflammatory markers were analyzed using enzyme-linked immunosorbent assays (ELISA): canine C-reactive protein (Abcam, Cambridge, MA) and matrix metalloproteinase-3 (Cloud-Clone Corp., Houston, TX).

Canine Brief Pain Inventory (CBPI) and Liverpool Osteoarthritis in Dogs (LOAD)

Questionnaires

Each dog owner completed CBPI and LOAD surveys on d -7, 14, 28 and 42. The CBPI is a validated survey for OA and osteosarcoma developed by researchers at the University of Pennsylvania (Brown et al., 2008). The survey consists of a total of ten questions, with the first nine rating the owner's perception of their dog's pain and how it interferes with the animal's daily routine over the past 14-d. The rating scale for each question ranges from 0 (no pain/interference) to 10 (extreme pain/interference). The last question is qualitative and asks the owner to rate the dog's QOL, ranging from poor to excellent. The LOAD survey is a validated survey for canine elbow OA and gait analyses. The survey consists of 10 questions about the

background and lifestyle information of the dog, followed by a series of 13 questions focused on the dog's mobility on a 5-point scale with descriptive terms.

Voluntary Physical Activity

Voluntary physical activity levels of each dogs were monitored throughout the entire study using HeyRex activity monitors (HeyRex Ltd., Wellington, New Zealand). Upon enrollment in the study (d -7), dogs were sent home with an activity collar to start recording their activity, and a modem to be connected to the internet router at home to receive and send all collected data to an online database. Activity data was monitored by a research personnel at a daily basis and any unusual activity data recorded due to monitor malfunction was notified to the owner to correct the problem. Activity collars were removed from the dog by study personnel on the last day of the study (d 42).

Statistical Analysis

All data were analyzed with SAS® (version 9.3; SAS Institute, Inc., Cary, NC) using the Mixed Models procedure with a repeated measures design. Proc Univariate was used to evaluate data normality. For data that were not normally distributed, log transformation was applied prior to statistical analysis. Means were separated using Fisher-protected least significant difference (LSD) with Tukey's adjustment to control for experiment-wise error. Data correlation was performed with R Studio (version 1.0.136; RStudio, Inc., Boston, MA) using Pearson correlation coefficients. Data were reported as means \pm SEM with statistical significance set at $P < 0.05$ and $P < 0.10$ considered as a trend.

RESULTS

Dog Population

Baseline LOAD survey data collected from all 82 dogs enrolled in the study are summarized in Figure 3.1, which provides information regarding the animal's mobility status and regular exercise level. Owner responses revealed that over 34% of dogs were suffering from mobility problems for more than 36 mo. About 76% of dogs were estimated to walk 0-2 km per week, with 72% going on one or no walk each day. Response to the type of exercise varied, with 34% of dogs being always on a lead while exercising, 26% being always off a lead, 23% mostly on a lead, 16% off a lead, and only 1% working. Responses to the type of terrain the dog experiences revealed that 59% of dogs exercised on level grass and during that exercise, the dog would walk on a lead (42%) or run freely (41%).

Baseline BW, BCS, and age of the 69 dogs that completed the study are listed in Table 3.1. In general, dogs were approximately 8 yr old, 32 kg BW, and slightly overweight (BCS – 6.1/9).

Canine Brief Pain Inventory (CBPI) and Liverpool Osteoarthritis in Dogs (LOAD)

Questionnaires

The results from the CBPI questionnaire for all dogs collected from d 7, 14, 28, and 42 are summarized in Table 3.2. Results of CBPI survey data showed some significant time effects, with average pain; pain as of right now; interference with the ability to rise from a lying position; interference with the ability to walk; interference with the ability to climb; pain severity score; and pain interference score decreasing ($P < 0.05$) over time. CBPI scores for pain at its least and interference with general activity tended to decrease ($P < 0.10$) over time.

Figures 3.2 to 3.15 summarize data from the LOAD survey collected from d -7, 14, 28, and 42. There were no significant treatment or treatment*time effects, but some time effects were observed for: who limits the extent of the exercise ($P = 0.0066$), how disabled is the dog with his/her lameness ($P = 0.0207$); how active the dog is ($P = 0.0484$); what effect exercise has on the dog's lameness ($P = 0.0152$); effect of cold, damp weather on the animal's ability to exercise ($P = 0.0015$), and the degree that the dog shows stiffness in the affected joint after a "lie down" following exercise ($P = 0.0400$). No other significant differences were observed.

Serum Chemistry and Inflammatory Markers

Serum chemistry data for all dogs are summarized in Table 3.3. All metabolites were within the reference range except for corticosteroid-induced alkaline phosphatase and total alkaline phosphatase, which were already higher than the upper reference range at d -7. Dogs fed the low dose of HIE tended to have lower ($P < 0.10$) blood sodium than dogs fed the low HIE dose. Serum bicarbonate concentration was greater ($P = 0.0175$) in the HIE-treated group compared to the control group. Serum total protein, albumin, globulin, calcium, potassium, and cholesterol concentrations increased ($P < 0.05$) and total bilirubin tended to increase ($P < 0.10$) over time. In contrast, serum sodium, sodium: potassium ratio, chloride, and glucose decreased ($P < 0.05$) over time. Table 3.4 presents inflammatory marker data for all dogs. Serum C-reactive protein (CRP) and matrix metalloproteinase-3 (MMP-3) were not different ($P > 0.05$) due to time. However, dogs fed the low dose of HIE tended to have higher ($P < 0.10$) CRP than dogs fed placebo or the high HIE dose.

Voluntary Physical Activity Data

Physical activity data collected from only 56 dogs were considered for statistical analysis due to issues in data quality. To be included in the analysis, activity data needed to contain at least 90% “quality days” of data recorded in the database. Data from dogs that had data integrity scores below the 90% threshold, which was commonly due to significant activity data loss, were excluded from analysis. Figure 3.16 presents activity data for each treatment throughout the 49 d of the study and figure 3.17 presents an average daily activity pattern for each treatment group. Time had a significant effect, but treatment and treatment*time effects were not significant. Weekly average voluntary physical activity data expressed in delta G for all treatments over the entire study, including data for the entire week (7 d), weekends only (2 d), and weekdays only (5 d), are summarized in Table 3.5 and Figure 3.18. Weekly; weekday; and weekend activity data were greater ($P < 0.05$) in dogs fed the low dose of HIE than dogs fed placebo or the high dose of HIE. Baseline activity data were highest in this group, however, these differences did not appear to be due to HIE treatment. Weekly, weekday, and weekend activity decreased ($P < 0.05$) over time. Figures 3.19 to 3.23 present the activity delta G measures for each activity type (run, walk, sleep, alert, and scratch) representing the average weekly, weekday, and weekend activity counts for each treatment. Weekend and weekday activity counts are presented in one graph to allow activity pattern comparison between weekday and weekend.

Correlation Analysis of Activity Data

Different activity patterns (run, walk, sleep, alert, and scratch) were correlated with pain severity score (PSS), pain interference score (PIS), pain scale provided by veterinarians, age, BW, BCS scored by a veterinarian, and BCS scored by lab personnel. Figures 3.24 to 3.28

provide correlation plots for all dogs (Figure 3.24), dogs over 29.94 kg BW (Figure 3.25 and 3.28), and under 29.94 kg BW (Figure 3.26 and 3.27), respectively. Age was negatively correlated with scratch ($P=0.03$; $r=-0.10$) and alert ($P=0.03$; $r=-0.13$) activity counts, while BW was negatively correlated with total activity counts ($P=0.02$; $r=-0.12$) in all dogs. In dogs with $BW>29.94$ kg, BW was positively correlated with alert activity counts ($P=0.01$; $r=0.04$). Age was negatively correlated with sleep ($P=0.02$; $r=-0.12$) and alert ($P=0.004$; $r=-0.30$); BCS by lab was negatively correlated with sleep ($P=0.02$; $r=-0.26$) and positively correlated with walk ($P=0.05$; $r=0.03$); BCS by vet was negatively correlated with sleep ($P=0.02$; $r=-0.27$), and walk ($P=0.02$; $r=-0.03$). For dogs with $BW<29.94$ kg, total activity was positively correlated with PIS ($P=0.02$; $r=0.21$) and negatively correlated with BW ($P=0.004$; $r=-0.12$).

DISCUSSION

OA is a progressive chronic disease that degenerates the joints of an animal, not only bringing pain and disability to the affected area but also lowering the overall QOL because of loss of function, reduced joint mobility, and increased pain. Currently, there is no cure for OA, but there are many potential treatment options aimed at managing pain, delaying disease progression, reducing joint instability and restoring near-normal joint function. Multimodal treatment approaches are needed for patients suffering from OA. Common treatment strategies include: pharmacological interventions, rehabilitation, surgery, weight loss, nutraceuticals, and alternative methods such as acupuncture or laser therapy. The treatment selected depends on the severity of OA.

Current pharmacological interventions, particularly NSAID, are commonly prescribed to help with pain management. However, reported side effects such as gastrointestinal

complications, ulceration, and negative impacts on kidney function have been a concern for animal care professionals and pet owners alike. There has been increased interest in nutraceuticals as alternatives to pharmaceuticals, with chondroitin sulfate (CS) and glucosamine sulfate (GS) being the most popular treatments for OA management. However, conflicting results have been reported for the efficacy of these nutraceuticals in canine OA treatment.

Several studies have reported disease-modifying effects of nutraceuticals (Wenz et al., 2017). A randomized, placebo-controlled, double-blinded study where OA was induced in 32 dogs using the Pond-Nuki model evaluated the effect of GS versus placebo by supplementing for eight weeks. Pond-Nuki is a model that to induce progressive degenerative changes of articular cartilage in a mechanically instable knee by transection of the anterior cruciate ligament (ACL). All dogs were assigned to four different sub-groups: 400 mg intra-articular GS, intra-articular placebo, 200 mg/kg BW of oral GS, and oral placebo. The non-operated stifle served as a control, and after 8 weeks of study, the medial and lateral tibial plateau and patella were histologically examined, and anatomic changes were quantified by light microscopy using Mankin score, which is a histopathological measure of OA severity in the cartilage. Results showed that Mankin score was decreased ($P < 0.002$) in the intra-articular GS group (8.1; range 7.9 to 8.8) compared to the intra-articular placebo group (13.9; range 11.6 to 15.9) and also decreased ($P < 0.002$) in the oral GS group (12.1; range 9.9 to 12.7) compared to the oral placebo (15.1; range 12.5 to 17.0). Mean Mankin score was lower ($P < 0.002$) in the intra-articular GS group compared to the oral GS group, concluding that GS significantly reduced histological signs of OA in Pond-Nuki-induced OA with intra-articular administration being more effective than oral (Wenz et al., 2017).

Although some studies report positive effects of GS and CS on canine OA, some of the studies provide conflicting results. Variability in the efficacy of these products is partly dependent on the source from which the products are derived (i.e., animal, plant), the production process, the purity, among other factors.

In recent years, there has been increased attention in novel sources of nutraceuticals, with these products being tested clinically to evaluate their benefits. The use of HIE as an alternative to antibiotics has been proposed in farm animals and been shown to reduce the risk of gastrointestinal infection in mice, poultry, piglets and calves (Diraviyam et al., 2014). Few reports exist in dogs, but it has been shown to positively affect fecal scores, fecal IgA concentrations, and response to viral infection in growing puppies (Reynolds and Knorr, 2006; Van Nguyen et al., 2006). Anecdotal reports claiming improvement in mobility and pain reduction from HIE supplementation exist, but no scientific evidence is available to support such claims.

The current study is the first clinical trial testing the efficacy of HIE in dogs with OA, with the objective of improving joint function, reducing clinical signs, and inflammatory markers. The test product was developed and patented by Trouw Nutrition USA (Highland, IL) (Kisic and Shipp, 2003). While this HIE supplier refused to provide many details about it, it is known to be a spray-dried product that originates from chickens that have been hyperimmunized with a vaccine comprised of one or more immunogens (specific immunogens unknown).

In the current study, pet owners were asked to complete a CBPI survey to assess their animal's pain throughout the study. Although it is a subjective scoring system, the same owner was asked to complete the surveys on all days of study for consistency. This survey method has been validated for pain severity score (PSS) and pain interference score (PIS) to provide an

objective and reliable measure of an animal's pain. We observed reduced average pain scores over time for all treatments. Improvements reported in the surveys over the 49-d study period are considered as positive changes. However, the HIE product was not shown to cause any observed changes.

Blood inflammatory biomarkers have been studied and recognized to be correlated to the presence of OA and disease progression. Biomarkers such as CRP and MMP are attributed to pro-inflammatory reactions and do not serve only as a detection purpose but also for targeted treatment options. A study examined 845 cases of women with OA and concluded that CRP concentrations were significantly increased in women with early knee OA, with even higher concentrations of CRP predicting disease progression over the coming 4 years (Belo et al., 2007). Similarly, MMP-3 expression was investigated in the synovium of knee joints at different stages in human OA patients. OA patient groups were divided into three subgroups according to disease severity: grade I (mild; n=30), grade II (moderate; n=30), and grade III (severe; n=30), and compared to a control (non-OA; n=30) group. Results of that study showed a higher ($P<0.005$) expression of MMP-3 in the OA group compared to controls, and a significant ($P<0.005$) difference among all OA subgroups. Though CRP and MMP-3 are validated methods for humans, literature supporting validity in dogs is still weak.

In this study, little difference was observed in inflammatory markers. Serum CRP tended to have the lowest concentrations in the control group compared to HIE-treated groups. Although a time effect was not observed, CRP results for the high HIE dose was highly variable. This could be an indication that somehow the treatment was not effective and there was another factor that exacerbated the inflammatory process or CRP is not an accurate biomarker in determining OA status in dogs.

Voluntary physical activity data in this study greatly varied over time as expected, but no treatment or treatment*time effects were observed. From observing overall activity measurements during the 49-d study period, it is difficult to make comparisons across the three treatments because baseline activity levels greatly differed among them. Although activity levels differed greatly, daily patterns of all three treatments peaked in early morning and mid-afternoon, and all three treatments had greater activity on weekends compared to weekdays. Even though that pattern was not associated with treatment intervention, the current study is the first to measure activity in dogs with OA using such monitors. Total activity and different activity types (run, scratch, alert, walk, and sleep) were correlated to PSS, PIS, pain scale provided by veterinarians, age, BW, BCS scored by lab personnel, and BCS scored by veterinarians for sub-analysis. Total activity counts had a negative relationship with BW, but it was not clear whether this was due to the OA disease condition in the animal, as pain scores did not show any correlation with measured activity.

In addition to the animal population studied, a significant limitation and difficulty experienced with this study stemmed from the lack of transparency from the supplier of the HIE test product. First, the specific immunogen(s) used to create the product and the typical antibody titers and nutrient composition of the product were not shared with the research team. Second, the specific HIE inclusion rate for each treatment was not provided. Third, although information regarding the quality and immunogenicity (e.g., antibody titers) of the test product was requested several times, the supplier refused to provide such information. Without knowing the inclusion level of HIE, it is difficult to calculate the dosage at which future studies should focus. Moreover, given the lack of information about the test products, it was impossible to determine whether HIE products in general are ineffective for relieving clinical signs of canine OA or if the

specific product provided to us was not of sufficient quality. Although HIE products of a different source, of higher quality, or of higher dosage may be of interest for future research, the results of the current study do not support the use of HIE for alleviating clinical signs associated with canine OA.

CONCLUSION

In summary, HIE was not shown to affect physical activity, blood biomarkers, or owner surveys of OA dogs in this study. There are many possible factors that may have contributed, such as the relatively small number of animals recruited for the study, the dosage of products tested, the short length of the study, or recruitment of animals with mild to moderate signs of OA. Dogs with more severe cases of OA that were not able to withdraw from interfering drugs were not eligible for the study. As this was a clinical trial, there was also great variation in breed, size, age, and OA severity in the animal population studied. Activity data collected were highly variable from home-to-home, as it is dependent on the owners' lifestyle and oftentimes their living conditions. The factors mentioned above may have led to increased variability in the different measurements and outcomes of this study and should be taken into consideration when conducting future studies.

Overall, the effects of HIE in this double-blind, placebo-controlled clinical study product remains inconclusive at this time. Further studies may be conducted to test a higher dosage of HIE product. A longer study length or altered method of supplementation (i.e., before or after meal) may also be considered in the future to determine if there is a minimum amount of time required to be absorbed or observe disease-modifying effects. In addition to owner's perception of pain in the animal, more objective outcomes could be considered to more accurately track

disease progression. Such options could include testing for additional inflammatory biomarkers as well as using other measurements such as joint-space narrowing in the animal. Activity measurement in a more controlled environment should also be considered to minimize possible variations due to the owner lifestyle.

TABLES AND FIGURES

Table 3.1. Baseline mean BW, BCS, and age per treatment for all dogs that completed the study

Variable	All	Treatment 1	Treatment 2	Treatment 3
Number of animals	69	22	24	23
Age	8.05 ± 3.4	8.08 ± 3.4	8.21 ± 3.3	8.23 ± 3.3
Body Weight (kg)	32.32 ± 11.28	32.29 ± 11.44	29.39 ± 10.91	34.58 ± 10.89
BCS Vet	6.13 ± 1.11	6.12 ± 1.12	6.21 ± 1.10	6.05 ± 1.10
BCS Lab	6.15 ± 1.11	6.14 ± 1.12	6.38 ± 1.10	5.90 ± 1.10

Table 3.2. Canine brief pain inventory (CBPI) scores of dogs on d -7, 14, 28 and 42 of the study

	TRT 1				TRT 2				TRT 3				SEM	P-Values		
	d -7	d 14	d 28	d 42	d -7	d 14	d 28	d 42	d -7	d 14	d 28	d 42		TRT	DAY	TRT* DAY
Pain at its worst	3.16	2.41	2.25	2.32	3.50	3.34	3.40	2.96	3.91	4.00	3.52	3.57	0.485	0.1211	0.1904	0.6821
Pain at its least	1.50	1.05	1.21	1.05	1.29	1.33	1.38	1.13	1.26	1.52	1.52	0.91	0.347	0.9702	0.0878*	0.4716
Pain on average	2.27	1.45	1.65	1.55	2.44	2.28	2.08	1.83	2.43	2.52	2.43	2.09	0.379	0.4203	0.0099 [†]	0.2882
Pain as of right now	1.82	1.32	1.36	1.09	2.00	1.94	1.92	1.71	2.17	1.91	1.78	1.65	0.394	0.5451	0.0196 [†]	0.9219
Interference of general activity	2.27	1.45	1.59	1.73	2.29	2.02	2.04	2.13	2.65	2.22	2.48	2.30	0.469	0.5567	0.0663*	0.9132
Interference of enjoyment of life	1.41	0.86	1.44	1.82	1.67	1.48	1.54	1.25	2.04	1.35	1.57	1.39	0.404	0.9074	0.1015	0.4997
Interference with ability to rise from lying position	2.91	2.14	2.24	1.86	2.83	2.18	2.50	2.46	3.78	2.65	2.65	2.61	0.479	0.5753	<.0001*	0.6921
Interference with the ability to walk	1.91	1.14	1.56	1.45	1.96	1.57	1.71	2.00	2.13	1.70	1.87	1.91	0.414	0.7465	0.0163*	0.885
Interference with the ability to run	2.95	1.95	2.20	2.45	2.33	2.29	2.21	2.33	3.13	2.70	2.74	2.43	0.537	0.7584	0.2214	0.724
Interference with ability to climb	3.23	2.00	2.29	2.41	3.21	2.75	3.04	3.17	4.04	2.73	2.70	2.96	0.581	0.6533	0.0005 [†]	0.5919
Overall quality of life	4.14	4.18	4.17	4.09	3.79	3.97	3.67	3.96	4.00	3.96	3.91	4.13	0.177	0.3841	0.2681	0.4562
Pain severity score	2.19	1.56	1.62	1.50	2.31	2.22	2.13	1.91	2.45	2.49	2.32	2.05	0.372	0.437	0.022*	0.6215
Pain interference score	2.45	1.59	1.89	1.95	2.38	2.05	2.17	2.22	2.96	2.21	2.42	2.27	0.432	0.6756	0.0012 [†]	0.8462

¹PSS (Pain severity score): Arithmetic mean of 4 questions rating the dog's pain intensity in the last 7 d.

²PIS (Pain interference score): Arithmetic mean of 6 questions rating how much the dog's pain interferes with its normal activity.

[†]Significance level at P < 0.05.

*Significance level at P < 0.10.

Table 3.3. Serum chemistry values for dogs on d -7 and 42 of the study

	Reference	TRT 1		TRT 2		TRT 3		SEM	P-values		
	Range ¹	d -7	d 42	d -7	d 42	d -7	d 42		TRT	DAY	TRT*DAY
Creatinine (mg/dL)	0.5-1.5	0.99	0.98	1.06	1.04	0.89	0.96	0.080	0.530	0.5258	0.1660
Blood urea nitrogen (mg/dL)	6-30	15.25	16.19	18.44	18.39	17.50	15.69	1.658	0.4607	0.6099	0.1919
Total protein (g/dL)	5.1-7.0	6.03	6.48	6.06	6.42	5.94	6.18	0.121	0.3626	<.0001 [†]	0.4731
Albumin (g/dL)	2.5-3.8	3.08	3.24	3.03	3.19	3.06	3.25	0.071	0.8386	<.0001 [†]	0.903
Globulin (g/dL)	2.7-4.4	2.95	3.24	3.03	3.23	2.88	2.93	0.098	0.1557	0.0012 [†]	0.171
Albumin:Globulin	0.6-1.1	1.08	1.02	1.02	0.99	1.08	1.13	0.047	0.2536	0.7118	0.1766
Calcium (mg/dL)	7.6-11.4	9.89	10.11	9.96	10.16	9.83	9.93	0.122	0.5221	<.0001 [†]	0.4339
Phosphorus (mg/dL)	2.7-5.2	3.83	4.01	3.81	3.88	3.87	3.84	0.163	0.9226	0.4494	0.7097
Sodium (mmol/L)	141-152	145.37	144.00	146.83	145.17	145.00	145.00	0.498	0.0777*	0.0016 [†]	0.0663
Potassium (mmol/L)	3.9-5.5	4.41	4.69	4.38	4.63	4.39	4.71	0.084	0.8595	<.0001 [†]	0.9001
Sodium:Potassium	28-36	33.13	30.88	33.83	31.61	33.19	30.75	0.646	0.4893	<.0001 [†]	0.9788
Chloride (mmol/L)	107-118	114.44	112.69	114.72	112.22	113.50	113.12	0.604	0.9448	<.0001 [†]	0.0534
Glucose (mg/dL)	68-126	102.38	92.50	102.33	90.78	94.44	91.00	3.559	0.4698	0.0018 [†]	0.3877
Total alkaline phosphatase (ALP; U/L)	7-92	148.75	176.13	78.50	102.28	159.87	178.44	48.922	0.4219	0.1006	0.9678
Corticosteroid-induced ALP (U/L)	0-40	95.75	103.19	45.67	67.06	121.25	147.31	43.035	0.4158	0.138	0.8144

Table 3.3. (Continued)

	Reference	TRT 1		TRT 2		TRT 3		SEM	P-values		
	Range ¹	d -7	d 42	d -7	d 42	d -7	d 42		TRT	DAY	TRT*DAY
Total alkaline phosphatase (ALP; U/L)	7-92	148.75	176.13	78.50	102.28	159.87	178.44	48.922	0.4219	0.1006	0.9678
Corticosteroid-induced ALP (U/L)	0-40	95.75	103.19	45.67	67.06	121.25	147.31	43.035	0.4158	0.138	0.8144
Alanine Aminotransferase (SGPT) (U/L)	8-65	51.75	59.06	47.56	56.50	42.63	54.56	12.439	0.9124	0.1663	0.9612
Gamma-glutamyltransferase (U/L)	0-7	3.44	3.19	3.28	2.83	3.75	3.56	0.500	0.6232	0.2941	0.9226
Total bilirubin (mg/dL)	0.1-0.3	0.25	0.29	0.25	0.26	0.21	0.29	0.028	0.7382	0.0566*	0.542
Cholesterol (mg/dL)	129-297	225.38	243.19	233.94	246.22	237.13	263.50	13.905	0.699	0.0003	0.4912
Triglycerides (mg/dL)	32-154	90.75	107.50	108.61	91.72	75.50	111.94	25.409	0.9713	0.5013	0.4629
Bicarbonate (mmol/L)	16-24	19.56	19.50	20.89	21.89	21.31	21.25	0.587	0.0175 [†]	0.4353	0.3942

¹University of Illinois Diagnostics Laboratory reference ranges.
[†]Significance level at P<0.05
*Significance level at P<0.10

Table 3.4 Serum C-reactive protein and matrix metalloproteinase-3 concentrations of dogs on -7 d and 42 d of the study

	TRT 1		TRT 2		TRT 3		SEM	TRT	DAY	TRT*DAY
	d -7	d 42	d -7	d 42	d -7	d 42				
C-reactive protein (ng/mL)	1430.18	1887.68	3607.97	3542.18	885.13	3643.45	891.65	0.0651*	0.1291	0.1815
Matrix metalloproteinase 3 (ng/mL)	8.84	8.60	9.38	8.38	8.70	9.20	1.02	0.7952	0.9805	0.9664
*Trend level at $P < 0.10$.										

Table 3.5. Average voluntary physical activity of dogs expressed as delta G for each treatment

	TRT 1						
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Weekly	4753	5359	4517	3979	4837	4389	3240
Weekday	3934	5360	4726	4017	4339	4342	3268
Weekend	6800	5357	3993	3886	6084	4509	3282
	TRT 2						
Weekly	6045	6444	5311	5696	6807	4717	4691
Weekday	5715	6569	5448	5389	5809	4750	5043
Weekend	6870	6165	4969	6462	9302	5466	4427
	TRT 3						
Weekly	3431	3826	2803	3196	3309	3271	2840
Weekday	2854	3771	2542	2725	3239	2911	2689
Weekend	4874	3964	3457	4656	3485	4170	3231

	SEM	Trt	Week	Trt*Day
Weekly	890	0.018 [†]	0.017 [†]	0.524
Weekday	821	0.022 [†]	0.027 [†]	0.697
Weekend	1368	0.022 [†]	0.024 [†]	0.569

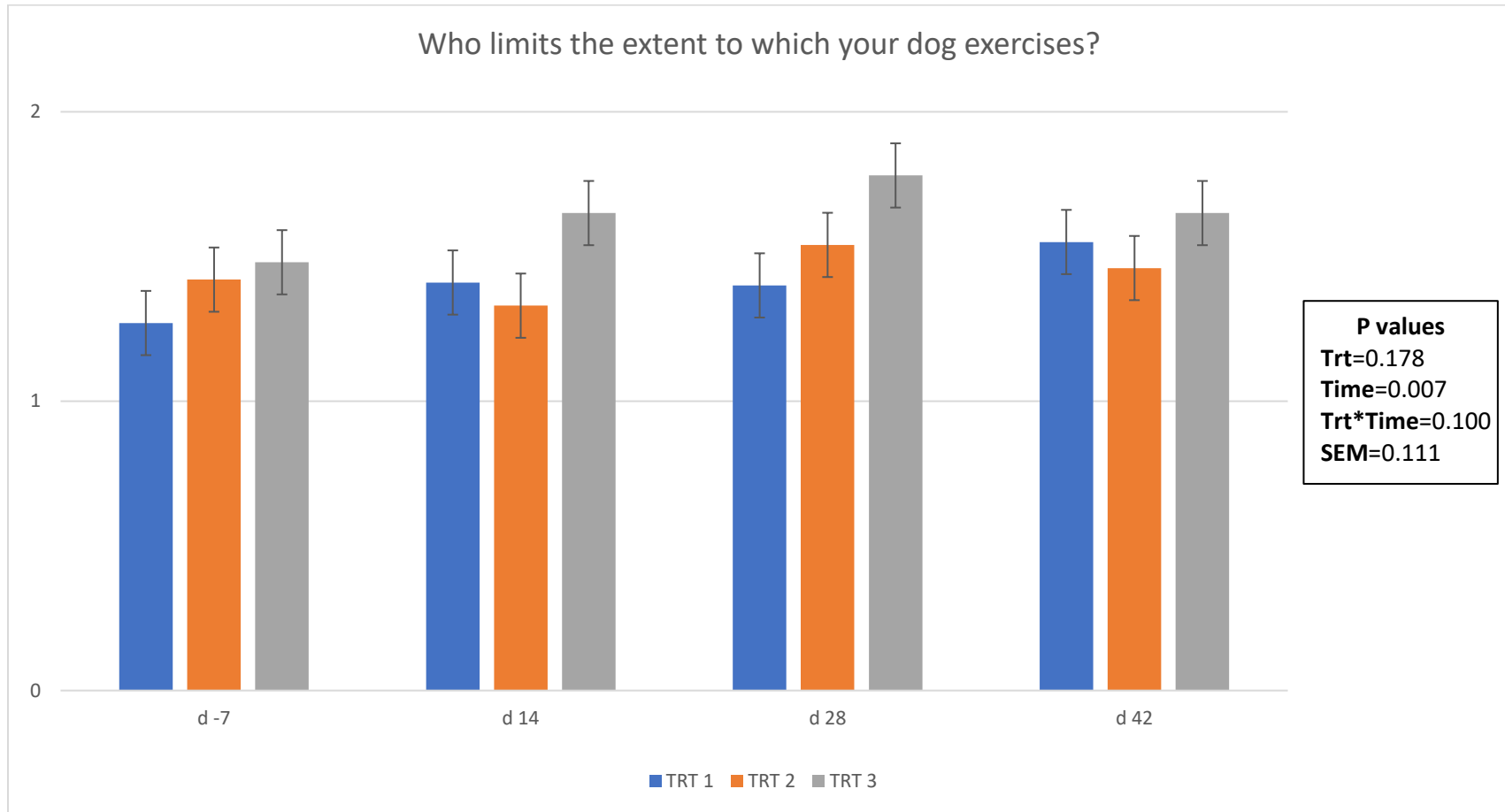
[†]Significance level at P < 0.05.

Figure 3.1. Baseline (d -7) Liverpool osteoarthritis in dogs (LOAD) survey data for background and lifestyle questions (% of responses)*



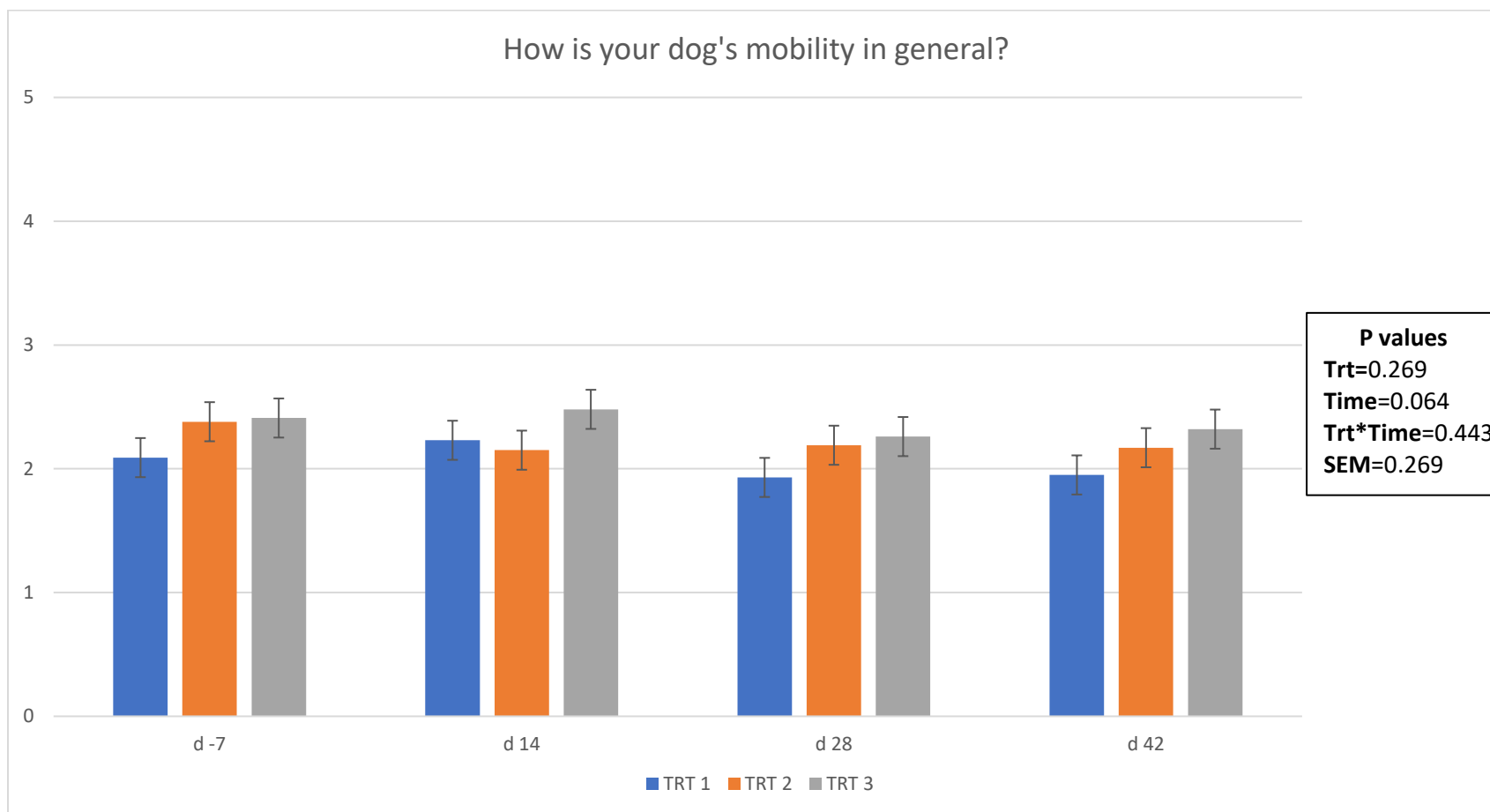
***Key:** **Mobility problems:** How long has your pet been suffering with his/her mobility problems? **Exercise:** In the last week, on average, how far has your dog exercised each day? **Walks:** In the last week, on average, how many walks has your dog had each day? **Exercise type:** What type of exercise is this? **Terrain:** On what sort of terrain does your dog most often exercise? **How dog is handled at exercise:** At exercise, how is your dog handled?

Figure 3.2 Owner response to Q9 of the Liverpool osteoarthritis in dogs (LOAD) survey*



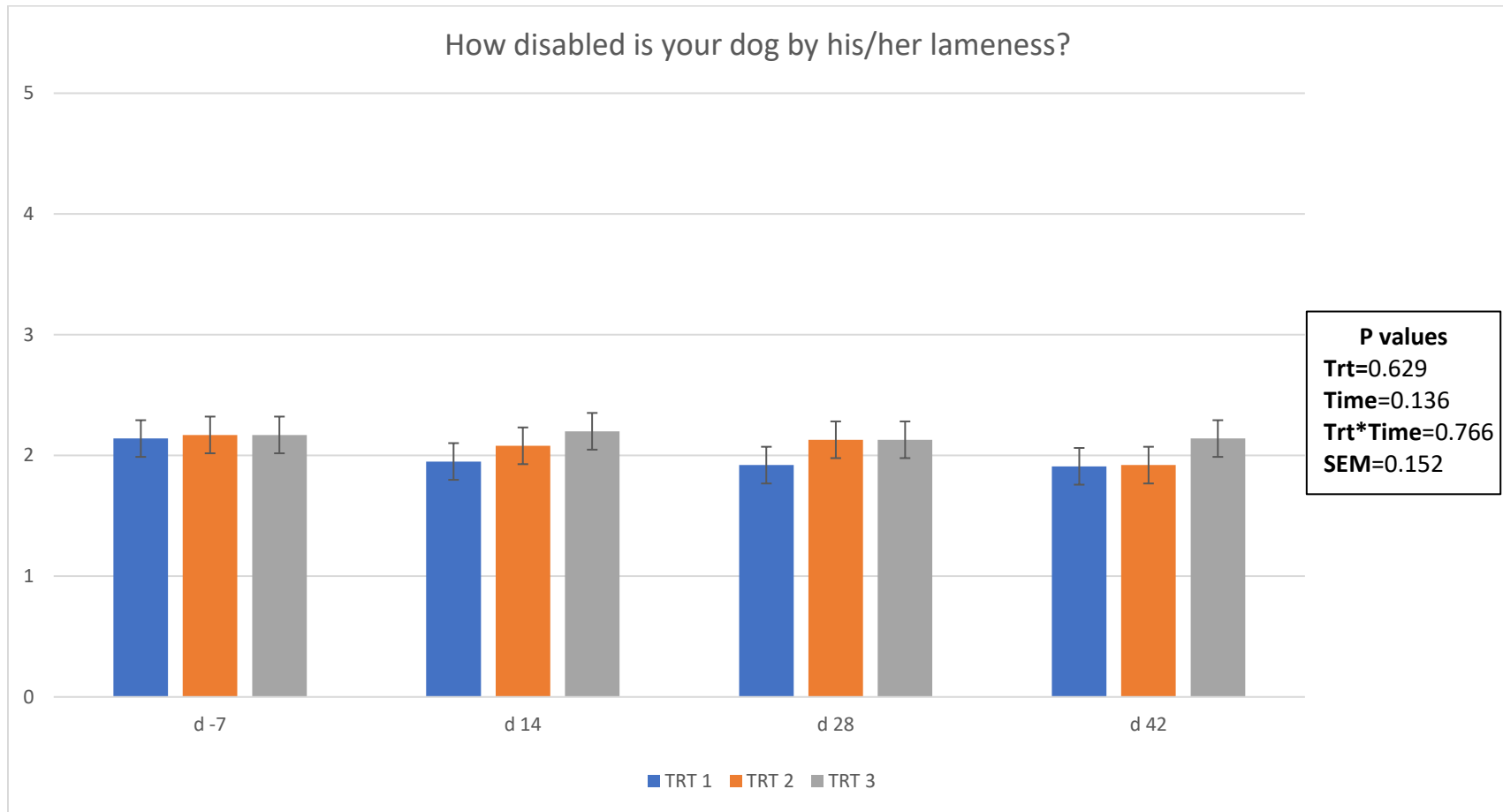
*Key: 1) You; 2) Your dog

Figure 3.3 Owner response to Q10 of the Liverpool osteoarthritis in dogs (LOAD) survey*



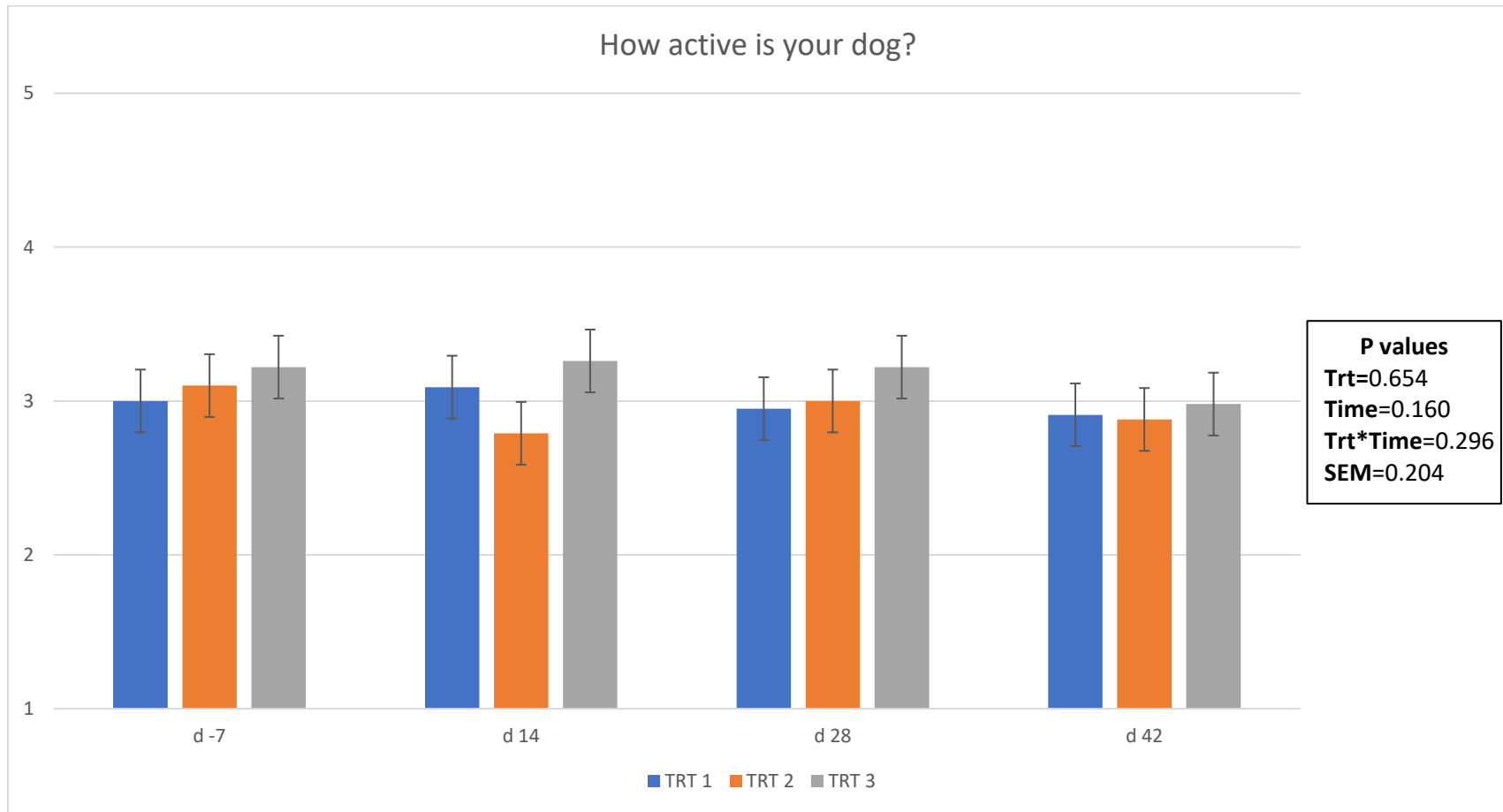
*Key: 1) Very good; 2) good; 3) fair; 4) poor; 5) very poor

Figure 3.4 Owner response to Q11 of the Liverpool osteoarthritis in dogs (LOAD) survey*



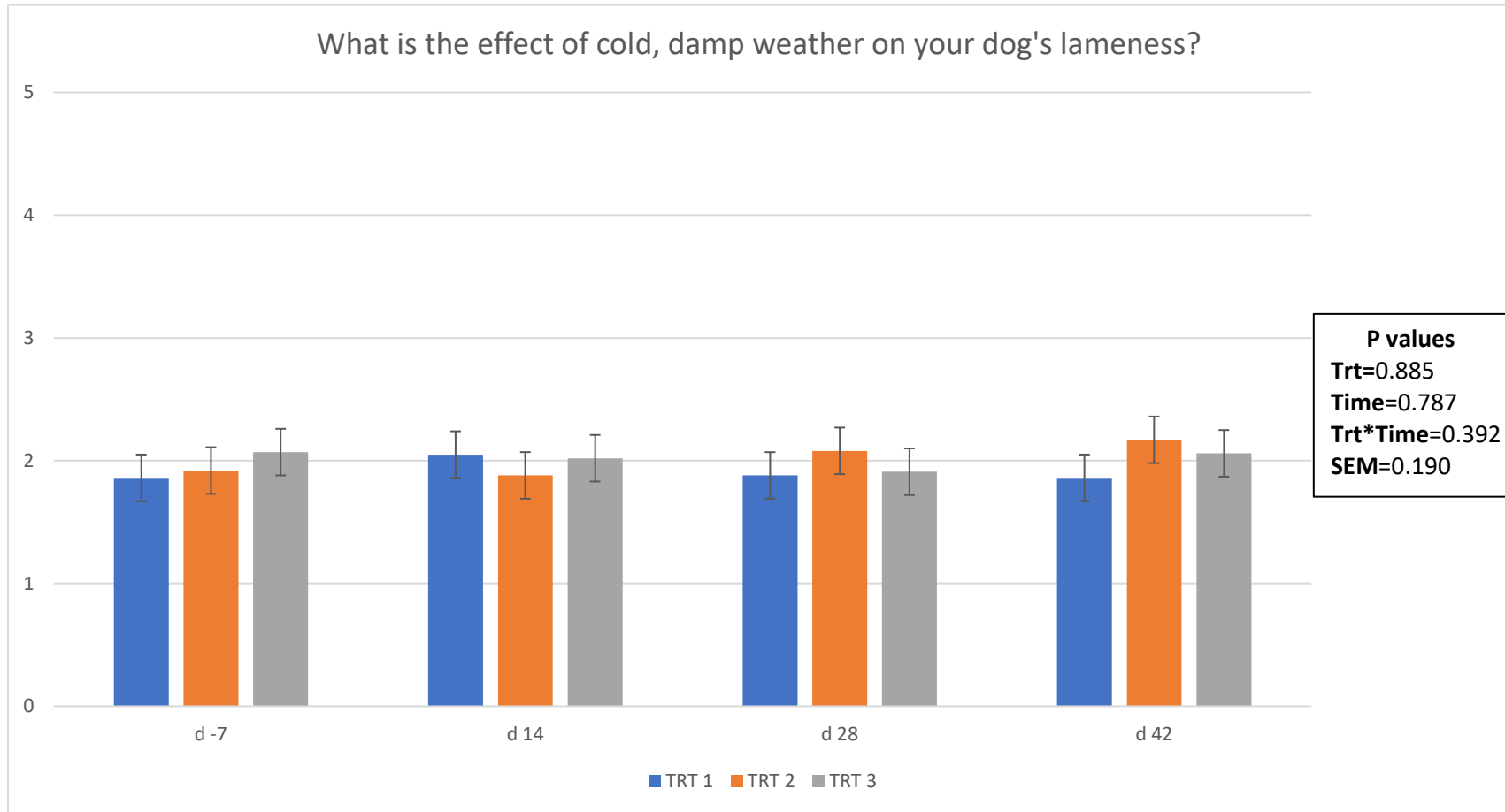
***Key:** 1)Not at all disabled; 2) slightly disabled; 3) moderately disabled; 4) severely disabled; 5) extremely disabled

Figure 3.5 Owner response to Q12 of the Liverpool osteoarthritis in dogs (LOAD) survey*



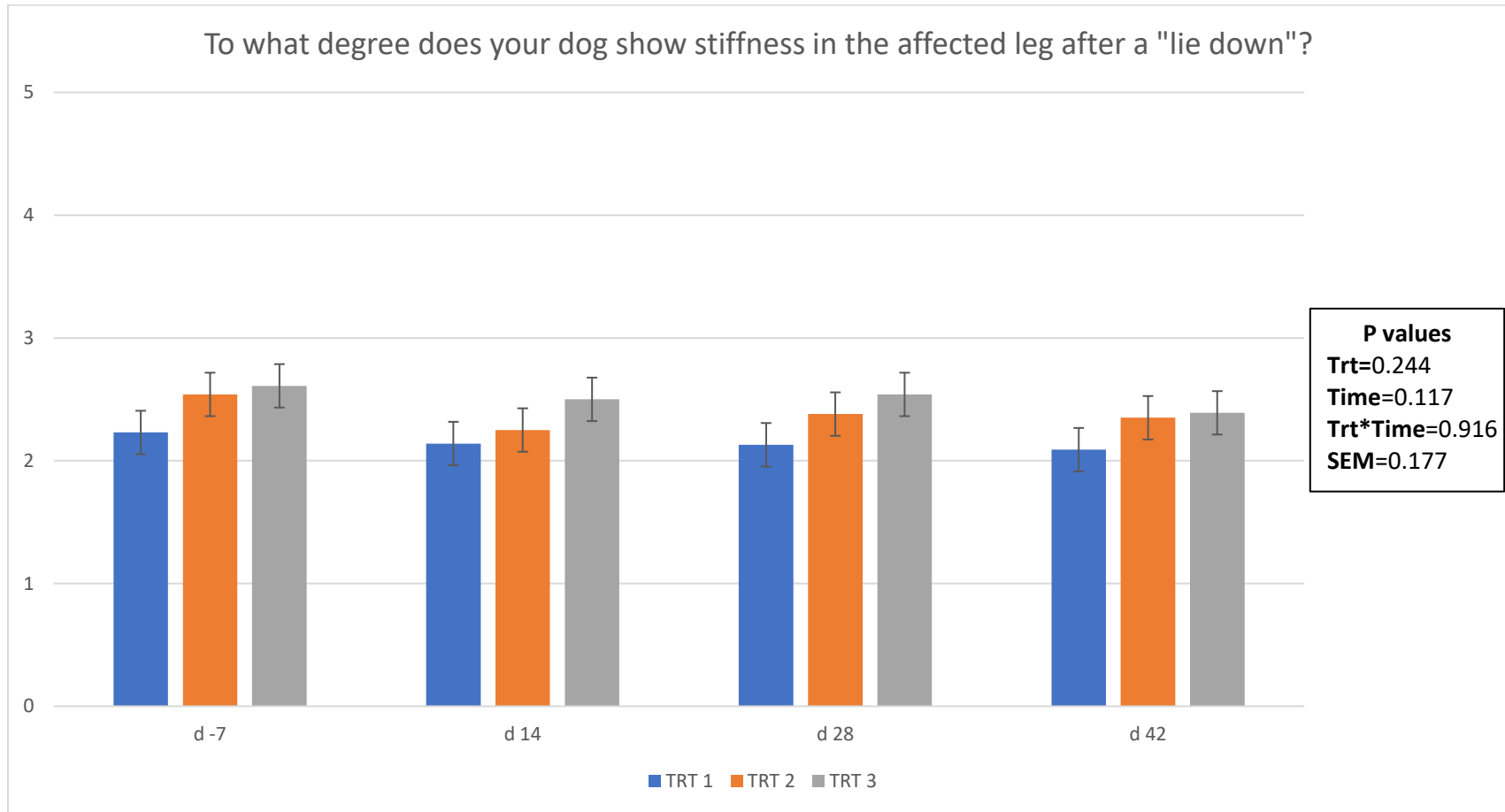
***Key:** 1) Extremely active ; 2) very active; 3) moderately active; 4) slightly active; 5) not at all active

Figure 3.6 Owner response to Q13 of the Liverpool osteoarthritis in dogs (LOAD) survey*



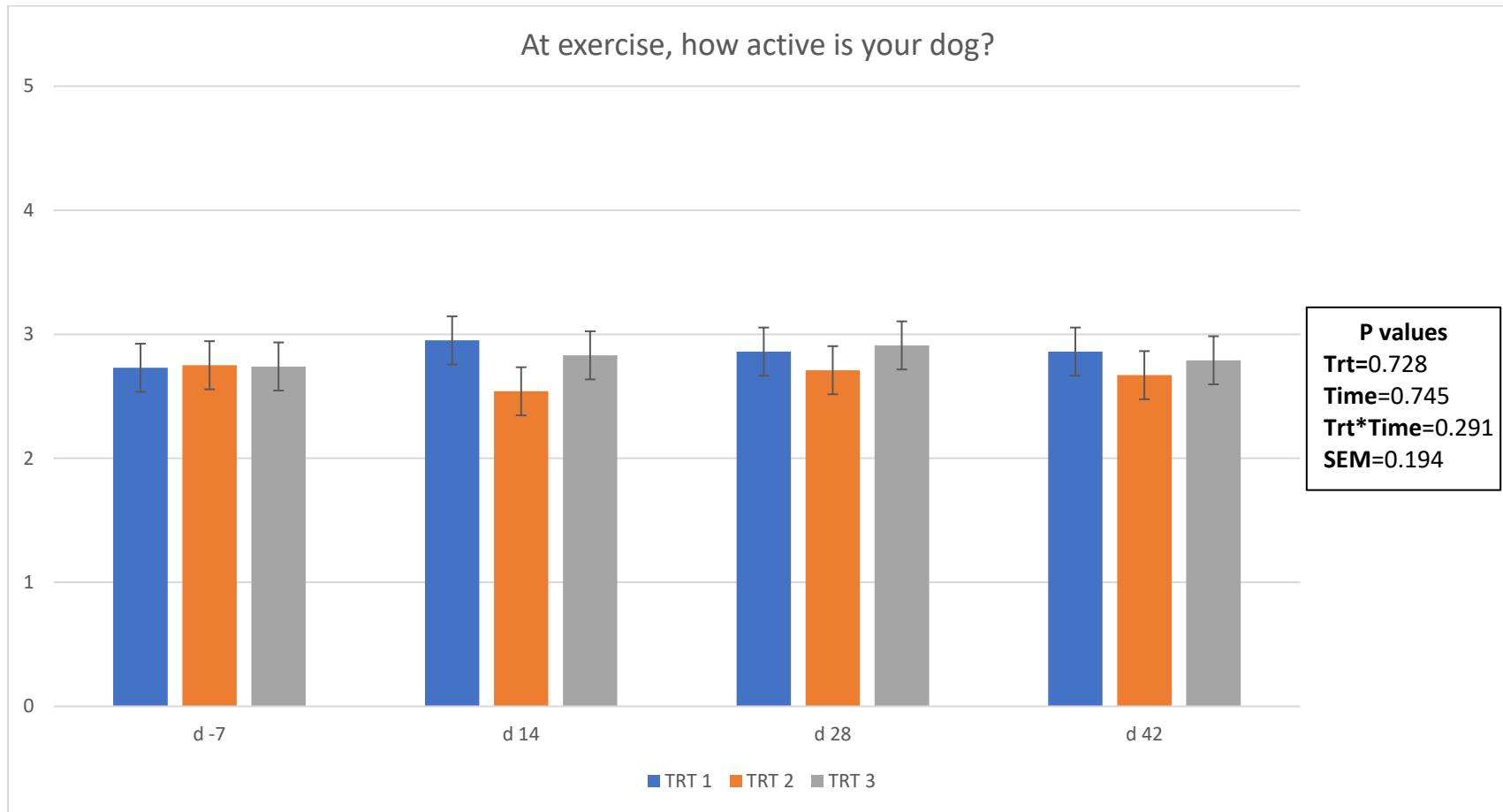
***Key:** 1) No effect ; 2) mild effect; 3) moderately effect; 4) severe effect; 5) extreme effect

Figure 3.7 Owner response to Q14 of the Liverpool osteoarthritis in dogs (LOAD) survey*



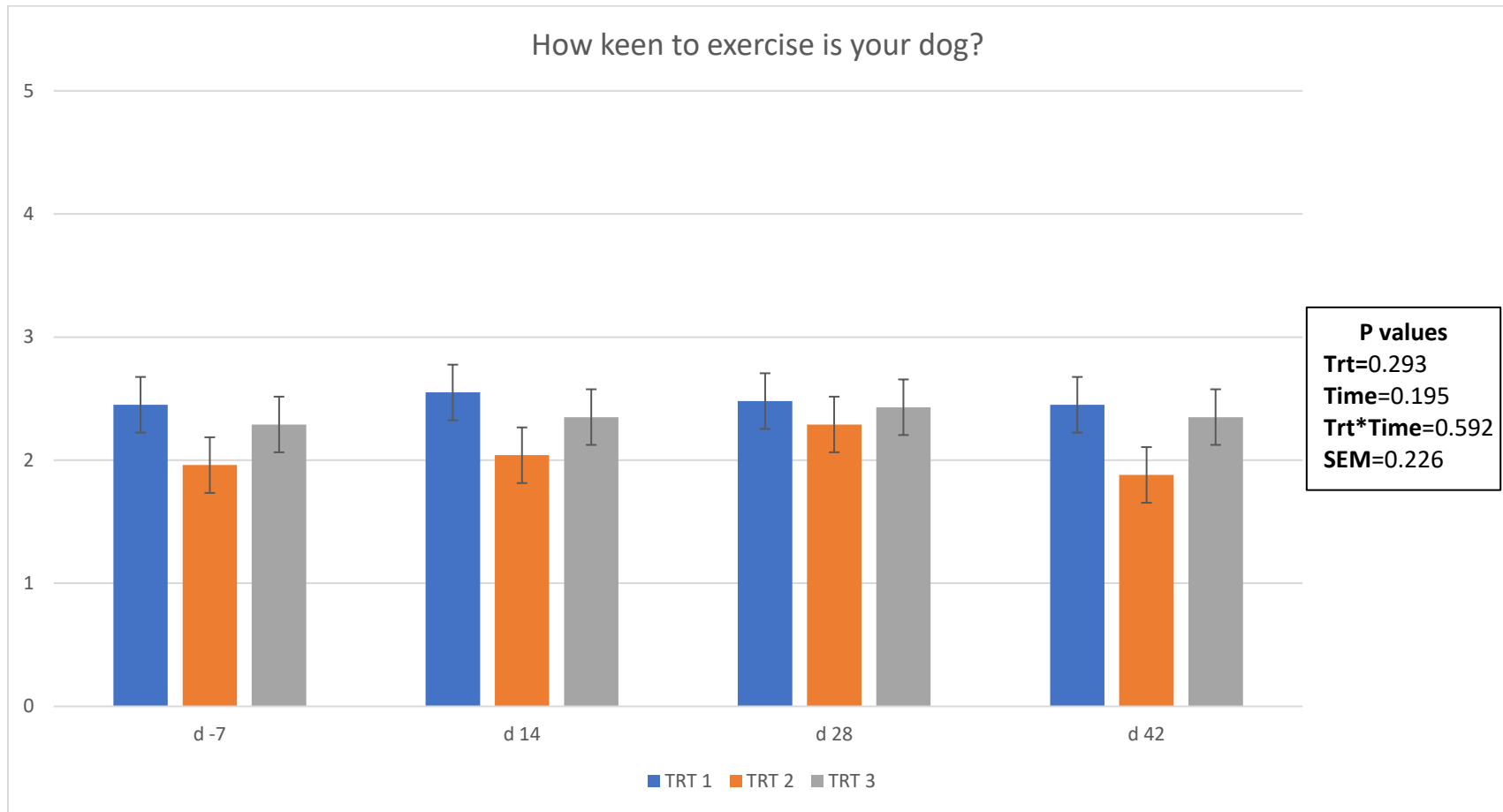
*Key: 1) No stiffness ; 2) mild stiffness; 3) moderately stiffness; 4) severe stiffness; 5) extreme stiffness

Figure 3.8 Owner response to Q15 of the Liverpool osteoarthritis in dogs (LOAD) survey*



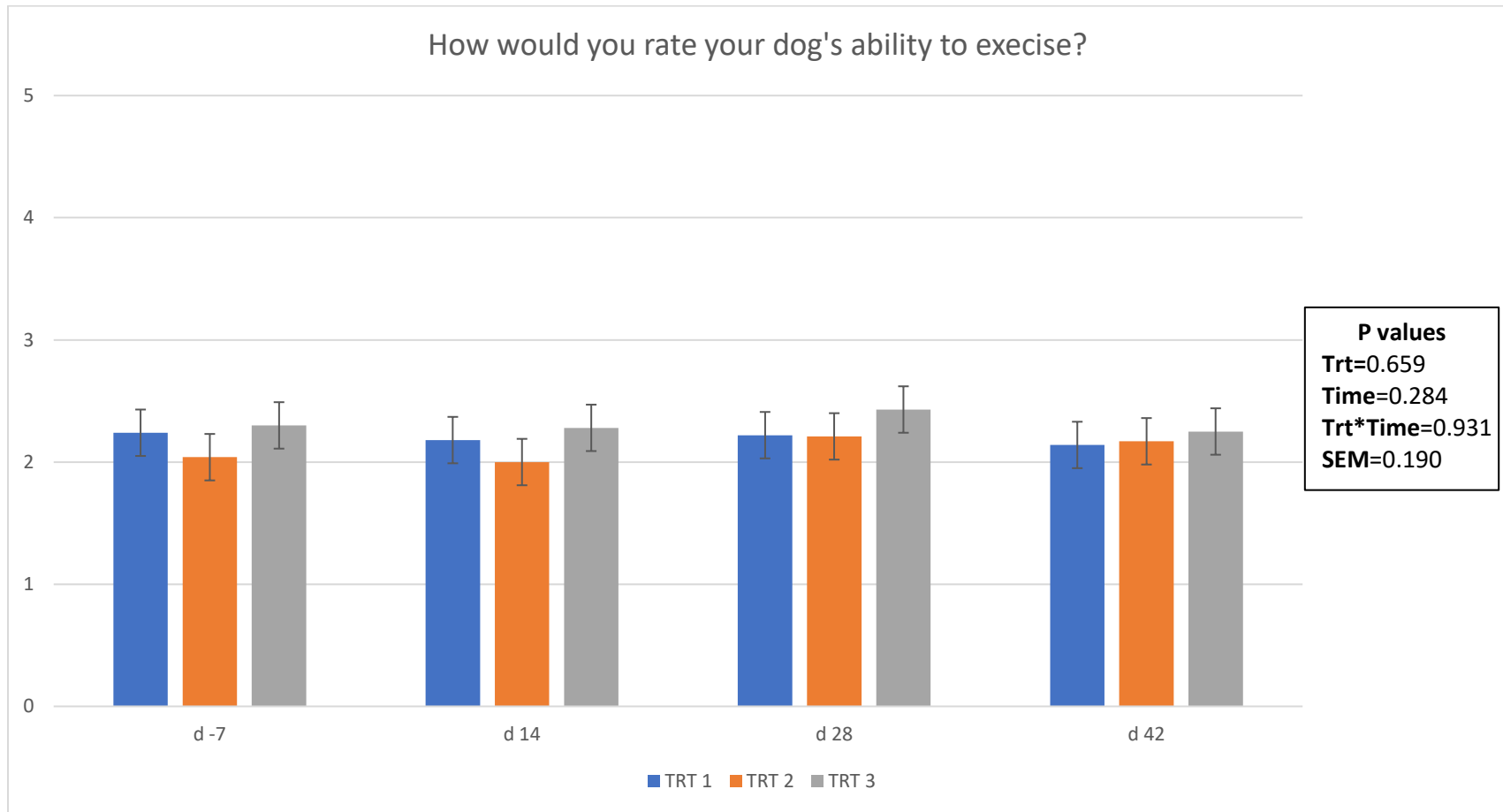
*Key: 1) Extremely active; 2) very active; 3) fairly active; 4) not very active; 5) not at all active

Figure 3.9 Owner response to Q16 of the Liverpool osteoarthritis in dogs (LOAD) survey*



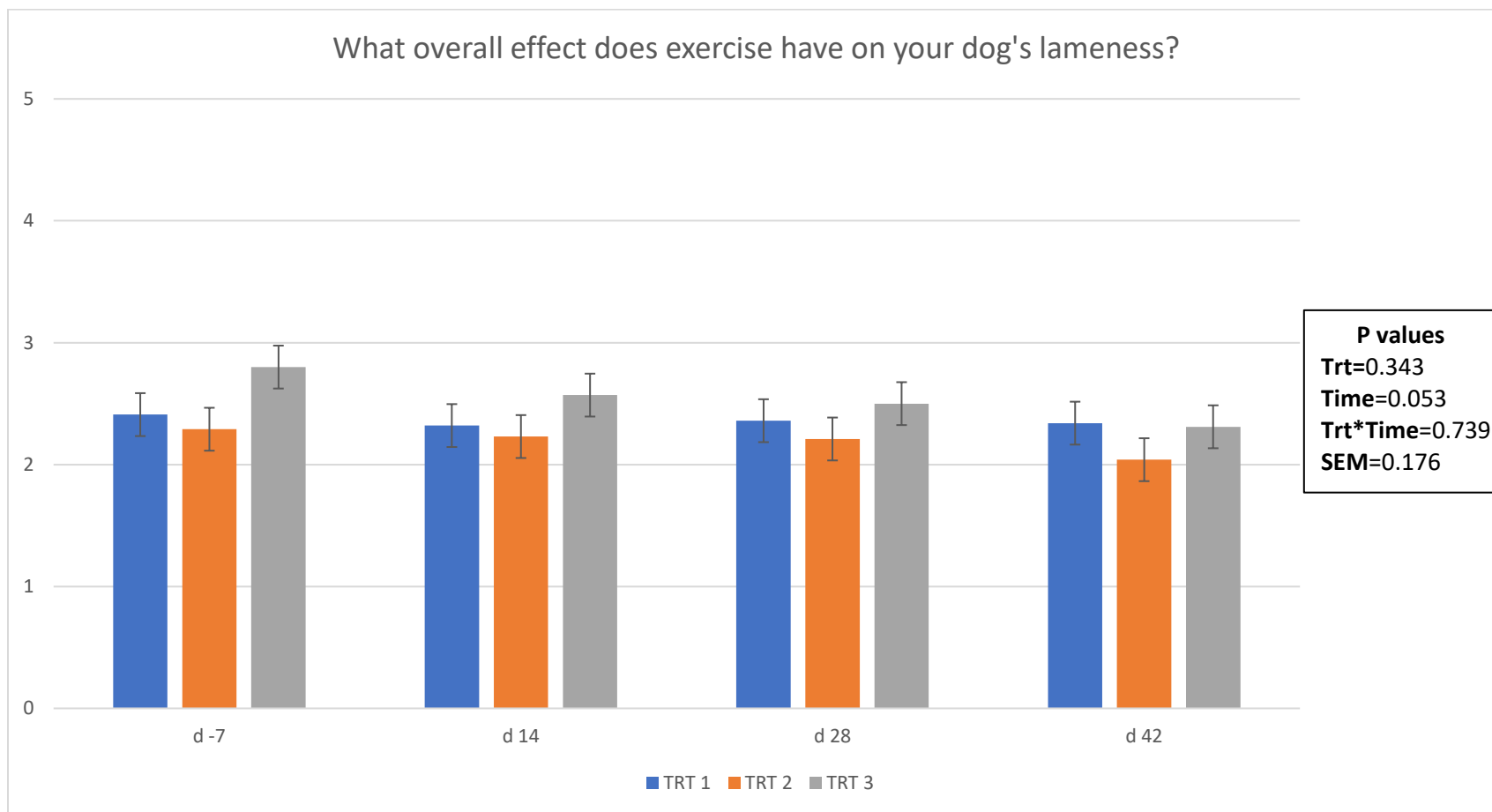
***Key:** 1) Extremely keen; 2) very keen; 3) fairly keen; 4) not very keen; 5) not at all keen

Figure 3.10 Owner response to Q17 of the Liverpool osteoarthritis in dogs (LOAD) survey*



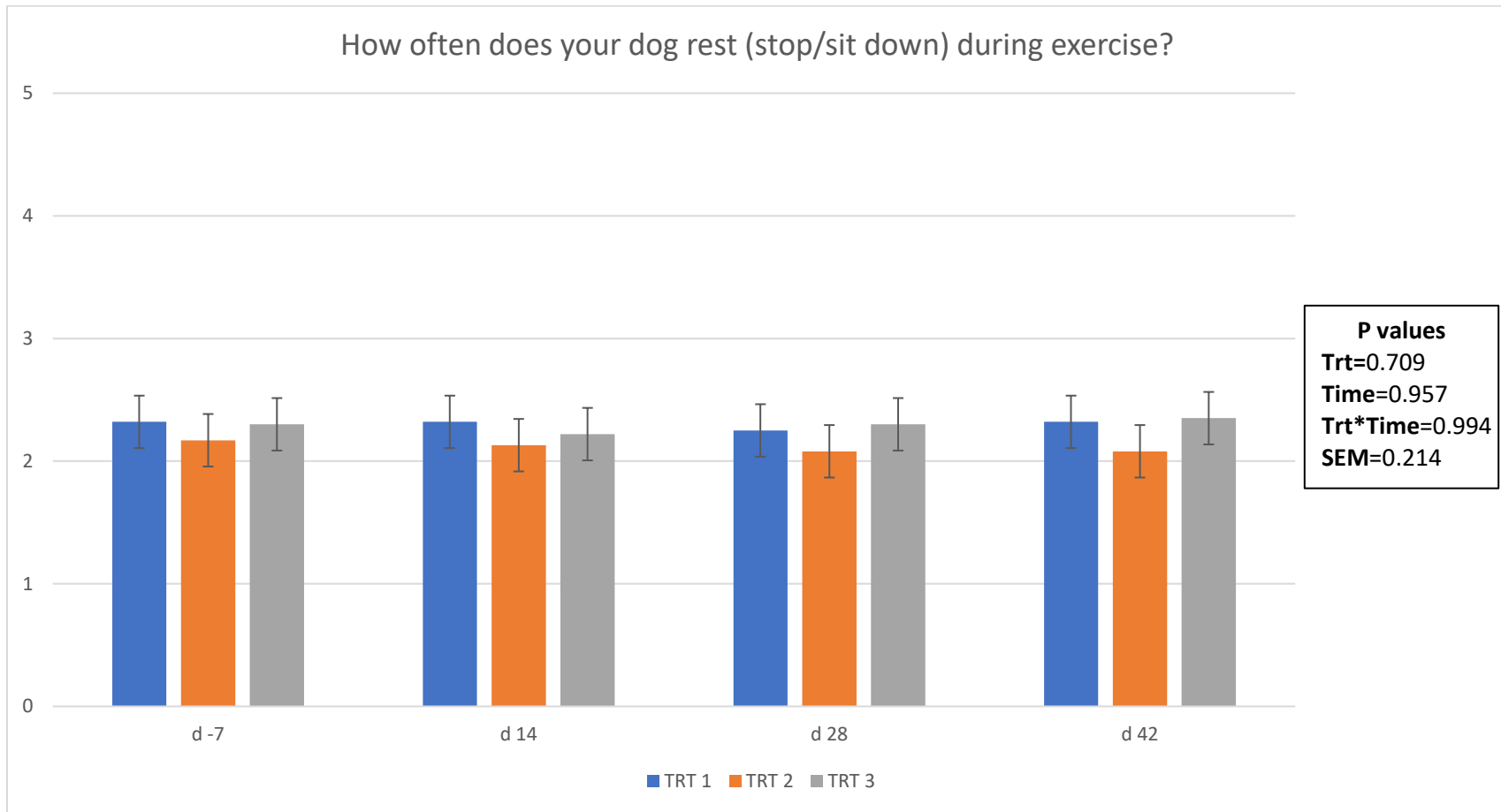
*Key: 1) Very good; 2) good; 3) fair; 4) poor; 5) very poor

Figure 3.11 Owner response to Q18 of the Liverpool osteoarthritis in dogs (LOAD) survey*



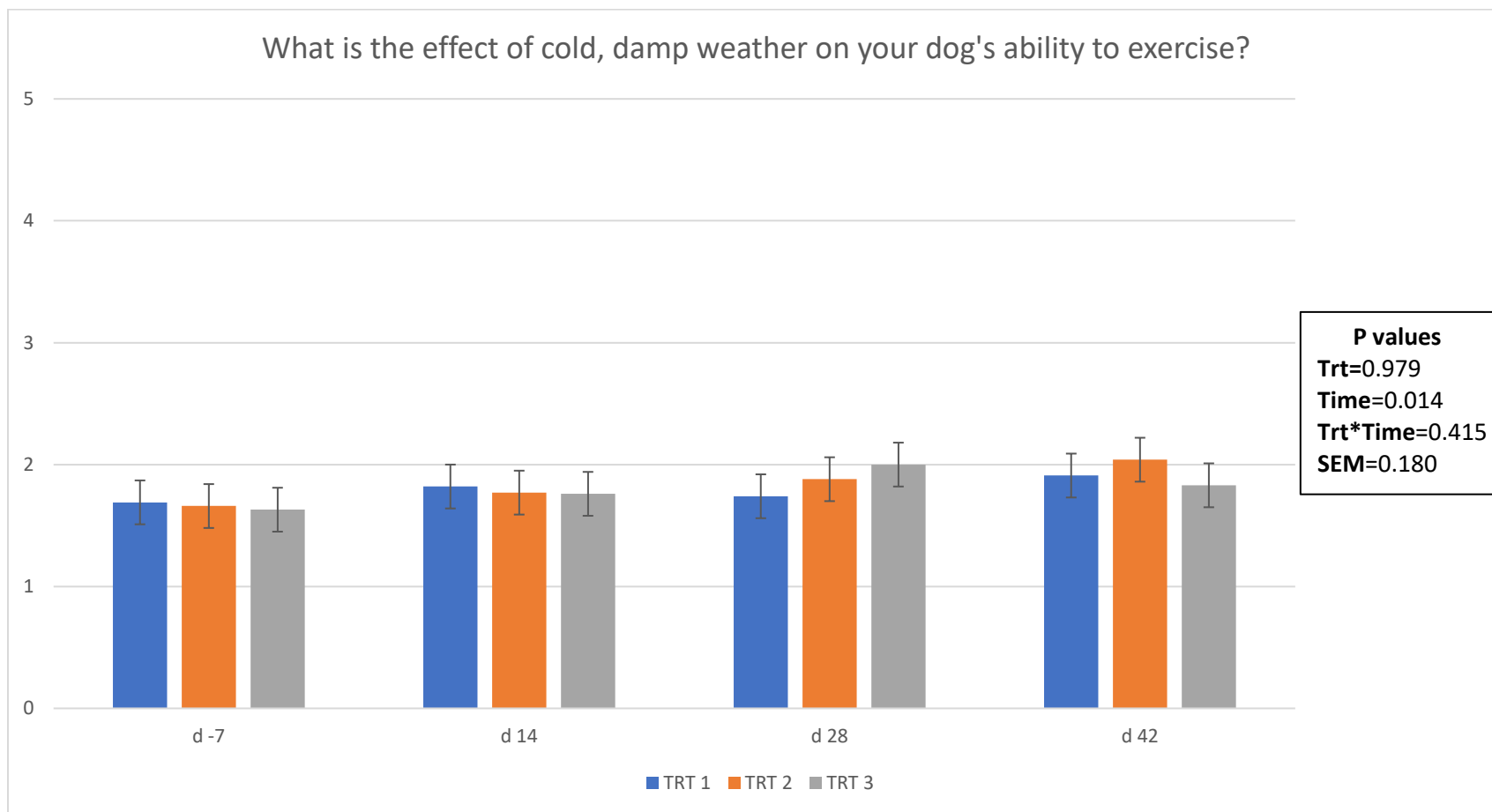
***Key:** 1) No effect; 2) mild effect; 3) moderate effect; 4) severe effect; 5) extreme effect

Figure 3.12 Owner response to Q19 of the Liverpool osteoarthritis in dogs (LOAD) survey*



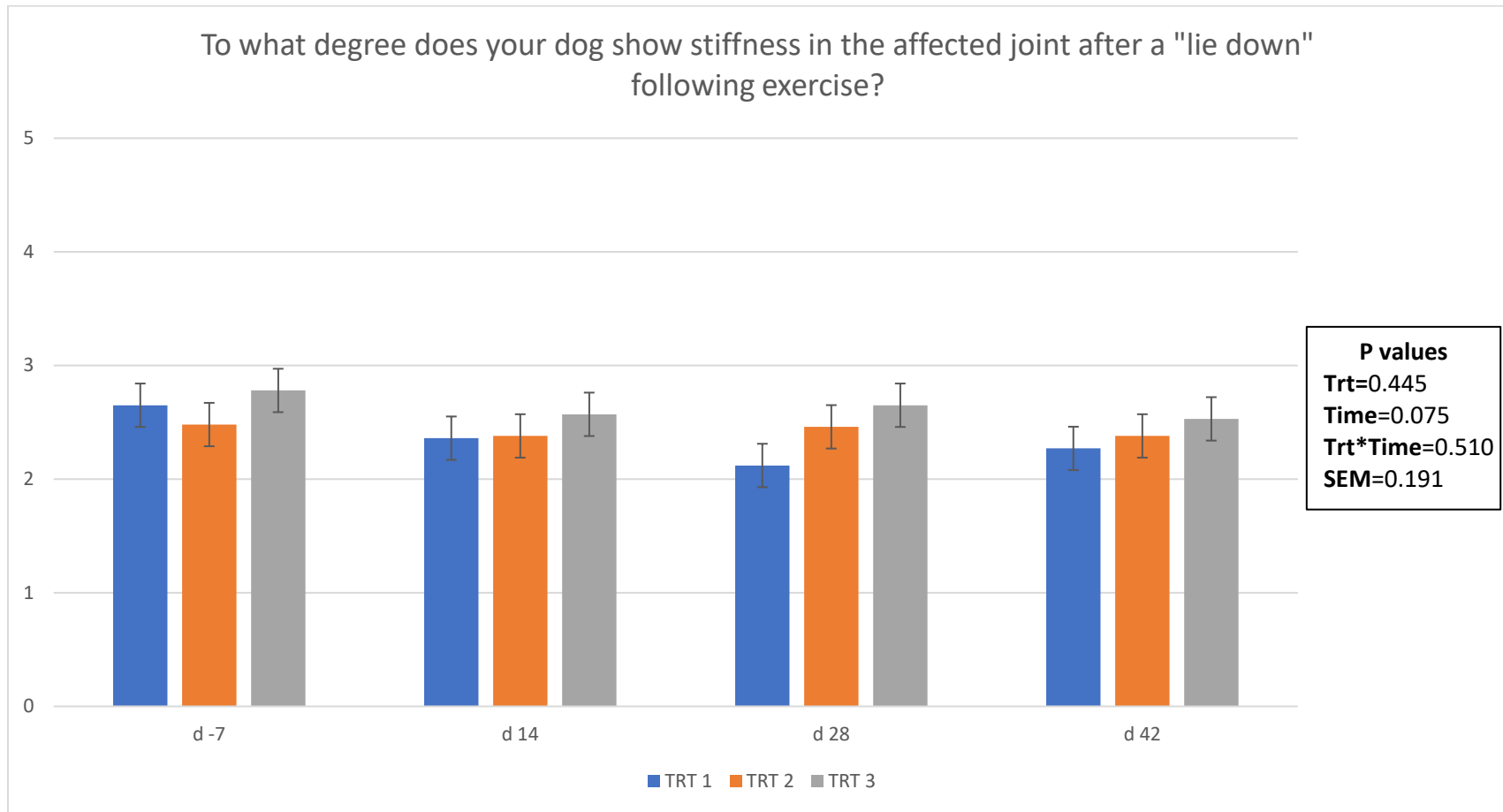
***Key:** 1) Never; 2) hardly ever; 3) occasionally; 4) frequently; 5) very frequently

Figure 3.13 Owner response to Q20 of the Liverpool osteoarthritis in dogs (LOAD) survey*



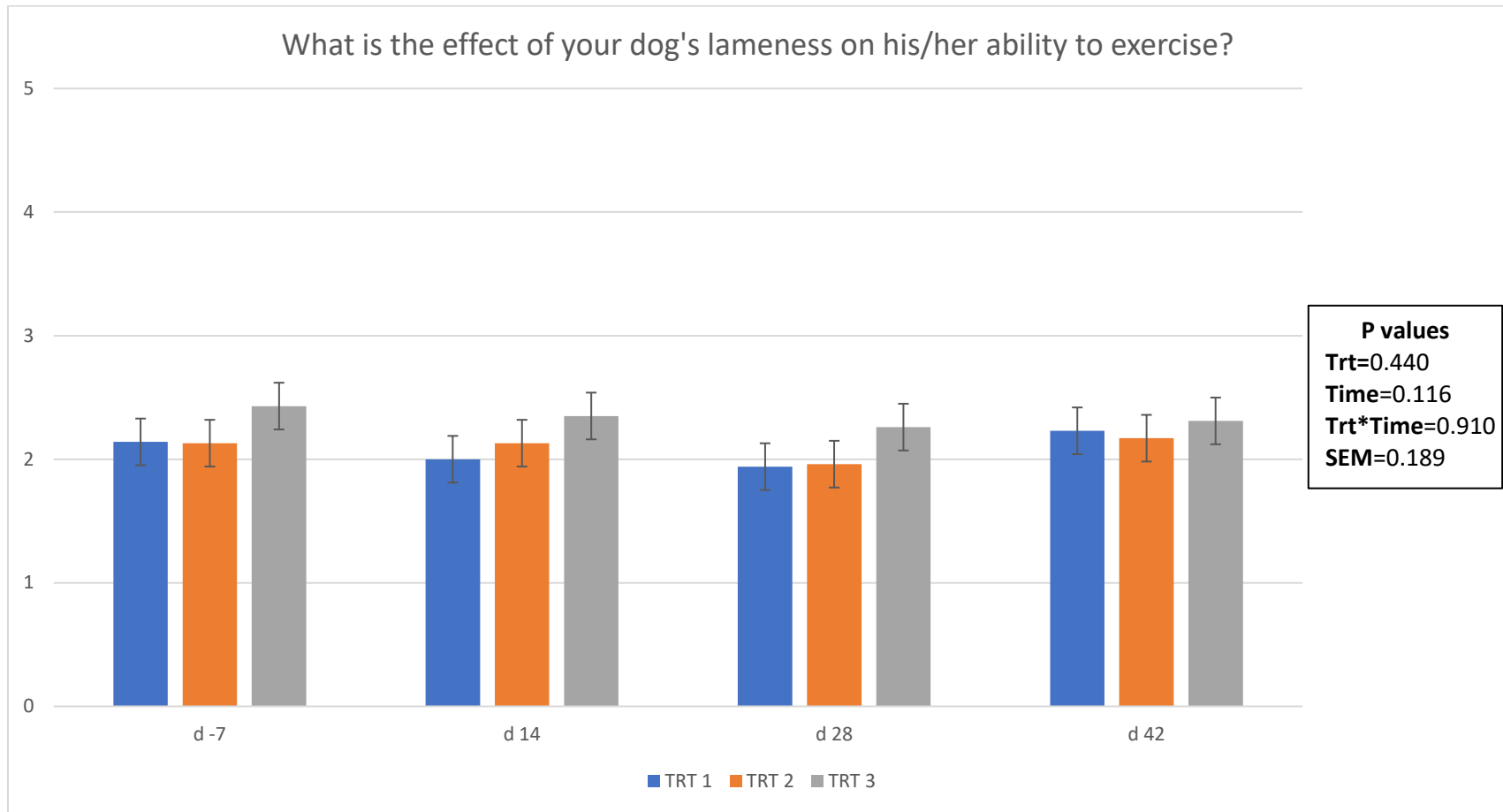
***Key:** 1) No effect; 2) mild effect; 3) moderate effect; 4) severe effect; 5) extreme effect

Figure 3.14 Owner response to Q21 of the Liverpool osteoarthritis in dogs (LOAD) survey*



***Key:** 1) No stiffness ; 2) mild stiffness; 3) moderately stiffness; 4) severe stiffness; 5) extreme stiffness

Figure 3.15 Owner response to Q22 of the Liverpool osteoarthritis in dogs (LOAD) survey*



***Key:** 1) No effect; 2) mild effect; 3) moderate effect; 4) severe effect; 5) extreme effect

Figure 3.16 Daily activity of dogs in Delta G Force over the entire study

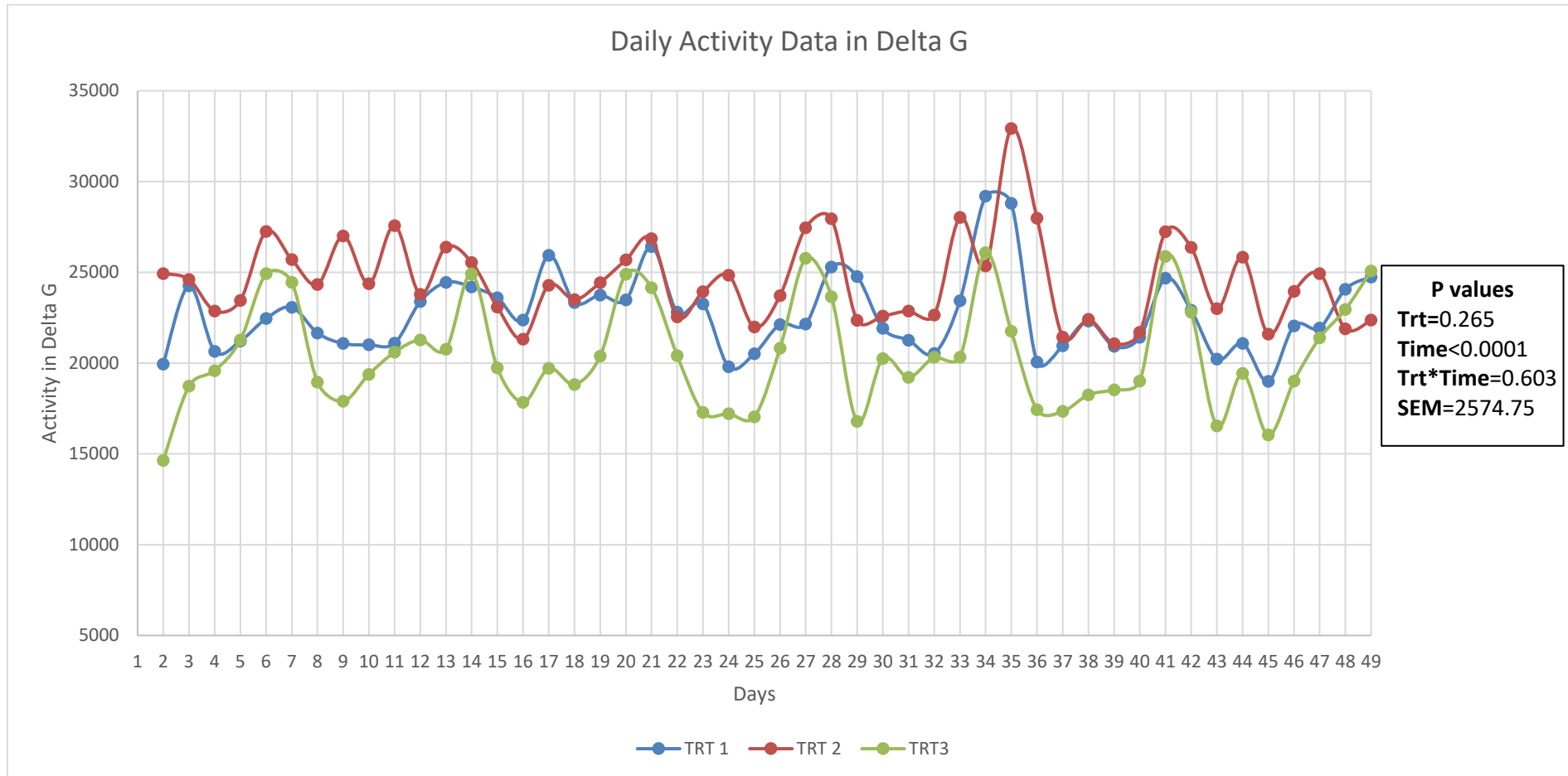


Figure 3.17 Average daily activity pattern of dogs in Delta G

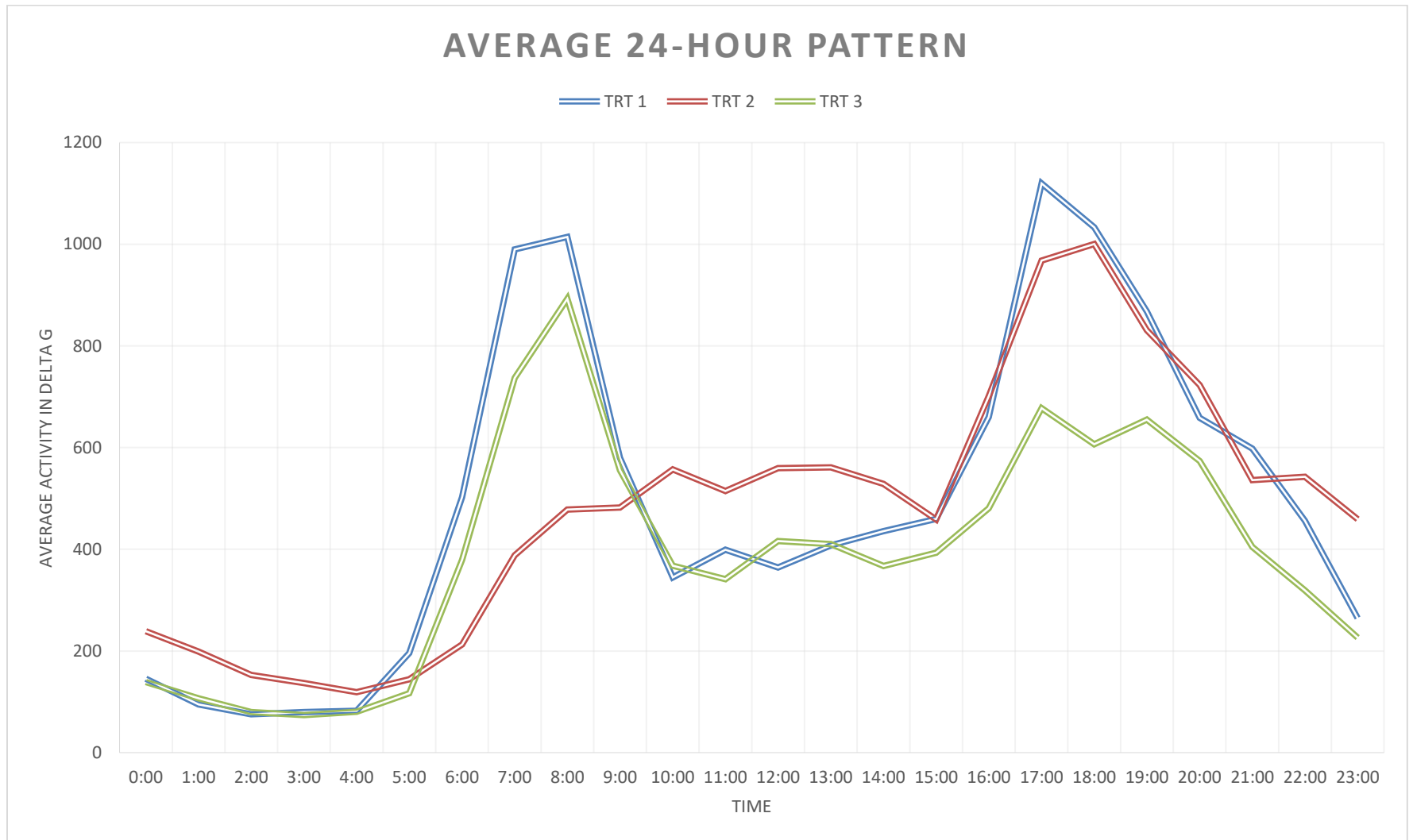


Figure 3.18 Average energy expenditure in Delta G Force by week

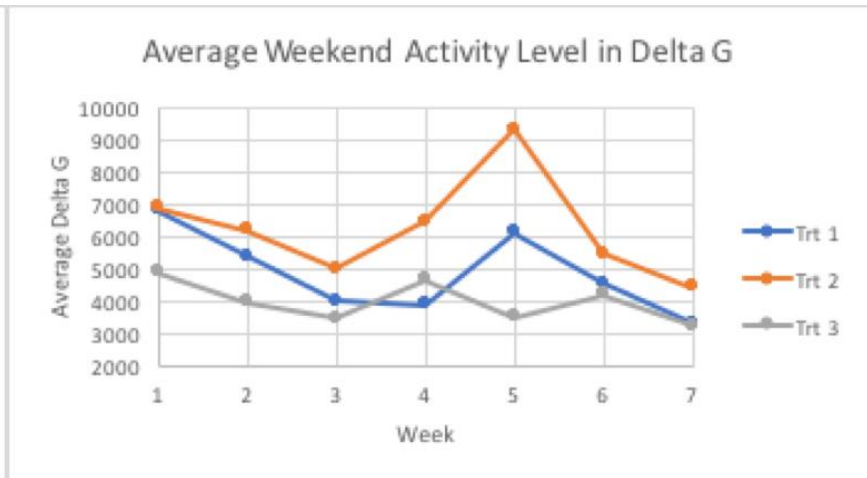
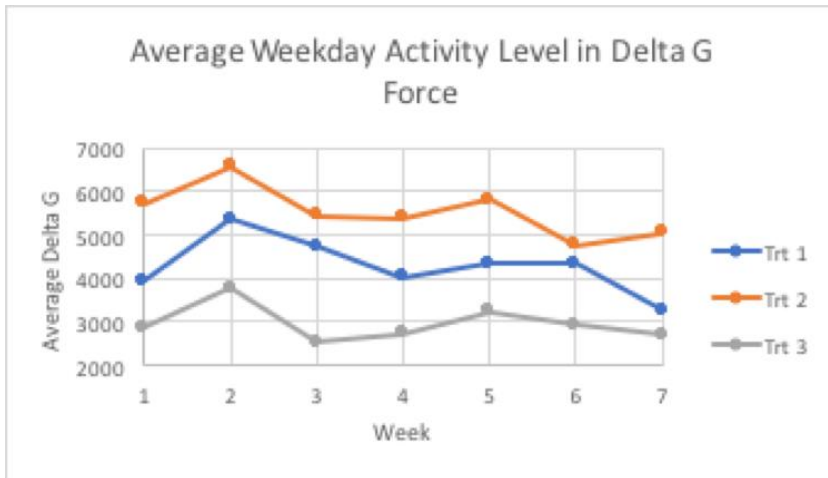
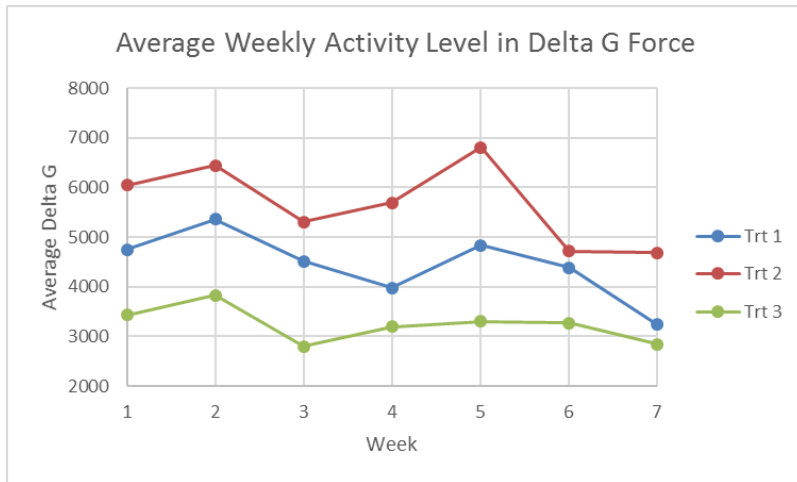


Figure 3.19 Average run delta G of dogs for the entire week (w), weekdays (wd), and weekends (wk)

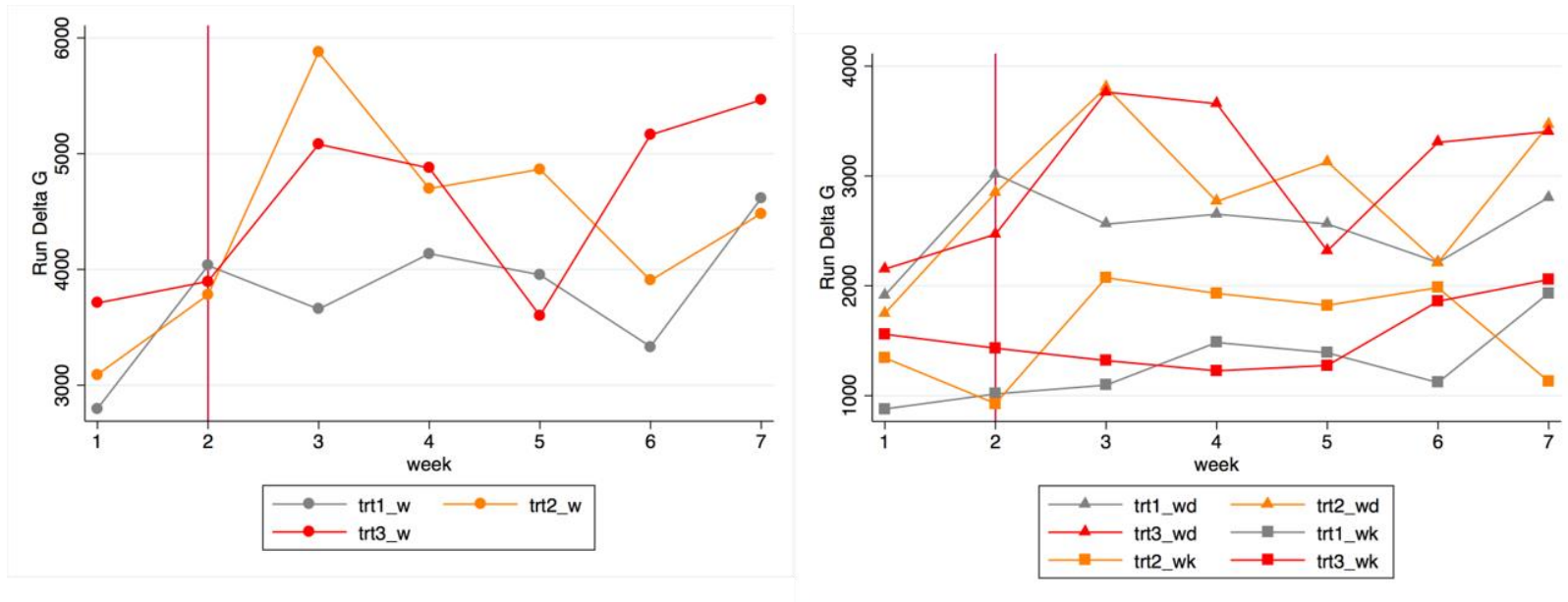


Figure 3.20 Average walk delta G of dogs for the entire week (w), weekdays (wd), and weekends (wk)

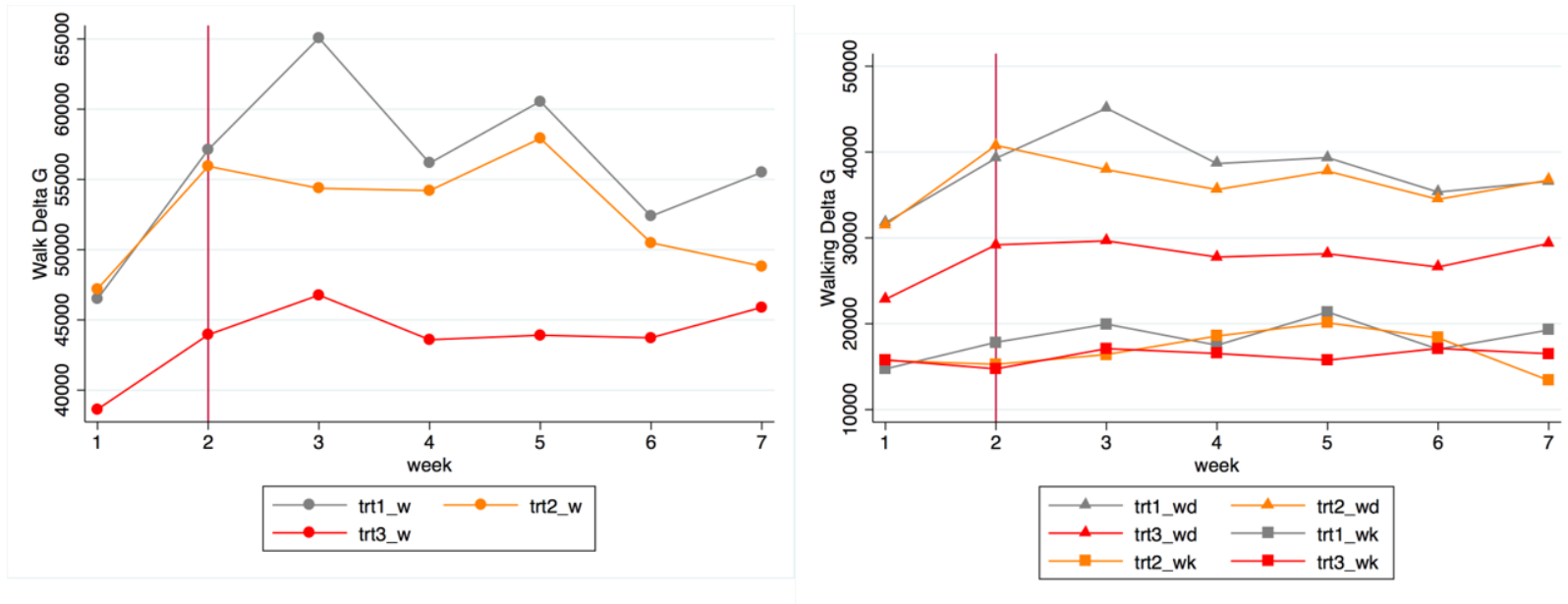


Figure 3.21 Average sleep delta G of dogs for the entire week (w), weekdays (wd), and weekends (wk)

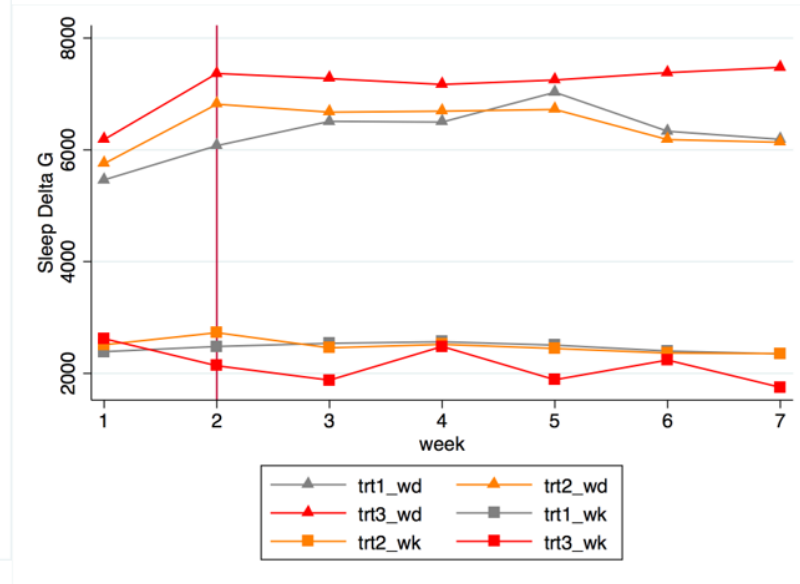
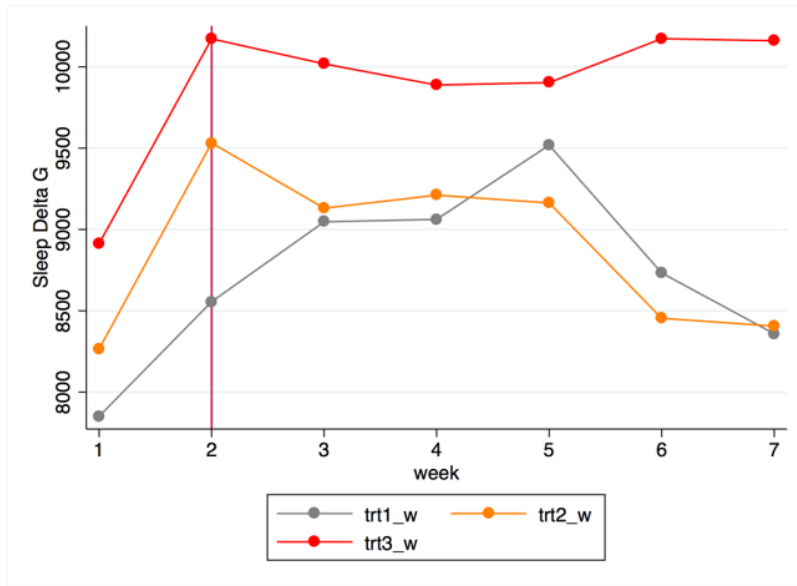


Figure 3.22 Average scratch delta G of dogs for the entire week (w), weekdays (wd), and weekends (wk)

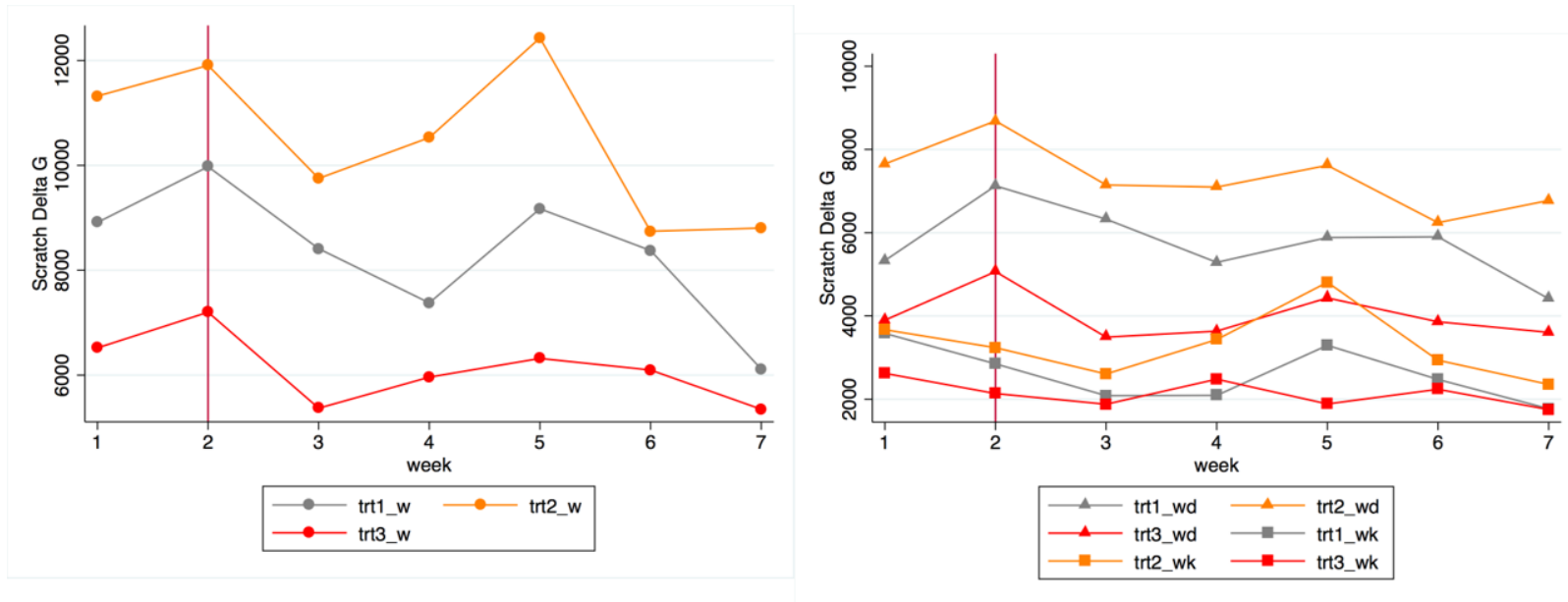


Figure 3.23 Average alert delta G of dogs for the entire week (w), weekdays (wd), and weekends (wk)

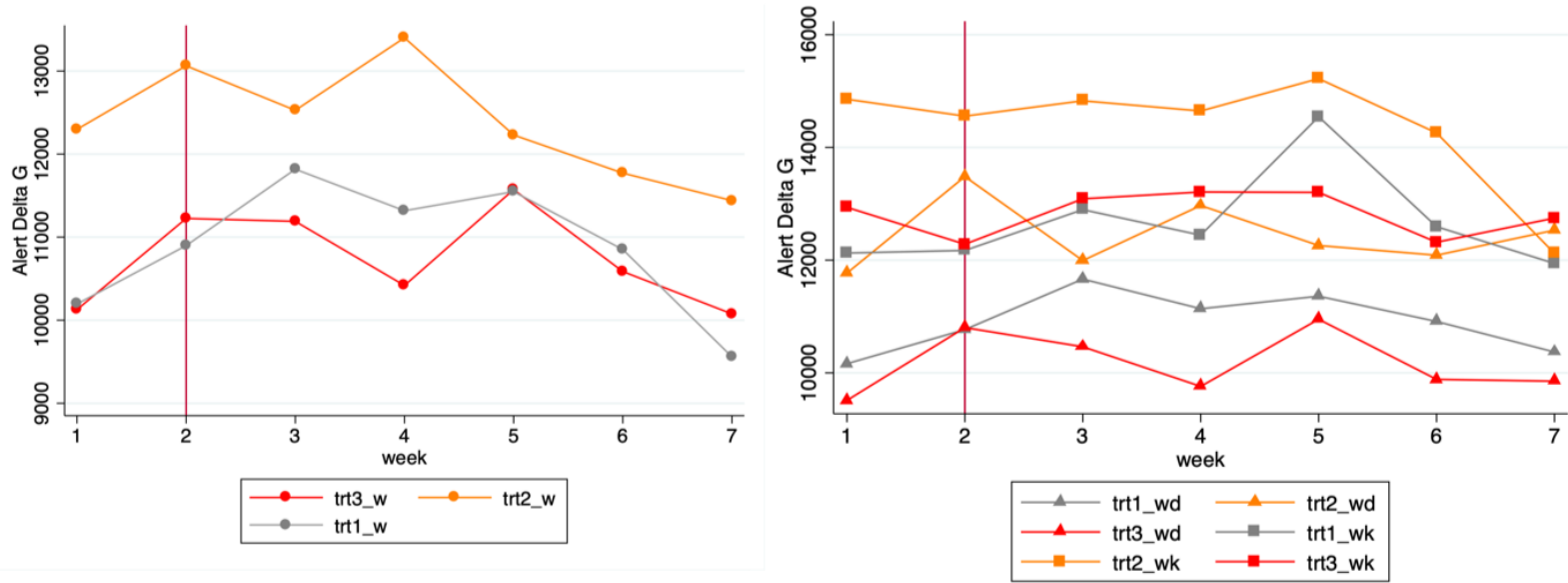
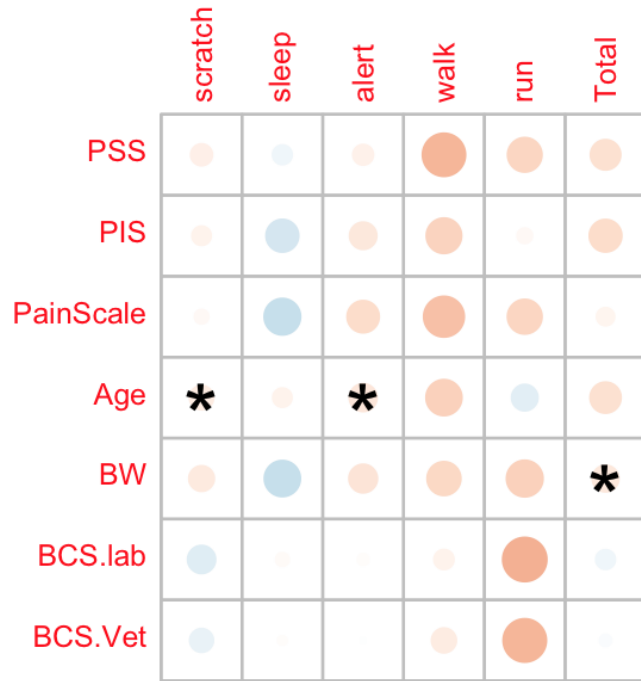


Figure 3.24 Correlations of physical activity (total, run, walk, alert, sleep, scratch) with pain severity score (PSS) by owner, pain interference score (PIS) by owner, pain scale by vet, age, BW, and body condition scores (BCS) assessed by a veterinarian and lab personnel for all dogs.

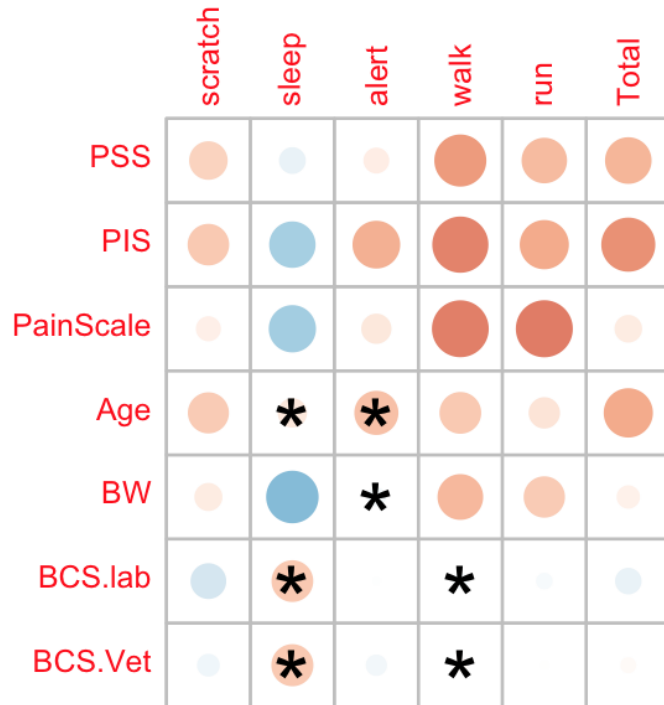


* represent $P < 0.05$

Red dots represents a positive correlation

Blue dots represent a negative correlation

Figure 3.25 Correlations of physical activity (total, run, walk, alert, sleep, scratch) with pain severity score (PSS) by owner, pain inventory score (PIS) by owner, pain scale by vet, age, BW, and body condition scores (BCS) assessed by a veterinarian and lab personnel for dogs BW>29.94kg.

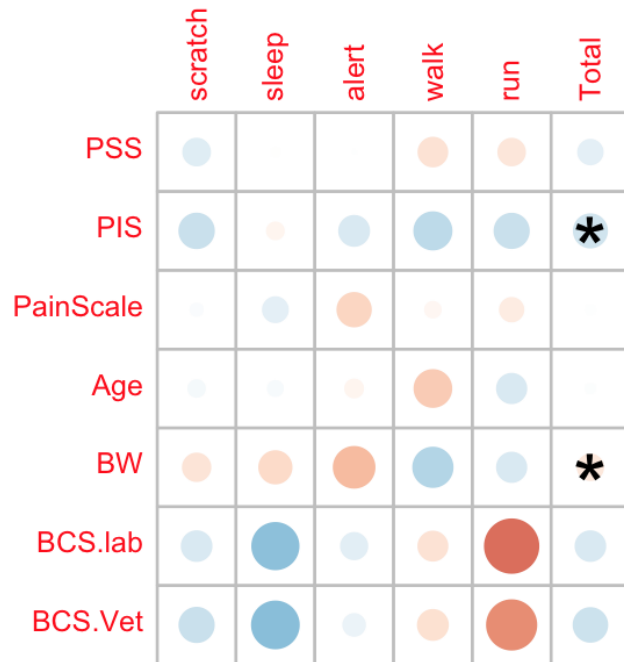


* represents $P < 0.05$

Red dots represent a positive correlation

Blue dots represent a negative correlation

Figure 3.26 Correlations of physical activity (total, run, walk, alert, sleep, scratch) with pain severity score (PSS) by owner, pain inventory score (PIS) by owner, pain scale by vet, age, BW, and body condition scores (BCS) assessed by a veterinarian and lab personnel for dogs BW<29.94kg.



* represents $P < 0.05$

Red dots represent a positive correlation

Blue dots represent a negative correlation

Figure 3.27 Correlation scatter plots for dogs BW<29.94 kg

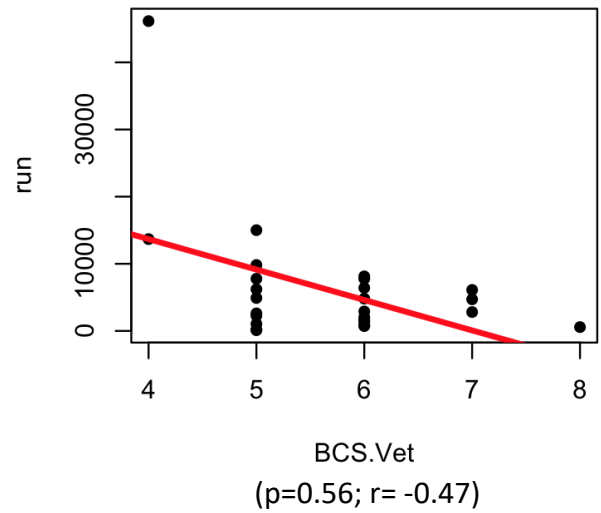
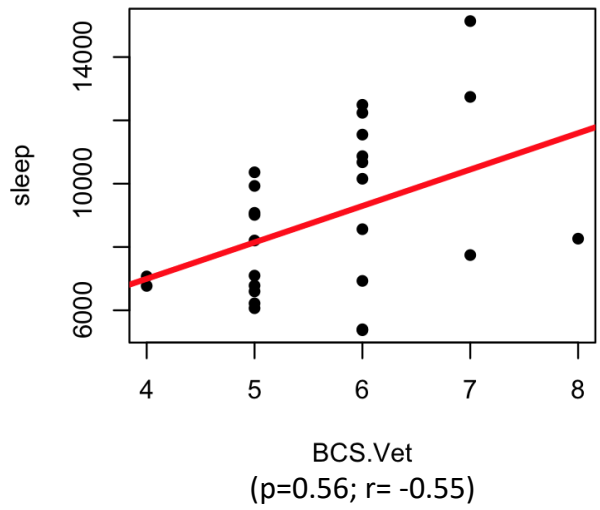
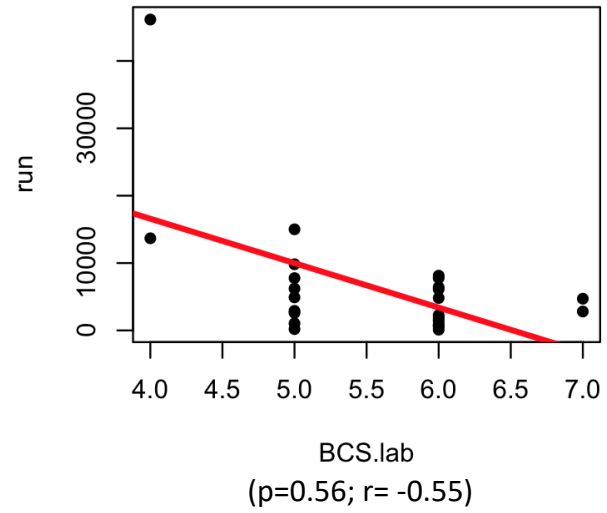
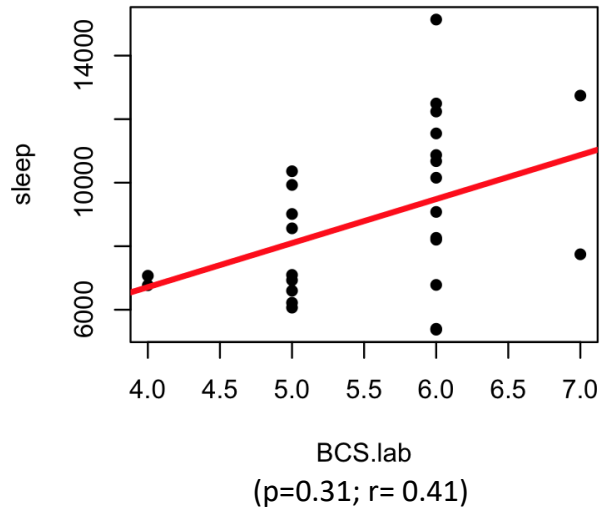


Figure 3.28 Correlation scatter plots for dogs BW>29.94 kg

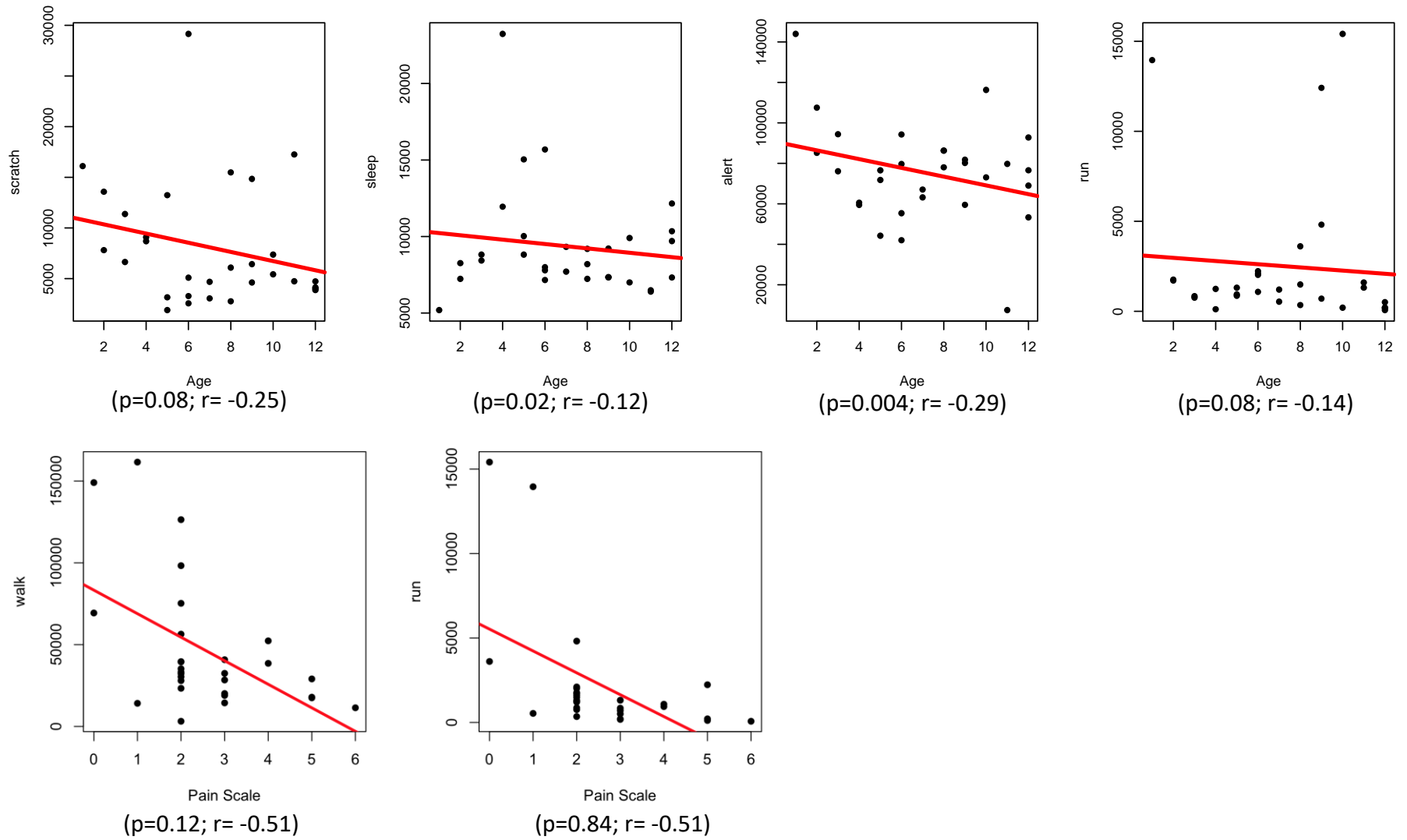
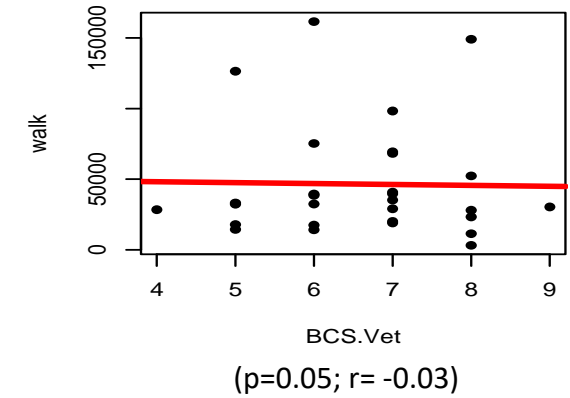
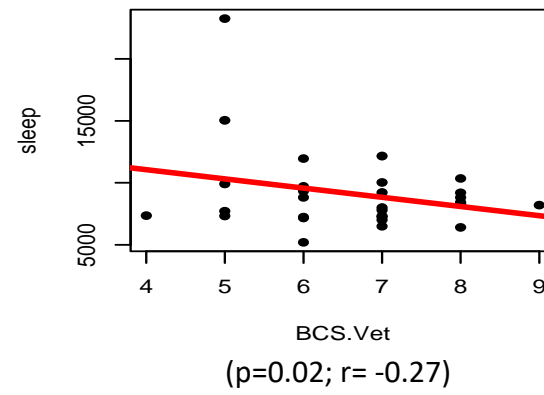
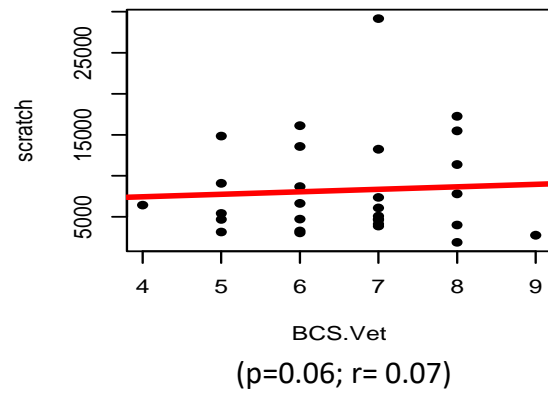
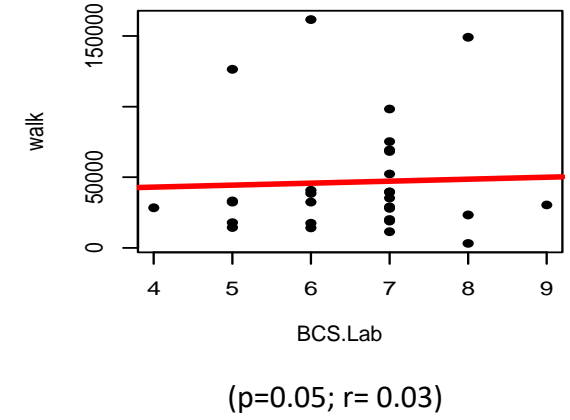
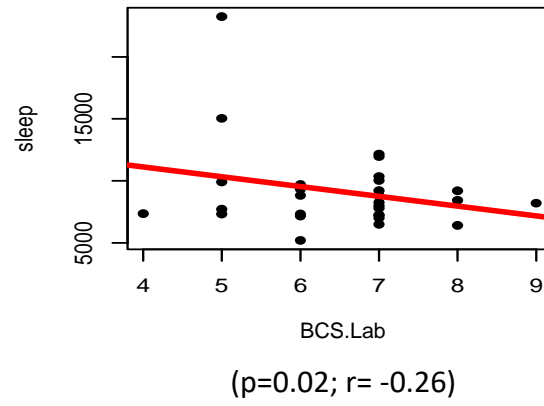
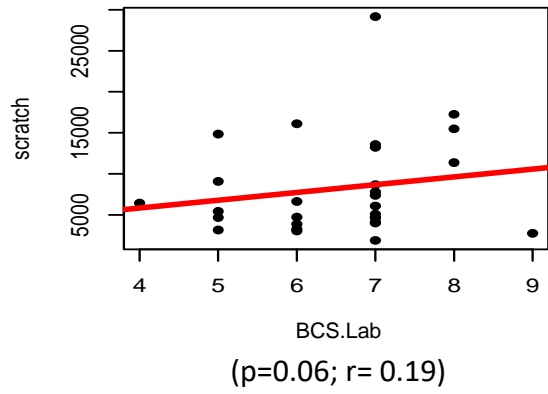


Figure 3.28 (Continued)



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