Synergistic anti-inflammatory activity of a novel combination of curcumin, quercetin and palmitoylethanolamide (PEA) in osteoarthritic dogs

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ABSTRACT

Curcumin and quercetin, along with endogenous compound palmitoylethanolamide (PEA), exhibit significant anti-inflammatory and immune modulating properties. As a nutraceutical, PEA assists in prevention and treatment of chronic inflammatory diseases, acting at several molecular targets.

The effects of the oral consumption of PCQ Pet™ , containing PEA, quercetin and curcuminoids in reducing inflammation and clinical signs of osteoarthritis in family-owned companion dogs was evaluated.

When given as a dietary supplement for 8-weeks to osteoarthritic (OA) dogs, PCQ reduced concentration of disease associated pro-inflammatory markers in some patients. Improvement in pain scores (Quality of Life, Pain Severity, and Pain Interference) from Canine Brief Pain Inventory (CBPI) was observed. In 4 out of 6 dogs, improvement in both peak vertical force (PVF) and vertical impulse (VI) in force plate analysis was found.

Outcomes from this case study provided insight on the potential benefits of PCQ Pet™ in an already established disease and how the supplementation can help in pain management, activity and improved gait, as well as in reduction of OA-associated inflammation. The novel formulation exhibits high applicability as a dietary supplement to be used alone or as an adjuvant to standard of care in chronic inflammatory conditions.

Demographics

The study population consisted of six companion dogs (free-living dogs in a home-setting). Table 1 shows the demographic data for the 7 enrolled dogs that finished the 8-week supplementation period. The average age and weight of enrolled dogs was 7.6 years and 29.2 kg.

Table 1. Demographic data of 7 dogs with osteoarthritis enrolled in the PCQ Pet™ pilot study

Dog #	Breed	Weight (kg)	Age (years)	Gender	Affected Limb
Dog 1	Pitbull	26.5	10	Female	Left elbow
Dog 2	German Shepard	27.6	6	Female	Right hip
Dog 3	Labrador	44.1	4	Male	Right elbow
Dog 4	Boxer	22	8	Female	Right Knee
Dog 6	English Springer Spaniel	20.2	10	Female	Right elbow
Dog 7	Blue Lacy	24.8	6	Male	Right elbow
Dog 8	Boxer	39.4	9	Female	Right knee

Canine Brief Pain Inventory (CBPI)

The Canine Brief Pain Inventory (CBPI) was developed as an owner-completed questionnaire designed to quantify the owner's assessment of the severity and impact of chronic pain in their dogs with osteoarthritis (Dorothy Cimino Brown, Boston, Coyne, & Farrar, 2010). This tool consists of four questions pertaining the pain severity (PSS – pain severity score) and six questions pertaining pain interference (PIS – pain interference score), i.e., how much the perceived pain interferes with the dog's regular activities (Dorothy Cimino Brown et al., 2010). A score of 1 represents no pain or no interference and a score of 10 represents extreme pain/interference. The CBPI also has a qualitative question about the animals' overall quality of life. This question is scored in a 5-point scale, from poor, fair, good, very good and excellent. The scores for each group of questions were averaged and reported as median and range, and a difference between baseline – follow-up was also calculated for each individual dog. According to the CBPI user guidelines, individual treatment success was defined as a reduction equal or greater than 2 for PI and equal or lesser than 1 for pain severity (Dorothy Cimino Brown, Bell, & Rhodes, 2013).

When analyzing each dog individually, all dogs except dog #2 showed reduction in pain scores. According to the CBPI guidelines, treatment was successful in reducing pain scores after 8 weeks (Figure 1).

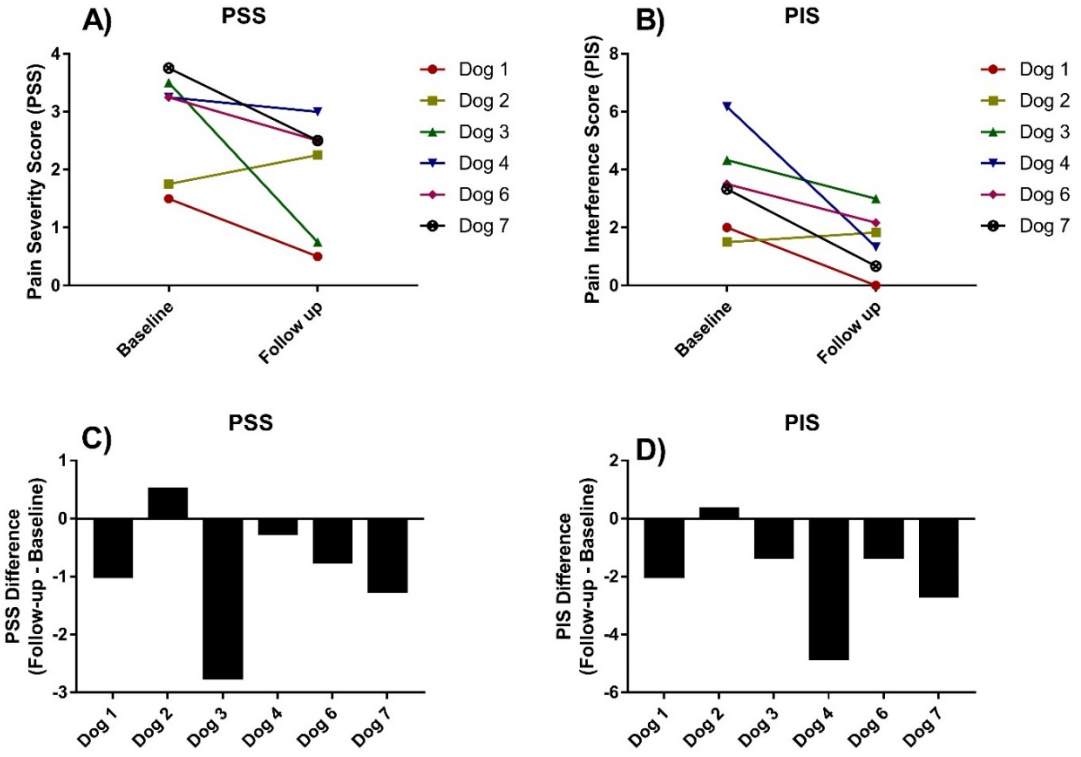

Figure 1. Canine brief pain inventory (CBPI) scores in dogs with osteoarthritis (OA) before and after dietary supplementation with the PCQ formulation for 8 weeks. A and B represents the scores given before and after the 8-week intervention for each dog. C and D represents the difference between follow-up and baseline pain scores (PSS and PIS) for each individual dog. A reduction ≥ 2 in PIS and ≥ 1 for PSS represent a successful treatment response.

When comparing CBPI median scores from baseline and follow-up visits, an overall reduction in both pain severity scores (PSS) and pain interference score (PIS) was observed after the 8-week supplementation period (Figure 2). For pain severity (PS), median scores were 3.25 and 2.38 at baseline and follow-up, respectively. For pain interference (PI), median scores were 3.42 and 1.58. A large range on values is expected, as baseline scores for each animal's pain is different and subjectively evaluated by each owner.

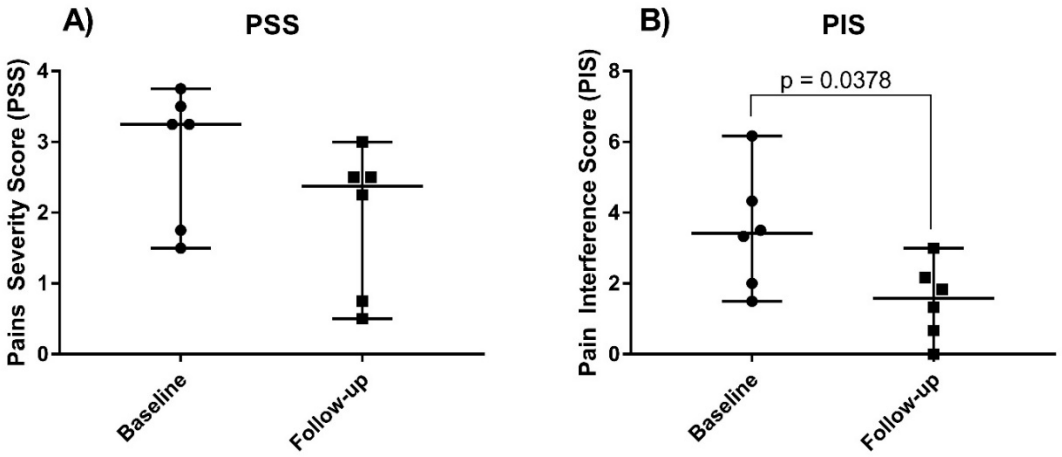

Figure 2. Median and range for CBPI Pain Severity Score (PSS) and Pain Interference Score (PIS) in 6 enrolled dogs. Indicated p value indicate statistical significance between baseline and follow-up visits.

Owners were also asked to rate the animals' overall quality of life (QOL) in a qualitative scale, that comprised 5 levels: poor, fair, good, very good and excellent. Even though the baseline score for each dog was different, overall medians were significantly higher ($p = 0.025$) at the follow up visit, indicating improvement in dogs' activities and perception of pain (Figure 3).

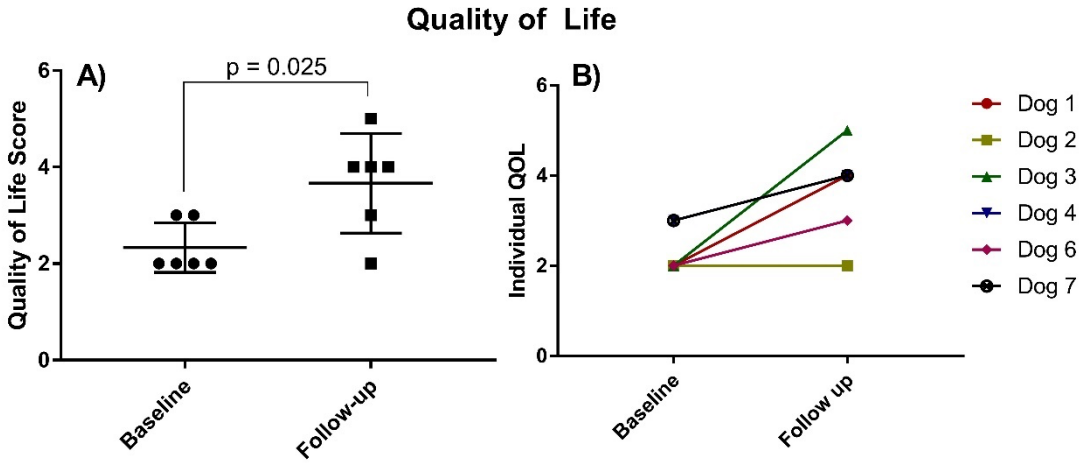

Figure 3. Canine brief pain inventory (CBPI) quality of life (QOL) scores for enrolled dogs. Scale is from 1 (poor) to 5 (excellent). A) Boxplots represent the median and range for baseline and follow-up visits (n = 6). B) Lines represent scores at baseline and follow-up visits. Indicated p-value indicate statistical significance (Paired t-test, $p < 0.05$).

CBPI is a common measurement of pain in studies assessing efficacy in dogs with OA. In a placebo-controlled clinical trial investigating the effects of a diet supplemented with curcuminoids extract, hydrolyzed collagen and green tea extract, researchers were able to detect differences between the control and the supplemented group, although no differences were seen within the supplement group after 3 months (Comblain et al., 2017). When evaluating the effects of a

commercially available joint supplement compared with carprofen in working dogs with OA, Alves et al. (2017) did not detect a clinical response based on CPBI scores.

Even though the CBPI is a validated tool to access pain is osteoarthritic dogs and widely used in several OA intervention studies (Alves et al., 2017; D. C. Brown, Boston, & Farrar, 2013; Dorothy Cimino Brown et al., 2013), it is important to consider other assessment tools and interpret outcomes accordingly. It is known that CBPI scores are not always correlated with force plate analysis results (D. C. Brown et al., 2013).

Force Plate Analysis

Force plate gait analysis is a valuable method to obtain objective data on limb loading in dogs (McLaughlin, 2001). In this study, dogs were trotted across the force plates by a single handler and five acceptable trials were obtained from each limb of each dog. Peak vertical force (PVF) is the largest of the orthogonal forces and is the one most commonly evaluated for gait analysis in animals.

Vertical impulse (VI) is also commonly reported and is represented by the area under the curve of a force over time graphical representation (McLaughlin, 2001). The mean values of PVF and VI of each dog were derived from the average PVF and VI of the first five valid trials on the most affected limb (specified in Table 9). Due to the fact that enrolled patient's most affected limb could be either a thoracic or pelvic limb, force plate data was converted into unitless values and reported as percent change from baseline (for each dog) to account for differences in thoracic vs. pelvic limb (Figure 4).

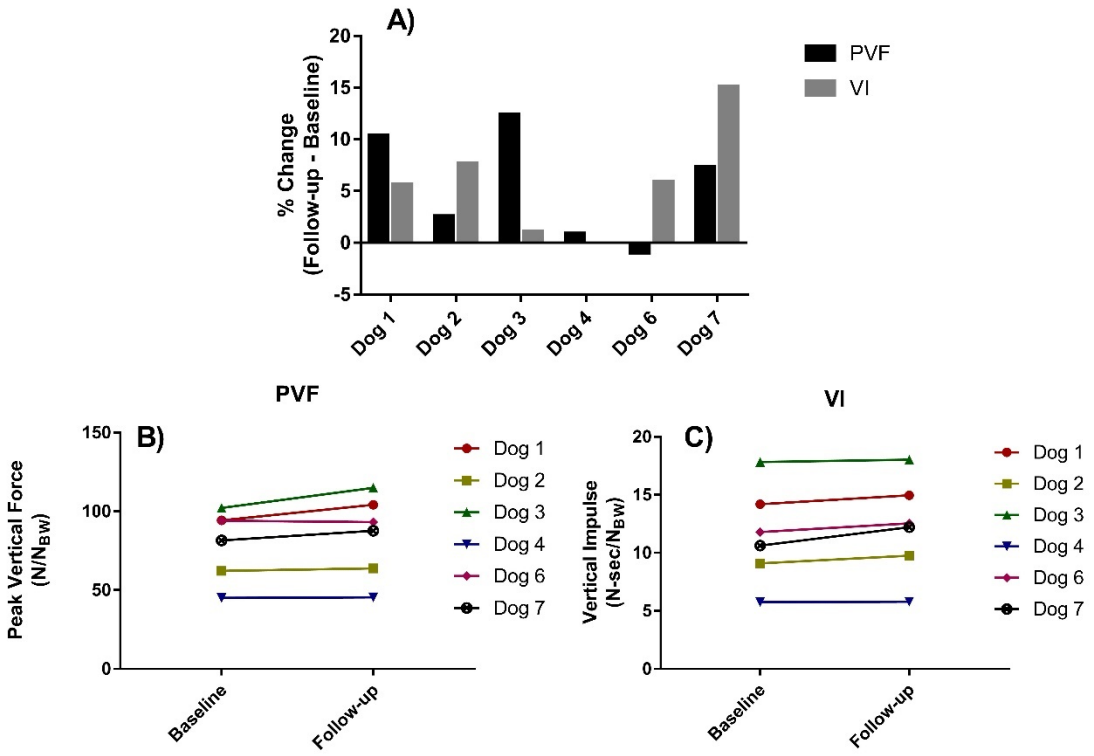

Figure 4. Force plate gait analysis of dogs with osteoarthritis (OA) before and after dietary supplementation with the PCQ formulation for 8 weeks. A) represents the percent (%) change of peak vertical force (PFV) and vertical impulse (VI) of the most affected limb of each dog compared to baseline. B) and C) represents the baseline and follow-up values obtained for PVF and VI in 6 enrolled dogs.

There was a positive percent change in 4 out of 6 of dogs for PVF and 5 out of 6 dogs for VI, after 8 weeks taking the supplement. Dogs affected by OA and experiencing pain tend to bear less weight on painful limbs, therefore an increase in PFV is an indication of improvement in pain and lameness. Similarly, an increase in VI indicates improvement in the force applied over time.

Moreau et al., 2014 detected significant increase in PVF in dogs (n = 13) taking a medicinal herb-based natural health product after 8 weeks, although no differences could be seen between treated and placebo-group at the 8-week time-point. On the other hand, Comblain et al., (2017) did not detect differences over time in PVF or VI in dogs (n = 21) taking a curcuminoid-collagen-green tea extract for 3 months. In these studies, even though it was not possible to always identify significant increase in PVF and VI specifically, positive effects observed in other outcome measurements (owner questionnaires and orthopedic evaluations) indicated that supplementation improved clinical signs of osteoarthritis. Based on overall favorable results obtained from this study, it is believed that a larger sample size would allow more confidence about the overall outcome.

A limitation of the force plate analysis is that it only evaluates the animals outside their normal environment and at one specific time point. Therefore, it is recommended that researchers use other outcome measures in addition to the force plate. The Canine Brief Pain Inventory, for example, is a validated questionnaire that quantifies owners' assessment of clinically relevant pain–related behaviors with the dog at its environment and over a larger period of time. Taken together,

these different assessment tools are able to better clarify the benefits of supplements and nutraceuticals in pain, lameness and overall quality of life of dogs with OA.

Serum inflammatory markers

With OA increasingly being considered an inflammatory disease, cytokines are being assessed as possible candidates for biochemical markers that could aid in determining severity of the disease, monitoring efficacy and safety of interventions as well as having the potential to act as a diagnostic and prognostic tool (Mabey & Honsawek, 2015). The synovial fluid is an important potential source of biomarkers in osteoarthritis (OA), as it reflects the biological environment of the joint, offering a direct measure of joint pathophysiology (Jayadev, Rout, Price, Hulley, & Mahoney, 2012). However, often OA joints do not offer sufficient SF volume to be collected for analysis. This study protocol included a joint fluid collection for detection of local inflammatory cytokines. Nevertheless, it was only possible to collect joint fluid from 2 dogs at the follow-up visit and no baseline sample. Therefore, concentrations of inflammatory markers in joint fluid could not be reported in this study. Alternatively, quantification of cytokines in serum was performed and can provide useful information about systemic inflammation associated with OA (Paquet et al., 2012; Stannus et al., 2010). It is important to consider that even though cytokine profiling in OA patients is an essential starting point, different inflammatory subtypes arise from affected joints (G. Ren et al., 2018).

Hematology

The clinical laboratory values for the blood panel were within normal limits during the study period of 8 weeks for most dogs (Table 8) (Willard & Tvedten, 2012). Values that were higher or lower than reference values are indicated in the table. There was no observed effect of dietary supplement on the hematological parameters.

CONCLUSION

Previously, we demonstrated that PEA, quercetin and curcuminoids are able to mitigate oxidative stress by reducing ROS and NO production, as well as to downregulate expression of inflammatory markers such as Cox-2, iNOS and TNF-α in RAW264.7 macrophages. Most importantly, a synergistic interaction was demonstrated between PEA and quercetin and PEA and curcuminoids in downregulation of IL-1b mRNA expression. Synergy parameters such as the combination index (CI), dose reduction index (DRI) and isobolograms for two different experimental designs were presented and indicated stronger synergy for the PEA and quercetin combination. These results provide evidence and rationale for the use of these ingredients in association in nutraceutical formulations targeting prevention and treatment of chronic inflammation and pain.

In this study, a formulation using PEA, quercetin and curcuminoids (PCQ Pet™) was developed and tested in a pilot study with family-owned osteoarthritic dogs. For the first time, PEA in combination with polyphenols was investigated as a potential treatment for osteoarthritis (OA) in dogs. Results indicated that an 8-week supplementation with PCQ Pet™ exerted beneficial effects, demonstrated by improvement in pain scores and gait, overall quality of life and also positively affecting pro-inflammatory markers in some dogs.

The exact mechanism through which PEA can counteract inflammation and pain in OA patients is yet to be elucidated, although Marini et al. (2012) highlight the ability of PEA to control mast cell degranulation as a potential mechanism. Turmeric and standardized curcuminoid extracts have been used as a nutritional supplement for osteoarthritis, both alone or as part of herbal formulations for humans (Panahi et al., 2014) and companion animals (Comblain et al., 2017; Musco et al., 2019; Sgorlon et al., 2016). Downregulation of pro-inflammatory cytokines and inhibition of cartilage degrading enzymes by inhibition of the NF-kB and AP-1 pathways, as well as exhibiting and radical scavenging properties are some of the proposed mechanism through which curcuminoids can assist in pain relief and reduction of inflammation in OA (Bello, Ayanda, Aworunse, & Olukanmi, 2017). Similarly, quercetin has a supportive effect in OA, by downregulating MMP-13, upregulating SOD and TIMP-1, as well as weakening the oxidative stress responses and inhibiting the degradation of cartilage extracellular matrix (Wei et al., 2019).

To date, this present study is the first to investigate the effects of a formulation containing PEA in dogs with osteoarthritis and moreover, the first to propose its combination with polyphenols for this purpose. This research focused on demonstrating the anti-inflammatory effect of PEA, quercetin and curcuminoids and the synergistic interaction that arises when these compounds are used in association. The beneficial effects of a combined formulation were demonstrated in a pilot animal trial. Further studies are still needed and can contribute to better understand the interaction between PEA and polyphenols, potential mechanisms of action and the overall disease modifying properties that these synergistic combinations might have on osteoarthritis (OA).

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