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Combining a joint health supplement with tibial plateau leveling osteotomy in dogs with cranial cruciate ligament rupture. An exploratory controlled trial

Filippo M. Martini [a], Anna Brandstetter de Bellesini [a], Alda Miolo [b],*, Laura Del Coco [c], Francesco P. Fanizzi [c], Antonio Crovace [d]

[a] University of Parma, Department of Veterinary Medicine, via del Taglio, 8, I-43126 Parma, Italy
[b] CeDIS, Science Information and Documentation Centre, Innovet Italia, via Einaudi, 13, I-35030 Saccolongo (Padova), Italy
[c] University of Salento, Department of Biological and Environmental Sciences and Technologies (DiSTeBA), Prov.le Lecce-Monteroni, 7, I-73100 Lecce, Italy
[d] University of Bari, Emergency and Organ Transplantation Department (D.E.T.O.), Veterinary Surgery Division, Piazza G. Cesare, 11, I-70010 Valenzano (Bari), Italy

ARTICLE INFO

ABSTRACT

Canine cranial cruciate ligament rupture (CrCLR) is a very common pathology. Surgical stabilization is the first choice treatment, although it does not fully eliminate the increased risk of osteoarthritis. This preliminary study was carried out to explore whether a newly formulated joint health supplement would benefit metabolic, clinical and radiographic changes in dogs with CrCLR surgically treated with tibial plateau leveling osteotomy (TPLO). Besides chondroitin sulfate and glucosamine hydrochloride, the studied supplement contained anti-inflammatory and antioxidant ingredients, the main ones being N-palmitoyl-D-glucosamine (Glupamid®) and quercetin. It was thus intended to target not only chondrodegenerative components of osteoarthritis, but also post-injury inflammatory response and oxidative stress of joint tissues. Thirteen dogs underwent TPLO and were randomly allocated to treatment (n = 6) and control groups (n = 7), the former receiving the joint supplement for 90 days. Lameness and radiographic osteoarthritis changes were scored before (i.e., baseline) and at 30 and 90 days post-surgery. Synovial fluid samples were collected from injured stifles at the same time points. Levels of representative metabolites were measured by proton nuclear magnetic resonance spectroscopy in a blinded fashion. In the metabolomic analysis, special attention was paid to lactate, due to its emerging recognition as a key marker of inflammation. In the last time period (from the 30th to the 90th day), lameness improved by a factor of 2.3 compared to control dogs. No significant difference was observed in the radiographic osteoarthritis score between groups. In the first postoperative month, lactate and creatine levels significantly dropped in treated compared to control dogs. Compared to surgery alone, combining the joint supplement with TPLO resulted in a trend to a better clinical outcome in the later time interval but did not influence osteoarthritis radiographic progression. A significantly better rebalance of joint microenvironment in the early time interval (baseline – 30 days) was shown by metabolomic analysis, thus suggesting that the study supplement could limit ongoing inflammatory responses.

1. Introduction

Rupture of the cranial cruciate ligament (CrCLR) is one of the most common orthopaedic problems in dogs and an important cause of hindlimb lameness and osteoarthritis (OA) [1]. Surgery is the gold standard to stabilize the stifle [2]. Among several techniques used to provide joint stability, tibial plateau leveling osteotomy (TPLO) is currently the most preferred [2,3], especially in medium- to large-breed dogs. Nonetheless, and like other surgical procedures [4,5], TPLO neither reduces nor halts progression of OA [6–9]. Therefore, the recommended treatment for CrCLR should also target secondary OA. Although the mechanisms leading to OA following ligament injury have not been fully established, chondrodegeneration and inflammatory changes are both considered important contributors [9–11]. Dietary supplements for joint

health have gained increased recognition as valuable management options within the combined therapy for OA [12]. The efficacy of joint supplements is clearly formulation-dependent, relying mainly on their respective composition (i.e., ingredients used, relative amounts, excipients, purity, etc). Since the 1990s, a wide variety of compounds with different chemical structures, bioavailability, mechanism of action and degree of purity have been introduced in the veterinary market as chondroprotective agents, i.e. substances specifically aimed to rebalance the metabolism of degenerating cartilage, by boosting the synthetic pathways (pro-anabolic effects) while inhibiting degradative responses (anti-catabolic effects) [13,14]. More recently, a new class of joint supplements has been developed, able not only to support or enhance the articular intrinsic repair capability (chondroprotection *sensu strictu*), but also exert anti-inflammatory and analgesic effects and improve the intrartricular oxidative status [12,15,16]. The joint supplement used in the present study as an add-on/complementary treatment belongs to this latter class. In this formulation, classical chondroprotective compounds (chondrointin sulfate and glucosamine) are combined with an antioxidant and anti-inflammatory agent to target both OA-associated inflammation and pain. N-palmitoyl-D-glucosamine and quercetin are the main constituents responsible for the latter activities, largely due to their anti-inflammatory, antioxidant and pain relieving effects [17–19].

Metabolomics is the "omic" technique aimed to study metabolic OA profiling (metabolome), and is considered a promising strategy to follow disease progression and evaluate the effect of disease-modifying therapies [20–22]. One of the most attractive high-throughput technologies for global screening of joint metabolites is high resolution proton nuclear magnetic resonance spectroscopy (^{1}H NMR) [23,24]. It is usually performed on synovial fluid samples, since metabolic joint products are most likely to accumulate earlier and at higher concentrations compared to other biological fluids (e.g., serum, urine) [25,26]. In evaluating the anti-inflammatory effects of the study supplement, particular attention was paid to synovial changes in lactate, since increased levels of this metabolite have been related to the degree of inflammation in metabolomic studies of joint disease [23,27].

This preliminary study was carried out to explore metabolic, clinical and radiographic outcomes in dogs with spontaneous ligament rupture surgically treated with TPLO and supplemented or not with a joint health supplement. Our working hypothesis is that addition of the study joint supplement to surgery may benefit joint metabolome, lameness and OA radiographic progression during the study period, in light of the purported chondroprotective and anti-inflammatory activities of said formulation.

2. Materials and methods

2.1. Animals

The study was designed as a 90-day, open-label, controlled preliminary trial, using an untreated group as control. Medium to large-breed client-owned dogs with naturally occurring unilateral CrCLR referred for TPLO were enrolled. The following inclusion criteria had to be met: (i) complete unilateral CrCLR, with no contralateral stifle problems as assessed clinically and radiographically; (ii) acute ligament injury, i.e., occurring less than 20 days before entering the study as determined by information provided by the owner or referring veterinarian; (iii) body weight between 15 and 55 kg; (iv) age between 12 months and 8 years; (v) mild to moderate stifle OA, corresponding to scores 1 and 2 on a 4-point radiographic grading system [28]. Exclusion criteria were any abnormality in the complete blood count and serum biochemical

profile, any history or evidence of previous stifle joint surgery, and other orthopaedic and neurologic diseases. Additional exclusion criteria were concomitant meniscal lesions (as confirmed by arthroscopic meniscal inspection before TPLO procedure), and concurrent treatment with non-steroidal anti-inflammatory drugs and corticosteroids. All owners gave informed consent before enrolment of their dog and approval from the local ethical committee was obtained. Breed, gender, age, body weight and clinical history of each dog were recorded.

2.2. Surgery

All surgeries were performed by the same surgeon (F.M.M.) according to the TPLO technique as described by Slocum and Slocum [29] and modified by Pozzi *et al.* [30] without meniscal release. The hind limb was clipped and prepared for surgery in a routine manner. A skin incision was made medially from approximately the distal fourth of the femur to the proximal third of the tibia. Stifle arthroscopy was performed before TPLO in all procedures. The jig was not applied [31] and a tibial osteotomy was made using an 18 mm, 24 mm or 30 mm biradial saw blade. The proximal tibial segment was then rotated according to Slocum's recommendations on the basis of the tibial plateau angle calculated from radiographs obtained prior to surgery. All osteotomies were stabilized with one or two anti-rotational Kirschner wires placed from the tibial crest directed caudo-distally to exit the caudal cortex of the proximal fragment. The osteotomy was secured by either a 3.5-mm SLOCUM TPLO plate, 3.5-mm small SLOCUM TPLO plate, 3.5-mm TPLO SYNTHES locking compression plate, 3.5-mm broad TPLO SYNTHES locking compression plate, 3.5-mm SECUROS TPLO plate or 3.0/3.5-mm FIXIN T Support, based on the size and temperament of the dog. The K-wires were removed after application of the bone plate. Postoperative prophylaxis consisted of cefadroxil (20 mg/kg q12h PO for 5 days), carprofen (3 mg/kg q24h PO for 7 days) and tramadol if needed (3 mg/kg q6h PO days 1–3). A modified Robert Jones was applied for 48 h postoperatively. All owners received written instructions concerning postoperative care and follow-up examinations. Instructions for postoperative confinement and the 8-week rehabilitation period were based on TPLO course recommendations [32].

2.3. Treatment

After TPLO, dogs were randomly allocated into two groups according to a simple randomization procedure. Group C (control) received no further treatment. Group T (treated) received the joint health nutraceutical,[1] administered daily at a dose of 1 tablet/25 kg b.w./PO for 90 days, starting on the day after surgery. Each tablet had the following composition: low molecular weight normosulfated chondroitin sulfate (NSCS 5/20 patented fraction), 200 mg; glucosamine hydrochloride, 300 mg; N-palmitoyl-D-glucosamine (Glupamid®), 100 mg; quercetin, 75 mg; Vitamin E, 50 mg; ω3 essential fatty acids, 50 mg.

2.4. Synovial fluid sample collection and preparation

Synovial fluid samples (0.5 mL each) were aseptically collected by arthrocentesis from the affected stifle joints before surgery (baseline visit, V0), and on days 30 and 90 after TPLO (V30 and V90, respectively). Samples were filtered using membranes of 0.8 μm porosity, centrifuged at approximately 17,000 *g* for 15 min in order to separate the cells and stored at −80 °C. The main metabolites present in canine synovial fluid samples were studied

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[1] Condrostress®, Innovet Italia Srl, Milan, Italy.

by ^{1}H NMR spectroscopy, in order to investigate potential differences between treated and control groups. This technique allows a one-shot, high-throughput multicomponent analysis of biological fluids with the advantage of not requiring sample pre-treatment [23,24,33]. Briefly, 420 μL of each sample were mixed with 280 μL of 0.23 M solution of 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) in deuterium oxide (Sigma-Aldrich Co, St. Louis, USA) and then poured into 5 mm diameter ^{1}H NMR tubes. DSS was used as a standard reference, both to calibrate the chemical shift scale (δ = 0.00 ppm) and to quantify the synovial fluid metabolites.

2.5. Proton NMR spectroscopy

All measurements were performed on a Bruker Avance III spectrometer (Bruker, Karlsruhe, Germany), operating at 400.13 MHz for ^{1}H observation and a temperature of 298.13 K, equipped with a BBI 5 mm direct detection probe incorporating a z axis gradient coil. For each sample, after a 5-min waiting period for temperature equilibration, a standard one-dimensional 1D zgpr, cpmgpr (Carr–Purcell–Meiboom–Gill spin-echo sequence, CMPG) and 2D 1H J-*resolved* NMR spectra were recorded, using the standard presaturation sequence for the water signal suppression. Spectra were obtained by the following conditions: zgpr pulse program, 32 K time domain, spectral width of 20.0264 ppm (8000 Hz), p1 (F1 channel-90° 1H transmitter pulse) 8.68 μs, pl1 −1.00 db (decibel), 16 repetitions; cpmgpr pulse program 32 K time domain, spectral width 20.0264 ppm (8000 Hz), p1 8.9 μs, pl1 −1.00 db (decibel), 256 repetitions; jresgppsqf pulse program 8 K time domain, spectral width 11.9851 ppm (4795.396 Hz), pl1 −1 db (decibel), 32 repetitions for 128 experiments. NMR data were processed using TopSpin 2.1 (Bruker) and visually inspected using Amix 3.9 (Bruker). The spectral region studied in all spectra was between 0.0 and 6.0 ppm. The metabolites (qualitative analysis) were assigned on the basis of 1D (cpmgpr) and 2D (1H *Jresolved*) NMR spectra analysis and compared with published data [33–36]. Qualitative and quantitative analysis of spectra was limited to those low molecular weight components which were most easily detectable because of their high concentration, or due to their resonance occurring in areas of the spectrum free of other signals. The presence of high molecular weight components (e.g., hyaluronic acid), producing background resonance broadened between 3 and 4 ppm; overlap with signals arising from other low molecular weight metabolites was minimized by particular devices, such as the initial sample filtration, application time of the excitation pulse and relaxation time. The relative concentration of each metabolite was obtained by calculating in the zgpr acquired spectra the integrals of the areas selected from time to time, referenced to a known concentration of DSS (prepared in such a proportion as to be comparable with that of metabolites present in the spectrum). From the concentrations obtained, relating to the number of protons in the molecule, absolute concentrations of metabolites were calculated as ratio of the molar concentration of DSS, and subsequently of the dilution factor used to prepare the sample [37]. All samples were processed by a technician who was blind to treatment. In view of the primary purpose of the study, only the most representative of all identifiable resonances for the joint compartment were analyzed. Particularly, synovial fluid levels of nine metabolites which are considered to give information about inflammatory and degradative status of the joint (lactate, pyruvate, acetate, N-acetyl groups, glucose, glycerol, choline, creatine and alanine) were quantified in each sample and compared between groups.

2.6. Orthopaedic and radiographic examinations

Each dog was examined for hindlimb lameness according to a previously described scoring system (Table 1), ranging from 1 to

Table 1
Lameness scoring system.

Score	Description
1	Stands and walks normally
2	Stands normally, slight lameness at walk
3	Stands normally, severe lameness at walk
4	Abnormal stance, slight lameness at walk
5	Abnormal stance, severe lameness at walk

Table 2
Radiographic osteoarthritis score (0 = absent, 1 = mild, 2 = moderate or 3 = severe for each feature, with an absolute 0–96 score for each stifle).

Stifle component	Radiographic feature
Patella	Apical osteophytes
	Basal osteophytes
	Cranial apical enthesiopathy
Femur	Trochlear groove periarticular osteophytes
	Supratrochlear lysis
	Fabellar (sesamoid) periarticular osteophytes
	Subchondral sclerosis
	Distal femoral condyle remodeling
	Subchondral cystic lucencies (central femoral metaphysis)
	Subchondral cystic lucencies (intercondylar fossa)
	Subchondral cystic lucencies (associated with a condyle)
	Lateral condylar periarticular osteophytes
	Medial condylar periarticular osteophytes
	Lateral collateral ligament enthesiopathy
	Medial collateral ligament enthesiopathy
Tibia	Cranial periarticular osteophytes
	Caudal periarticular osteophytes
	Subchondral cystic lesions
	Condyle remodeling
	Central tibial plateau osteophytes
	Medial periarticular osteophytes
	Lateral periarticular osteophytes
	Medial subchondral sclerosis
	Lateral subchondral lesions
	Intercondylar eminence osteophytes
Soft tissues	Joint effusion and/or capsular joint thickening
	Lateral soft tissue thickening
	Medial soft tissue thickening
Other aspects of the joint	Intracapsular mineralized osseous fragments
	Enthesiophytes at the patellar ligament insertion on the tibia
	Meniscus mineralization
	Intercondylar avulsion fragments

5 [38]. Lameness was assessed before surgery (V0) and on days 30 and 90 after TPLO (V30 and V90, respectively). At the same time points, orthogonal radiographs (caudocranial and mediolateral views) of the affected stifle joint were obtained. For the mediolateral view; the stifle was flexed at 90 degrees [29]. An OA score for each stifle was determined as previously described [28] by evaluating joint structures (patella, femur, tibia, and surrounding soft tissues) for 32 specific radiographic features, including but not limited to osteophytes, enthesiopathy, subchondral cystic lesions and soft tissue thickening (Table 2). Each feature was weighted equally and scored as 0 = absent, 1 = mild, 2 = moderate or 3 = severe, thus giving an absolute OA score for each stifle ranging from 0 to 96.

2.7. Data analysis

The mean, median and standard deviation (SD) values of demographics were used for descriptive purposes. The mean, median and standard error (SE) values of ^{1}H NMR synovial metabolites, OA and lameness scores were calculated at all time points. For

Table 3
Demographics of the enrolled dogs by group.

Group	Breed	Gender	Age (months)	Weight (kg)
C	Pointer	F	66	16.7
	Dogue de Bordeaux	M	72	50
	Labrador Retriever	F	84	28
	Mixed breed	F	90	23
	Italian Mastiff	M	17	48
	Boxer	M	87	36
	Labrador Retriever	SF	84	33.7
	Mean	–	*71*	*33.6*
	Range	–	*17–90*	*16.7–50*
	SD	–	*25*	*12.3*
T	Mixed breed	SF	24	25
	Labrador Retriever	F	84	39.5
	Beagle	CM	72	30
	Labrador Retriever	F	72	32.6
	Dogue de Bordeaux	SF	69	55
	Labrador Retriever	M	94	40.5
	Mean	–	*69*	*37.1*
	Range	–	*24–94*	*25–55*
	SD	–	*24*	*10.5*
P		*0.72*	*0.87*	*0.60*

Abbreviations: F = female; SF = spayed female; M = male; CM = castrated male; C = control group; T = treated group.
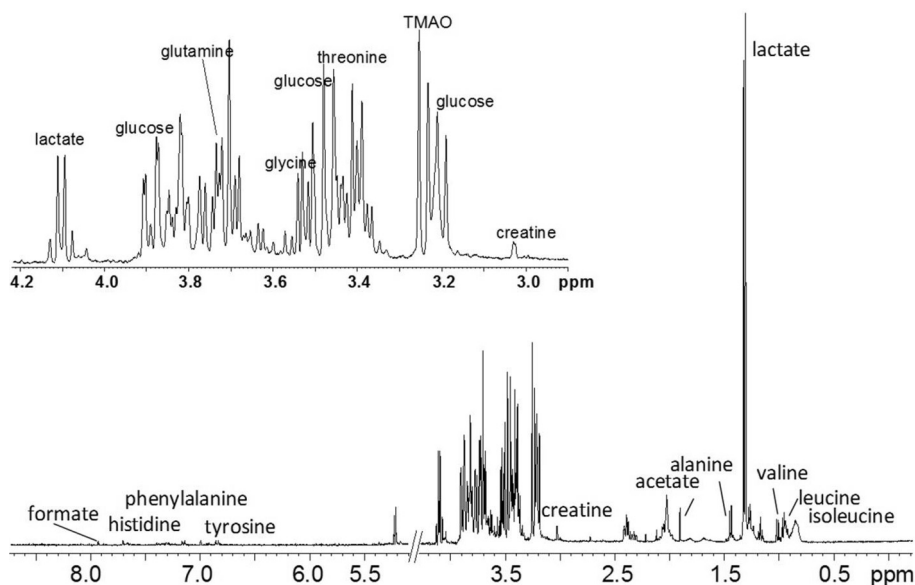

Fig. 1. Carr–Purcell–Meiboom–Gill spin-echo sequence (cpmgpr 1D) spectrum and expansions in the range 4.2–3.0 ppm showing signals of the main metabolites present in canine synovial fluid samples.

the statistical analysis of the effect SE was preferred to SD since it better represents the variability of the means. The generalized linear mixed model for repeated measures was used to analyze mean global differences between the two groups. A Kruskall-Wallis *post hoc* test with Bonferroni-Holm P correction for family-wise error was performed to analyze single differences between groups at study intervals (preoperative and at one and three months postoperative). Due to the ordinal-level nature of the orthopaedic evaluation (i.e., 5-point scoring system) Wilcoxon and Kruskal-Wallis tests were used for analyzing the treatment effect and the difference between groups, respectively. Data were analyzed using SAS v9.2 (SAS Institute, Cary, NC, USA). The significance threshold was set at 0.05. Exact *P* values are reported.

3. Results

Thirteen dogs were enrolled in the study. Demographic characteristics are summarized in Table 3. All dogs had acute lameness lasting between 7 and 20 days. There was no significant difference between groups in terms of body weight ($P = 0.60$), gender distribution ($P = 0.72$) and age ($P = 0.87$) (Table 3).

3.1. Synovial fluid metabolites

In total, 39 ^{1}H NMR spectra were acquired (13 dogs × 3 time points). Relevant peaks, related to the studied metabolites, in a typical synovial fluid cpmgpr 1D spectrum, are shown in Fig. 1. Of the analyzed metabolites, lactate and creatine showed significant differences between groups and are detailed below.

3.1.1. Lactate

Lactate was detected in the spectral range of 4.0–4.2 ppm. The mean delta of lactate synovial fluid concentration between baseline and V30, i.e. one month after surgery, was negative in T dogs (-5.86 M/l × 10^{-4}) and positive in C dogs (10.51 M/l × 10^{-4}) (Fig. 2a). In this time interval the difference between groups was
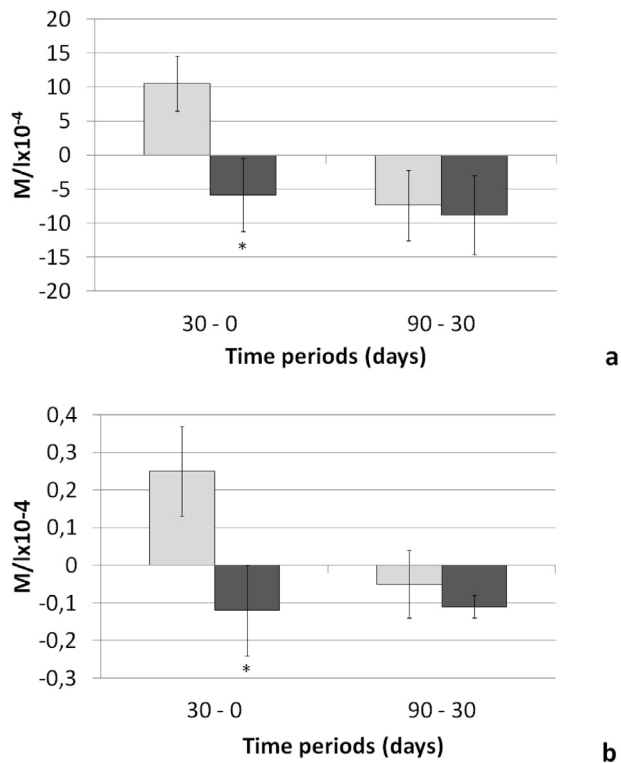
Fig. 2. Mean delta synovial concentration of lactate (a) and creatine (b) in the treated (dark grey) and control (light grey) groups. *P < 0.05.

statistically significant (P = 0.03), while it was not in the later one (-7.40 M/l $\times$ 10^{-4} and -8.85 M/l $\times$ 10^{-4} for the C and T groups, respectively, Fig. 2a).

3.1.2. Creatine

Creatine was detected in the spectral range of around 3.3 ppm. As observed for lactate, the mean delta concentration for creatine in the first post-operative month (V0-V30) was statistically significantly different between groups (P = 0.04, Fig. 2b). In particular, the mean delta of creatine synovial fluid concentration was negative in group T dogs (-0.12 M/l $\times$ 10^{-4}) and positive in C group dogs (0.25 M/l $\times$ 10^{-4}, Fig. 2b). In the second time interval (V30-V90), mean delta values failed to show any significant difference between groups (-0.05 M/l $\times$ 10^{-4} and -0.11 M/l $\times$ 10^{-4} in the C and T groups, respectively, Fig. 2b).

3.2. Radiographic findings and lameness score

There was no significant difference in the mean OA scores over time by group (Table 4) either in terms of total score (P = 0.70) or on each stifle component score (for single P values see Table 4).

The mean lameness score significantly improved over time, regardless of treatment (P = 0.011 and P = 0.010 for C and T groups, respectively, Fig. 3a). There was no significant difference between groups (P = 0.60, Table 5). Thirty days after surgery, 3/6 (50%) and 4/7 (57%) dogs in the T and C groups, respectively, scored 1 on the lameness scoring system, corresponding to "stands and walks normally". At the following visit (V90) of those dogs that could have further improved, 2/3 (67%) of the T group and 1/3 (33%) of the C group actually showed a lower lameness score (i.e., score = 1). Finally, in the last time period (V30-V90), the improvement achieved by treated dogs was 2.3-fold higher compared to control dogs (Fig. 3b).

4. Discussion

This work is the first exploratory study evaluating the effects of combining a joint health supplement with TPLO in dogs with naturally-occurring unilateral CrCLR. The findings suggest that the supplement utilized favoured a rapid rebalance of the joint microenvironment, mainly on the inflammatory side. Consistent with the metabolic aspect of the present study's hypothesis, the decrease in synovial fluid concentrations of both lactate and creatine was significantly higher in the treated compared to control dogs during the first month after surgery. Lactate is an end-product of anaerobic glycolysis and its synovial fluid levels are increased during canine OA [27,34] and human joint inflammation [39], probably the result of a predominantly anaerobic metabolism due to increased oxidative stress and low-grade inflammation. The development of inflammation following CrCLR is confirmed by the significant increase of a variety of pro-inflammatory cytokines [40] and the occurrence of histological signs of inflammation [41]. The effect on lactate observed here thus suggests an early anti-inflammatory effect exerted by the study supplement. This effect is consistent with previous findings showing that combining a supplement similar to the one used here with reconstruction surgery significantly limited the increase of lactate compared with surgery alone [33].

To the best of our knowledge this is the first observation of a rebalancing effect on the levels of creatine in synovial fluid. Creatine is considered an intermediate of muscle energy metabolism that increases during OA, both in horses and dogs [34,42]. The significantly higher decrease that we found in the treated group compared to controls would be consistent with an eventual rebalancing effect on muscle metabolism. Taken together, the results on synovial fluid metabolites suggest that, when associated with TPLO, the supplement used here has the potential to speed up rebalance of the joint microenvironment compared to surgery alone, particularly in terms of inflammation. The study joint supplement consisted of a mixture of feed materials (Regulation (EU) No 68/2013 on the Catalogue of feed materials and following amendments), principally normosulfated low molecular weight chondroitin sulfate, N-palmitoyl-D-glucosamine and quercetin. N-palmitoyl-D-glucosamine (Glupamid®) is a congener of the parent aliamide molecule palmitoylethanolamide, a broad-acting anti-inflammatory and neuroprotective lipid mediator [43]. It is the amide of palmitic acid and glucosamine and is thought to exert not only the characteristic chondroprotective effects of glucosamine [12] but also the anti-inflammatory/anti-nociceptive activities of aliamides [44,45]. In particular, N-palmitoyl-D-glucosamine down-modulates mast cell behavior – the so called ALIA (Autacoid Local Injury Antagonism) mechanism [46]. In this context it is rather interesting, as synovial mast cell hyperreactivity is viewed as a potential new target for the treatment of joint inflammation and pain [11].

In an experimental model of OA, N-palmitoyl-D-glucosamine significantly relieved joint pain [17]. The studied supplement also contains quercetin, an anti-oxidant flavonoid able to significantly limit oxidative damage in canine chondrocytes [47], protect joints from inflammation [19], inhibit overproduction of reactive oxygen species and reduce pro-inflammatory cytokines [18]. When co-micronized with palmitoylethanolamide, quercetin decreases inflammation and relieves pain in inflammatory and osteoarthritic pain models [48]. Conceivably, N-palmitoyl-D-glucosamine and quercetin contained in the supplement used here may be responsible for the early anti-inflammatory effect observed in treated dogs compared to control, as reflected by changes in synovial fluid levels of lactate and creatine during the first postoperative month. Unfortunately this effect did not correspond to a better

Table 4
Radiographic osteoarthritis scores at study time points.

		V0	V30	V90	P
Patella					
	Mean	3.90	4.00	4.40	*0.80*
C	Median	4.00	4.00	5.00	
	SE	0.40	0.44	0.43	
	Mean	3.80	4.30	4.70	
T	Median	4.00	4.00	5.0	
	SE	0.48	0.42	0.42	
Femur					
	Mean	15.43	17.86	20.00	*0.45*
C	Median	13.00	16.00	18.00	
	SE	2.46	2.41	1.89	
	Mean	13.83	17.00	18.33	
T	Median	13.50	16.50	17.50	
	SE	1.01	0.93	1.23	
Tibia					
	Mean	17.71	20.71	22.57	*0.87*
C	Median	17.00	19.00	22.00	
	SE	2.12	1.19	0.69	
	Mean	15.83	18.83	20.33	
T	Median	14.50	19.50	22.00	
	SE	1.74	1.33	1.38	
Soft tissues					
	Mean	6.14	6.57	6.14	*0.98*
C	Median	6.00	6.00	6.00	
	SE	0.46	0.48	0.46	
	Mean	5.67	6.17	5.67	
T	Median	6.00	6.00	6.00	
	SE	0.80	0.91	0.67	
Other aspects of the stifle joint					
	Mean	0.43	1.43	1.57	*0.12*
C	Median	0.00	1.00	1.00	
	SE	0.30	0.30	0.30	
	Mean	0.00	0.33	1.00	
T	Median	0.00	0.00	1.00	
	SE	0.00	0.21	0.45	
OA Total Score					
	Mean	43.57	50.57	54.71	*0.70*
C	Median	39.00	47.00	52.00	
	SE	5.18	3.86	2.94	
	Mean	39.17	46.67	50.00	
T	Median	38.00	47.50	53.00	
	SE	3.29	2.45	2.72	

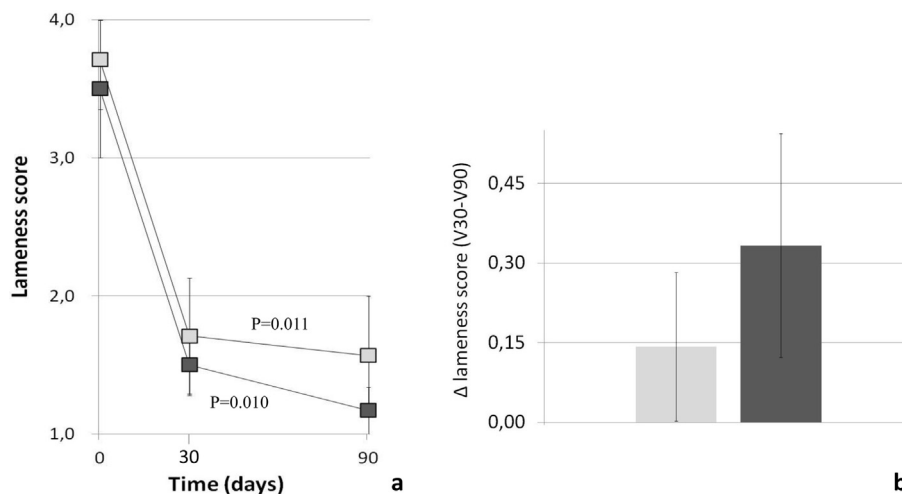Abbreviations: C = control group; T = treated group.

Fig. 3. (a) Mean (±SE) lameness score over time in the treated (dark grey squares) and control (light grey squares) groups. (b) Lameness score improvement (±SE) in the V30-V90 period in the control (light grey) and treated (dark grey) groups.

Table 5
Lameness scores at study time points.

		V0	V30	V90	P
C	Mean	3.71	1.71	1.57	0.60
	Median	4.00	1.00	1.00	
	SE	0.360	0.421	0.429	
T	Mean	3.50	1.50	1.17	
	Median	4.00	1.50	1.00	
	SE	0.500	0.224	0.167	

Abbreviations: C = control group; T = treated group.

radiographic outcome in the treated compared to control dogs. The hypothesis of a favorable influence of the study supplement on OA progression was thus not supported by these findings. This could depend, in part, on the longer times usually required to achieve structurally (radiographic) detectable changes after TPLO surgery [8,28,49,50] and also after administration of joint supplements [12]. As radiographic analysis only visualizes the effect on bony structures, short-term changes would not have been detected. Longer study duration and/or larger sample size could have helped in revealing the difference.

A statistically significant reduction in lameness score was observed in both the treated and control groups. This finding is consistent with previous observations concerning improvement in joint function starting 4–6 weeks after TPLO [51]. It is, however, interesting to note that during the last observation period (i.e., V30-V90) the improvement achieved by treated dogs was 2.3-fold higher compared to control dogs. In other words, this functional improvement was 130% higher with respect to control dogs. Although not reaching statistical significance, the magnitude of this difference could suggest an adjunctive clinical benefit of the supplement over surgery and support, in principle, our hypothesis.

The principal limitation of the present study is its small sample size, largely due to challenges in recruitment and strict inclusion/exclusion criteria. For example, this factor might have prevented uncovering findings such as other synovial metabolite differences. A further limitation is the absence of synovial fluid sampling from the contralateral healthy stifle. Although ethical and technical issues (both considered mandatory) prevented arthrocentesis of healthy joints, the diseased to healthy stifle ratio/subtraction could have minimized intrasubject variability. Last, but not least, is the relatively short duration of the observation period (3 months) chosen to encourage owner compliance. These caveats notwithstanding, we show that associating the study dietary supplement for joint health with TPLO results in a significantly better metabolic outcome within the joint compartment at one month postsurgery, suggesting an early down-modulation of inflammatory responses. Moreover, a trend to better clinical outcome in the later time interval was also observed. Clearly, studies on larger sample sizes and longer durations will be needed to confirm these initial observations. Nevertheless, these preliminary findings might have interesting implications in the management of CrCLR if one considers that low grade inflammation may worsen or even precede related chondrodegeneration [52,53] and is considered a risk factor for some postoperative TPLO complications [54].

5. Conclusions

This study provides the first evidence that, when combined with TPLO, a joint supplement containing N-palmitoyl-D-glucosamine and quercetin (besides chondroprotective substances) results in an early improvement of the joint inflammatory microenvironment. Although not statistically significant, the more than twofold improvement in lameness score during the last

observation period (30–90 day interval) points to a possible functional advantage of the add-on nutraceutical treatment. Given the non-pharmacologic nature of the study supplement and these interesting, albeit preliminary, findings, use of joint health nutraceuticals targeting inflammation besides chrondrodegeneration should be considered in the combined treatment to canine OA.

Competing interests

Alda Miolo is an employee of Innovet Italia Srl. No conflicts of interest have been declared by other authors.

Funding

Acknowledgements

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