A randomised, double-blinded, placebo-controlled study on the efficacy of a unique extract of green-lipped mussel (*Perna canaliculus*) in horses with chronic fetlock lameness attributed to osteoarthritis

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Keywords: horse; lameness; green-lipped mussel; *Perna canaliculus*; osteoarthritis; degenerative joint disease; Biolane

Summary

**Reasons for performing study:** Lyophilised products from green-lipped mussel (*Perna canaliculus* [LPPC]) are used to orally treat horses with osteoarthritis (OA). However, no randomised, controlled or double-blinded studies on the efficacy of this treatment in horses have been reported to date.

**Objective:** To investigate the effects of a unique LPPC (Biolane) in improving clinical signs of OA in the fetlock.

**Methods:** Data were analysed from 26 horses with primary fetlock lameness in a controlled, randomised and double-blinded, multi-centre clinical trial. The study design was a partial crossover with a washout period and consisted of 19 horses treated with LPPC and 20 with a placebo. Horses were dosed orally with 25 mg/kg bw/day LPPC or placebo for 56 days. Efficacy was evaluated by clinical assessment of lameness, passive flexion, pain, swelling and heat in the affected joint. Relationships between variables were analysed using an ordinal logistic model with random effects for horse and horse x treatment according to a modified intention-to-treat analysis.

**Results:** Clinical evaluation of horses with a fetlock lameness treated with LPPC showed a significant reduction in severity of lameness (P<0.001), improved response to the joint flexion test (P<0.001) and reduced joint pain (P = 0.014) when compared with horses treated with placebo.

**Conclusions:** The LPPC significantly alleviated the severity of lameness and joint pain and improved response to joint flexion in horses with lameness attributable to OA in the fetlock.

Introduction

Osteoarthritis (OA) and associated lameness is the most common cause of early retirement of pleasure and performance horses (Rossdale *et al.* 1985; Todhunter 1992). It has also been described as a perennial limitation to the utility and well-being of performance horses (Pearson and Lindinger 2009). Common practice is to treat horses early using rest, supplements and medication (McIlwraith and Vachon 1988). Well studied noninvasive therapies with minimal side effects are needed for treatment of degenerative joint disease in horses (Pearson and Lindinger 2009). Clinical trials in man and dogs have demonstrated the efficacy of lyophilised products from *Perna canaliculus* (LPPC) in treating osteoarthritic conditions (Audeval and Bouachacourt 1986; Kendall *et al.* 2000; Pollard *et al.* 2006). In *vitro* data show that LPPC has a range of anti-inflammatory activities including inhibition of tumour necrosis factor-alpha (TNFα), Cox-2 expression, prostaglandin E2 (PGE2), Phospholipase A2 (PLA2), Oxygen Radical Absorbance Capacity (ORAC), antioxidant capacity, lipolytic and fibrinolytic activities (Rainsford and Whitehouse 1980; Cheras *et al.* 2005).

Materials and methods

**Study design**

The study design was randomised, placebo controlled, double-blinded, multi-centre and partial crossover. The hypothesis was that clinical signs of OA in the fetlock joint would improve in horses treated with LPPC but not in horses treated with placebo. An animal ethics committee constituted according to New Zealand law approved the design. Horses were only enrolled once an informed owner consent form had been signed.

Veterinary clinical examination was chosen for the evaluation of lameness and based on the American Association of Equine Practitioners (AAEP) scale (Kester 1991) that enabled the design to specify the baseline variables required for inclusion. Keegan *et al.* (2010) showed that clinical evaluation of lameness resulted in a 93.1% agreement between clinicians, when the AAEP score was >1.5. For consistency only one veterinarian assessed each horse during the study.

The number of horses required for the study was a conservative estimate based upon previous studies of nutraceuticals in equine joint disease (Pearson and Lindinger 2009) and large enough to include a parallel group (Hills and Armitage 1979). There were insufficient previous data on LPPC in horses to generate a high-quality power calculation.

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A partial crossover study design was chosen because of the difficulty in recruiting horses that met the enrolment criteria. As horses were enrolled in the study, each was randomly allocated to a treatment group. Fifteen horses were enrolled again after the washout period had been completed; the second treatment was the alternative to the first treatment. Of those 15 horses that participated in both arms, 8 participated in the LPPC arm first, followed by the placebo and 7 participated in the placebo arm first, followed by the LPPC. In addition, 7 eligible horses enrolled only in the LPPC group and 7 only in the placebo group.

Crossover studies have both advantages and disadvantages in terms of clinical study design quality (Hills and Armitage 1979; Senn 2002; Troy 2006). This study sought to minimise the disadvantages of crossover design. Firstly, as LPPC is proposed to alleviate the signs of OA and requires continual dosage over time rather than affecting a cure, it is an appropriate modality for the crossover design. Secondly, treatment with the placebo was hypothesised to have no effect on clinical signs. Thirdly, a significant washout period was specified.

The pharmacokinetics of the LPPC and green-lipped mussel products in general are unknown, therefore a conservative estimate of washout period was made in order to minimise any carryover effect. Similar studies used washout periods of between 7 and 20 days (Eddington et al. 2001; Pearson 2003; Du et al. 2004; Kvaternick et al. 2007; de Grauw et al. 2009). The washout period was at least 70 days for the crossover horses, with the exception of 2 who were given the placebo first. It was shortened to 36 and 43 days for these horses in order for the owner to be available to re-enrol. Allowance for this was made by communication between the owner and study monitor without revealing the nature of the initial treatment. In addition, the monitor initiated all appointments between owner and investigating veterinarian. The investigators did not track or record the duration between successive treatments so that blinding of the study was fully maintained. The washout periods averaged 133 and 80 days, with ranges of 89–216 and 36–129 days for the LPPC and placebo groups, respectively. Washout periods varied largely due to availability of the owner to participate in a second period of the study. After the washout period, the horses had to again satisfy all the inclusion criteria.

**Horses**

Thirty horses of varying breeds, age, geographical location and sex enrolled in the study. They were randomly assigned to either the LPPC or placebo group (Fig 1; Moher et al., 2010). The cohort was balanced with respect to these parameters (Table 1). The horses were all healthy for age other than presenting with lameness. Horses were grazed at pasture and maintained outdoors, as is normal practice in New Zealand. Owners were instructed to feed the horse to maintain constant bodyweight and to continue exercising or resting the horse.

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**Fig 1: The progression of horses through the phases of the randomised, partial crossover clinical trial. LPPC = lyophilised products from Perna canaliculus.**

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TABLE 1: Cohort demographics

<table>
<thead>
<tr>
<th>Breed</th>
<th>LPCC group (n = 19)</th>
<th>Placebo group (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/weight/sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average age (years)</td>
<td>18.7</td>
<td>19.8</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>7–30</td>
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<td>Median age (years)</td>
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<tr>
<td>Average weight (kg)</td>
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<td>473</td>
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<tr>
<td>Weight range (kg)</td>
<td>350–550</td>
<td>350–600</td>
</tr>
<tr>
<td>Median weight (kg)</td>
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<td>480</td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Gelding</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Age/weight/sex</td>
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<td></td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
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<tr>
<td>Thoroughbred</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
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<td>3</td>
<td>3</td>
</tr>
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<td>1</td>
</tr>
<tr>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Crossbred</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Warmblood</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

LPCC = lyophilised products from Perna canaliculus.

Inclusion and exclusion criteria

Inclusion: Horses had lameness attributable to OA in a metacarpophalangeal or metatarsophalangeal (fetlock) joint as defined by mild to moderate lameness (grade 1–4 on the AAEP lameness scale). The affected joint was radiographed according to standard veterinary technique. Radiographs were assessed for swelling, presence and size of osteophytes, narrowing of the joint space, sclerosis or lysis of the bone underlying the joint cartilage and fractures. For inclusion, intra-articular anaesthesia of the fetlock joint had to result in at least 50% improvement in the severity of lameness. Lameness was required to be present for at least 3 months prior to enrolment in order to exclude horses with acute soft tissue injuries and or synovitis. Horses were included if the owner provided informed consent and was deemed to be willing and capable of complying with the requirements of the study.

Exclusion: Horses were excluded if they had been medicated with:
- Glucosamine, chondroitin, stallion extract, polysulphated glycosaminoglycan or pentosan polysulphate during the 10 weeks prior to enrolment.
- Corticosteroid or nonsteroidal anti-inflammatory drugs during the 6 weeks prior to enrolment.
- Multiple treatments of pentosan polysulphate or polysulphated glycosaminoglycan or hyaluronic acid during the 6 months prior to enrolment.
- Horses were excluded if they had a grade 5 lameness, infectious OA, severe OA or fractures evident radiographically or if they presented with concurrent disease likely to influence the assessment of lameness due to OA. Pregnant mares were excluded as were horses expected to gain or lose weight significantly over the duration of the study.

Post inclusion removal: Any horse that failed to conform to the inclusion and exclusion criteria was excluded from the study cohort. Horses that developed a disease state or were given a medication likely to affect study outcomes were also removed from the cohort.

Double-blinding procedure: Blinding of veterinary investigators and horse owners was maintained throughout the study and LPCC and placebo formulated with identical physical appearances and distributed in identical code labelled containers. The coding information remained secure until the conclusion of the study.

Treatment: The LPCC contained 100% lyophilised pure green-lipped mussel extract (Biolane) with flavouring. The placebo contained 97% microcrystalline cellulose with nonactive colours and flavour to mimic green-lipped mussel.

The dose of the LPCC and placebo was 25 mg/kg bwt using a scoop that delivered 7.5 g. A 300 kg horse received one scoop per day. A >300–≤500 kg horse received 1.5 scoops daily and horses over 500 kg received 2 scoops daily. The treatment was added to supplementary feed. The owners recorded the date and amount of treatment given on each occasion. The study monitor collected and weighed treatment containers after 28 and 56 days of treatment to determine owner compliance with dosing requirements.

Bioavailability and pharmacokinetic parameters for LPCC and other green-lipped mussel products in general are unknown. The dose was based on previous work. Studies in cats and dogs used 25–31 mg/kg bwt (average) (Orima et al. 1998, unpublished; Pollard et al. 2006) and in man generally 10–21 mg/kg bwt per day (Gibson et al. 1980; Kendall et al. 2000), although one study used a dosage of 30 mg/kg bwt (Audeval and Bouchacourt 1986). From these data, a dosage of 25 mg/kg bwt was deemed appropriate for this study.

Toxicity studies showed that LPCC exhibited no toxicity in rats at a single dose of 8 g/kg bwt or repeated dosages of 2 g/kg bwt for 14 days and can be considered to be nontoxic (Miller 1981, unpublished). It has been used extensively and safely in man and animals for over 30 years.

The treatments were administered daily for 56 days ± 3 days with a number of exceptions; 13 horses were treated for 60–71 days (10 horses in the placebo group and 3 horses in the LPCC group) due to scheduling difficulties between owners and veterinary investigators. One horse (LPCC group) was only treated for 48 days.

Physical examination

A veterinary lameness examination assessed the site and severity of the lameness and graded it according to the AAEP 6-point scale (0–5). The veterinary investigator performed the examination as follows: the horse was evaluated while walking on a loose lead then circled left and right at a walk in a 6 m circle. If the lameness was not evident at a walk then the horse was trotted on a loose lead then circled left and right in a straight line towards and away from the investigator. If the lameness was still not apparent, the horse was trotted in a 10 m circle. After identifying the painful limb, the horse was examined for swelling, heat and pain to localise and score the lameness. The investigation continued if the investigator considered the findings were congruent with OA in the fetlock.

A passive flexion test was performed, flexing the joint for 60 s after which the horse was immediately trotted-off. The investigator...
flexed the joint maximally while supporting the hoof but did not apply extra force to accentuate the flexion. The flexion test was scored according to a 5-point scale (following flexion: 0 = no response to flexion, 1 = a few lame steps only, 2 = consistent lameness, 3 = exaggerated lameness, 4 = minimal weightbearing). The severity of joint pain was also graded on a 5-point scale (0 = no pain evident on joint palpation; 1 = horse responds mildly, e.g. turns head; 2 = moderate response, e.g. pulls limb away; 3 = marked response to palpation; 4 = palpation not possible due to severity of pain). Degree of swelling was also assessed on a 5-point scale (0 = no swelling evident on inspection of the joint; 1 = swelling of joint subtle and discernable only on close inspection; 2 = moderate swelling of joint; 3 = marked swelling; 4 = massive swelling).

Following this, 4–7 ml of 2% mepivacaine was aseptically injected into the joint space of the affected joint. The affected fetlock was radiographed and assessed for swelling, size of joint space, changes in the subchondral bone, the presence and size of osteophytes and presence of fracture. If the diagnosis of OA with no complicating factors was made then the horse was included in the study. Finally, each horse’s weight was recorded to ensure appropriate dosage of the LPPC and placebo. These assessments were performed both at enrolment and the end of the study for each horse, with the exception of radiography, which was carried out only at enrolment.

Horses were stratified for severity of lameness (lameness scores 1–2 and lameness scores 3–4) before initial treatment was randomly allocated to ensure the treatment groups were balanced for severity of lameness.

Data analysis

Student’s t test and Chi-square analysis were used to compare the demographic data in the 2 treatment groups. For breed, a randomisation test was used because of the low numbers in the study. To assess efficacy, the primary variable was clinical improvement in lameness. Pain, swelling and response to flexion were considered secondary variables. Data analysis was performed on the primary lameness variable and secondary variables and then also using a composite measure according to Koene et al. (2010) as follows: a reduction of at least one in lameness score or combined reduction of at least 3 among the scores for pain, flexion and swelling. The level of significance was taken as P<0.05.

For the individual outcome measures (lameness, flexion, pain and swelling) ordinal logistic models (Piepho and Kalka 2003) with random effects for horse and horse x treatment and fixed effects for before vs. after treatment, initial severity of lameness and treatment order (first vs. second), plus treatment x initial lameness and treatment x treatment order interactions were fitted to all observations (before and after treatment) using WinBUGS (version 14).5

For the composite measure, a hierarchical generalised linear model analysis with a Bernoulli distribution, random effect for horse and fixed effects for treatment, first vs. second treatment and initial lameness grade, plus their interactions was undertaken using Genstat (version 11 for Windows)4. The null hypothesis was no effect of treatment whereas the alternative hypothesis was that a difference would be observed between the placebo and treatment groups.

**Table 2: The number of horses with each lameness severity categorical score in the lyophilised products from Perna canaliculus (LPPC) and placebo treatment groups at Day 0**

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>LPPC Categorical score</th>
<th>Placebo Categorical score</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAEP lameness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Categorical score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Post treatment</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Post treatment</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Placebo group</td>
<td>(n = 19)</td>
<td>(n = 20)</td>
</tr>
</tbody>
</table>

**Table 3: The number of horses with each categorical score before and after treatment with the lyophilised products from Perna canaliculus (LPPC) and placebo for each of the 3 outcome variables - lameness, flexion and pain**

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>LPPC Categorical score</th>
<th>Placebo Categorical score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Post treatment</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Post treatment</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Placebo group</td>
<td>(n = 19)</td>
<td>(n = 20)</td>
</tr>
</tbody>
</table>

**Results**

The LPPC and placebo groups were consistent with regard to the demographic parameters (Table 1). There were no differences in age, weight, sex or breeds between the groups.

**Lameness examination scores**

Table 2 describes the number of horses that completed the study in each group and their lameness scores at enrolment.

The lameness data in Figure 2 and Table 3 shows that none of the 19 horses in the LPPC group developed worsening of the lameness score, whereas 7 remained the same and 12 showed an improvement. In contrast, of the 20 horses in the placebo group, 6 showed worsening of the lameness score, 11 remained the same and 3 showed an improvement. The improvement in lameness scores of horses given the LPPC was significantly greater than the changes in lameness scores in horses treated with the placebo (P<0.001).

There was no significant interaction between treatment effect and initial severity of the lameness (P = 0.171).

Joint flexion scores were recorded for 18 of the 19 horses in the LPPC group. As shown in Table 3 and Figure 2, none showed worse response to flexion, 6 stayed the same and 12 improved. The comparable figures for the 20 horses in the placebo group were 5, 7 and 8 respectively. The improvement in flexion scores in horses given the LPPC treatment was significantly greater than in those horses given the placebo (P<0.001). Pain variables were recorded for 18 of the 19 horses in the LPPC group. The categorical data for pain demonstrated a significantly greater reduction in pain for the horses treated with LPPC than the placebo (P = 0.014). The categorical data for swelling showed no
There is significant improvement in the lameness scores of LPPC-treated horses.

There is significant improvement in the flexion scores of LPPC-treated horses.

There is significant improvement in the pain scores of LPPC-treated horses.

Fig 2: The degree of change in the categorical scores of horses treated with lyophilised products from Perna canaliculus (LPPC) and placebo for each outcome variable - lameness, flexion and pain.
significant reduction for either treatment and no difference between the groups (P = 0.382).

Using the composite measure incorporating lameness, swelling, heat and pain variables (Koen et al. 2010), the LPPC group demonstrated a significantly greater improvement when compared with the placebo group (P = 0.019).

The data analysis showed no significant interactions between the treatment effect and initial degree of lameness for all the outcome variables (P = 0.110–0.425) or the order of treatment (for the horses treated 2 times) (P = 0.200–0.399).

Figure 2 shows the degree of change in the categorical scores of horses treated with LPPC and placebo for each outcome variable - lameness, flexion and pain.

Discussion

The objective of this study was to evaluate the effect of an LPPC on clinical signs of OA in horses. The study was based on recruitment of animals displaying a lameness arising primarily from the fetlock. The dietary supplement on trial was a commercially available product made in New Zealand from green-lipped mussel. A ruling from the European Food Safety Authority (2009) states that green-lipped mussel extracts are not uniform in character. The green-lipped mussel ingredient used in this trial is identified as Biolane1, a unique New Zealand product made using a proprietary process.

Green-lipped mussel products including LPPC are often classed with glucosamine-based nutraceuticals (GBNs), as therapeutic agents. A review of the literature reporting on the effects of GBNs in alleviating lameness in horses has recently been published (Pearson and Lindinger 2009). These authors concluded that with only one exception, the studies evaluating equine GBNs failed to meet rigorous standards of scientific enquiry: double blind, placebo-controlled study designs. The results reported here are from a partial crossover study that was randomised, double-blinded, multi-centre and placebo controlled. Using the scoring system proposed by Pearson and Lindinger (2009), this study easily exceeded the suggested threshold figure of 60% for providing confidence in interpreting data generated in equine lameness studies. The veterinary lameness examination data indicated that horses with OA of the fetlock treated with 25 mg/kg bwt LPPC, showed a significant reduction in severity of lameness (P<0.001), improved response to flexion (P<0.001) and reduced pain scores (P = 0.014) when compared with the data from horses given the placebo.

A modified intention-to-treat analysis (mITT) was used to preserve the integrity of the randomisation and minimise nonrandom error (Sainani 2010; Schulz et al. 2010). The only datasets excluded from the data analysis were 3 from horses that developed medical conditions and 2 from horses enrolled with lameness of the pastern and knee rather than fetlock (Fig 1). A sensitivity analysis was performed, as a classic ITT analysis, where all excluded horses were included to an n = 30 dataset as per Figure 1 and an imputation method used where missing data points were recorded as the last observation carried forward. Analysis of this dataset identified the same effects as significant (or not significant) as the mITT, showing that no bias was introduced by excluding the 4 horses as discussed, supporting the mITT analysis. For instance, in the ITT analysis treatment, effect for lameness was P<0.001, flexion P<0.001 and pain P = 0.024; interactions between treatment effect and initial lameness had P values between 0.091 and 0.337.

The symptoms of OA can be influenced over time by a variety of extrinsic factors, including weight gain and day-to-day activity. The study design minimised these and, although the possibility that lameness scores may have been affected by extrinsic factors cannot be completely ruled out, the placebo-controlled, double-blinded nature of this study makes this unlikely.

Although it was confirmed that the source of fetlock pain was intra-articular in all enrolled horses, it was possible that capsulitis or synovitis were present rather than OA. However, 14 horses in each group had radiographic signs of OA in the affected fetlock (reduced joint space and/or subchondral bone lysis or sclerosis). For the horses with normal radiographs, one horse in the LPPC group had duration of lameness of 6 months and one horse in the placebo group had been lame for 4 months prior to enrolment. The inclusion criteria stated that horses must have been lame for at least 3 months and other than these two all horses with normal radiographs had been lame for at least a year. The chronicity of the lameness makes a diagnosis of OA more likely. Diagnostic arthroscopy to confirm the presence of OA was beyond the scope of the study.

Carryover between treatment and placebo groups is always a concern in a crossover design. A significant washout period of at least 70 days was included in the study design to minimise any carryover effect. Furthermore, an analysis of the data for any interaction of first vs. second treatment failed to demonstrate an effect of treatment order. Accordingly, the authors believe that a significant carryover effect was not present.

Reviews of other studies of green-lipped mussel (Perna canaliculus) products have concluded that there are variable results for the efficacy of these products in the literature and that further work needs to be carried out (Cobb and Ernst 2006; Brien et al. 2008). Notably these reviews critically evaluated study design and analysis and concluded that there are shortcomings in these areas in the published literature. The authors have sought to address many of these issues in this study. Also of note are the variations in preparations of green-lipped mussel extracts, as heat-treated, cooked, chemically stabilised and lipid extracts are all classed as green-lipped mussel (GLM) in the literature and in reviews. The LPPC used in this study is distinctive from these other preparations in the processing technology used; producing a product demonstrated in vitro models to possess a range of active components (Cheras et al. 2005) and putatively retaining many of the naturally active components in their native form.

Conclusion

This study demonstrated that the unique LPPC treatment (Biolane1), administered orally at a dosage of 25 mg/kg bwt significantly alleviated the severity of lameness, joint pain and improved response to joint flexion in affected fetlock joints as assessed by veterinary lameness examinations.

Authors’ declaration of interests

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Manufacturers’ addresses

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2Imperial College and MRC, UK.
3VSN International Ltd, Hemel Hempstead, UK.

References