Full Length Research Paper

**Ovopet® a new and effective treatment to decrease inflammation, pain and lameness in competing trotters**

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Received 18 October, 2019; Accepted 7 January, 2020

The use of nutritional supplements for joint problems has received much attention in recent years. At this sight, Ovopet®, an innovative ingredient from eggshell membrane that ensures the supply of nutrients necessary for the repair of joints together with anti-inflammatory compounds of natural origin, appears as a natural alternative supplementation for the treatment of joint problems in horses. Thus, the aim of this study was to evaluate the effects of oral supplementation with Ovopet® (eggshell membrane) in trotting horses with mild joint disease during the competing period to assess changes in some locomotor and physiological markers. Twenty French trotters positively identified as irregular or slightly lame were recruited and randomly assigned to either placebo or treatment group. The evaluations were done at days 0 and day 60 after the beginning of the treatment. The lameness, measured by the Equinosis® Q with Lameness Locator® was improved at low and medium speed in the horses intaking Ovopet® while it was kept stable or worsened in the placebo group. The improvement in lameness could be related to a decline in pain in the treated group. Similarly, the minimum, mean and maximum heart rate were lower at day 60 compared to day 0 in Ovopet® group. The heart rate variability whereas, was higher in Ovopet® compared to placebo. Lower heart rate variability is associated with increased pain. At the sight of the present results, Ovopet® appears as an alternative treatment to decline inflammation, pain and to improve the lameness of competing horses.

**Key words:** Eggshell membrane, inflammation, lameness, ovopet®, trotter.

INTRODUCTION

Across all equestrian disciplines, one of the most consistent and major reasons for the loss of animals is in relation to lameness and musculoskeletal injury (Cogger et al., 2008; Dyson, 2002; Murray et al., 2010). Moreover, there is a strong relationship between the horses’ athletic ability and its resulting economic worth (Rogers et al., 2012). But this athleticism, the intense exercise, may contribute to chronic inflammatory state, poor physical performance and the onset of chronic diseases especially in their joints (Brenner et al., 1999).

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When joint problems occur, the athletic horse may not perform to his normal capability, or, worse, may become lame, so the importance of preventing and treating joint disease appears as crucial in equine athletes. In treating the horse with joint disease, there are two basic objectives; to reduce the symptoms of the disease (particularly pain and inflammation), and to decrease or stop the progression of the disease (McIlwraith, 2010). While some treatments may just decrease the symptoms of joint disease, others can decrease disease progression particularly if they can reduce the severity of joint inflammation for a period of time. Reducing chronic joint inflammation can protect the tissues of the joint (especially the cartilage), thereby reducing the progression of some joint disease (Comblain et al., 2016). Based on this concept, it makes sense to take a proactive approach to the management of equine joint disease (Dyson et al., 2008).

In equine athletes, the use of nutritional supplements oriented to joint problems has received much attention in recent years as preventive or as treatment therapy for joints diseases. Most common joint supplements use a combination of glucosamine, chondroitin and Methyl Sulfonyl Methane to reduce inflammation and to help manage osteoarthritis in horses (Oke and McIlwraith, 2008, 2010). They give to chondrocytes, the building blocks they need to synthesize the cartilage extracellular matrix and they also exert anti-inflammatory effects (Comblain et al., 2016). Nevertheless, some studies using those supplements showed low evidence for efficacy (Pearson and Lindinger, 2009).

At this sight, Ovopet®, an innovative ingredient from eggshell membrane appears as a natural supplement for the treatment of joint problems in horses as it has been shown in dogs (Aguirre et al., 2018). Ovopet® is an all-natural eggshell membrane manufactured by Eggnovo S.L. via a patented process. Ovopet® is a natural ingredient obtained from the inner membrane that covers the shell of the egg. Eggshell membranes are composed of fibrous proteins such as collagen types I-V-X (Wong et al., 1984; Arias et al., 1991), glucosamine (Picard et al., 1973), hyaluronic acid (Long et al., 2005), glycosaminoglycans like dermatan sulphate and chondroitin sulphate (Baker and Balch, 1962; Jingwen, 2012), and other components including lysozyme (Hincke et al., 2000), ovotransferrin (Gautron et al., 2001), elastine (Starcher and King, 1980) and sulfur-bearing amino acids. The need of supplements containing sulfur compounds for recovery after intensive exercise is known (Wither et al., 2017). Ovopet® contains naturally the above cited components, all of them being important constituents of joints and playing a crucial role in their health, mobility, flexibility and recovery (Comblain et al., 2016). Ovopet® ensures the supply of nutrients necessary for the repair of joints together with anti-inflammatory compounds of natural origin, arising as a potential alternative supplementation for equine athletes. Thus, the purpose of this study was to evaluate the effects of oral supplementation with Ovopet® (eggshell membrane) in trotting horses with mild joint disease during the competing period to assess changes in some locomotor and physiological markers.

MATERIALS AND METHODS

Horses and the experimental design

Twenty French trotters (7 females, 9 geldings and 4 males) were recruited in two different stables in Normandy, France. The horses ranged from 2 to 7 years old (mean age of 4 ± 0.4 years). They were positively identified as irregular or slightly lame by a veterinary surgeon (lameness score > 2 based on the lameness grading system developed by the American Association of Equine Practitioners). All horses were fit enough to perform an exercise test on a race track.

Pairs of horses were constituted on the basis of similar initial mean score of lameness. Then the horses of each pair were randomly assigned to either placebo or treatment group. The double-blind placebo-controlled study was carried out by EQUI-TEST, an applied research centre of Equine Exercise Physiology, during October 2017 and January 2018. This period corresponds to a high intensity training to prepare qualification and competing period for trotters. Horses were exercised every day using a mix of five different exercises: (1) Promenade exercise: Light exercise at walk and at slow trot on a bridle path. (2) Jogging exercise: Moderate trot exercise on a bridle path. (3) Parcours exercise: Moderate intensity trot over a similar distance to races on a racetrack with a final sprint. (4) Interval type exercise: Fast trot sessions with sprint phases on the racetrack. (5) Free exercise on a sandy paddock. The training and nutrition schedules were equivalent for both groups. The evaluations were done at day 0 and day 60 after the beginning of the treatment. The study procedures are in adherence to the EU Directive 2010/63/EU for animal experiments.

Food supplement under investigation

The dietary supplement used in this study was Ovopet® (Villatuerta, Eggnovo, Spain) consisting of eggshell membrane separated from eggshells. Ovopet® was introduced in a micro-granulated supplement with the following formulation: Ovopet®, lucerne, barley, Lithothamnum calcareum algae, carob, molasses, vanilla cream aroma, green anise and corn starch. The placebo supplement contained the same recipe unless Ovopet® that was substituted by calcium carbonate and Lithothamnum calcareum algae.

Both supplement batches were tested by the laboratory “Laboratoire des Courses Hippiques” to check the absence of 11 contaminants (dopants) prohibited in French races: Caffeine, theobromine, atropine, theophylline, scopalamine, morphine, methylbufotenine, dimethyltryptamine, bufotenine, hordenine and thebaine). All of the contaminants were absent in both batches.

The palatability was observed and noted by stable workers to verify the correct intake of treatments.

Lameness evaluation

The Equinosis® Q with Lameness Locator® (Columbia, MI, USA)
Figure 1. The global index score with horses held in hand, at slow, medium and high speed after 60 days of treatment is represented. Black bars represent the placebo treatment and dotted bars the treatment with Ovopet®. Values are mean ± SD (n = 10 for Ovopet®; n = 10 for placebo).

Resting cardiac evaluation

Heart rate variability (HRV) describes variations of both instantaneous heart rate (HR) and inter-beat (RR) intervals. The variations in HR were evaluated by calculating the mean HR and the square root of the mean squared differences of successive RR intervals (RMSSD). The resting HRV evaluation is based on the recording of cardiac activity of each horse for at least one hour, at rest in a box. Measurements were done with the Team Pro System (Polar Electro Oy, Finland). The analysis of recordings for HRV was done with the software Kubios (Finland), during the five minutes with the lowest HR.

Statistical analysis

Statistical analysis of the diverse parameters was performed. Differences between placebo and Ovopet® group and between baseline (day 0) and final data (day 60) were analysed by unpaired t-test. Results are shown as mean ± standard deviation (SD). Statistical significance was considered when P < 0.05.

RESULTS

Lameness on foot and at trot were determined in both groups, placebo and Ovopet® on Day 0 and Day 60, employing the Equinosis® Q with Lameness Locator® that quantifies the total value of lameness measured through the Global Index. Three different exercise tests were made: A low speed test at 30 km/h, a medium speed test at 36 km/h and a high-speed test at 40 km/h; a trial on foot with the horse held in hand was also done. The results of lameness at all speeds (Figure 1) did not reach...
Comparing each treatment separately, the RMSSD increased in horses intaking Ovopet® although the changes lack to reach significance. The tendency of RMSSD to increase was of 64.9% in Ovopet® group comparing the end of the study with the baseline. The placebo group whereas, showed a decreasing trend of -6.3% between the end and the beginning of the treatment (Figure 3).

**DISCUSSION**

Joint pain and lameness are common causes of poor performance, diminished quality of life and early retirement in equine athletes (Dyson et al., 2008). The intense exercise may set chronic inflammatory state and chronic diseases with the consequent economic burden (Brenner et al., 1999). Therefore, the identification and use of nutritional supplements with proven effects to decrease the severity or progression of joint discomfort appears as crucial for equine athletes.

Oral joint health supplements are the most popular nutritional supplement for horses and among them, glucosamine, chondroitin sulphate and methylsulfonylmethane are regularly employed ingredients for equine joint problems (Oke and McIlwraith, 2008, 2010). Their use in horses with osteoarthritis has been supported by diverse clinical trials (Hanson et al., 1997; Forsyth et al., 2006). Nevertheless, other studies refuse their efficacy (Pearson and Lindinger, 2009; Higler et al., 2014).

In the present study the efficacy of a novel dietary supplement, Ovopet® (obtained from eggshell membranes), was tested in equine athletes for the decline of lameness and pain. Joint pain is one of the most common causes of lameness in the horse. Moreover, a decrease in performance does not always correlate to a quantifiable lameness that can be localized.
with the human eye (McCracken et al., 2012). Therefore, the use of a lameness locator for the objective lameness quantification, that has been proven to be proper to measure lameness in horses, arises as important (Equinosis et al., 2019; Keegan, 2010). Results showed an improvement in lameness higher than 13% at slow and medium speeds in Ovopet® treatment while the placebo treatment was kept stable or worsen in lameness (Figure 1). At high speed, whereas, the lameness of horses intaking Ovopet® was kept stable while the placebo treatment worsens around 10%. Less lameness could be related to a decline in pain.

Additionally, the HRV was measured. Resting HRV changes are reported in chronic pain measurements in humans (Adlan et al., 2017). It has been shown that patients with inflammatory joint disease have cardiac parasympathetic autonomic dysfunction which is related to inflammation (Provan et al., 2017). Lower HRV, measured as RMSSD, is associated with increased inflammation and pain (Adlan et al., 2017). RMSSD is strongly backed by research and is considered the most relevant and accurate measure of Autonomic Nervous System activity (De Giorgio et al., 2010).

The present results, although failed to be significantly different, showed that there was a tendency of a decline in RMSSD in placebo group whereas in the horses intaking Ovopet® the contrary was observed. Ovopet® has previously shown an anti-inflammatory effect in dogs with hip dysplasia (Aguirre et al., 2018). One of the symptoms associated to intense exercise is the inflammation and chronic diseases in joints (Brenner et al., 1999). At the sight of the present results, Ovopet® appears as an alternative treatment to decline inflammation, pain and to improve the lameness of competing horses.

In conclusion, we found that Ovopet® decreases lameness in horses at slow and medium speed. The tendency of HR to decline together with the increase in the HRV (RMSSD) could be in relation with a decrease of global chronic pain in Ovopet® intaking horses. Moreover, the effects of Ovopet® in reducing the inflammation in dogs have been previously proven (Aguirre et al., 2018). Altogether, Ovopet® appears as an effective ingredient for horse nutritional supplements to help reduce the pain associated to excessive joint use or to osteoarthritis. Further research is needed to fully understand the

Figure 3. The square root of the mean squared differences of successive RR intervals (RMSSD) after 60 days of treatment is represented. Black bars represent the placebo treatment and dotted bars the treatment with Ovopet®. Values are mean ± SD (n = 10 for Ovopet®; n = 10 for placebo).
mechanism involved in the supplementation with Ovopet®.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors want to thank EquiTest Physiologie du Sport for the conducted research.

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