

# A randomised placebo controlled double blind study on the effect of subspecies of rose hip (*Rosa canina*) on the immune system, working capacity and behaviour of horses

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## Abstract

The aim of this study was to investigate whether rose-hip had any effects on the immune system, working capacity or behaviour of horses in exercise training. Forty-four horses, all trotters, were included in a double-blind, placebo controlled trial and were supplemented with either rose-hip (*Rosa canina*) LitoVET, or a placebo for 3 months. The results from this study suggest that rose-hip LitoVET is effective as an anti-inflammatory and anti-oxidative agent in horses and improves vitamin C status.

*Keywords: rosehip, anti-oxidant, anti-inflammatory, performance*

## Introduction

A standardized powder produced from the seeds and husk of certain subspecies of rose-hip by HybenVital, Langeland, Denmark, has shown anti-inflammatory properties and improves the flexibility of joints in humans (Winther *et al.*, 2005). The powder also reduces pain and the consumption of pain killers such as paracetamol and NSAID in humans suffering from musculoskeletal diseases such as osteoarthritis and rheumatoid arthritis (Warholm *et al.*, 2003; Willich *et al.*, 2010; Winther *et al.*, 2005). It has also been documented that this standardised powder, as well as a certain galactolipid named GOPO isolated from the same product, inhibits the break-down of cartilage and up-regulates the genes responsible for the generation of collagen and aggrecan, two main ingredients of cartilage (Schwager *et al.*, 2008). The present herbal remedy has therefore been suggested as a possible disease-modifying agent in joint disease.

The present study aimed to investigate whether LitoVet, a HybenVital rose hip powder, especially developed for horses, would affect the immune system, working capacity and behaviour of horses in exercise training. As rose-hip is also noted for its high content of vitamin C, the ability of a daily supplementation with rose hip to affect the plasma concentration of vitamin C in horses was also investigated. This aspect of the research was of particular interest, as it has been shown that horses can develop vitamin C depletion, as a result of strenuous exercise and that the uptake of synthetic vitamin C, added orally to their daily diet is very poor (Snow *et al.*, 1987).

## Materials and methods

Seventy-four horses, that were in active trotting training were recruited to a double-blind, placebo controlled trial. All of the horses were located at the same training yard and were subject to a common training program. Trotters were used in this study as they often develop osteoarthritis early in life, as a result of their intensive training program and performance on an oval track. The horses were allocated to either of two groups supplemented with LitoVET or placebo. The allocation of horses

to these groups was achieved using block randomisation. Within each block of 3 horses, two were allocated to the LitoVet group and one to the placebo group. The horses were fed LitoVET (210 g daily as a dry powder added to their feed) and the placebo group were fed the same amount of a placebo powder with a similar taste, odour and colour. The mean age of the horses was  $7\pm 2.4$  and  $6.8\pm 2.1$  years for the LitoVET and placebo group, respectively. Mean bodyweight was  $432.8\pm 16.9$  and  $431.7\pm 17.9$  kg for the LitoVET and placebo group, respectively. The horses were supplemented with either LitoVET or placebo for a period of three months.

During the period of supplementation some horses were lost from both the LitoVet and placebo groups due to unrelated circumstances resulting in a smaller group of horses 44 that completed the trial, 29 horses that were fed the LitoVET and 15 horses that were fed the placebo for the period of supplementation. The horses underwent a standardised training regime during the trial that consisted of about 45 minutes of exercise 4 times per week including low intensity warm up and cool down exercise interspaced with 6-8 interval period of high intensity but sub-maximal exercise.

Venous blood samples from the jugular vein were drawn for the various analyses between 7-8 am at the same time relative to feeding and training just before the start of the trial (pre) and after 3 months of supplementation (post) with either LitoVET or placebo. The anti-inflammatory capacity was estimated using the chemotaxis of neutrophil leucocytes using a Boyden chamber (Kharazmi and Winther, 1999). Anti-oxidant capacity was estimated, by the release of antioxidative anions from neutrophils using chemiluminescence (Kharazmi and Winther, 1999). Vitamin C in serum was estimated using established photometric methodology (Hausman Lench and Lewis, 1961).

Working capacity was estimated as the time to run 1000 meters (in seconds) during actual races all conducted on the same track. Horses were placed in these races on a random basis and were competing against other horses that were not taking part in this study. Behaviour was evaluated using a questionnaire, which was completed by the staff responsible for the daily care of the horses. As the study was double-blinded, neither the staff with daily care of the horses, the veterinarians, or the trainers responsible for the exercise regime knew which horses were supplemented with the LitoVet or placebo.

Within group (LitoVET or placebo) statistical analysis of neutrophil chemotaxis and serum vitamin C was carried out using the nonparametric Wilcoxon test. This non parametric statistical test was also used to analyse the time to run 1000 metres prior to and following supplementation within the LitoVET or placebo groups. Statistical comparisons between the placebo and LitoVET supplemented groups was carried out using a non parametric Man Whitney test. The positive or negative responses to the trainer survey was analysed statistically using the non parametric sign test. All data are presented as mean  $\pm$  SD.

## Results

During supplementation with LitoVET, neutrophil chemotaxis declined from  $30.4\pm 14.0$  to  $9.0\pm 13.5$  (number of cells migrating per unit time) ( $P<0.004$ ) indicating enhanced anti-inflammatory activity (Figure 1). There was no significant difference observed in neutrophil chemotaxis in the placebo group  $31.5\pm 12.1$  to  $33.7\pm 12.9$  (number of cells migrating per unit time). There was no placebo data for the washout period as the horses were released from the study.

LitoVET also significantly improved the anti-oxidative capacity, when estimated as chemiluminescence ( $P<0.05$ ). Prior to the period of supplementation there was no significant difference in the 1000 meter run time between the placebo and LitoVET groups ( $P=0.263$ ). Horses supplemented with LitoVET shortened their time to run 1000 meters significantly ( $78.3\pm 2.6$  to  $77.2\pm 2.4$  seconds), which was equivalent to a mean decline of  $1.1\pm 1.5$  seconds ( $P=0.02$ ).

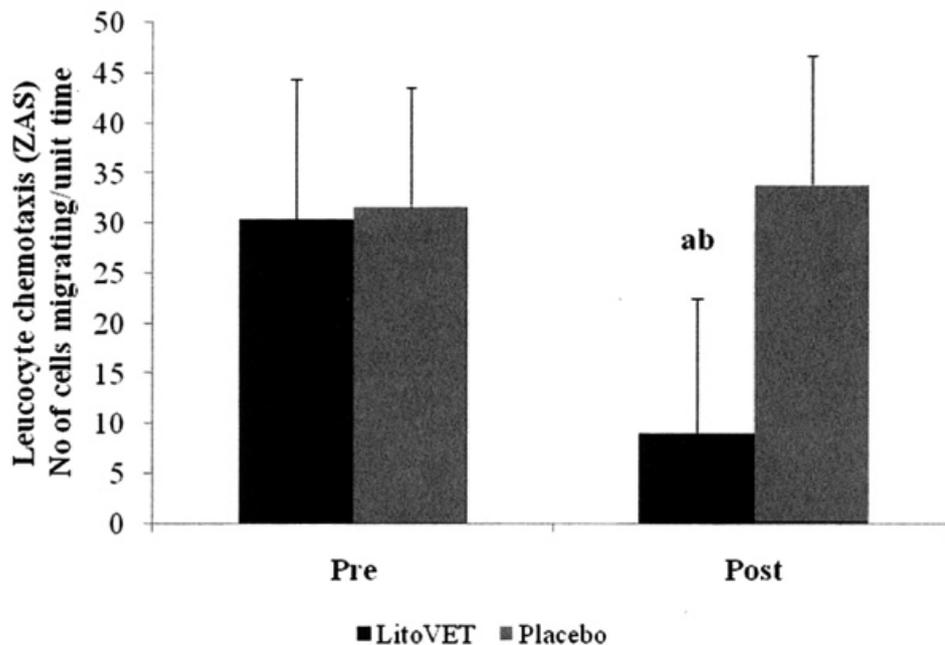


Figure 1. Effect of supplementation with rose hip (*Rosa canina*) LitoVet (210 g daily) on leucocyte chemotaxis (ZAS) (mean  $\pm$  SD) before (pre), after 3 months supplementation (post).

<sup>a</sup> Significantly different from pre within group ( $P < 0.01$ ); <sup>b</sup> significantly different from corresponding group for a given time ( $P < 0.05$ ).

In contrast, the mean time to run 1000 meters in the placebo supplemented horses increased from  $77.0 \pm 2.1$  to  $77.3 \pm 2.3$  seconds, however this was not a statistically significant change. Whilst there was a numerical difference in time to run 1000 meters between the LitoVET and placebo group following supplementation, this did not attain statistical significance ( $P < 0.075$ ).

Trainers were also asked as part of a questionnaire whether the horses were more lithe or supple and easier to work the day after strenuous exercise and were asked to respond with either yes, possibly or absolutely not. In the supplemented group 19 out of 27 horses (70%) indicated that their horses were, or possibly were more supple, whereas this figure was significantly lower in the control group (8 out of 15 or 53%,  $P < 0.05$ ). In addition, supplementation of the diet with 210 g of LitoVet also resulted in a mean increase in the serum vitamin C level of 17% two hours following feeding, Figure 2 ( $30.9 \pm 4.5$  vs.  $36.2 \pm 5.2$   $\mu\text{mol/l}$ ,  $P < 0.05$ ). There was no significant change in the serum vitamin C concentration in response to normal feeding in the placebo group of horses ( $29.9 \pm 3.7$  vs.  $30.7 \pm 3.0$  vs.  $29.9 \pm 3.4$   $\mu\text{mol/l}$ ).

## Discussion

The present data suggests that LitoVET works as an anti-inflammatory and anti-oxidative agent in horses and is effective at increasing the vitamin C level in serum within 2 hours of supplementation. In addition, horses supplemented with LitoVET were also reported, by their staff and trainers, to be more free moving or supple the day after strenuous exercise.

Prior to supplementation there was no significant difference in the time taken to run 1000 meters between the LitoVET and the placebo group. In contrast to the placebo group, there was a decrease in the time taken to run 1000 meters in the LitoVET group following 3 months supplementation. The anti-inflammatory and antioxidative action of LitoVET may have had a beneficial effect on tissue microtrauma or inflammation which can be associated with the exercise training (Evans, 2000), thus improving the time to complete 1000 meters through maintenance of free movement. However, whilst this data supports the possibility of a restoration of exercise performance in the LitoVET

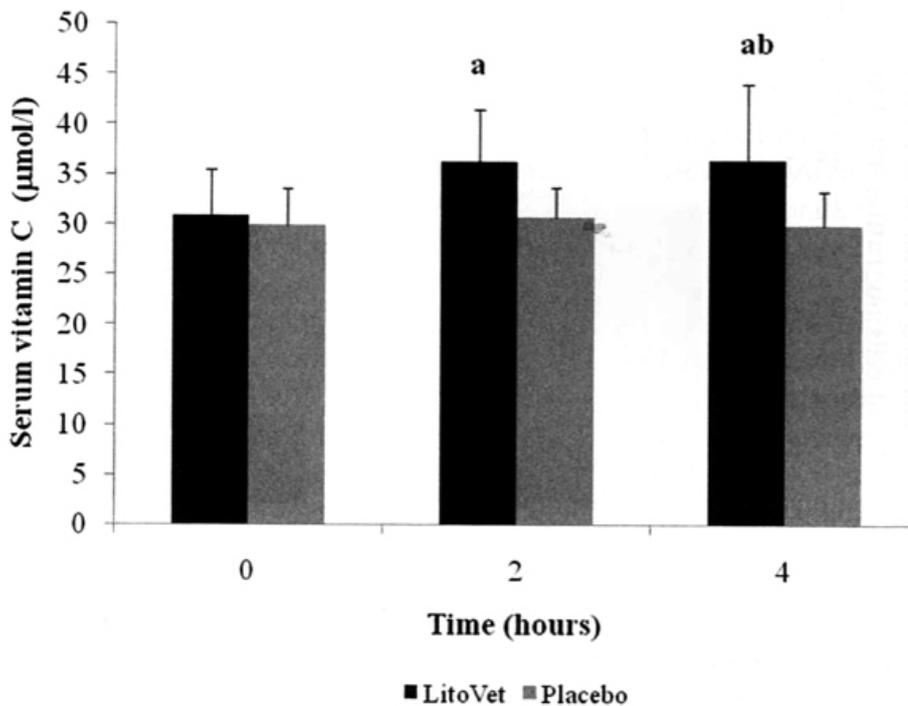


Figure 2. Effect of supplementation with rose hip (*Rosa canina*) LitoVet (210 g single dose ) on serum vitamin C (mean  $\pm$  SD,  $\mu\text{mol/l}$ ) concentration 2 and 4 hours following feeding on a single day. <sup>a</sup> Significantly different from time 0 within group ( $P < 0.05$ ); <sup>b</sup> significantly different from corresponding group for a given time ( $P < 0.05$ ).

group, the magnitude of change in run time was small and confounding factors training effect, or differences in the competitive nature of the timed races cannot be discounted as contributory factors.

Similar effects have previously been shown in humans suffering from different types of joint diseases (Warholm *et al.*, 2003; Willich *et al.*, 2010; Winther *et al.*, 2005). It has also been reported, that rose-hip is by far the strongest antioxidant in comparison to the numerous amounts of different plants, berries and fruits that have been analysed (Halvorsen *et al.*, 2002)(Halvorsen *et al.*, 2002). The present data also indicate that rose-hip probably due largely to its vitamin C content functions as an anti-oxidant in horses.

It has been reported that sportsmen, as well as trotters are sometimes given NSAID's to alleviate pain and stiffness the day after strenuous exercise. This medication is associated with undesirable side effects such as gastric ulceration and bleeding and its use is also precluded during racing under the prohibited substance rules of most racing jurisdiction. It is therefore of interest that in the present study this subspecies of rose-hip powder, which has been shown to have a strong anti-inflammatory capacity in humans, seems to exert a similar effect in horses but without any observable effect on gastrointestinal function, although any effects of long term administration require investigation. Equally the results of this trial need to be interpreted with care as the effects observed are likely to be dependent on the rose hip sub-species due to a varying level of the galactolipid GOPO.

The improved serum vitamin C level observed after 2 hours following supplementation is potentially beneficial, especially given the poor availability of conventionally used forms of vitamin C such as ascorbic acid in horses (Deaton *et al.*, 2003; Snow and Frigg, 1990). Whilst the present data shows potential, a larger double-blind study in horses is warranted in order to consolidate the effects of LitoVet rosehip powder.

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