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Bovine colostrum supplementation optimises earnings, performance and recovery in racing Thoroughbreds

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RESEARCH ARTICLE

Abstract

Bovine colostrum (BC) is the first milk produced by cows after calving and contains numerous beneficial substances for the immunity and development of the newborn calf. Because of the growth and immune factors in BC, it has become an attractive supplement for use by athletes to support immunity and health during athletic performance. In order to evaluate the effects of oral BC supplementation on equine athletes, this study evaluated the earnings, performance, recovery and incidence of upper respiratory infections (URTI) in racing horses. The study design was a randomized cross-over racing performance study. 21 horses in race training were randomly assigned to train and compete with or without BC supplementation. After each horse competed in three races, it was crossed over to the other group, allowed a three week washout period, and then competed in three additional races. Horses in public training stables of 3 participating trainers were used. Race performance as determined by earnings, Bloodstock Research Information System (BRIS) speed figures, recovery as determined by number of days between races and incidence of upper respiratory tract disease was recorded. 11 horses completed the study. There was no effect of the order of BC supplementation on the measured variables. Horses on BC supplementation earned \$ 2,088 more purse money per race, than when unsupplemented ($P=0.016$), and ran an average of 5 BRIS speed points higher ($P=0.03$). Horses returned to racing on average 7.5 days faster (16.9 days vs 24.4 days, $P=0.048$). There were no URTI among the horses on BC supplementation and two infections while not on BC supplementation (z -test, $P=0.11$). Statistical analysis showed that horses recovered more quickly, earned three times more money and raced better as judged by BRIS scores while competing with BC supplementation. BC supplemented horses also experienced fewer URTI, although this effect was not significant.

Keywords: bovine colostrum, horse, performance, brisnet speed figures, upper respiratory tract infections

1. Introduction

Bovine colostrum (BC) is the first milk produced by cows after calving. BC contains immunoglobulins, other immune factors, growth factors and nutrients necessary for optimal immunity, support and long term performance of the neonatal calf. More recently, BC has been used as a nutritional supplement in humans for both immune support and for optimising athletic performance. In humans, BC

has been shown to be effective in reducing the incidence of influenza (Cesarone *et al.*, 2007), and upper respiratory tract infections (URTI) in children (Patel and Rana, 2006), and in athletes (Brinkworth and Buckley, 2003). In humans, BC has been found to improve endurance cycling performance (Coombes *et al.*, 2002) and to enhance recovery and improve performance following sequential exercise sessions (Buckley *et al.*, 2002; Shing *et al.*, 2006).

Horse racing is a highly competitive sport in which trainers and veterinarians strive to maintain their animals in optimal health. Dietary supplements that support immune responses and thereby optimise the health, welfare and athletic performance of horses are potentially of great benefit to the racing industry. While the effect of BC dietary supplementation may be related to reported increases in plasma immunoglobulin (IGF)-1 in humans (Mero *et al.*, 1997), no such BC supplementation increases in IGF-1 occurs in horses associated with the BC supplementation protocol reported herein (Fenger *et al.*, 2014).

Given these considerations, this study was undertaken to determine if BC has a beneficial effect on the incidence of URTI and athletic performance in Thoroughbred racehorses.

2. Materials and methods

Study facilities and animals

Privately owned Thoroughbred racehorses in race training in Central Kentucky were used throughout. Horses were housed and trained according to standard racing stable management procedures at a Thoroughbred training facility in Lexington, KY, USA. Horses were stabled in 3.7×3.7 m stalls bedded in straw. Training consisted of trotting approximately 1.6 to 2.4 km (1 to 1.5 miles) and galloping approximately 1.6 to 2.4 km (1 to 1.5 miles) either on the dirt racetrack or a turf exercise field five days a week, with about one breezing day (fast gallop approaching racing speeds for 0.6 to 1.0 km (3/8 to 5/8 mile) per week and one day of walking only per week. Races were chosen according to the standard operating procedures of the 3 trainers involved. The study protocol was approved by the institutional animal care and use committee of Equine Integrated Medicine PLC and for all horses participating in the study, the owner or his or her designee (i.e. the trainer) provided informed consent.

Bovine colostrum

The colostrum used in this trial was produced under USDA and CFIA permits for commercial sale as a veterinary biologic. Excess colostrum was collected from dairies licensed to produce milk for human consumption and stored frozen (-18 °C) until processing. The colostrum was then thawed at room temperature until softened sufficiently to be pooled following pulverisation by an ice crusher, then pasteurised using a proprietary method (Saskatoon Colostrum Company, Ltd., Saskatchewan, Canada) developed to eliminate pathogenic contaminants while maintaining the integrity of the immune factors and spray dried. The spray dried colostrum was tested for retention of potency using an radial immunodiffusion method for IgG and for safety using standard microbiological methods for total plate counts, coliforms and *Salmonella*. The BC

powder was then packaged by Animal Healthcare Products and Packaging, Inc. (Winchester, KY, USA) according to good manufacturing practices protocol before being shipped to the study location.

Experimental design

All racehorses in the three participating trainers' control were included in this cross-over study. Trainers were previously known to one of the investigators (CF) to be reliable and able to comply with the study procedures. Horses were randomly assigned to the BC supplementation first or no BC supplementation first groups. Horses in the treatment group received 100 g BC daily as a feed top dress for at least weeks prior to the first race and until 3 races were completed, at which point they had a 3 week washout period, followed by three races without BC supplement. Horses in the no BC supplementation first group had three races, followed by at least a 3 week acclimation period on 100 g BC daily, which period continued until 3 races were completed. Horses which were claimed, sold or retired prior to completing the 6 race regimen were excluded from the performance data analysis. All horses were included in the URTI incidence data. Horses that completed the study were all 3 or 4 year olds, with 3 geldings, a male and 2 females in the no-treatment first group and four females and a gelding in the treatment first group.

Because of the differences in racing class, time of year, track surface and condition, the times or finish positions of the races were not considered to be useful for analysis. Racing performance was analysed based on the BRIS speed figure for each race; Bloodstock Research Information System (BRIS) speed figures were generated by an independent organisation (Bloodstock research Information System, Lexington, KY, USA; www.brisnet.com) and recorded for each of the six races for each horse. This standardised number represents a racing score independent of other factors. The amount of money earned for each race was similarly recorded. The total amount of money earned was averaged for each horse for the three races in each group. Recovery days between races were similarly averaged for all BC days and all non-BC days for each horse. Layoffs associated with injuries were excluded from the recovery data. Upper respiratory tract infections were defined as a nasal discharge and cough, which was diagnosed by endoscopy to be due to either lymphoid hyperplasia or guttural pouch infection.

Data analysis

The analysis of variance for a cross-over design, controlling for period, sequence, and horse, was fit to the data. Normality in each of the independent variables was determined by visual inspection of normality plots and Anderson-Darling test of normality, and analysed using a

general linear model (Proc GLM; SAS Institute, Cary, NC, USA). When normality assumptions were not fulfilled, the data were transformed before analysis to appropriately meet model assumptions. Normality assumptions were met for the analysis of average BRIS speed figures, money earned in the treatment and control races, and for the square root transformed recovery days. F-tests were used to determine the presence of carryover and sequence effects. Incidence of URTI was analysed using a z-test.

3. Results

A total of 21 racehorses were originally enrolled in the study, with 11 horses completing the study. Seven horses were sold before completing 6 races and 3 were 2 year olds which never started during the study period. Because the horse sales and 2 year olds were independent of the assigned treatment, the 10 horses not contained in the analysis are considered missing at random and hence are unlikely to unduly bias the results presented here. Of the 11 horses completing the study, 2 horses were excluded from the recovery analysis owing to injuries, which affected the days off between races. An ex-post power study was performed on the cross-over design used to analyse these data. Across all response variables, a maximum of 10 horses was necessary for least 80% power, hence the 11 horses considered in this study is sufficient for average horse effects. Further studies, using a greater number of horses, would be required to find different effects of bovine colostrum across genders. However, gender stratification is shown in the figures to demonstrate that there appears to be no difference between genders.

A standard linear, normal model was fit to the data using the appropriate analysis of variance model for a cross-over design, controlling for period, sequence, and horse. For each response variable we investigated departures from normality with both visual inspections and an Anderson-Darling test of normality. Neither earnings nor BRIS numbers deviated from the assumption of normality, however, Recovery (days between races) failed the normality test, so a square root transformation was performed before analysis. No response variable showed a sequence effect (earnings, $P=0.48$; BRIS numbers, $P=0.12$; recovery, $P=0.33$) or carry-over effect (earnings, $P=0.16$; BRIS numbers, $P=0.53$; recovery, $P=0.24$), thus suggesting our cross-over model is not inappropriately fit to these data. Earnings were higher ($P=0.016$) with least squares means of \$ 3,241 for horses while receiving BC supplementation compared to \$ 1,153 for horses while not on BC (Figure 1). The data also show an improvement in speed (BRIS number, $P=0.03$) with horses running a least squares mean of 67 while racing on BC and 62 when not on BC (Figure 2). Horses also returned to racing 7.5 days quicker (16.9 days vs 24.4 days, $P=0.048$) while supplemented with BC (Figure 3). In this study, no statistically significant effect of BC supplementation could be identified on the incidence of URTI ($P=0.11$), likely because of the small number of test animals and the relatively small incidence of overt URTI in this cohort.

4. Discussion

Our data demonstrated a clear improvement in athletic performance in horses receiving BC supplementation. Horses raced faster (higher BRIS speed figures) with

Figure 1. Average race earnings (US\$, $P=0.016$) for all horses with or without bovine colostrum (BC) supplementation (randomly jittered along the x-axis for clarity). The horizontal bars across each treatment represents the least squares mean as predicted by the model fit to the data shown.

Figure 2. Average BRIS speed ratings ($P=0.03$) for all horses with or without bovine colostrum (BC) supplementation (randomly jittered along the x-axis for clarity). The horizontal bars across each treatment represents the least squares mean as predicted by the model fit to the data shown.

Figure 3. Average recovery days between races for the second and third races with or without bovine colostrum (BC) supplementation for all horses (randomly jittered along the x-axis for clarity). The horizontal bars across each treatment represents the least squares mean as predicted by the model fit to the data shown. Two horses were excluded from analysis because of illness or injury. Statistical analysis was performed on transformed data ($P=0.048$), but non-transformed data are shown here.

aggregate greater earnings of \$ 70,455 over the course of this study while supplemented with BC. BRIS speed figures are calculated based on a computed algorithm which takes into account racetrack, surface, track condition, quality of the other horses in the race, and other factors contributing to the racing performance of a horse in a given day (www.brisnet.com). This is a performance metric which is comparable across all races on all days and is computed for all horses in North America. BRIS speed figures translate into approximately 1 point per 1-1.5 lengths in a race, with the value of a length decreasing as the race distance increases to accurately reflect racing effort. Therefore, the 5 BRIS speed figure improvement in racing performance in horses on BC supplementation reflects an approximately 5 length improvement in racing performance.

The performance effects of dietary supplementation with BC in human athletes is mixed. No improvement in performance was seen in a small double-blind study in healthy men and women undergoing aerobic and resistance training (Antonio *et al.*, 2001), although there was an increase in lean body mass. Similarly, no performance effect was seen in endurance exercise in male and female hockey players (Hofman *et al.*, 2002), rowing ability in elite female rowers (Brinkworth *et al.*, 2002), or a single exercise-to-exhaustion test (Buckley *et al.*, 2002). On the other hand, a small improvement in a 2 h endurance time trial in cyclists after an 8-week supplementation period has

been reported (Coombes *et al.*, 2002), and a recent study found increased leg press strength when older adults were supplemented with BC (Duff *et al.*, 2014).

The mechanism of athletic performance improvement in humans has not been determined, but has been suggested to be associated with an increase in serum IGF-1 concentration (Mero *et al.*, 1997). However, the magnitude of the increase of IGF-1 seen in the Mero study was small, did not exceed the normal range, and has not been repeatable by other investigators (Buckley *et al.*, 2002; Coombes *et al.*, 2002; Duff *et al.*, 2014). Similarly to these latter studies, there is no increase in IGF-1 in Thoroughbred race horses associated with BC supplementation (Fenger *et al.*, 2014). This indicates that any improvement in performance seen with BC in these horses was independent of an IGF-1 effect.

One possible mechanism could be related to other cytokines in BC, such as interleukin (IL)-1 β , IL-6, tumour necrosis factor alpha (TNF- α), interferon-gamma (INF- γ) and IL-1 receptor antagonist (IL-1ra), any of which may have an effect on athletic performance. While most cytokines would typically be degraded in the digestive tract, it has been suggested that cytokines in BC may be protected by some unidentified mechanism, resulting in their survival through the digestive process and absorption into the circulation (Davison, 2012). The role of inflammatory cytokines, such as IL-1 β , INF- γ , IL-6 and TNF- α during exercise is to send critical signals to exercising muscles that reactive oxygen species and other indicators of stress are forthcoming. These critical signals set off a cascade of reactions mediated by nuclear factor kappa-beta (NF- $\kappa\beta$), which moderates adaptation to exercise (Radak *et al.*, 2013). If these cytokines are absorbed from the BC, then this adaptation may be the mechanism of the improved performance during exercise.

Post-exercise recovery was improved with BC supplementation in our subjects. Horses were able to race back 7.5 days quicker, reflecting a shorter recovery time after a maximal racing effort in our study. This is a similar finding to studies in humans. In an 8-week, double-blinded, placebo-controlled study, BC supplementation did not improve performance on an exercise-to-exhaustion test. However, it did improve performance on a repeat test 20 min later (Buckley *et al.*, 2002). In cyclists performing high intensity exercise tests with short recovery times, BC supplementation also benefitted recovery (Shing *et al.*, 2006). The improved recovery may be the result of a reduction in oxidative stress in skeletal muscle during exercise while supplemented with BC (Appukutty *et al.*, 2013).

In our study, there was no difference in incidence of overt URTI in horses while BC supplemented. However, this was likely due to an overall low rate of overt URTI in this cohort. In humans, BC supplementation decreased the incidence of influenza (Cesarone *et al.*, 2007), URTI in

children (Patel and Rana, 2006), and in athletes (Brinkworth and Buckley, 2003). Intense exercise training is well known to cause immunosuppression in humans (Smith, 2003) and horses (Davis *et al.*, 2007) which can manifest as URTI. This has been suggested to result from a suppression of TH-2 cell-mediated immunity by an exercise related increase in the TH-1 immune response (Smith, 2003), and an increase in natural killer (NK) cells (Wong *et al.*, 2014). In human studies, BC supplementation has multiple immune system effects in athletes, including increasing salivary immunoglobulin A concentrations in runners (Crooks *et al.*, 2006), improvement in the recovery of neutrophil function and maintenance of salivary lysozyme concentrations in cyclists (Davison and Diment, 2010), and preventing the TH-2 immune suppression associated with exercise in cyclists (Shing *et al.*, 2007). While our URTI data did not attain statistical significance, the overall incidence of overt URTI was low in our group. This was likely the result of the requirement of the crossover model for 6 or 7 sequential races. Often, younger horses, which are more susceptible to overt URTI, fail to make this number of races sequentially without substantial layoffs. Nonetheless, the only overt URTI were in the horses during the non-BC supplemented period, and it is likely that subclinical infection also occurs, which may have detrimental performance effects. The mechanism of decreased incidence of URTI in athletes supplemented with BC is likely associated with an immune modifying effect of the BC supplementation, presumably offsetting the transient immunosuppression associated with intense exercise. An immune modifying effect may have influenced performance and recovery in this study, although this cannot be unequivocally shown with these data.

The horse is physiologically unique in that as soon as it begins to exercise it fails to fully oxygenate its blood (Fenger *et al.*, 2000), suffering from 'exercise induced hypoxemia'. The stroke volume of blood travels so rapidly across the pulmonary circulation that there is insufficient time for complete oxygenation across the thin alveolar-capillary membrane. Inflammation in the airways as may be caused by subclinical infection acts to exacerbate 'exercise induced hypoxemia' in horses, by thickening this alveolar-capillary membrane. Demonstrating this phenomenon, Sánchez *et al.* (2005) showed that the faster a horse runs the greater the oxygen deficit in the blood delivered to the muscles, and that this deficit is exacerbated by airway inflammation. Any intervention that reduces airway inflammation may be expected to immediately and proportionally improve oxygen delivery to the exercising musculature and thus equivalently improve racing performance, and may have been the mechanism of the performance benefit seen in the present study.

In conclusion, BC can be supplemented to racehorses for the purpose of optimising athletic performance and recovery of the animal. Further studies to elucidate the

optimal dose, extent and specific mechanisms of immune modifying effect of BC in exercising horses are warranted.

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