7-2016

Enhanced Bovine Colostrum Supplementation Shortens the Duration of Respiratory Disease in Thoroughbred Yearlings

Clara K. Fenger
Equine Integrated Medicine

Thomas Tobin
University of Kentucky, ttobin@uky.edu

Patrick J. Casey
University of Aukland, New Zealand

Edward A. Roualdes
California State University - Chico

John L. Langemeier
Equine Integrated Medicine

See next page for additional authors

Enhanced Bovine Colostrum Supplementation Shortens the Duration of Respiratory Disease in Thoroughbred Yearlings

Digital Object Identifier (DOI)
https://doi.org/10.1016/j.jevs.2016.03.012

Notes/Citation Information

Published in *Journal of Equine Veterinary Science*, v. 42, p. 77-81.

Authors

Clara K. Fenger, Thomas Tobin, Patrick J. Casey, Edward A. Roualdes, John L. Langemeier, Ruel Cowles, and Deborah M. Haines

Journal of Equine Veterinary Science 42 (2016) 77–81

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Enhanced Bovine Colostrum Supplementation Shortens the Duration of Respiratory Disease in Thoroughbred Yearlings

Clara K. Fenger [a,*], Thomas Tobin [b], Patrick J. Casey [c], Edward A. Roualdes [d,1], John L. Langemeier [a], Ruel Cowles [e], Deborah M. Haines [f,g]

[a] Equine Integrated Medicine, PLC, Georgetown, KY
[b] Gluck Equine Research Center, University of Kentucky, Lexington, KY
[c] Mont Liggins Charitable Trust, Research Centre in Reproductive Medicine, University of Auckland, Auckland, New Zealand
[d] Department of Statistics, University of Kentucky, Lexington, KY
[e] Gunston Hall, Lexington, KY
[f] Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[g] The Saskatoon Colostrum Co. Ltd, Saskatoon, Canada

ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form 11 March 2016
Accepted 16 March 2016
Available online 5 April 2016

Keywords:
Bovine colostrum
Respiratory tract infections
Horses
Immune modulator
Supplement

Abstract

Bovine colostrum (BC) is used in humans as a nutritional supplement for immune support and has been shown to reduce Respiratory disease (RD). Other nutritional supplements, minerals and vitamins including mannan oligosaccharides (MOS), zinc and vitamins A, C and E have also been used for immune support. The aim of this prospective blinded randomized clinical trial was to evaluate the effects of a BC, MOS, zinc and vitamin based enhanced bovine colostrum supplement (BCS) on incidence and duration of RD occurring in yearling horses. 109 yearlings on two Thoroughbred farms in Central Kentucky were randomly assigned to treatment or placebo groups. Yearlings were supplemented once daily for 17 to 25 weeks with 100 g of a high quality commercial BCS (containing 50 g BC) or a full fat soy flour placebo, which were applied as a "top-dress" to feed. Yearlings were observed daily and evaluated weekly for signs of RD. All yearlings completed the study. The proportion of the study period during which each yearling exhibited illness was considerably shorter for BCS yearlings (least squares mean = 23% of the study period) than placebo yearlings (least squares mean = 34% of the study period, $P = .002$). The average duration of illness was shorter for BCS yearlings (1.96 weeks) than placebo yearlings (4.39 weeks, $P < .0001$). There was no statistical difference in the incidence of RD in these study yearlings.

1. Introduction

Bovine colostrum (BC) is the first milk produced by cows after calving. Bovine colostrum contains immunoglobulins, other immune factors, growth factors and other nutrients necessary for optimal immunity, maturation, and support of the neonatal calf. Recently, BC has become increasingly popular as a nutritional supplement in humans for immune support. In humans, BC has been shown to be effective in prevention of influenza [1], upper respiratory tract infections in children [2], upper respiratory tract infections in athletes [3], and to improve the humoral immune response to vaccination [4]. This effect is not limited to humans: Bovine colostrum supplementation enhances the immune response in dogs to canine distemper vaccine [5].

* Corresponding author at: Clara K. Fenger, Equine Integrated Medicine, PLC, 4904 Ironworks Rd, Georgetown, Kentucky 40324.
E-mail address: drfenger@hotmail.com (C.K. Fenger).
[1] Current Address: Department of Mathematics and Statistics, California State University, Chico, Chico, CA 95929, USA.

0737-0806/$ – see front matter
http://dx.doi.org/10.1016/j.jevs.2016.03.012

The effect of natural substances on the immune response is not limited to BC. Mannan oligosaccharides (MOSs) modify the cytokine response of pulmonary macrophages in pigs [6]. Zinc has a myriad of direct and indirect effects on the immune system, via both its role as a cofactor in over 300 enzymes, its direct effects on leukocytes [7] and on the humoral immune response [8]. Vitamins A [9], C [10], and E [11] have well-described benefits on immune function in animals.

Equine respiratory disease (RD) is a significant cause of morbidity in horses, and young horses are significantly more susceptible to RD than older horses [12]. Respiratory disease is the second leading cause of missed training days in racehorses and is responsible for almost a quarter of lost training days [13]. Influenza, herpesviruses, and rhinitis viruses are among the most common causes of viral respiratory infection in horses, whereas *Streptococcus zooepidemicus*, *Actinobacillus* or *Pasteurella* spp, coagulase negative *Staphylococcus*, or nonhemolytic *Streptococcus* are the most common cause of bacterial respiratory infection, with younger horses (2 years of age and less) highly susceptible to primary viral disease and secondary bacterial superinfection [12]. Supplements that support immune function and mitigate these infections would benefit the equine industry.

This study was undertaken to determine if a BC; MOS; zinc; and vitamins A, C, and E containing supplement (ImmHand EQ Animed, Winchester, KY) has a disease-sparing effect for RD in Thoroughbred yearlings.

2. Materials and Methods

2.1. Study Facilities and Animals

Privately owned Thoroughbred yearlings residing on two farms in Central Kentucky were used throughout. These yearlings consisted of all farm yearlings, both resident foaled and purchased from sales before and during the study period. There was no quarantine instituted for horses added to the farm during the study period. Horses were housed and fed according to farm management procedures and identified by mare names on halter name tags, which consisted of either a brass name plate or a cattle ear tag affixed to the halter with the name indicated on the tag. All horses were vaccinated and dewormed according to farm protocols, which included a primary series of vaccinations for Eastern, Western, and West Nile Equine Encephalitis viruses, tetanus, botulism, influenza, and rhinopneumonitis with boosters in March. Deworming consisted of a 2-month rotational deworming program of pyrantel pamoate, anthelcide, and ivermectin during the study period. Horses were stabled in $12' \times 12'$ stalls bedded in straw, shavings, or bluegrass bedding. On farm A, yearlings were brought into the barn and fed separately twice daily. Fillies were housed in a communal paddock and colts in an adjacent paddock, and treatment and placebo groups were commingled. On farm B, yearlings were brought into the barn and fed once daily 5 days per week and fed outside in communal feeders once daily 5 days per week and twice daily for the remaining 2 days. On farm B, colts and fillies were similarly separated, but the placebo and treatment groups were not commingled. Groups were housed separately in adjacent paddocks. The distribution of the yearlings among farms, treatment groups, and gender is shown in the Table. The study protocol was approved by the Institutional Animal Care and Use Committee of Equine Integrated Medicine PLC, and the owner or his or her designee (i.e., the farm manager) provided informed consent for all horses participating in the study.

2.2. Bovine Colostrum

The colostrum used in this trial was produced under USDA and CFIA (Canadian Food Inspection Agency) permits for commercial use as a veterinary biologic. Excess colostrum was collected from dairies licensed to produce milk for human consumption and stored frozen until processing. The thawed colostrum was pasteurized using a proprietary method (Saskatoon Colostrum Company, Ltd, Sasketchewan, 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 a 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 into a blended nutritional supplement with MOSs; vitamins A, C, and E; zinc; and folic acid (ImmHand EQ) in a GMP facility (Animal Healthcare Products and Packaging, Inc, Winchester, KY) before being shipped to the study location. Placebo was soy flour, selected to match color, and consistency of the test article. No attempt was made to match the protein, fat, or other dietary ingredients because the supplement comprised less than 3% of the total diet and was not considered to have a substantial impact on the balance of the macronutrients of the diet.

2.3. Experimental Design

All yearlings housed on the two farms were included in this randomized blinded prospective placebo controlled study. Horses were randomly assigned to the treatment or placebo groups. Horses in the treatment group received 100 g ImmHand EQ (containing 50 g BC) (BCS group) as a feed top dress once daily, and horses in the placebo group received 100-g soy flour (P group) as a feed top dress once daily. On farm A, the supplement or placebo was fed once daily in the morning feed. On farm B, The supplement or placebo was fed once daily in the morning feed which was offered when the horses were in the stalls 5 days per week and in the communal feeders 2 days a week when they did not come into the stalls. Supplementation was initiated in the yearlings in mid-January, or when the horse was added to the farm, and discontinued in June, unless the horse left the farm before June. On farm A, the study period for the yearlings averaged 22 weeks (range, 17–23), and on farm B, the study period averaged 22.6 weeks (range, 6–26).

2.4. Variables Recorded

Yearlings were observed daily by farm personnel and assessed once weekly for evidence of respiratory tract

infections, including epiphora, nasal discharge, and cough. The farm manager and yearling managers were trained by the study principle investigator (C.K.F.), and either manager performed all observations. The incidence (number of times yearlings were affected during the study) and duration of illness (number of sequential weeks during which yearlings were observed to be affected) were recorded. Any missed observations were omitted from the analysis. Antibiotic or other treatment for RD was intended to be instituted if yearlings became anorectic, which did not occur in any yearlings during this study.

2.5. Data Analysis

The analysis of variance, controlling for nonrandom influences of farm and gender, was fitted 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 analyzed using a general linear model (PROC GLM SAS Institute Inc, Cary, NC).

3. Results

A total of 109 yearlings were in the study. Three response variables were measured: one/proportion of the study period each yearling was sick (proportion), two/mean number of weeks affected per incident per yearling (weeks/incident), and three/incidence rate of sickness during the study period (incidence). Using PROC GLM in SAS (SAS Institute Inc, Cary, NC), the response variables were estimated using a completely randomized block design, with blocks consisting of both gender and farm. Because the treatment groups were not balanced, Type 3 sums of squares were used throughout.

After controlling for both gender and farm, analysis of variance F-tests indicated differences by treatment groups of the response variables proportion (P value = .002, Fig. 1) and weeks/incident (P value < .0001, Fig. 2), but no statistical significance for incidence (P value = .41, Fig. 3). The least squares means for proportion was 0.23 for BCS group and 0.34 for P group. The yearlings in the BCS group exhibited signs of respiratory illness for less than one-quarter of the study period, whereas yearlings in the P group had signs of respiratory illness for one-third of study period. Thus, yearlings in the BCS group were affected with RD for shorter durations (1.96 weeks) during the study period despite experiencing the same incidence of RD during this time as compared to yearlings in the P group (4.39 weeks).

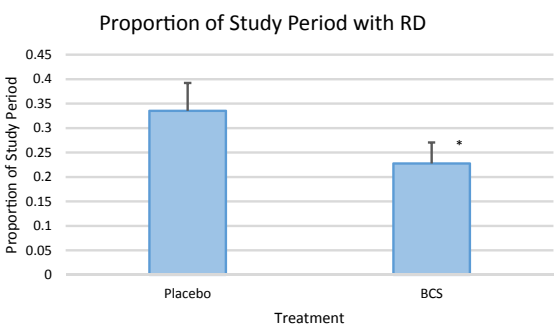

Fig. 1. Proportion of the study period, which was the number of weeks during which the yearlings exhibited respiratory disease divided by the total number of study weeks for that individual for the placebo and BCS groups: least squares means + 1.96 standard error (* indicates significant difference from placebo). RD, respiratory disease; BCS, bovine colostrum supplement.

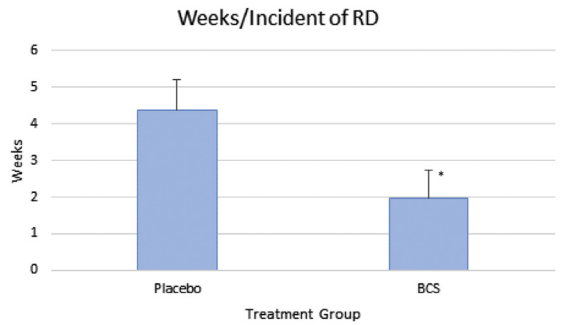

Fig. 2. Average number of weeks duration of each incident of respiratory disease in yearlings, which was the number of weeks with respiratory disease divided by the umber of incidents of respiratory disease: least squares means ±1.96 standard error (* indicates significant difference from placebo). RD, respiratory disease.

The residuals from the models, proportion, weeks/incident, and incidence are not normally distributed, as tested by the Anderson–Darling test for normality. However, analysis using the central limit theorem provides reassurance about the estimated parameters because the sample sizes for the overall models were large (n = 109) with 56 yearlings in treatment group BCS and 53 in treatment group P. Only in the model fitted to the response variable weeks/incident did any signs of heteroskedasticity (variance was not equal between the two groups) exist and was predominantly due to a few outliers, discussed further below.

The heteroskedasticity found in the model for the response variable weeks/incident was largely due to three horses, all in the placebo group, with studentized residuals greater than 3. Furthermore, for both response variables proportion and incidence, one colt in the BCS group had an extraordinarily large studentized residual of 3.3 for proportion and 4.5 for incidence. The models were refitted for all three response variables without their respective

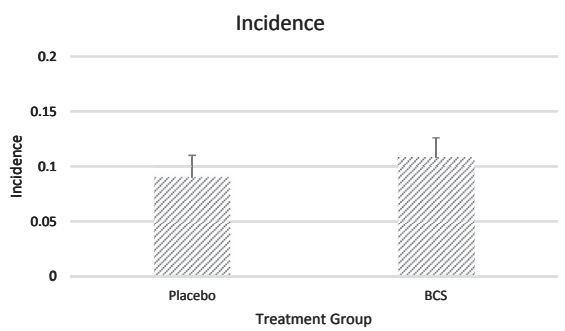

Fig. 3. Incidence of respiratory disease among yearlings, which was the number of times yearlings exhibited respiratory disease divided by the number of animals in the study: least squares means ±1.96 standard error.

outliers, and no qualitative differences from our findings above were identified.

3.1. Discussion

This study demonstrated that daily use of a BCS (ImmHand EQ), a feed supplement product containing BC; MOS; zinc; and vitamins A, C, and E resulted in a lower proportion of time affected with RD and shorter duration of illness per event in Thoroughbred yearlings. This effect is consistent with findings in humans receiving BC oral supplementation. Several studies in people have documented the effects of BC supplementation on the incidence and/or severity of RD, including a lower incidence of influenza-like symptoms [1,14], respiratory tract symptoms in children [2], and in athletes [3]. These studies demonstrated a decreased incidence of RD with BC supplementation, whereas our study demonstrated a decreased number of days affected, but no difference in incidence of RD between supplemented and placebo yearlings. One possible explanation for this disparate finding in RD incidence between the species is the management differences between horses and humans. Humans can avoid contact with their peers when there is contagious RD in a group, whereas horses are maintained in comingled herds, and there were no isolation procedures in place when horses became affected on either of the two farms. However, a clear disease sparing of the BC effect was evident by the shortened duration of RD per incident in the yearlings being fed the immune enhanced formulation in this study.

Additional ingredients in ImmHand EQ may have been responsible for the observed RD sparing effect. Mannan oligosaccharides are functional carbohydrates which are not digested, but exert their action principally within the intestinal tract. Mannan oligosaccharide effects include local binding of type-1 fimbria of pathogenic bacteria, such as salmonella and *Escherichia coli* [15], and local effects in the intestines of increasing protective mucus production in sea bass [16], and altering cytokine profiles in pigs [6]. Despite the localization of the MOS within the intestinal tract, there is evidence that MOS directly stimulates alveolar macrophages, indicating that it exerts some effect on pulmonary immunity [6].

Zinc effects on the immune system include its role as cofactor in over 300 enzymes, direct and indirect effects on leukocytes [7]. Zinc has long been identified to have antiviral properties against herpes viruses [17] and rhinoviruses [18] in humans. Similar to the RD among yearlings in this study, zinc administration in rhinovirus infected humans resulted in a decreased duration of RD [18], suggesting that zinc may have played an important role in mitigating disease in our study. A relationship has been identified between zinc supplementation and decreased risk of equine herpes myelitis [19], further suggesting that zinc may play a similar role in horses as in humans against respiratory viruses.

No attempts to identify the etiologic agent(s) of the RD were made in our group, which is a drawback to our study. The identification of RD was by visual inspection, which makes the identification of the initial serous nasal discharge associated with viral infection difficult. Typically, by the time a mucopurulent nasal discharge is identified, the primary viral insult cannot be identified. However, the most likely viral agents are influenza, equine rhinitis virus, Equine Herpes Viruses (EHV-1, EHV-2, and EHV-4) [20], whereas bacterial pathogens would most likely be *S. zooepidemicus*, *Actinobacillus* or *Pasteurella* spp, coagulase negative *Staphylococcus*, or nonhemolytic *Streptococcus* [12]. Transmission of these pathogenic agents is by droplet inhalation, and BC has been shown to increase salivary IgA in athletes [21], which may have contributed to the mechanism by which the BCS mitigated disease in Thoroughbred yearlings. All yearlings had been vaccinated with a primary series of vaccinations in the preceding year and were boostered during the study, which would have confounded any analysis of viral titers, so such analysis was not performed.

Any contribution of the BC to the RD disease-sparing effects could be due to the direct action of colostral antimicrobial factors or to indirect effects of cytokines and growth factors potentially enhancing the innate or acquired immune response [22]. Direct effects could be due to antibacterial and antiviral activities of molecules such as lactoferrin and lactoperoxidase that could directly interfere with transmission of infectious agents in the respiratory tract following oral or nasal exposure. Indirect effects on the immune response could be due to impacts of high levels of growth factors such as insulin-like growth factor 1 (IGF1) and epidermal growth factor (EGF) or to regulatory events from impacts of colostral cytokines (interleukin [IL]-1beta, IL-2, IL-6, IL-17, tumor necrosis factor-alpha, interferon-gamma, IL-10) on the cytokine network that regulates immune responses.

In human studies, BC has multiple immune system effects in athletes, including increased salivary IgA in runners [23], improvement in the recovery of neutrophil function and maintenance of salivary lysozyme concentrations in cyclists [24], and prevention of the TH-2 immune suppression associated with exercise in cyclists [25]. When used in combination with vaccination, BC has enhanced the humoral response to vaccine in both humans [4] and dogs [5]. The mechanisms of the effects of this BC containing supplement on the horses in this study were not investigated; however, they are presumably similarly associated with an immune modifying effect of the BC or direct antimicrobial effects in the upper respiratory tract.

As many as 16% of young Thoroughbreds are sidelined as a result of RD, and it is the second leading cause of missed training days [13]. A previous study [26] showed no effect of BC on RD in horses in race training, but the authors felt that this was likely a result of the low incidence of RD in this cohort of horses. The authors hypothesized that investigating an age group that experienced a higher rate of RD would produce more meaningful results, which led to the present study in younger individuals more susceptible to RD. In addition, the previous study included only BC and not the other ingredients in the BCS used here (MOSs; vitamins A, C, and E; zinc; and folic acid), which likely contributed to the immune effect.

4. Conclusion

A BC-based supplement was highly beneficial for reducing the duration of RD in Thoroughbred yearlings and

provides a useful nonpharmacologic alternative for reducing the adverse impact of RD in Thoroughbred yearlings.

Acknowledgments

The authors thank Animal Healthcare Products and Packaging, Inc and Saskatoon Colostrum Company for providing funding ImmHand EQ and funding for this project. Published as Kentucky Agricultural Experiment Station Article 16-14-005 with approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experimental Station.

Dr C.K.F. is a paid consultant for Saskatoon Colostrum Company and Animal Healthcare Products and Packaging, Inc. Dr D.M.H. is an officer and employee of Saskatoon Colostrum Company.

References

[1] Cesarone M, Belcaro G, Di Renzo A, Dugall M, Cacchio M, Ruffini I, Pellegrini L, Del Boccio G, Fano F, Ledda A, Bottari A, Ricci A, Stuard S, Vinciguerra G. Prevention of influenza episodes with colostrum compared with vaccination in healthy and high-risk cardiovascular subjects: the epidemiologic study in San Valentino. Clin Appl Thromb Hemost 2007;13:130–6.
[2] Patel K, Rana R. Pedimune in recurrent respiratory infection and diarrhoea–the Indian experience–the pride study. Indian J Pediatr 2006;73:585–91.
[3] Brinkworth G, Buckley J. Concentrated bovine colostrum protein supplementation reduces the incidence of self-reported symptoms of upper respiratory tract infection in adult males. Eur J Nutr 2003;42:228–32.
[4] He F, Tuomola E, Arvilommi H, Salminen S. Modulation of human humoral immune response through orally administered bovine colostrum. FEMS Immunol Med Microbiol 2001;31:93–6.
[5] Satyaraj E, Reynolds A, Pelker R, Labuda J, Zhang P, Sun P. Supplementation of diets with bovine colostrum influences immune function in dogs. Br J Nutr 2013;110:2216–21.
[6] Che TM, Johnson RW, Kelley KW, Dawson KA, Moran CA, Pettigrew JE. Effects of mannan oligosaccharide on cytokine secretions by porcine alveolar macrophages and serum cytokine concentrations in nursery pigs. J Anim Sci 2012;90:657–68.
[7] Rink L, Gabriel P. Zinc and the immune system. Proc Nutr Soc 2000; 59:541–52.
[8] Shinde P, Dass RS, Garg AK, Chaturvedi VK, Kumar R. Effect of zinc supplementation from different sources on growth, nutrient digestibility, blood metabolic profile, and immune response of male Guinea pigs. Biol Trace Elem Res 2006;112:247–62.
[9] Brown CC, Noelle RJ. Seeing through the dark: new insights into the immune regulatory functions of vitamin A. Eur J Immunol 2015;45: 1287–95.
[10] Sorice A, Guerriero E, Capone F, Colonna G, Castello G, Costantini S. Ascorbic acid: its role in immune system and chronic inflammation diseases. Mini Rev Med Chem 2014;14:444–52.
[11] Pekmezci D. Vitamin E and immunity. Vitam Horm 2011;86:179–215.
[12] Wood JL, Newton JR, Chanter N, Mumford JA. Association between respiratory disease and bacterial and viral infections in British racehorses. J Clin Microbiol 2005;43:120–6.
[13] Bailey CJ, Reid SWJ, Hodgson DR, Rose RJ. Impact of injuries and disease on a cohort of two- and three-year-old thoroughbreds in training. Vet Rec 1999;145:487–93.
[14] Belcaro G, Cesarone MR, Cornelli U, Pellegini L, Ledda A, Grossi MG, Dugall M, Ruffini I, Fano F, Ricci A, Stuard S, Luzzi R, Grossi MG, Hosoi M. Prevention of flu episodes with colostrum and Bifivir compared with vaccination: an epidemiological, registry study. Panminerva Med 2010;52:269–75.
[15] Spring P, Wenk C, Dawson KA, Newman KE. The effects of dietary mannan oligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella challenged broiler chickens. Poult Sci 2000;79:205–11.
[16] Torrecillas S, Montero D, Caballero MJ, Pittman KA, Custódio M, Campo A, Sweetman J, Izquierdo M. Dietary mannan oligosaccharides: counteracting the side effects of soybean meal oil inclusion on European sea bass (Dicentrarchus labrax) gut health and skin mucosa mucus production? Front Immunol 2015;5(6):397.
[17] Eby GA, Halcomb WW. Use of topical zinc to prevent recurrent herpes simplex infection: review of literature and suggested protocols. Med Hypotheses 1985;17:157–65.
[18] Mossad SB. Effect of zincum gluconicum nasal gel on the duration and symptom severity of the common cold in otherwise healthy adults. QJM 2003;96:35–43.
[19] Traub-Dargatz JL1, Pelzel-McCluskey AM, Creekmore LH, Geiser-Novotny S, Kasari TR, Wiedenheft AM, Bush EJ, Bjork KE. Case-control study of a multistate equine herpesvirus myeloencephalopathy outbreak. J Vet Intern Med 2013;27:339–46.
[20] Gilkerson JR, Bailey KE, Diaz-Méndez A, Hartley CA. Update on viral diseases of the equine respiratory tract. Vet Clin North Am Equine Pract 2015;31:91–104.
[21] Mero A, Kahkonen J, Nykanen T, et al. IGF-I, IgA, and IgG responses to bovine colostrum supplementation during training. J Appl Physiol 2002;93:732–9.
[22] Rathe M, Müller K, Sangild PT, Husby S. Clinical applications of bovine colostrum therapy: a systematic review. Nutr Rev 2014;72: 237–54.
[23] Crooks C, Wall C, Cross M, Rutherfurd-Markwick K. The effect of bovine colostrum supplementation on salivary IgA in distance runners. Int J Sport Nutr Exerc Metab 2006;16:47–64.
[24] Davison G, Diment B. Bovine colostrum supplementation attenuates the decrease of salivary lysozyme and enhances the recovery of neutrophil function after prolonged exercise. Br J Nutr 2010;103: 1425–32.
[25] Shing C, Peake J, Suzuki K, Okutsu M, Pereira R, Stevenson L, Jenkins D, Coombes J. Effects of bovine colostrum supplementation on immune variables in highly trained cyclists. J Appl Physiol 2007; 102:1113–22.
[26] Fenger CK, Tobin T, Casey PJ, Roualdes EA, Langemeier JL, Haines DM. Bovine colostrum supplementation optimises earnings, performance and recovery in racing Thoroughbreds. Comp Exerc Physiol 2014;10:233–8.