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CAN FEED CAUSE A POSITIVE BLOOD TEST IN RACEHORSES? SOME RECENT INFORMATION ON THE EFFECT OF DIETARY SUPPLEMENTS ON PLASMA tCO_2 CONCENTRATION IN HORSES

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An extensive amount of published scientific research has established that the measurement of plasma total carbon dioxide (tCO_2) concentration is a scientifically valid method for detecting the administration of alkalinizing agents to horses. However, questions exist as to whether various common management and nonmanagement factors (e.g., electrolyte supplementation, ration formulation, environmental temperature, breed, etc.) inadvertently cause an elevation in plasma tCO_2 concentration. This paper will review some of the recent research conducted at Rutgers and elsewhere on the effects of various feeding practices, in particular, electrolyte and feed supplements on plasma tCO_2 concentrations. Information gained will be useful to trainers and veterinarians who wish to avoid having their animals inadvertently exceed established threshold limits.

Milkshakes and Their Detection

In recent years, sodium bicarbonate and other alkalinizing agents have been administered to horses with the goal of buffering the decrease in pH or acidosis that occurs with high-intensity exercise. This practice, commonly referred to as "milkshaking," has become more sophisticated and the administration of "other alkalinizing agents" is perceived as a threat to the integrity of the sport of racing (Rose and Lloyd, 1992; Irvine, 1992; Lloyd and Rose, 1992; Hinchcliff et al., 1993) and a potential threat to the health and welfare (Roelofson, 1992; Frey et al., 1995; Rivas et al., 1996) of the equine athlete. Therefore, racing agencies throughout the world have supported research to develop methods to detect the administration of alkalinizing agents (Irvine, 1992; Lloyd and Rose, 1992; Slocumbe et al., 1995; Auer, 1993; Lorimer, 1998).

Extensive published scientific data has established the validity of the use of plasma total carbon dioxide concentration (tCO_2) by itself to determine if a horse has been given an alkalinizing agent (Auer, 1993; Lloyd and Rose, 1993; Slocumbe et al., 1995; Frey et al., 1999; Greene et al., 1999; Kauffman et al., 1999). Put simply, Lloyd and coworkers explain that plasma tCO_2 concentration is a measure of bicarbonate together with dissolved carbon dioxide (Lloyd et al., 1992). Lloyd further states that the actual bicarbonate concentration is about 95% of tCO_2 value.

This strong statistical relationship between bicarbonate concentrations and plasma tCO_2 concentration is a fact that is detailed in basic physiology and biochemistry textbooks (Tenney, 1970; Martin, 1981; Guyton, 1981) and has been accepted as valid in several other papers that detail the validity of using plasma tCO_2 concentrations to detect the administration of alkalinizing agents.

The aforementioned scientific data, published in internationally recognized scientific journals and proceedings, has made the use of tCO_2 alone an accepted method in racing jurisdictions both in the United States (Maryland, New Jersey, Illinois, New York, etc.) and throughout the world (Australia, New Zealand, Hong Kong, etc.). This testing is performed either before or after a race; however, in many jurisdictions, prerace sampling has fallen out of favor, since it was found that the timing of administration of an alkalinizing agent allowed some horses to circumvent the test. Postrace testing can be affected by intense exercise, which causes an acidosis and a decrease in plasma tCO_2 concentration. However, the work of Frey et al. (1995) demonstrated that plasma tCO_2 concentrations return to prerace levels within one hour of the race. Therefore, regulations have established a postrace threshold on samples taken a minimum of one hour after the cessation of exercise. In several states, including New Jersey, postrace testing is used to identify horses that may have been administered a substance that alters plasma tCO_2 concentration. Another effective paradigm used in some states is to have all horses report to a secured paddock area several hours before major races. In this format prerace testing has been shown to be effective as racing officials can monitor the horses and any overages can be handled as scratches.

The threshold limits used in New Jersey were established after a study of more than 250 horses. Lorimer and coworkers (1998) demonstrated that the mean plasma tCO_2 concentration measured in New Jersey Standardbred horses on an off day, when they were not likely to have been given an alkalinizing agent, was ~30 mMol/L. The highest reading of tCO_2 was 34 mMol/L, a value well below the threshold established in New Jersey. Several other studies of hundreds of horses (Rose and Lloyd, 1992; Lloyd and Rose, 1992, Auer et al., 1993) conducted around the world have consistently demonstrated that, in nonmilkshaked populations, the mean plasma tCO_2 concentration averages ~30 mMol/L with very little variation around that mean. This makes sense physiologically as blood pH and acid:base status is regulated. For optimum cellular function, a horse's body will "fight" to maintain acid:base homeostais around a set point with narrow variation. Based on measured population data, many racing jurisdictions (e.g., Maryland, Illinois, New Jersey, etc.) have used concentrations that are 4 standard deviations from the norm to establish a threshold of 37 mMol/L (39 mMol/L for horses on Lasix) and to ensure against false positives.

In New Jersey and other jurisdictions, regulations have been established in consultation with horse owners and trainers that allow the horse owner to place a horse into quarantine, where plasma tCO_2 concentration can be measured under controlled conditions. Almost all horses placed into quarantine exhibit plasma

tCO_2 concentrations near to the mean of the normal population; thus, elevated plasma tCO_2 concentrations previously measured postrace are usually due to either the deliberate or inadvertent administration of an alkalinizing agent. It is the latter circumstance that was of concern in recent research conducted at Rutgers and elsewhere.

Are There Naturally High Plasma tCO_2 Concentrations?

One defense that trainers, horse owners, and veterinarians have used has been that their animal has a naturally high plasma tCO_2 concentration (Vine, 1998). To this end, several papers have looked at common conditions affecting plasma tCO_2 concentration. Slocumbe and coworkers (1995) demonstrated that there is only a very minor, statistically significant, variation across time. More importantly, their data demonstrated that those "cyclical" variations were physiologically insignificant, and even the difference between the highest and lowest points in the day would not push a normal horse near to the established threshold. Slocumbe et al. (1995) further demonstrated that alterations in breathing only cause minor alterations in plasma tCO_2 concentrations. Irvine (1992) and Slocumbe et al. (1995) demonstrated that excitement does not affect plasma tCO_2 concentration. Irvine further demonstrated that there was no effect of sex or age on bicarbonate status. Slocumbe et al. (1995) demonstrated that transportation did not affect plasma tCO_2 concentration. Several studies, including that of Frey et al. (1995), have demonstrated that blood pH and bicarbonate concentrations return to prerace or pre-warm-up levels by one hour postexercise. Those studies are the basis for collecting postrace/postexercise samples at least one hour after exercise. Finally, it is well established that Lasix alters plasma tCO_2 concentration. That is why most states allow a higher plasma tCO_2 for horses on Lasix.

Effect of Dietary Manipulation

Another area of concern to horse owners is whether their feed can cause an inadvertently high plasma tCO_2. Part of the rationale for these concerns has been based on a report suggesting that horses in Australia appeared to have higher plasma bicarbonate concentrations when compared to North American horses. The Australian data suggests that pasture-fed horses and horses fed diets with coarse roughage may have slightly higher plasma bicarbonate concentrations compared to horses fed higher quality forage (Kauffman, et al., 1999). Upon closer examination, however, the difference between pasture feeding and formulated feeds was minimal. Furthermore, an examination of data from several studies from Australia and New Zealand demonstrates that the normal mean value for plasma tCO_2 concentrations in racehorses in those countries was ~30 mMol/L.

Several other studies have examined manipulation of the protein, fat, and starch concentration of rations given to horses. Graham-Thiers et al. (1999) reported

on the effects of high versus low protein diet on differences in plasma bicarbonate concentrations. While there were differences in HCO_{3-} and acid:base status during exercise, there were no differences due to treatment before or at 30 minutes after exercise. This suggests that manipulating protein concentrations in the ration should not affect prerace or postrace markers of bicarbonate status. Taylor et al. (1995) examined the effects of dietary fat on acid-base variables before and during exercise, before and after training. They reported that there were no effects of feeding increased dietary fat on venous pH or plasma bicarbonate concentrations. Interestingly, data are mixed as far as the effects of carbohydrates/starch. Work by Ralston et al. (1993) reported a decrease in blood pH due to starch intake with dietary cation-anion difference (DCAD) held constant. Mueller et al. (1999), however, found that blood HCO_{3-} concentration was greater in horses that consumed diets containing a high DCAD compared to horses that had consumed diets with a low DCAD. They also found that starch intake or source had no significant effect on blood HCO_{3-} concentration. One should thus take care when using a ration formulation that alters the DCAD. Depending upon the ingredients, that manipulation does appear to affect blood pH and blood HCO_{3-} concentrations and could push a horse's plasma tCO_2 concentration upwards. Nevertheless, when one looks at the values presented in published studies, the variation due to DCAD manipulation can only account for a small portion of an increase in plasma tCO_2 concentration. With reference to the effect of diet on plasma tCO_2 concentrations, the studies mentioned above are limited as the researchers have only looked at acid:base status in general terms and have not focused on the effects of feeds on plasma tCO_2 concentration. Furthermore, they have not examined changes in postexercise plasma tCO_2 concentrations as measured in most racing jurisdictions. However, one recent report by Kauffman et al. (1999) has examined the effects of various common dietary regimens on plasma tCO_2 concentrations.

In that study, Kaufman et al. (1999) tested the effect of five different rations on plasma tCO_2 concentrations. One ration treatment consisted of pasture, and another consisted of a pellet formulated with a coarse forage and grain combination similar to that used in Australia. The other three diets were pelleted with varying alfalfa and grain combinations representing 100%, 60%, and 40% alfalfa. Mean plasma tCO_2 concentrations ranged from 25.6 mMol/L to 29.1 mMol/L. The authors found that for the most part these dietary manipulations caused a very minimal change or no change in plasma tCO_2 concentration. They also demonstrated that, while pasture horses had slightly higher plasma tCO_2 concentrations, the difference was minor. The authors did report a transient effect when changing from one diet to another. Thus, one should take care when introducing a new feed. One should also note that the diets used in the study above were carefully formulated to exclude ingredients that could have an alkalinizing effect. Some ration formulations can have bicarbonates and other ingredients that can alter DCAD and/or have an alkalinizing effect that can have the potential to alter plasma tCO_2 concentrations. Horse owners and trainers should

check their feed label to make sure that their ration does not contain ingredients such as bicarbonate or other alkalinizing agents that could be a problem.

Recent Rutgers Equine Science Center Studies

Effect of Electrolyte Supplements. It has become extremely popular for horse owners/trainers to give their animals a variety of electrolyte supplements to replace salts lost in the sweat. In theory, the simplest products on the market may actually have an acidifying effect and should not be a worry. However, many of the newer electrolyte supplements have a long list of ingredients, and some of these ingredients may have an alkalinizing effect. Researchers at Rutgers have recently conducted a series of studies to test the hypothesis that several common electrolyte supplements (Lyte-Now, Stress-Dex, Summer Games, Electroplex, Enduramax, Acculytes, Perform n' Win) would alter preexercise and postexercise plasma tCO_2 concentration in normal healthy horses. Horses were tested before and after a simulated race test performed on a high-speed treadmill. It was found that the electrolyte supplements did not alter plasma total carbon dioxide concentrations in unfit Standardbred horses. However, one should caution that the products mentioned above are just a few of the many products on the market. Some electrolyte supplements do contain ingredients (such as sodium bicarbonate) with an alkalinizing effect. Thus, an owner or trainer should check the label before giving electrolytes to his horse or wait to supplement until after the postrace testing has been performed.

Dietary Supplements. As mentioned above, diet manipulations have been hypothesized to have the potential to affect acid:base status in the horse. Inadvertent elevation of tCO_2 through dietary changes can thus be a potential problem for racehorse trainers/owners. Another study was performed at Rutgers to test the hypothesis that dietary supplements used in horses in New Jersey (Omelene 200™, Strategy™, Drive™) would alter plasma tCO_2 concentrations before and after exercise. That research demonstrated that none of the diets (Omelene 200™, Strategy™, Drive™) examined had an effect on plasma tCO_2 concentrations measured before and after a simulated race test.

Exercise Training. Plasma tCO_2 concentrations measured in unfit horses in the two Rutgers studies were slightly higher than those reported for fit racehorses (Lorimer, 1998). We speculated that the repeated challenge of exercise training and racing may chronically lower plasma tCO_2 concentrations in the fit horse. To test that hypothesis, a third study was conducted to compare the plasma tCO_2 concentrations measured in unfit horses with eighteen horses that were being moderately trained as part of another experiment. Each group underwent similar housing, feeding, and watering protocols. Moderately trained horses had a significantly lower plasma tCO_2 concentration compared to the untrained horses,

and the mean (31.4 mMol/L) for the trained horses was similar to previously reported data (Lorimer, 1998) for fit racehorses sampled on an off day when they were unlikely to have been given an alkalinizing agent or milkshake. These results are similar to data reported by Irvine (1992).

Quarantine-Induced Detraining. Another area of concern in New Jersey has been the effect of the limited amount of exercise performed by horses during quarantine. Some horse owners have suggested that the limited exercise and stall rest associated with quarantine may have an effect on plasma tCO_2 concentration. Data indicated that two days of detraining does not affect plasma tCO_2 concentration.

Conclusions

Data demonstrate that normal healthy horses defend blood pH, blood bicarbonate concentration, and plasma tCO_2 concentration. Studies to date have documented minimal changes due to normal diet or electrolyte supplementation; however, alterations in DCAD or addition of alkalinizing agents to a ration may influence plasma tCO_2 concentration. Trainers and owners are cautioned to examine the label of ingredients for any product they may give their horses.

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