FixINE®

DIGESTIVE PROBIOTICS

TECHNICAL INFORMATION

- Clostat
- Kemin Technical Literature
- Diamond V XPC
- ButiPearl Z EQ

GUT H3ALTH SOLUTIONS
KNOCK OUT

CLOSTAT®

CLOSTAT® contains a proprietary, patented strain of *Bacillus subtilis*, PB6. PB6 is a probiotic that helps improve intestinal health and integrity in an array of animals — including horses. It does this by maintaining a healthy balance of microflora in the gut and by helping develop a natural defense against harmful bacterial pathogens. These factors contribute to the overall wellbeing and performance of the horse.

THE SCIENCE BEHIND CLOSTAT

The PB6 in CLOSTAT is a unique, naturally occurring microorganism. Kemin has more than 200 internal research and development (R&D) documents referencing PB6 trial work. This work indicates that PB6 is more robust than other strains, as it is proven to survive in the horse gastrointestinal (GI) tract and maintain efficacy when pelleted.[1] In addition, PB6 exhibits a strong inhibition against *Clostridium* isolates, as well as a variety of other horse-specific pathogenic bacteria.[2]

WHY FEED CLOSTAT?

The lining of the gut not only absorbs nutrients, it also provides a barrier against pathogens, parasites and toxins. PB6 secretes an active substance which helps maintain the balance of microflora in the gut. When balanced, good bacteria help keep pathogenic bacteria under control.[3] When unbalanced, pathogens can multiply and damage the gut lining, resulting in leaky gut syndrome. Harmful substances can then cross the barrier and infect the horse. By supporting the microbial balance in the intestinal tract of your horse, **CLOSTAT can help improve gut health, which can lead to improved athletic performance and general wellbeing.**

THE CLOSTAT CONFIDENCE

Proven efficacy against horse-specific pathogens, including a variety of *Clostridium* species

Demonstrated safety in foals and adults

Stable when blended with other feed ingredients

Maintains efficacy in pelleted feeds

Survives in the horse GI tract

More than 200 internal R&D documents referencing PB6 research

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Effect of CLOSTAT® and ButiPEARL® Z EQ on Non-steroidal Anti-Inflammatory Drugs (NSAID)-Induced Gastrointestinal Inflammation in Horses

Abstract

This study was conducted to determine the effect of CLOSTAT® and ButiPEARL® Z EQ (BPZ EQ) on equine gut health parameters prior to and during NSAID-induced inflammation. Phenylbutazone was used as the NSAID. Thirty horses were randomly assigned to one of 3 treatments: control (no NSAID and no BPZ EQ + CLOSTAT), Phenylbutazone (BUTE; 4.4 mg/kg every 24 hrs; no additive), and BUTE + BPZ EQ (4 g/hd/d) + CLOSTAT (4 g/hd/d) on -14 Day of Treatment (DOT). On 1 Day of Treatment (DOT), BUTE was administered using an oral paste as a carrier for BUTE. Gastroscopy for stomach ulcers and circulating rDNA for bacterial abundance were measured prior to and during BUTE administration. BPZ EQ + CLOSTAT decreased squamous and glandular ulcers scores (during challenge) and 16s rDNA (prior to challenge) compared to the control/BUTE. These results indicate that the combination of BPZ EQ + CLOSTAT can provide a protective effect to the intestinal barrier. Further investigation is needed using a more enterically-challenged model.

Introduction

Butyric acid and zinc play an important role in key biological processes affecting animal health and performance. Research has shown that butyric acid and zinc positively influence the structural integrity of the intestinal barrier through various mechanisms affecting different processes. BPZ EQ is an encapsulated butyric acid and zinc product that is released in a controlled manner along the intestinal tract. In addition, CLOSTAT is a probiotic that contains a unique, patented strain of *Bacillus subtilis* PB6. Although the benefits of BPZ EQ and CLOSTAT are known, there are no studies looking at the effect of these products combined in an equine *in vivo* model. Thus, the objective was to evaluate the effect of BPZ EQ and CLOSTAT using a NSAID-induced intestinal inflammation model in horses.[1, 2]

Experimental Design

Thirty-six horses were randomly selected from over 70 available horses at Texas A&M University. Three horses were matched based on age (+/- 2 years), breed, sex and weight (+/- 100 pounds) and randomly assigned to one of 3 treatments:

1. Control (no NSAID and no BPZ EQ + CLOSTAT)
2. Phenylbutazone (BUTE; 4.4 mg/kg every 24 hrs; no additive)
3. BUTE + BPZ EQ (4 g/hd/d) + CLOSTAT (4 g/hd/d)

The matching and assignment of treatments was performed 11 more times, so there was a total of 12 horses per treatment. When the horses were moved to the assigned pen pasture, they were put on a basal diet during the acclimation period for 14d (-28d to -14d DOT). On -14d DOT 10 horses were assigned to BPZ EQ + CLOSTAT by top dressing their basal diet with pellets containing the products (0.5 lb/hd/day). The Control and BUTE groups received the same top dressing of pellets, but the pellets did not contain BPZ EQ and CLOSTAT.

On 1 DOT, oral paste containing BUTE was given to the BUTE only and BPZ EQ + CLOSTAT treatment groups and oral paste containing no added BUTE was given to the Control treatment group. The BUTE (or just oral paste) was given every 24hr up to 10 DOT. BUTE was given during the feeding time in their individual stalls. Blood and feces were taken on 3, 5, 7 and 10 DOT (during BUTE administration) and at 15 DOT of the experiment (no BUTE).

Metrics Measured

Gastroscopy was performed on all horses on d1 and d10 of the experiment. Squamous scoring was based on a previously published scoring system: 0= intact normal mucosa, 1= intact mucosa with reddening and/or hyperkeratosis, 2= small single or small multifocal ulcers, 3= large single or large multifocal ulcers, 4= extensive (often coalescing) ulcers with areas of deep ulceration.[2] Glandular ulcers were scored using the same method.

Blood was collected on -14d, d1, d3, d5, d7, d10 and d15 from each horse to determine changes in the bacterial 16S rDNA gene. Quantification of the bacterial 16S rDNA gene in blood has been used as a marker for loss of GI barrier function and bacterial translocation in people with inflammatory bowel disease and in animal models of GI diseases.[3-5]

Statistical Analysis

Data were analyzed using JMP® (SAS, Cary, NC) with significance set at P< 0.05. Gastroscopy (10d) scores were compared using ANOVA across treatments, and score proportions was compared with a chi-square test. Relative 16S rDNA concentrations were compared using repeated measures ANOVA for 0-15d data points across treatments (challenge period). In addition, ANOVA comparisons were made between -14d and 0d (pre-treatment). Since BUTE and Control were considered the same (no challenge), they were combined as one group (Control/BUTE vs. BPZ EQ + CLOSTAT). Contrasts were used to determine treatment differences.

Results

All horses consumed > 95% of the therapeutic and/or placebo with enthusiasm suggesting no concerns with palatability. There were no differences in body weight among groups during the pre-treatment period (-14d and 0d) nor during the challenge period (data not shown).

Gastroscopy

No horses had evidence of squamous ulcers on 0 DOT. On 10 DOT, there was a significant difference in average squamous scores with BUTE compared to Control, with BPZ EQ + CLOSTAT being the intermediate (Figure 1A, P=0.02). There was an association with treatment to squamous scores using Chi Square test, P=0.04 (Figure 1B).

No horses had evidence of glandular ulcers on day 0. There were treatment differences with average glandular scores (Figure 2A), and no treatment association with scores according to Chi-Square test on 10d (Figure 2B).

Figure 1A

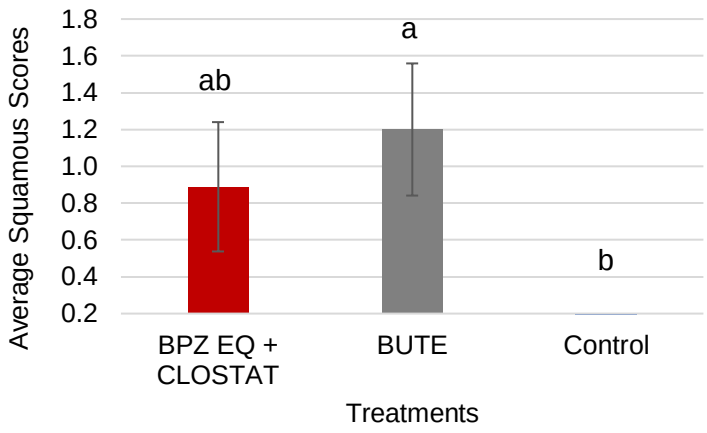

Figure 1B

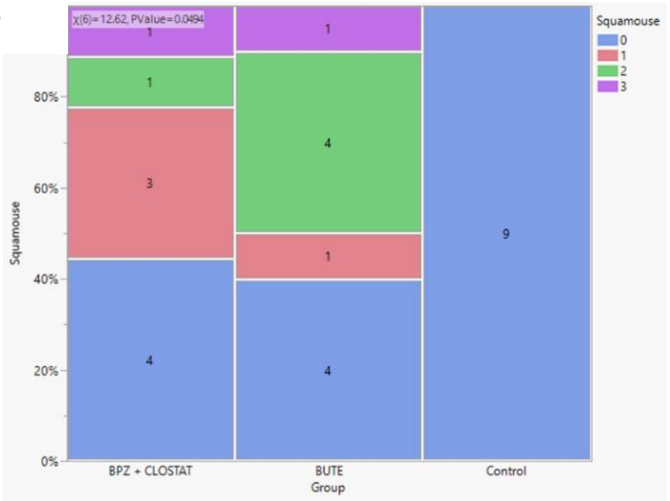

Figure 1. Average (1A) and Chi-Square (1B) Squamous scores at 10 DOT in control horses and horses challenged with either phenyl butazone or challenged and supplemented with BPZ EQ and CLOSTAT for 10 days. Avg ± SE. [a,b]Superscripts indicate significant differences between treatments.

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Figure 2A

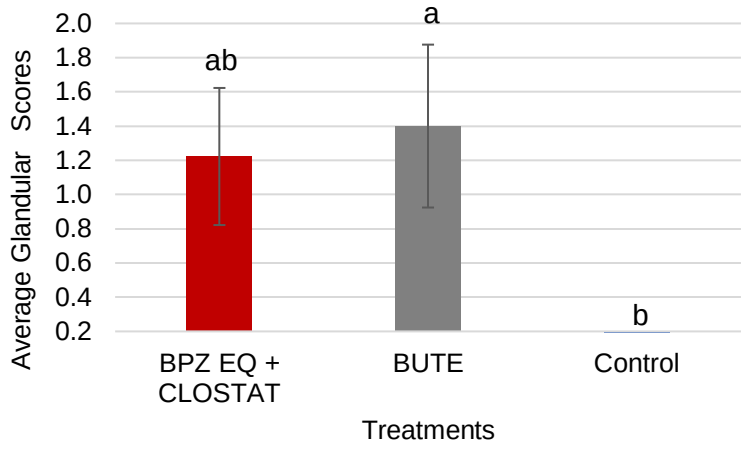

Figure 2B

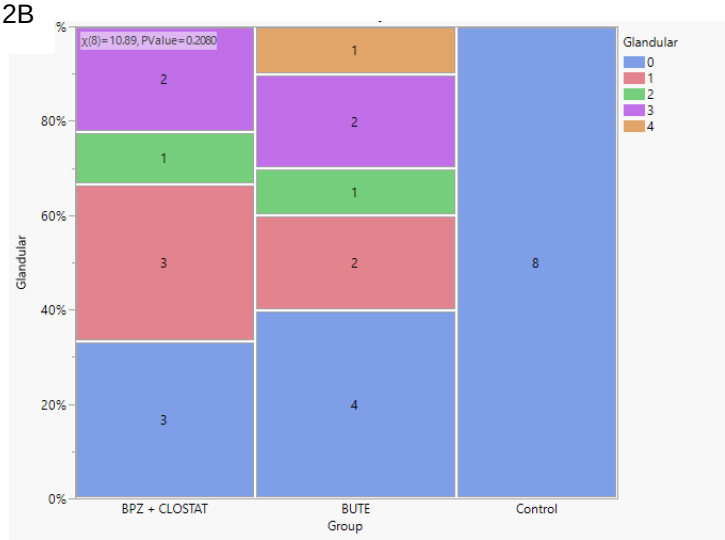

Figure 2. Average (2A) and Chi-Square (2B) Glandular scores at 10 DOT in control horses and horses challenged with either phenyl butazone or challenged and supplemented with BPZ EQ and ClOSTAT for 10 days. Avg ± SE. [a,b]Superscripts indicate significant differences between treatments. *One control horse was removed from the statistical analysis for non-conformity.

Circulating 16s rDNA:

There was no difference among treatments with 16s rDNA during the challenge period (0 to 15 DOT; Figure 3). During the pretreatment period (-14 to 0 DOT), there was a signficant difference between control (control and BUTE combined) and BPZ EQ + CLOSTAT at 0d (P= 0.0003; Figure 4).

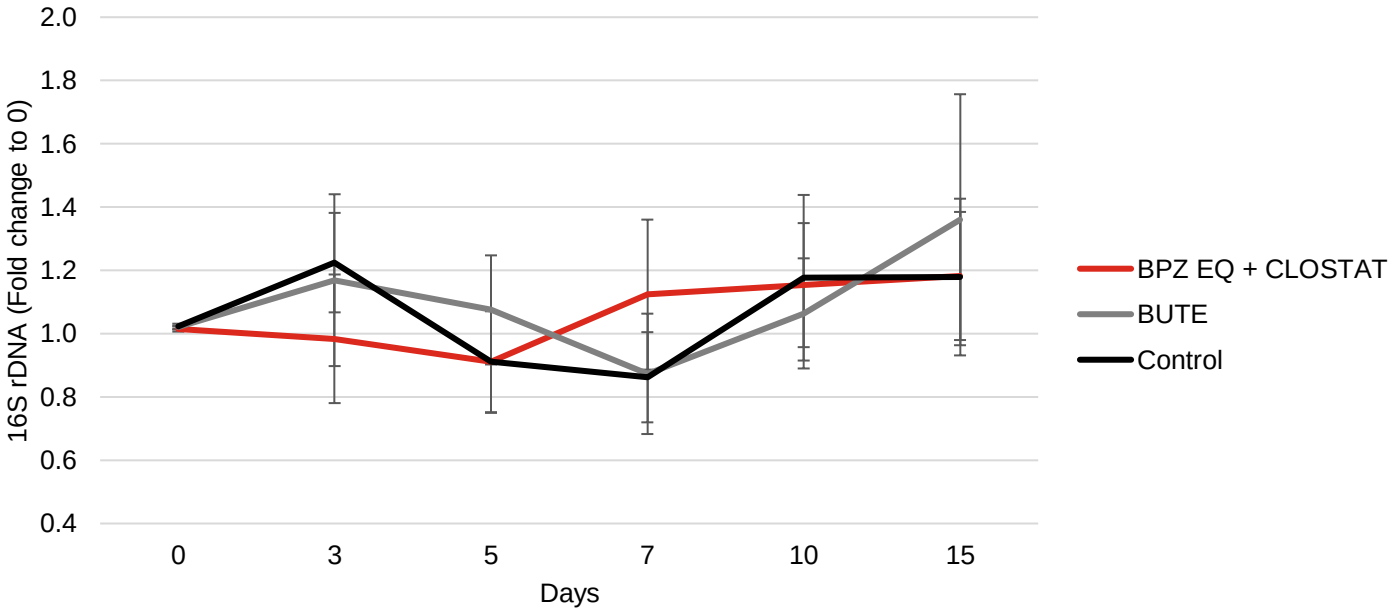

Figure 3. Fold Change of 16S rDNA for each treatment within the challenge period. Avg ± SE

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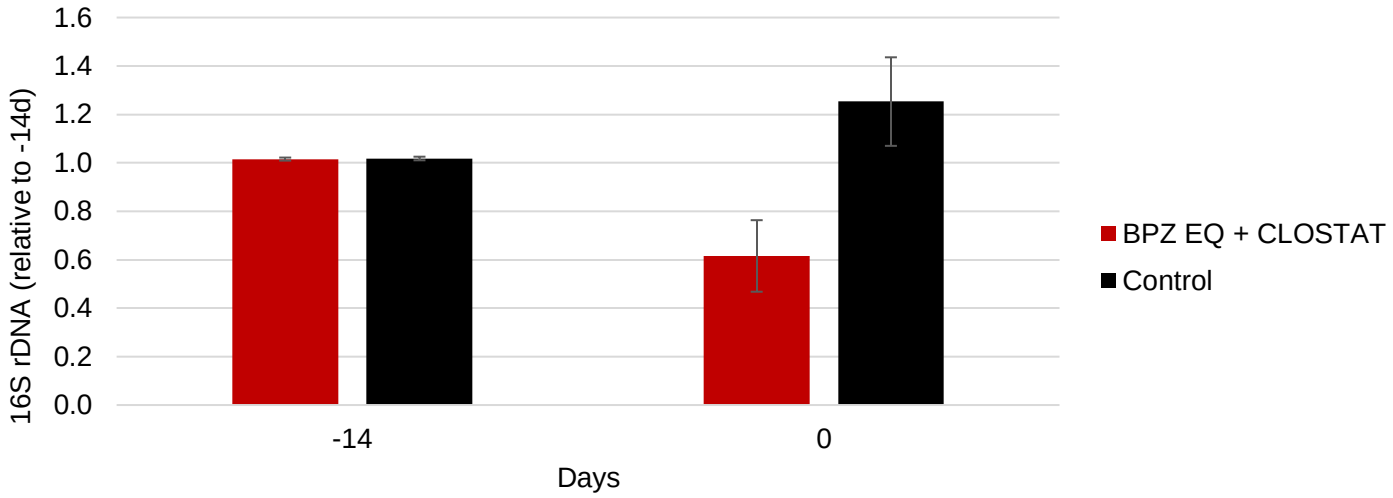

Figure 4. Fold Change of 16S rDNA for each treatment prior to challenge.
Control and BUTE horses were combined for ANOVA analysis. Avg ± SE Contrasts: -14d Control vs. 0d Control, P =0.15; -14d BPZ vs. 0d BPZ EQ, P=0.10; 0d Control vs. 0d BPZ EQ, P=0.003

Conclusions

BPZ EQ + CLOSTAT was palatable based on normal eating behavior of the horses, and no changes in body weight across treatments were observed during the testing period. Overall, BPZ EQ + CLOSTAT showed an effect on squamous and glandular ulcers (during the challenge period) and 16s rDNA (prior to challenge) compared to the control and/or BUTE. These results indicate that the combination of BPZ EQ + CLOSTAT provides a protective effect to the gut barrier under NSAID induced challenge. In order to investigate further on the effect of BPZ EQ + CLOSTAT combination on gut health, enterically-challenged horses need to be used.

References

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6. Effect of CLOSTAT and ButiPEARL Z EQ on Non-steroidal Anti-Inflammatory Drugs (NSAID)-Induced gastrointestinal Inflammation in Horses -TD-22-8083

BARASTOC

FOOD FOR THOUGHT

TECHNOLOGY INSIDE
Diamond V
Formulated with Diamond V® XPC

- DIAMOND V® XPC -

All-natural, fermentation-based product for use in equine diets, Diamond V XPC, is produced using proprietary anaerobic fermentation technology of Saccharomyces cerevisiae. This unique fermentation process produces metabolites that promote robust digestive health and improve the resilience and functions of the immune system. Research has shown that Diamond V XPC supports:

- ☑ Improved gut morphology
- ☑ Feed digestibility and efficiency
- ☑ Recovery from work, injury or stress
- ☑ Immune function by reducing systemic inflammation and antioxidant status
- ☑ Digestive health by promoting a healthy balance of bacteria in the lower gastrointestinal tract

In terms of the equine performance of athletes' horses, Diamond V has been shown to affect the nutrients digestibility, nitrogen retention (higher protein digestibility) and exercise parameters. Dietary energy fuels a horse to perform with both speed and endurance. Research results have shown that the inclusion of Diamond V in horse diets increases digestible energy by improving the nutrients' digestibility. Research has shown that feeding Product affects plasma fatty acid levels, plasma lactic acid concentrations, hemoglobin and packed cell volumes of the blood, and horses' overall heart rate during and after exercise.

Lipid metabolism (turnover) is correlated to concentrations of FFA in the blood. Plasma FFA concentrations increase during and immediately following exercise. Research reported that Diamond V supplemented horses had a slower rise in plasma FFA rates during an exercise period indicating a more efficient uptake of FFA by the working muscles. In another study, scientists reported that FFA levels in animals following exercise remained elevated, suggesting that dietary supplements of Diamond V Product may enhance the oxidation of free fatty acids, especially during the recovery period. This indicates a possible conversion of energy usage from muscle glycogen to fat utilization.

BARASTOC

FOOD FOR THOUGHT

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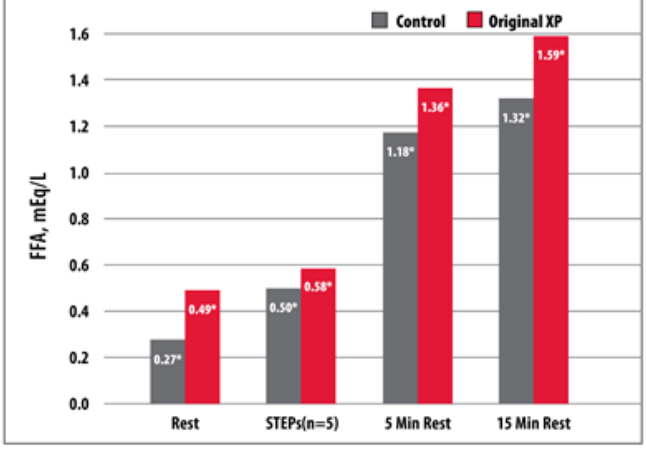
A research result confirmed in a standardized exercise test that Diamond V supplementation in horses increased blood FFA concentrations significantly at every STEP and during REST periods before and after exercise (Figure 1). The results indicate increased fat utilization and a sparing of blood glucose in the exercised horse.

Blood plasma lactate levels are proportional to the rate of intramuscular production of lactic acid from exercise intensity and duration in horses. In essence, lactate is produced throughout the exercise. Blood lactate is an assessment of muscle metabolism and is related to fatigue.

FIGURE 1
Effect of Diamond V Original XP on free fatty acid (FFA) concentration



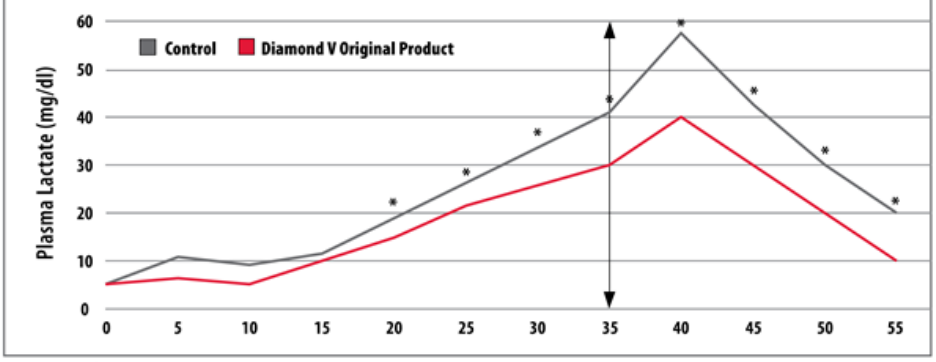
Wickler et al, 2002.
* $P < 0.05$

Diamond V exhibited significantly smaller and slower increases in plasma lactate concentration (Figure 2). This effect was more remarkable and became statistically significant as the length of exercise increased from 20 minutes or longer. Researchers reported a numerical reduction in blood lactate levels during the exercise portion of a standardized exercise test in horses. Any treatment that decreases the lactic acid production in the muscle would potentially increase the athletic capacity of the animal.

BARASTOC

FOOD FOR THOUGHT

FIGURE 2
Effect of Diamond V Original Product on plasma lactate levels in horses exercising for 35 minutes, followed by 20 minutes resting period



Glade and Campbell-Taylor, 1990.
* $P < 0.01$

In horses, lower heart rates indicate relatively reduced cardiac output and therefore increased efficiency in energy metabolism and oxygen utilization. This leads to a greater capacity for performing a given amount of work by the animal. A 35-minute exercise trial showed significantly lower heart rates in horses supplemented with Diamond V during the first 5 and final 10 minutes of the workouts (Glade et al., 1990). In another study,

it reported that the velocity required to maintain 160 beats per minute at the 5th minute of exercise averaged 15.26 and 16.21 feet per second in control and Diamond V supplemented horses, respectively (Miller-Graber et al. 1994). This means that the horses fed Diamond V maintained the same heart rate while running faster than unsupplemented horses. Lowered heart rates in responses to workloads are a good indication of the relative improved fitness of horses.

ButiPEARL™ Z EQ

GUT H3ALTH SOLUTIONS
BUILD UP

ButiPEARL™ Z EQ — the first product of its kind on the market — provides supplemental butyric acid and zinc to help strengthen the intestinal tract of the horse.

WHY FEED ButiPEARL Z EQ?

Strengthening the lining of the gut is vital to the health and performance of the horse, as it:

- ✔ Improves nutrient absorption
- ✔ Provides a strong barrier against pathogens, parasites and toxins

If the intestinal barrier is compromised — either through damage of the cells that make up the lining or by breakdown of the tight junctions — harmful substances can cross into the bloodstream. This barrier damage is typical of horses under stress and is often referred to as Leaky Gut Syndrome (LGS).

Providing two powerful nutrients (butyric acid and zinc) directly to the cells that make up the intestinal barrier can lead to a lower incidence of LGS, thereby improving the performance, behavior and overall health of your horse.

THE SCIENCE BEHIND ButiPEARL Z EQ

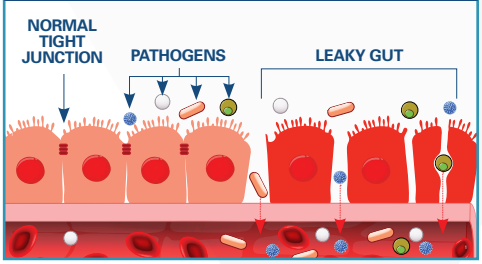
The unique encapsulation technology used to make ButiPEARL Z EQ ensures that the target nutrients are released slowly throughout the entire intestinal tract of the horse. Through this timely release process, the tight junctions are coated and strengthened — improving the integrity of the intestinal lining.

- ✔ Butyric acid functions:
  - Strengthen tight junctions[1,2]
  - Provide energy source used in the growth and development of the intestinal lining[3]
  - Support immune function[4]
  - Reduce gut inflammation[4]
- ✔ Zinc functions:
  - Strengthen tight junctions[5]
  - Expedite wound healing[6]

ButiPEARL Z EQ BENEFITS

- **Provides two important nutrients that play a vital role in gut health — butyric acid and zinc**
- **Encapsulation technology allows for release throughout the upper and lower gut**
- **Improves overall gut health, which can lead to improved performance and wellbeing**

BUILD A BARRIER AGAINST LEAKY GUT



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