

Effect of Coated Probiotic and Butyric Acid on Non-steroidal Anti-Inflammatory Drugs (NSAID)-Induced Gastrointestinal Inflammation in Horses

Abstract

This study was conducted to determine the effect of Coated Probiotic and Butyric Acid 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 PB and BA), Phenylbutazone (BUTE; 4.4 mg/kg every 24 hrs; no additive), and BUTE + Butyric acid (4 g/hd/d) + Probiotic (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. butyric Acid and Probiotic 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 Butyric Acid + Probiotic can provide a protective effect to the intestinal barrier. Further investigation is needed using a more enterically-challenged model.

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. Butyric acid is an encapsulated butyric acid and zinc product that is released in a controlled manner along the intestinal tract. In addition, Probiotic contains a unique, patented strain of *Bacillus subtilis* PB6. Although the benefits of Butyric Acid and Probiotic 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 Butyric Acid and Probiotic 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 Butyric Acid + Probiotic)
2. Phenylbutazone (BUTE; 4.4 mg/kg every 24 hrs; no additive)
3. BUTE + Butyric Acid(4 g/hd/d) + Probiotic (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 Butyric Acid + Probiotic 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 Butyric Acid and Probiotic.

On 1 DOT, oral paste containing BUTE was given to the BUTE only and Butyric acid + Probiotic 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. Butyric Acid + Probiotic). 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 Butyric Acid + Probiotic 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).

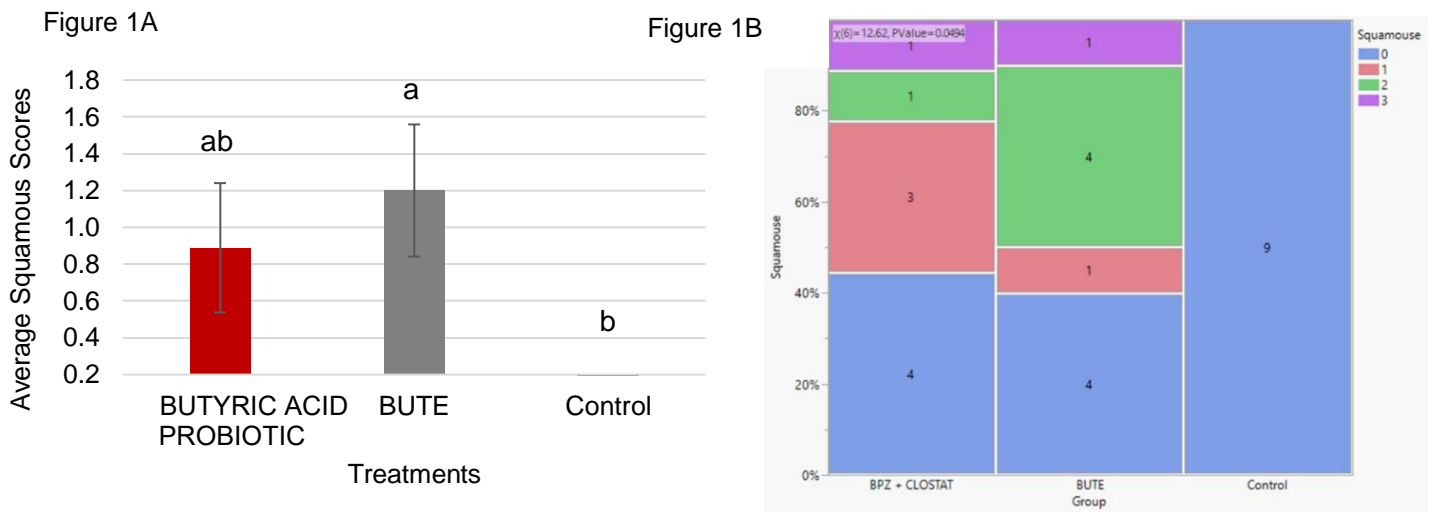


Figure 1. Average (1A) and Chi-Square (1B) Squamous scores at 10 DOT in control horses and horses challenged with either phenyl butazone or challenged and supplemented with Butyric acid and Probiotic for 10 days. Avg ± SE. ^{a,b}Superscripts indicate significant differences between treatments.

Figure 2A

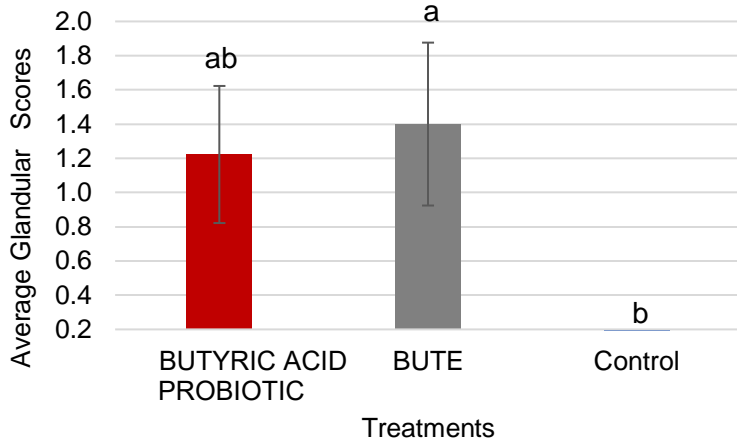


Figure 2B

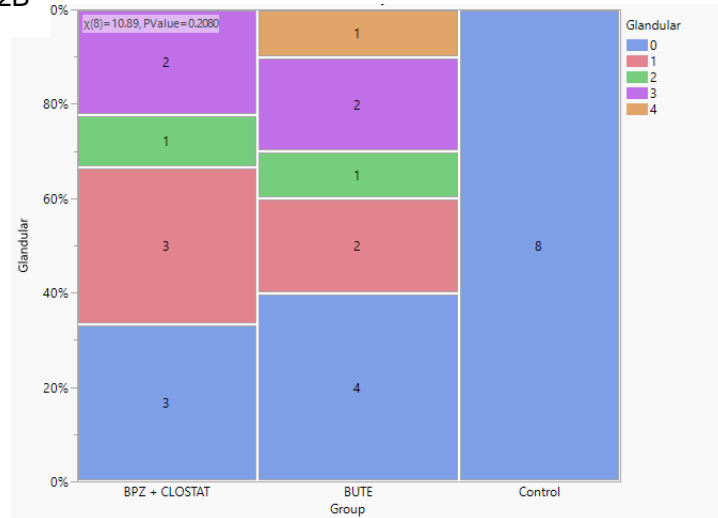


Figure 2. Average (2A) and Chi-Square (2B) Glandular scores at 10 DOT in control horses and horses challenged with either phenyl butazone or challenged and supplemented with Butyric Acid and Probiotic for 10 days. Avg ± SE. ^{a,b}Superscripts indicate significant differences between treatments. *One 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 significant difference between control (control and BUTE combined) and Butyric acid + Probiotic at 0d (P= 0.0003; Figure 4).

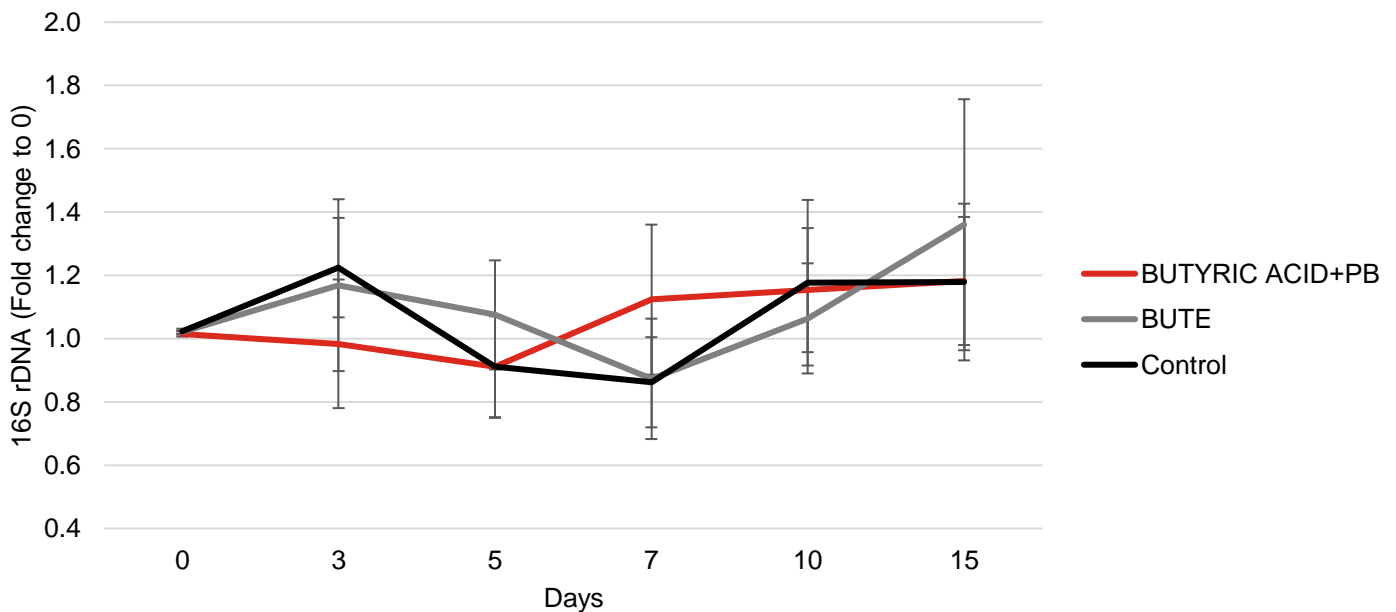


Figure 3. Fold Change of 16S rDNA for each treatment within the challenge period. Avg ± SE

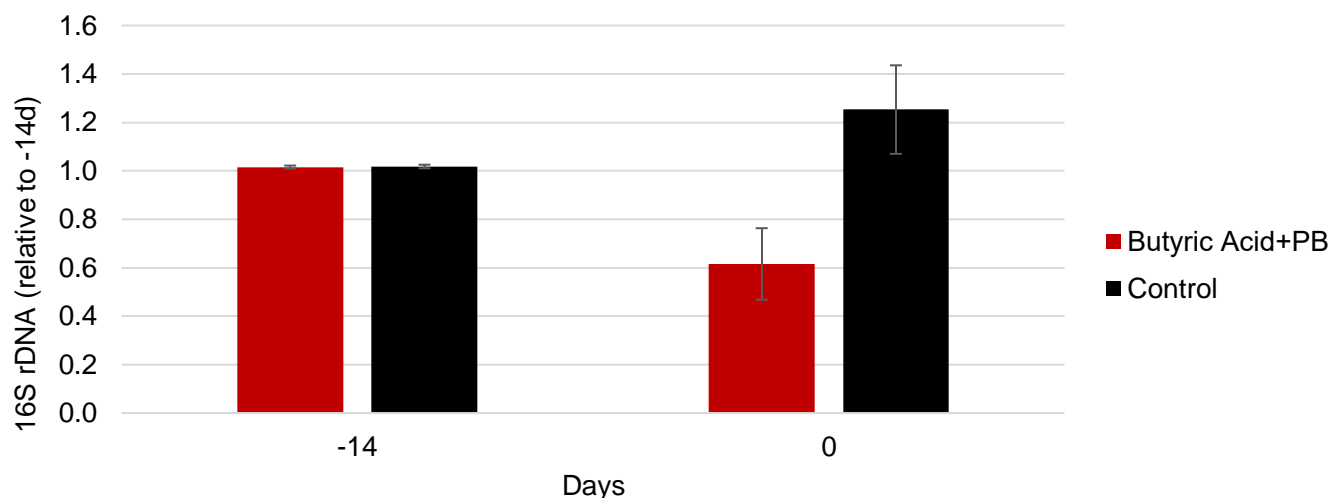


Figure 4. Fold Change of 16S rDNA for each treatment prior to challenge.

Control and BUTE horses were combined for ANOVA analysis. Avg \pm 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

Butyric acid + Probiotic 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, Butyric acid + Probiotic 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 Butyric acid and Probiotic provides a protective effect to the gut barrier under NSAID induced challenge. In order to investigate further on the effect of Butyric acid and Probiotic combination on gut health, enterically-challenged horses need to be used.

References

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