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Evaluation of the Protective Effects of Butyric Acid and Zinc on Swine Intestinal Epithelial Cells Under Inflammatory Challenge and Heat Stress

Inflammatory challenges and heat stress are encountered by livestock and poultry regularly.

Both stressors have immense potential to decrease growth and production performance.

By adding encapsulated butyric acid and zinc at 500 ppm to diets, it is possible to:

- *Reduce the negative effects of inflammatory challenge and heat stress on growth performance.*
- *Reduce the cost per weight gained.*

This study shows butyric acid and zinc may reduce animal stress and may improve feed efficiency with a lower cost per pound of gain.

ABSTRACT

Butyric acid and zinc supplementation help maintain intestinal integrity and barrier function which is beneficial to livestock and poultry2,3. An encapsulated source of butyric acid and zinc (BPZ) was used in this study in a 2:1 ratio. Measuring the transepithelial resistance (TER) under a lipopolysaccharide (LPS) inflammatory challenge and heat stress conditions over a period of 36-48 hours, BPZ along with other comparable treatments were tested for their protective effects toward intestinal epithelial barrier function using pig intestinal epithelial cells (IPEC-J2). BPZ improved the TER under each stress condition indicating protective effect against inflammatory challenge and heat stress ($P < 0.05$).

Encapsulated source of butyric acid and zinc in MicroPEARLS®.

INTRODUCTION

Zinc is an essential trace mineral which is necessary for the normal growth and performance of animals[2]. Zinc has been shown to be involved in the activity of more than 300 enzymes and has the potential to influence immune function and intestinal health[4]. Zinc is typically supplemented as zinc sulfate in animal diets but other forms of zinc are available including zinc propionate and zinc oxide. There is a possibility the alternative forms of zinc may be more beneficial during a stress condition, such as inflammatory stress or heat stress, rather than during the normal growth period. Butyric acid is a short chain fatty acid usually synthesized in the hindgut by bacterial fermentation of non-digestible fibers[5]. Apart from serving as an energy source, butyric acid has been shown to have beneficial effects toward intestinal health including maintaining villus height and crypt depth, balancing the normal microbial flora, and maintaining osmotic balance[3]. Butyric acid is commonly supplemented in livestock and poultry feed as a combination of either butyric acid and sodium or butyric acid and calcium[6].

It was hypothesized combining butyric acid and zinc would have beneficial effects toward the intestine, particularly if supplemented during a stress condition, like inflammatory challenge or heat stress. Butyric acid and zinc effects were tested in an *in-vitro* cell culture model. Transwell plates offer an opportunity in which intestinal epithelial cells can be grown on a membrane filter. The cells differentiate into a fully functional state and form the apical and basolateral compartments as well as tight junctions by using this method[7]. BPZ was tested using IPEC-J2 grown on transwell plates under various conditions to study its protective effects on intestinal integrity and barrier function.

MATERIALS AND METHODS

Inflammatory challenge and heat stress. IPEC-J2 cells were seeded onto polycarbonate filter cell culture chamber inserts and TER was measured using an epithelial voltohmmeter (World Precision Instruments Inc., Sarasota, FL). When the cells

attained peak TER, approximately 9 days post confluence, cells were treated with zinc sulfate, zinc chloride, butyric acid and zinc or calcium chloride for 36-48 hours. Ten (10) µg/mL LPS was used to stimulate an inflammatory challenge. The treatments were co-incubated with LPS and TER was measured over 36-48 hours. For the heat stress experiments, the cells were incubated either at 37°C or at 41.5°C.

RESULTS

Experiments were conducted with 100 µM zinc and different butyric acid and zinc sources commonly used in livestock and poultry feed. The cells were subjected to an inflammatory challenge with bacterial LPS at a 10 µg/mL dose (Fig. 1). Statistical differences were observed after 24 hours, where BPZ and zinc sulfate had numerically higher values at 24 hours. At 36 hours, BPZ TER was statistically higher than all the other treatments indicating a protective effect.

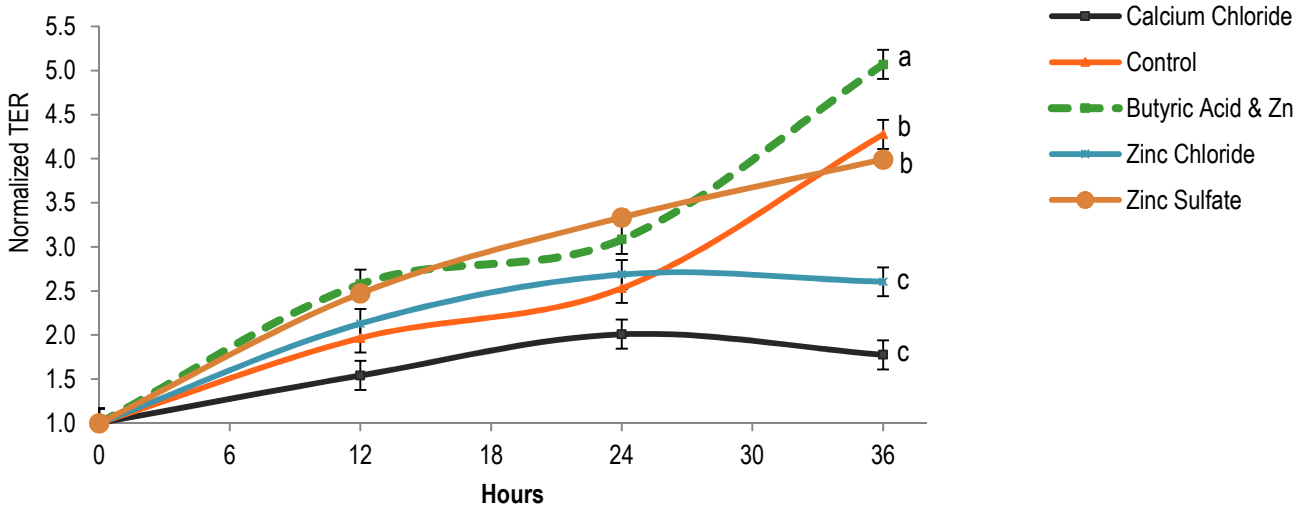

Figure 1. Effect of 100 µM BPZ on IPEC-J2 cells under inflammatory challenge conditions. Cells were treated with 10 µg/mL LPS to stimulate the inflammatory challenge. Different superscripts (a, b, c) indicate a significant difference in values.

Since heat stress is a major issue affecting the intestine of livestock and poultry, BPZ was also tested in a heat stress model with the IPEC-J2 cells. Twelve hours after heat stress, BPZ had a similar TER as Control-TN group. Control-HS had the lowest TER and zinc sulfate had intermediate TER values. At 36 and 48 hours, Control-TN and Butyric acid and Zinc-HS had the highest TER, indicating the protective effect of BPZ which is equivalent to thermoneutral cells (Fig. 2).

In commercial applications, zinc is typically supplemented as zinc sulfate and is present in the vitamin trace mineral premix. BPZ can be added on top of the zinc sulfate already present in the mineral mix. The next experiment was performed in which the treatments were added on top of zinc sulfate under heat stress conditions (Fig. 3).

CONCLUSIONS

Butyric acid and zinc have been shown to benefit the intestine in normal and stress conditions. Efficacy of BPZ was tested using intestinal epithelial cells under normal, inflammatory challenge and heat stress conditions. The data presented provides evidence BPZ helps to protect the cells from inflammatory challenge as well as heat stress. Under inflammatory challenge and heat stress conditions, BPZ treated cells performed better than other treatment groups. This indicates that beneficial effects of butyric acid and zinc can be obtained through a combination product of BPZ.

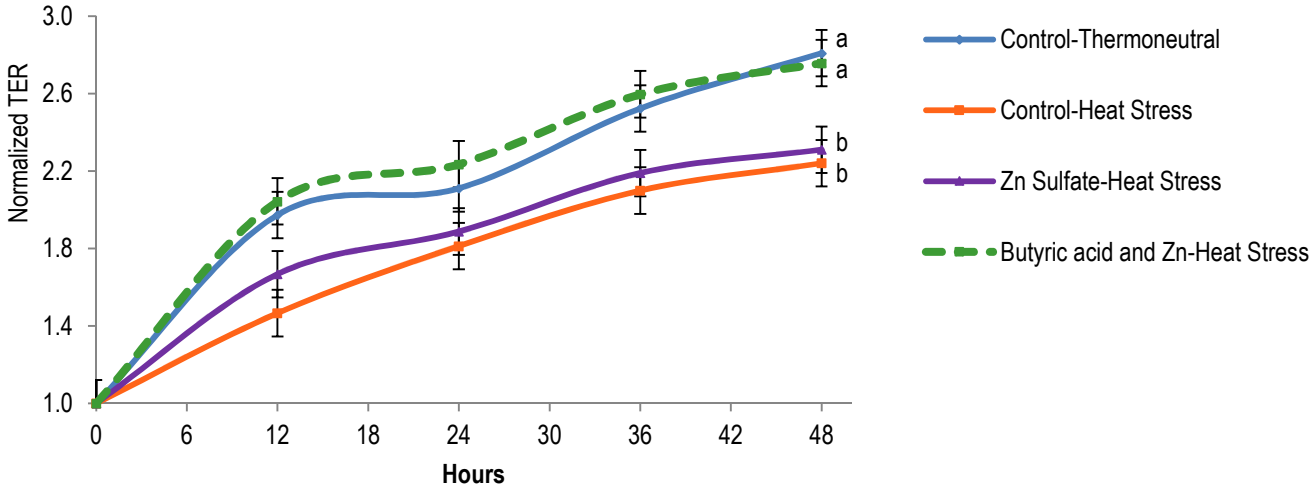

Figure 2. Effect of 300 µM concentration of different test compounds on IPEC-J2 cells under heat stress conditions. Cells were treated with the test compounds and incubated at 41.5°C except for the Control-Thermoneutral treatment which was incubated at 37°C. Different superscripts (a, b) indicate a significant difference in values.

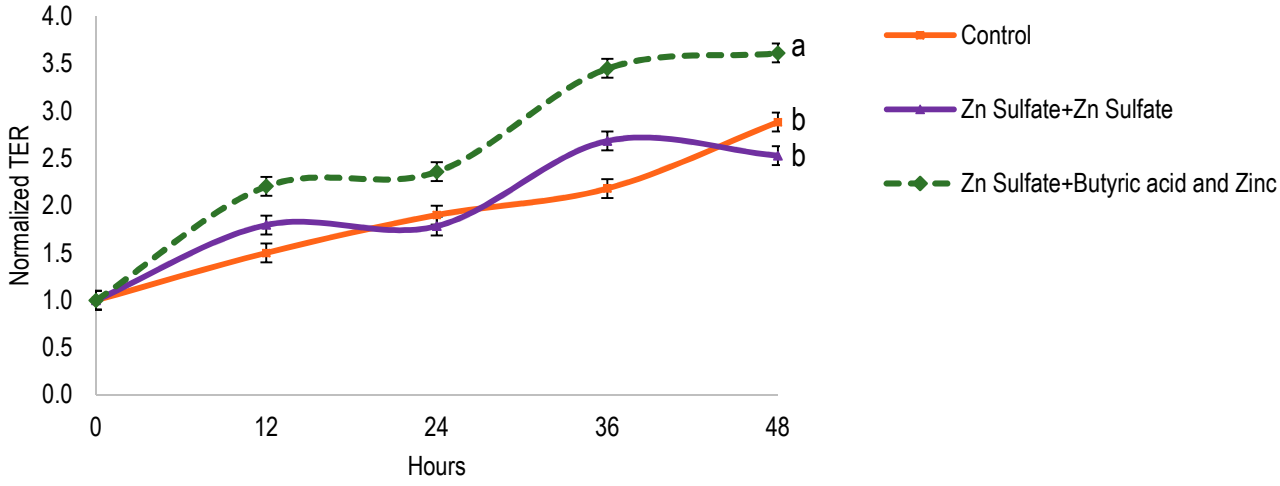

Figure 3. Effect of 300 µM concentration of different test compounds on IPEC-J2 cells under heat stress conditions added on top of zinc sulfate. Cells were treated with the test compounds and incubated at 41.5°C. Different superscripts (a, b) indicate a significant difference in values.

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