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Effects of supplemental zinc concentration on cellulose digestion and cellulolytic and total bacterial numbers *in vitro*

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ABSTRACT

Effects of various zinc (Zn) concentrations on cellulose digestion and cellulolytic and total bacterial numbers were studied *in vitro*. All fermentation tubes were inoculated with a 1:10 dilution of whole rumen contents and used either 0.0075 or 0.015 g/ml purified cellulose as substrate. When Zn was added at six different concentrations: 5, 10, 15, 20, 25 and 50 μg/ml, using either 0.0075 or 0.015 g/ml cellulose, the 50 μg/ml concentration reduced cellulose digestion ($P<0.01$) at 24 h of incubation. However, cellulose digestion was similar to the control tubes at 48 h. At 24 h, addition of Zn at 50 μg/ml to the medium containing 0.0075 g/ml cellulose lowered cellulose digestion ($P<0.016$), but did not affect cellulolytic and total bacterial concentrations. By 48 h, cellulolytic bacterial concentrations in the control tubes were lower ($P<0.001$) than in the Zn supplemented tubes. Total bacterial concentrations were also lower ($P=0.076$) for control tubes. Using 0.015 g/ml cellulose and Zn concentrations of 0, 50, 100 and 150 μg/ml, cellulose digestion after 24 h was lower than the control for all tubes with added Zn ($P<0.01$). By 48 h, cellulose digestion was similar in tubes containing 0 and 50 μg/ml concentrations of Zn, while digestion remained lower with concentrations of 100 and 150 μg/ml ($P<0.01$). After 72 h, only the 150 μg/ml Zn concentration had less cellulose digested ($P<0.05$). Final pH values followed a similar pattern: the higher the Zn concentration, the less decrease in pH. Concentrations of cellulolytic bacteria were lower ($P<0.001$) in all tubes with Zn after 24 h

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incubation, but their concentration in the control tubes decreased markedly between 24 and 48 h, presumably due to low pH and substrate limitation. No differences were observed in total bacterial concentrations at any fermentation time period. The decreased cellulolytic bacterial concentration in the tubes with high Zn levels may be due to decreased utilization of cellulose by the bacteria, rather than a toxic effect of high Zn on the bacteria. Possibly Zn has an inhibitory effect on the cellulolytic enzymes produced by the bacteria.

1. Introduction

Zinc is an essential trace mineral for all forms of life because of its fundamental role in gene expression, cell development and replication as well as its presence in many enzymes. Zn is required in the diet of both ruminants and rumen microorganisms for proper metabolic function. Zn generally is added to diets to ensure that nutritional requirements are met, however the dietary Zn supply for ruminants often exceeds actual requirements. Zn concentrations of 500 mg/kg of diet will cause toxicity in beef cattle (National Research Council, 1980), however most livestock species tolerate concentrations as high as 1000 mg/kg in the diet (McDowell, 1992). Miller et al. (1989) demonstrated that feeding lactating Holstein cows diets containing 1000 ppm of supplemental Zn as zinc sulfate had no adverse effects on milk production, feed intake, body weights, animal health or reproduction. Bateman et al. (2004) suggested that supplementing Zn well above requirements could alter ruminal fermentation to capture increased feed energy as volatile fatty acids. These reports suggest that ruminants can be fed diets containing high Zn without adverse effects. In addition, recent studies have demonstrated that supplementations of organic and inorganic combinations of Zn enhance performance, and improve health and reproduction of ruminants when supplemented at or above six times the recommended level for sheep, goat and dairy cows (Gaynor et al., 1988; Hatfield et al., 1995; Malcolm-Callis et al., 2000; Eryavuz et al., 2002; Salama et al., 2003; Spears, 2003; Kellogg et al., 2004).

Ruminal cellulose digestion is a complex microbial process that involves adhesion of microbial cells to cellulose, cellulose hydrolysis, and fermentation of the resulting cellodextrins and sugars to VFA, methane, and CO_2 (Pell and Schofield, 1993). Cellulolytic bacteria are known to first attach to plant particles and then to produce enzymes that slowly degrade the plant cell wall structure. If cellulose digestion decreases in the rumen, this can increase gut-fill and subsequently decrease voluntary intake when animals consume a diet based on forage (Russell, 1998). At this time, very little is known about the effects of zinc supplementation on ruminal cellulose digestion. Although the influence of zinc sulfate on cellulose digestion (Martinez and Church, 1970; Bonhomme et al., 1979) and rumen protozoal concentrations (Froetschel et al., 1990) has been investigated, none of these studies attempted to associate cellulose digestibility in the diet with Zn levels or with the concentrations of cellulolytic and total bacteria. The objectives of our study were to determine *in vitro* the concentrations of Zn that are toxic to the bacteria.

2. Materials and methods

2.1. Media and culture

The basal purified cellulose medium used in experiment 1 contained the following ingredients per 100 ml: 15 ml each of mineral solutions I and II of Bryant and Burkey (1953), 0.1 ml of a 0.1 g/100 ml resazurin solution, 25 ml of a 3 g/100 ml suspension of 24 h ball-milled cellulose (Sigmacell-20; Sigma, St. Louis, MO, USA), 40 ml of rumen fluid (the supernatant obtained from centrifugation at $1000 \times g$ for 10 min), 3.33 ml of 12 g/100 ml Na_2CO_3 and 1.67 ml of 3 g/100 ml cysteine hydrocholoride. Eight-milliliter aliquots were tubed under O_2-free CO_2 into 16 by 150-mm culture tubes, closed with rubber stoppers, and autoclaved in racks at 121 °C for 20 min (Dehority, 1969). An additional 1 ml of either