Original paper

EFFECTS OF CADMIUM ON RUMEN FERMENTATION AND NUTRIENT DIGESTIBILITY USING DUAL FLOW CONTINUOUS CULTURE SYSTEM

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Abstract

This experiment was conducted to investigate the effects of different doses of cadmium on fermentation characterization and nutrient digestibility using dual flow continuous culture system. Eight dual-flow, continuous culture fermenters were used in 2 replicated periods of 10 d (7 d of adaptation and 3 d of sampling). Fermenters were inoculated with a composited ruminal fluid from 3 beef steers fed a high concentrate diet for at least 2 mo before the beginning of the trial. Anaerobic conditions were maintained by the infusion of N_2 at a rate of 40 mL/min. Temperature (38.5°C) and liquid (10%/h) and solid (5%/h) dilution rates were kept constant. Fermenters were fed daily with 120 g dry matter in three equal portions. Treatments arranged as complete randomized block design with control and addition three levels of cadmium (0.1, 1 and 10 mg/l) to the high concentrate diet (15:85 forage to concentrate ratio). Organic matter, dry matter, NDF and ADF digestibility significantly decreased with the addition of 1 and 10 mg/l cadmium to the culture media compered to control treatment ($P<0.05$). Cadmium significantly decreased total volatile fatty acids concentration, acetate proportion and acetate to propionate ratio. Propionate and butyrate proportion increased significantly in response to cadmium addition ($P<0.05$). Concentration of NH3-N was significantly decreased in 1 and 10 mg/l cadmium treatments compared with control treatment. The results of this study suggest that cadmium in doses of 1 mg/l or more had strong inhibitory effect on mixed rumen microorganisms.

Key words: *Cadmium, Digestibility, Rumen fermentation, Toxicity.*

Introduction

Heavy metals are well known to be toxic to most organisms when present in excessive concentrations (Giller et al., 1998). Cadmium, a heavy metal, is a member of group IIb in the periodic table of elements which is present in soils, sediments, air and water (Stoeppler, 1991). Cadmium (Cd) is a non-essential heavy metal which accumulates in mammals and is potentially toxic to both humans and animals. It accumulates in many agricultural crops, mainly as a result of phosphate fertilizer and sewage sludge use, and presents a significant

risk to humans consuming high levels of food products from hyper accumulators, such as offal products derived from grazing animals (Wilkinson et al., 2003). Cadmium is transported into microorganisms by the energy-dependent manganese or magnesium transport systems (Nies and Silver, 1999). Cd is highly toxic (Babich and Stotzky,1977). It competes with and replaces other functional metals inside cells (Hughes and Poole, 1989). It also brings about the denaturation of proteins, inhibits bacterial respiration and proton–solute cotransport, and causes single-stranded breaks in cellular DNA (Cunningham and Lundie, 1993). Many microorganisms have evolved mechanisms to tolerate and overcome Cd toxicity. A major mechanism for heavy metal resistance involves alterations in the membrane transport system of an organism, resulting in the reduction or denial of entry of Cd into the organism (Laddaga et al., 1985). Alternatively, the intracellular or extracellular sequestration of heavy metals by adsorption to cell walls (Mullen et al., 1989) or by binding to a specific biopolymer results in tolerance to heavy metal toxicity (Kurek et al., 1991). Heavy metals can be stimulatory, inhibitory, or even toxic for biochemical reactions, depending on their concentrations (Gikas and Romanos, 2006). The presence of many metals is required in trace amounts for the activation or functioning of many enzymes and coenzymes. Excessive amounts, however, can lead to inhibition or toxicity (Juliastuti et al., 2003). The various mechanisms of metal toxicity in microorganisms are (1) substitutive ligand binding, (2) redox reactions with sulfur groups, (3) Fentontype reactions, (4) inhibition of membrane-transport processes, and (5) electron siphoning (Harrison et al., 2007). The aim of this study was to detect the toxic effect of cadmium on rumen microbial fermentation and nutrient digestibility using dual flow continuous culture system.

Material and methods

Apparatus and experimental design

Eight dual-flow continuous culture fermenters (Hoover et al., 1976) were used in 2 repeated periods of 10 d each (7 d of adaptation, 3 d of sampling). Fermenters were inoculated with a composited ruminal fluid taken after slaughter from 3 beef cattle fed a high-concentrate diet. Donor animals were therefore different in each period. Temperature (38.5°C) and liquid (0.10/h) and solid (0.05/h) dilution rates were maintained constant. Anaerobic conditions were maintained by the infusion of N2 at a rate of 40 ml/min. Artificial saliva (Weller and Pilgrim, 1974) was continuously infused into flasks and contained 0.4 g/l of urea to simulate recycled N. Fermenters were fed 97g of DM/d in 3 equal portions, at 08:00, 16:00, and 24:00 h.

Treatments arranged as complete randomized block design with control and addition three levels of cadmium (0.1, 1 and 10 mg/l) to the high concentrate diet (15:85 forage to concentrate ratio).

Sample collection, processing and chemical analysis

During sampling days, collection vessels were maintained at 4°C to impede microbial action. Solid and liquid effluents were mixed and homogenized for 1 min, and a 500-ml sample was removed via aspiration. Upon completion of each period, effluent from the 3 d of sampling was composited and mixed within fermenter and homogenized for 1 min. Subsamples were taken for total N, ammonia-N, and VFA analyses. The remainder of the sample was dried. Dry samples were analyzed for DM, ash, NDF and ADF.

Dry samples were ashed overnight at 550°C in a muffle furnace (AOAC 1990; ID 942:05). Ether extract was analyzed by Soxhelet, and total N was determined as described by AOAC (1990; ID 976.05) procedures. The NDF were analyzed sequentially by the

detergent system (Van Soest et al., 1991). Samples for VFA were prepared using 4-methylvaleric acid (Aldrich Chemical Company, Milwaukee, WI, USA) as the internal standard. The analysis was performed by GLC using a polyethylene glycol nitroterephthalic acid-treated capillary column. A 4-ml subsample of filtered fluid was acidified with 4 ml of 0.2 N HCl and frozen. Samples were centrifuged at 25000 × g for 20 min, and the supernatant was analyzed for ammonia-N (Chaney and Marbach, 1962).

Statistical analysis

Data were analyzed as a randomized block design using PROC MIXED of SAS (version 8.1; SAS Inst., Inc., Cary, NC). Differences in average between treatments were declared at P <0.05 using Tukey's multiple comparison test, and least squares means for treatments are shown.

Results and discussion

Table 1. *Effects of Cadmium on Apparent nutrient digestibility in continuous culture*

Parameters	Control	0.1 mg Cd/l	1.0 mg Cd/l	10 mg Cd/l	SEM	P value
DM	53.2[a]	52.1[a]	50.8[b]	22.6[c]	1.40	0.001
OM	47.8[a]	46.4[a]	45.3[b]	18.4[c]	1.01	0.001
NDF	40.1[a]	39.6[a]	36.2[b]	14.3[c]	2.12	0.001
ADF	38.4[a]	38.2[a]	34.9[b]	11.6[c]	2.33	0.001

[a,b,c] Means within a row with different superscripts differ (P < 0.05)

Addition of 1 and 10 mg Cd/l significantly decreased apparent dry matter, organic matter, NDF and ADF digestibility. Low level of Cd (0.1 mg Cd/l) had no significant effect on nutrient digestibility. It has been reported that sensitivity of ruminal bacteria to heavy metals is depends on bacterial species and conditions which affect their metabolic activity (Faixová and Faix, 2002; Mihaliková et al., 2009). Individual species of rumen bacteria differed from one another in their sensitivities to various heavy metals (Forsberg, 1978). Salem et al (2010) reported when the inhibitory effect of Cd was examined on separated bacterial and protozoal fractions, it was more inhibitory to bacteria.

Although Cd is extremely toxic, some bacteria can overcome its adverse effects or resist its presence at low concentrations. Divalent ion transport systems are normally required to transport essential metals such as magnesium, phosphate, and sulfate (Laddaga et al., 1985). Nutrient metal transport systems are often up-regulated in times of need or starvation. An adverse consequence of this is the co-transport of other cations that may be toxic to the organism. Sensitive bacteria can accumulate 3 to 15 times more Cd(II) than resistant bacteria (Laddaga et al., 1985). Cadmium resistance occurs through all of the biochemical resistance mechanisms with the exception of enzymatic detoxification. This metal will not move out of the cell; instead they will remain to undergo oxidation back to its original form (Nies, 1992). Resistance to Cd(II) can be mediated by chromosomes, plasmids, or transposons (Lebrun et al., 1994). The most prominent metal resistance system for Cd(II) is by effux pumps. The reduction in nutrient digestibility at levels of 1 and 10 mg Cd/l concentration showed that mixed rumen anaerobic microorganisms could not tolerate these levels of heavy metal and the digestion process corrupted by addition of Cd. The results is supported by the investigation conducted by Yue et al., (2007) reported severe decrease in anaerobic digestion of cattail by the 1.6 mg/l Cd addition to the culture medium.

Table 2. *Effects of Cadmium on total and individual volatile fatty acids (VFA) and ammonia nitrogen in continuous culture*

Parameters	Control	0.1 mg Cd/l	1.0 mg Cd/l	10 mg Cd/l	SEM	P value
Total VFA, m*M*	112.1[a]	105.2[b]	103.6[b]	41.3[c]	5.3	0.001
VFA, mol/100 mol						
Acetate	52.5[a]	44.6[b]	41.3[bc]	36.9[c]	2.35	0.004
Propionate	24.4[d]	28.9[c]	30.5b[c]	33.4[a]	1.97	0.009
Butyrate	17.2[c]	19.9[b]	21.2[b]	24.0[a]	1.38	0.021
Valerate	2.35	3.44	3.88	3.49	0.71	0.039
Isovalerate	3.61[a]	3.07[a]	3.14[a]	2.14[b]	0.58	0.019
C2:C3[1]	2.15[a]	1.54[b]	1.36[bc]	1.11[c]	0.17	0.001
NH3-N, mg/100 ml	13.1[a]	10.5[b]	5.45[c]	4.12[c]	1.39	0.001

[a,b,c] Means within a row with different superscripts differ ($P < 0.05$); [1]C2:C3 = acetate to propionate ratio

Addition of Cd affected total VFA concentration, and the molar proportions of acetate and propionate ($P < 0.05$; Table 2). Total VFA concentration decreased with increasing level of Cd in the culture medium. Molar proportion of acetate (mol/100mol) was greatest in the control diet (52.5) followed by 0.1 mg Cd/l (44.6), 1 mg Cd/l (41.3) and 10 mg Cd/l (36.9) having the lowest acetate proportion among treatments. Propionate concentration was lowest in the control treatment and greatest in 10 mg Cd/l, and consequently acetate to propionate ratio was highest and lowest in control and 10 mg Cd/l treatment, respectively.

Data are lacking investigating the effects of Cd on VFA concentration and proportion. The results are probably due to the effects on different microorganisms fermenting different substrates. Forsberg (1977) reported resistance in *Streptococcus bovis* and *Megasfera elsdenii* to mercury. These microorganisms are able to ferment glucose to propionate. However at low concentrations, Cd had a stimulatory effect on some ruminal enzymes (Faixová and Faix, 2002). Nies (1999) showed that resistance to Cd in bacteria is based on Cd efflux and our results suggest that, due to their high toxic effects, Cd has limited beneficial effects on rumen bacteria. More research in needed for conclusion.

Cd addition significantly decreased NH3-N concentration. Reduction in urease activity of rumen microorganisms in the presence of Cd reported by Faixova and Faix (2002). In the conditions of the present experiment, it is likely that the reduced protein degradation may be related to the reduction in the digestibility of fiber associated with the protein within feeds. The undigested fiber within feeds will reduce the access of bacteria and enzymes to the protein, and therefore reduce protein degradation (Devant et al., 2000).

Conclusion

Cadmium addition to the culture medium significantly decreased nutrient digestibility and VFA production. The results of this study suggest that cadmium has strong inhibitory potential on mixed rumen microorganisms and feedstuff pollution with this metal could seriously interfere with normal rumen microbial fermentation.

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