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International journal of Advanced Biological and Biomedical Research

Volume 1, Issue 12, 2013: 1652-1659

Investigating the effects of cadmium on rumen microbial fermentation and nutrient digestibility using gas production

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ABSTRACT

Effects of different levels of cadmium (0.1, 1, 2, 4, 8, 16, 32, 64, 128, 256 mg/l of culture medium) were evaluated using gas production technique. In the glass vials used to measure gas production 30 mg of buffered rumen fluid (2:1 ratio of rumen fluid: buffer) poured in glass and cultured at 38.6 temperature. Volume and pressure of produced gas were measured with syringes and digital pressure meter at different times and exponential model were used to determine the gas production parameters. The results of the experiment indicated that using different levels of cadmium sulfate caused a significant reduction in b parameter, partitioning factor, metabolizable energy and digestion of organic matter. Addition of 0.1, 1, 2, 4 and 16 mg cadmium/l did not affect c parameter in comparison with the control treatment. The results of this study suggest that cadmium in higher levels of 8 mg per liter had strong inhibitory effect on rumen anaerobic microorganisms' activity.

Keywords: Cadmium, rumen fermentation, gas production, dual continuous culture

1. 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 hyperaccumulators, 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 1989; Perry and Silver 1982). Cd is highly toxic (Babich and Stotzky 1977; Bowman et al. 1990). 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

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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 gas production.

2. Material and method

2-1. Feed samples

In vitro gas production technique was used for evaluating the effect of cadmium sulfate on rumen fermentation. Substrate used for fermentation was diet consisted of 85 percent concentrate. Feed ingredients and chemical composition of the diets are given in Table 1.

Table 1 Diet ingredient and chemical composition

Feed items	Quantity (%)
Alfalfa	15.0
Corn	26.0
Barley	42.6
Soybean meal	15.0
Lime	0.50
Vitamin and mineral supplements	0.60
Salt	0.30
Total	100
Chemical composition	
Crude protein	16.5
Organic matter	94.8
NFC	57.2
NDF	20.3
ADF	10.5
Forage NDF	6.8
Metabolizable energy (Mcal/kg dry matter)	2.52

2-2. In vitro gas production

Samples (300 mg) were weighed into 100 ml calibrated glass vials. Buffered mineral solution (Menke and Steingass, 1988) was prepared and placed in a waterbath at 39◦C under flushing with CO2. Rumen fluid was collected before the morning feeding from ruminally fistulated, nonlactating, no pregnant Holstein cow. Rumen fluid was pumped with a manually operated vacuum pump and transferred into two pre-warmed thermos flasks, combined, filtered through two layers of cheesecloth and flushed with CO2.

Rumen fluid was added to the buffered mineral solution, which was maintained in a water bath at 39◦C, and combined. About 30 ml of buffered rumen fluid (ratio 1:2 rumen fluid and buffered mineral solution, respectively) was dispensed into glass vials containing the feeds. After closing the silicon tube at the glass vials, glass vials were gently shaken and tubes opened to remove gas by syringe to achieve complete removal of gas. This initial volume recorded. Volume and pressure of produced gas were measured with syringes and digital pressure meter at different times and exponential model were used to determine the gas production parameters.

2-3. Equations and computation of gas production

Rate and extent of gas production was determined for each feed by fitting gas production data to the nonlinear equation P= b(1−e−ct) (Orskov and Mcdonald, 1979), where P is the volume of gas produced at time t, b the potential gas production (ml g−1 DM), and c the fractional rate of gas production.
Metabolizable energy (megajoul/Kg dry matter) and in vitro organic matter digestibility (percent) were calculated by Menke et al (1979) equation.
ME (MJ/Kg DM) = 2.2 + 0.136 GP + 0.057+0.0029 CP2
IVOMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651 Ash
DM: dry matter, CP: percent of crude protein, A: ash percent, GP: milliliter pure gas production of 300 milligram dry sample after 24 h incubation.
Microbial mass (MP) production was calculated followed (blummel et al, 1997).
MP (mg / g DM) = mg TDS-(ml gas × 2.2mg / ml)
TDS: Truly degraded substrate

2-3-2. pH and Partitioning factor (PF)

pH was measured at the end of incubation by pH meter. The partitioning factor (PF), which is mg of truly digested OM (TDOM) divided by the ml of gas produced in vitro at the time of half asymptotic gas production (t1/2), was proposed by Blümmel et al. (1997) as an index of the partitioning of TDOM between microbial biomass and fermentation gases during fermentation.
2-4. Statistical analysis
Data of gas production was analysed using completely randomised design by GLM procedure of SAS 9.2 (2009) software. Mean comparison was conducted using Duncan multiple range test method (at 5% level).

3. Result and discussion

3-1. Gas production potential and rate of gas production

The gas production potential of the diet with addition of different doses of cadmium (0.1, 1, 2, 4, 8, 16, 32, 64, 128 and 256 mg/l) after 96 hours incubation are shown in table 2. The gas production potential was significantly decreased with increasing level of cadmium in comparison with a control treatment ($P<0.05$). Maximum level of cadmium (256 mg/l) showed minimum gas production potential. Levels more than 8 mg/l cadmium strongly decreased gas production potential, but the inhibitory potential of levels lower than this level was much less in comparison with the higher level. The rate of gas production significantly decreased with addition of 32, 64, 128 and 256 mg Cd/l in comparison with the control diet ($P<0.05$).
Gas production is considered as a useful indicator for monitoring an anaerobic rumen microorganism's activity (Hickey et al., 1989), and was employed to monitor the effect of the cadmium on rumen microorganisms in this study. Levent Altas (2009) investigated on methane-producing anaerobic granular

sludge in term of adding cadmium. It was indicated that cadmium has a negative effect on methane production which supports the results of this study. In an experiment using sulfate salts, it was observed that the concentration of 700 mg/l Cu (II) caused 50% gas reductions (Wong and Cheung, 1995) which approve the results of current study. These results support our experiment and in the other hand approved cadmium severely toxicant in rumen culture.

Table 2 Effect of different levels of cadmium on gas production parameter

Cadmium level (mg/l)	Fermentation Parameter		Gas value in different time (hours)			
	b	c	24	48	72	96
0	76.92±2.7[a]	0.04045±0.0219[a]	51.66[a]	69.9[a]	73.4[a]	74.8[a]
0.1	57.28±1.2[b]	0.03919±0.0404[a]	39.10[b]	47.3[b]	52.8[b]	56.5[b]
1	55.22±2.2[b]	0.04751±0.0043[a]	39.86[b]	46.5[b]	52.5[b]	55.7[b]
2	56.14±3.6[b]	0.04539±0.0030[a]	39.87[b]	47.7[b]	52.5[b]	56.3[b]
4	56.58±1.5[b]	0.04176±0.0028[a]	39.80[b]	47.3[b]	52.7[b]	56.1[b]
8	56.56±2.3[b]	0.04007±0.0029[a]	29.93[c]	43.0[b]	53.2[b]	58.6[b]
16	19.41±4.1[c]	0.04032±0.0265[a]	10.03[d]	16.9[c]	25.0[c]	35.7[c]
32	16.31±3.7[d]	0.02149±0.0034[bc]	7.60[d]	9.7[cd]	11.6[d]	14.8[d]
64	14.95±2.5[d]	0.02434±0.0034[b]	9.03[d]	9.5[cd]	10.0[d]	13.5[d]
128	18.68±2.3[d]	0.01278±0.0169[c]	7.53[d]	9.6[cd]	10.5[d]	13.6[d]
256	6.1±4.2[e]	0.02460±0.0037[b]	1.83[e]	2.6[d]	8.7[d]	11.8[d]
SEM	2.38	0.0036	1.8	1.6	1.7	1.4

b: gas production potential; c: rate of gas production; SEM: Standard Error of Mean

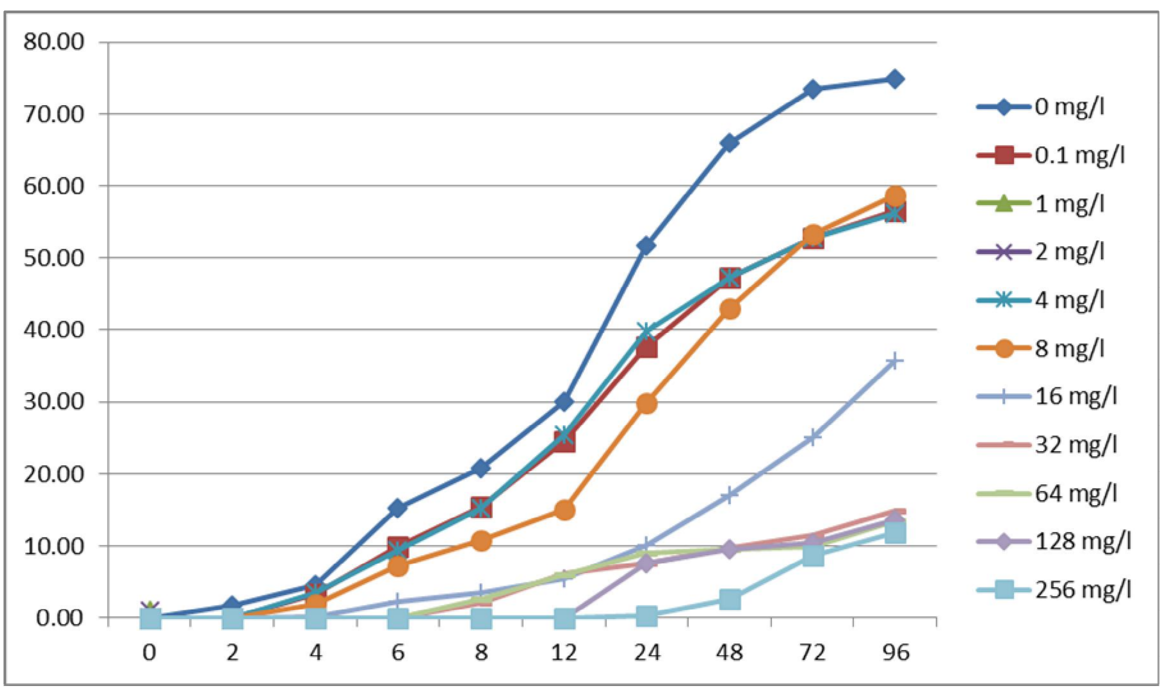

Figure 1 cumulative gas production at different levels of cadmium in different incubation time

3-2. Digestibility, partitioning factor and pH medium

The results of adding different levels of cadmium on in vitro organic matter digestion (IVOMD), truly digested organic matter (TDOM) and metabolizable energy (ME) after 24 h incubation is shown in table 3. IVOMD in all cadmium levels decreased in comparison with control treatment (P<0.05) and lowest IVOMD was observed at 256 mg/l cadmium (22.45 g/100 g organic matter). Addition of cadmium significantly decreased ME in all levels in comparison with control treatment (P<0.05). Minimum ME was observed by addition of highest level of cadmium (3.3 mg/kg organic matter). There was a significant decrease in IVOMD with cadmium addition in comparison with control treatment. Highest value of IVOMD was in the control treatment (314.35 mg) and minimum value of that was on 256 mg/l cadmium (18.33 mg).

Table 3 Effects of different levels of cadmium on gas production parameter

Cadmium level (mg/l)	TDOM (mg)	IVOMD (g/100 g organic matter)	ME (Megajoule/kg organic matter)
0	314.35[a]	68.7[a]	10.1[a]
0.1	228.23[b]	56.2[b]	8.2[b]
1	201.58[c]	57.5[b]	8.4[b]
2	200.09[c]	58.2[b]	8.5[b]
4	173.98[d]	58.2[b]	8.4[b]
8	146.62[e]	49.4[c]	7.1[c]
16	110.83[f]	31.7[d]	4.4[d]
32	38.16[g]	29.5[d]	4.1[d]
64	33.36[g]	30.8[d]	4.3[d]
128	28.58[g]	29.5[d]	4.1[d]
256	18.3[g]	24.4[e]	3.3[e]
SEM	7.9	1.7	0.2

Yue et al (2007) reported that methane production in the presence of the 1.6 mg/l cadmium relative to the control, stimulate rumen microorganisms, whereas with an increasing metal dosage inhibited the rumen microorganisms and cause methane reduction. Our results support parts of these researchers work which is increasing cadmium led to toxicity for rumen microorganisms, nevertheless in the present study lower dosage impairment bacteria activity. These researchers stated that cadmium as a microelement can boost bacteria growth. In another study Kuo and Genthner (1996) found that addition of Cr (VI) at 0.01 mg/l increased the biodegradation rates of phenol (177%) and benzoate (169%), while Cd (II) and Cu (II) at 0.01 mg/l enhanced biodegradation rates of benzoate (185%) and 2-chlorophenol (168%), respectively. Addition different levels of cadmium lead to decline and finally decreasing digestibility of organic matter. Partitioning factor (PF), is mg of TDOM divided by the ml of gas produced in vitro at the time of half asymptotic gas production (t1/2), was proposed by Blümmel et al., (1997) as an index of the partitioning of TDOM between microbial biomass and fermentation gases during fermentation. Cadmium addition significantly decreased partitioning factor in comparison with a control treatment. There was a ceiling of PF and biomass in a control treatment (8.4 and 232 mg/l, respectively) and lowest value of these parameters were seen in 256 mg/l (3.1 mg/l and 5.34 mg/l, respectively). Diets with higher PF would be expected to increase proportion of TDOM used for biomass. Control treatment had the highest PF which means main part of a diet produce microbial protein by microorganisms and by increasing cadmium level microbial protein decrease which supports gas production and digestibility obtained data in this work. Blummel et al. (1997) suggested that the PF of diets can be used as an index to differentiate them on the basis of efficiency of feed OM conversion into microbial biomass.

Table 4 effect of different levels of cadmium on fermentation after 96 hours incubation

Treatment*	Partitioning factor	Biomass (milligram)	Biomass Efficiency (%)
0	8.4[a]	232.0[a]	0.73[a]
0.1	8.06[b]	166.0[b]	0.72a
1	7.23[c]	140.2[c]	0.70[b]
2	7.1[c]	138.0[c]	0.68[b]
4	6.2[d]	112.2[d]	0.64[c]
8	6.0[d]	94.1[e]	0.62[d]
16	5.6[e]	67.8[f]	0.61[d]
32	5.1[f]	21.8[g]	0.57[e]
64	4.8[f]	18.4[g]	0.54[f]
128	4.2[g]	13.6[g]	0.47[g]
256	3.1[h]	5.3[h]	0.28[h]
SEM	0.6	5.7	0.008

*cadmium level (mg/l)

The results of medium pH after 96 h incubation are shown in table 5. Treatments with different levels of cadmium showed a significant reduction in comparison with control treatment ($P<0.05$).

Table 5 Effects of different levels of cadmium on pH

cadmium level (mg/l)	pH
0	6.81[a]
0.1	6.51[cb]
1	6.57[b]
2	6.54[bc]
4	6.60[b]
8	6.57[bc]
16	6.54[bc]
32	6.61[b]
64	6.54[bc]
128	6.45[bc]
256	6.38[c]
SEM	0.04

* Cadmium level (mg/l)

At low pH, cell wall ligands were closely associated with the hydronium ions H3O+ and restricted the approach of metal cations as a result of the repulsive force. As the pH increased, more ligands such as carboxyl, phosphate, imidazole and amino groups would be exposed and carried negative charges with subsequent attraction of metallic ions with positive charge and biosorption onto the cell surface. Crist et al. (1981) also suggested that zero-point charge, or isoelectric point, would be found at pH 3.0 for the algal biomass. Above this algal cells would have a net negative charge. The ionic state of ligands will be such as to promote reaction with metal ions. This would lead to electrostatic attractions between positively charged cations such as cadmium (II) and negatively charged binding sites. The rise in culture medium pH was not according to our expectation. Volatile fatty acid production is one of the most important influential factors for end point fermentation pH in gas production. Reduction in digestibility and cumulative gas production does not support the decrease in medium pH. Data are lacking about the effects of heavy metals on rumen pH ad more research is needed for conclusion.

Conclusion

Cadmium had strongly effects on gas production parameters and with a further increase metal concentration would reduce methane 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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