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In vitro Assessment of Adsorbents to Counteract Lead Toxicity in Ruminal Fermentation

Research Article

S. Azadbakht[1], A.A. Khadem[1] and M.A. Norouzian[1*]

[1] Department of Animal Science, College of Abouraihan, University of Tehran, Tehran, Iran

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*Correspondence E-mail: manorouzian@ut.ac.ir

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ABSTRACT

An *in vitro* gas production technique was used to evaluate the effects of different adsorbents on the gas production parameters of lead-exposed diet. Ruminal fluid, obtained from fistulated sheep (3-4 years of age and 40±5 kg BW), and experimental diets samples, 200 ± 0.2 mg DM, were incubated in 100 mL glass bottles. To each bottle, one of the following treatments was applied: 1) control (no Pb and adsorbent), 2) 15 mg/kg DM Pb as Pb acetate and no adsorbent, 3) 15 mg/kg DM Pb as Pb acetate and 3% activated carbon, 4) 15 mg/kg DM Pb as Pb acetate and 3% nanoclay and 5) 15 mg/kg DM Pb as Pb acetate and 3% bentonite. Supplementation of ruminal medium with 15 ppm Pb significantly (P<0.01) decreased total and rate of gas production at all incubation times. Rate and total gas production were significantly (P<0.05) higher for nanoclay compared to control and the other adsorbents treatments. Lead inclusion decreased organic matter digestibility (OMD), net and metabolizable energy contents and short chain fatty acids production (P<0.01). Among the adsorbents, nanoclay showed to be more effective on ruminanl fermentation parameters and improved rate and total gas production.

KEY WORDS adsorbent, lead, rumen fermentation, sheep.

INTRODUCTION

Both primary and secondary lead–zinc smelters have been implicated in producing lead toxicity in cattle, buffalo and goat reared around these industrial units (Radostits *et al.* 2000; Swarup *et al.* 2006). The higher lead levels in animals reared around such industrial activities are mainly due to ingestion of pasture contaminated with lead as well as inhalation of lead particles (Mohajeri *et al.* 2014). These toxic trace elements hinder rumen fermentation, thereby decreasing the productivity of ruminants. Forsberg (1977) showed that the elements can causing 50% inhibition of *in vitro* rumen fermentation. Ions of heavy metals may also inhibit activity of some ruminal enzymes (Faixova and Faix, 2002). Despite of this fact that the effect of heavy metal contamination on rumen fermentation has been previously reported (Faixova and Faix, 2002; Forsberg, 1977), based on our survey it appears no literature on the effect of adsorbents supplementation on ruminal fermentation parameters of sheep chronically exposed to lead contamination. Therefore, the present study was aimed to assess the effect of different adsorbent supplements on *in vitro* ruminal fermentation parameters.

MATERIALS AND METHODS

In this study an *in vitro* gas production technique (Menke *et al.* 1979) was used to estimate fermentation parameters, organic matter digestibility (OMD), net energy (NE) and metabolizable energy (ME) contents and short chain fatty acids (SCFA) production. Ruminal fluid obtained from 4 fistulated sheep (3-4 years of age and 40±5 kg BW) 3 h after morning feeding was mixed and strained through 4 layers of cheesecloth into a pre-warmed thermos and transported to the laboratory. The lambs were fed a total mixed ration (60:40 forage:concentrate; DM basis) and 0.6% mineral and vitamin premix. The lambs were fed twice daily at 0700 and 1900 hours and had free access to water.

Incubation medium was prepared as described by Menke *et al.* (1979). Sample from experimental diets, each of 200 ± 0.2 mg DM, were incubated in 100 mL glass bottles in which 30 mL of the incubation medium was added. To each bottle, one of the following experimental treatments was applied: 1) control (no Pb and adsorbent), 2) 15 mg/kg DM Pb as Pb acetate and no adsorbent, 3) 15 mg/kg DM Pb as Pb acetate and 3% activated carbon, 4) 15 mg/kg DM Pb as Pb acetate and 3% nanoclay and 5) 15 mg/kg DM Pb as Pb acetate and 3% bentonite.

Samples were incubated in triplicate and cumulative gas production was monitored at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 h post-incubation. Three bottles with incubation medium only, were used as blanks to correct the gas production values for gas release from the rumen contents. The gas production data were fitted to the following model introduced by France *et al.* (2000):

$$A= b \times [1 - e^{-c(t-L)}]$$

Where:
A: volume of gas production at time t.
b: asymptotic gas production (based on mL/200 mg DM).
c: rate of gas production per hour from the slowly fermentable feed fraction b.
Time lag (L): discrete lag time prior to gas production.

The rate of gas production (RGP) at 4 and 6 h was calculated from recorded volumes of gas produced before and after these times (Vázquez-Armijo *et al.* 2011). For example, RGP at 4 h was calculated as:

RGP 4 h [(mL/g DM)/h)]= (volume of gas produced at 6 h-volume of gas produced at 2 h) / (4×sample weight (mg))

OMD, ME (Menke *et al.* 1979) and net energy (NE) (Menke and Steingass, 1988) contents were estimated according to the equations given below:

OMD (%)= 14.88 + 0.8893 $IVGP_{24}$ + 0.448 CP (%DM) + 0.651 A (% DM)
ME (MJ/kg DM)= 2.20 + 0.136 $IVGP_{24}$ (mL/200 mg DM) + 0.057 CP (% DM)
NE (MJ/kg DM)= 0.101 $IVGP_{24}$ + 0.051 CP (% DM) + 0.11 EE (% DM)

Where:
$IVGP_{24}$: 24 h *in vitro* gas production volume.
CP: crude protein.
A: ash.
EE: ether extract contents of the feed sample.

SCFA were estimated by the equation of Makkar (2005):

SCFA (mmol/g DM)= 0.0222 (mL gas at 24 h) - 0.00425

Data were analyzed as a completely randomized design using the MIXED procedure of SAS (2004). Duncan multiple range test was used to detect statistical significance between treatments using a significance level of 0.05.

RESULTS AND DISCUSSION

Rate of gas production (mL/200 mg DM) in different incubation times are presented in Table 1. Lead-polluted non-supplemented batch (group 2) had lower rate of gas production compared to the groups supplemented with adsorbents and the control (P< 0.01).

Gas production volumes (mL/200 mg DM) in different incubation times of experimental groups are shown in Table 2. Supplementation of ruminal medium with 15 ppm Pb has significantly (P<0.01) decreased total gas production at all incubation times. Gas production volumes were significantly (P<0.05) higher for nanoclay compared to control and other adsorbents containing groups (Table 2 and Figure 1).

Gas production parameters (b, c) and calculated amounts of OMD, ME, NE and SCFA are presented in Table 3. Supplementation of lead adsorbents improved gas production parameters (P<0.01). Among the different adsorbents, the nanoclay was the most effective one on ruminanl fermentation parameters. However, there were no significant differences between the different treatments regarding the fractional rate of gas production (c). Lead inclusion led to reduction in OMD, NE, ME contents and SCFA production (P<0.01).

Heavy metals prevent rumen fermentation, thereby decreasing the productivity of ruminants. Forsberg (1977) believed that these elements may be inhibitory to both the fermentative activity and the growth of microorganisms present in the rumen.

In this study, inclusion of lead to ruminal fermentation bottleshad an reducing effect on the measured fermentation parameters.

Table 1 Effect of adsorbents addition to lead-exposed diets on rate of gas production at different incubation times

Incubation time	Treatment[1]					SEM	P-value
	1	2	3	4	5		
RGP 4 h	11.4^{c}	9.6^{d}	12.2^{bc}	14.1^{a}	11.9^{bc}	2.2	< 0.01
RGP 6 h	9.9^{c}	8.5^{d}	10.6^{b}	12.8^{a}	10.1^{b}	2.4	< 0.01
RGP 8 h	8.9^{c}	8.4^{c}	11.2^{ab}	12.5^{a}	10.0^{b}	2.1	< 0.01
RGP 12 h	6.5^{c}	7.0^{c}	9.7^{ab}	10.8^{a}	9.0^{ab}	1.9	< 0.01
RGP 24 h	2.9^{c}	2.1	3.1^{ab}	3.4^{a}	2.9^{ab}	0.4	< 0.01
RGP 48 h	2.9^{c}	2.8^{d}	4.0^{a}	3.9^{a}	3.7^{b}	0.5	< 0.01
RGP 72 h	0.5^{b}	0.6^{b}	1.0^{a}	1.0^{a}	0.9^{a}	0.1	< 0.01

[1] Treatment: 1) control (no Pb and adsorbent); 2) 15 mg/kg DM Pb as Pb acetate and no adsorbent; 3) 15 mg/kg DM Pb as Pb acetate and 3% activated carbon; 4) 15 mg/kg DM Pb as Pb acetate and 3% nanoclay and 5) 15 mg/kg DM Pb as Pb acetate and 3% bentonite.
RGP: rate of gas production in milliliters per 200 mg DM per hour at 4, 6, 8, 12, 24, 48 and 72 h after incubation.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 2 Effect of adsorbents addition to lead-exposed diets on total gas production (mL) at different incubation times

Incubation time	Treatment[1]					SEM	P-value
	1	2	3	4	5		
GP 2 h	9.1^{b}	7.0^{c}	8.9^{b}	12.5^{a}	9.6^{b}	2.3	< 0.01
GP 4 h	15.2^{b}	11.9^{c}	15.3^{b}	19.5^{a}	16.1^{b}	2.8	< 0.01
GP 8 h	23.7^{b}	19.1^{c}	24.1^{b}	30.2^{a}	24.5^{b}	3.5	< 0.01
GP 12 h	30.5^{c}	26.0^{d}	34.0^{b}	40.6^{a}	32.8^{b}	4.5	< 0.01
GP 16 h	35.0^{c}	30.9^{d}	40.4^{b}	48.3^{a}	39.6^{b}	5.2	< 0.01
GP 24 h	44.2^{c}	36.2^{d}	48.4^{b}	58.1^{a}	47.7^{b}	5.8	< 0.01
GP 48 h	55.2^{b}	45.5^{c}	61.1^{b}	71.2^{a}	59.6^{b}	6.4	< 0.01
GP 72 h	59.6^{c}	50.6^{d}	68.5^{b}	78.0^{a}	66.3^{b}	6.4	< 0.01
GP 96 h	60.6^{c}	52.2^{d}	71.7^{b}	81.5^{a}	69.1^{b}	6.5	< 0.01

[1] Treatment: 1) control (no Pb and adsorbent); 2) 15 mg/kg DM Pb as Pb acetate and no adsorbent; 3) 15 mg/kg DM Pb as Pb acetate and 3% activated carbon; 4) 15 mg/kg DM Pb as Pb acetate and 3% nanoclay and 5) 15 mg/kg DM Pb as Pb acetate and 3% bentonite.
GP: gas production (in milliliters per 200 mg at 2, 4, 8, 12, 16, 24, 48, 72 and 96 h after incubation).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

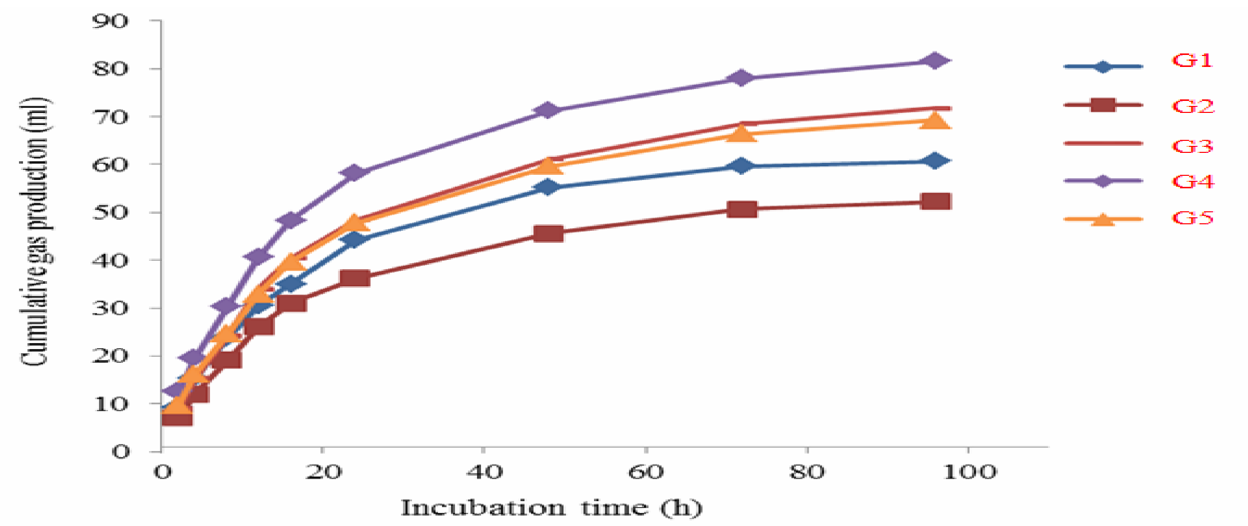

Figure 1 Gas production of lead-exposed diets supplemented with adsorbents: G1) control (no Pb and bentonite); G2) 15 mg/kg DM Pb as Pb acetate and no adsorbent; G3) 15 mg/kg DM Pb as Pb acetate and 3% activated carbon; G4) 15 mg/kg DM Pb as Pb acetate and 3% nanoclay and G5) 15 mg/kg DM Pb as Pb acetate and 3% bentonite

Table 3 Effect of adsorbents addition to lead-exposed diets on fermentation parameters and energy and fatty acids production

Factor	Treatment[1]					SEM	P-value
	1	2	3	4	5		
b (mL/g DM)	60.3^{c}	50.9^{c}	69.7^{b}	79.3^{a}	67.7^{b}	5.8	< 0.01
c (h)	0.05^{9}	0.057	0.053	0.059	0.054	0.006	0.74
OMD (%)	61.4^{b}	54.2^{c}	65.1^{b}	73.7^{a}	64.5^{b}	5.2	< 0.01
ME (MJ/kg DM)	9.0^{c}	7.9^{d}	9.6b^{c}	10.9^{a}	9.5b^{c}	0.8	< 0.01
NE (MJ/kg DM)	6.1^{b}	5.1^{c}	6.5^{b}	7.7^{a}	6.5^{b}	0.6	< 0.01
SCFA (mmol/g DM)	0.97^{b}	0.80^{c}	1.07^{b}	1.28^{a}	1.05^{b}	0.13	< 0.01

[1] Treatment: 1) control (no Pb and adsorbent); 2) 15 mg/kg DM Pb as Pb acetate and no adsorbent; 3) 15 mg/kg DM Pb as Pb acetate and 3% activated carbon; 4) 15 mg/kg DM Pb as Pb acetate and 3% nanoclay and 5) 15 mg/kg DM Pb as Pb acetate and 3% bentonite.
b: asymptotic gas production (in milliliters per 200 mg DM); c: fractional rate of gas production (per hour); OMD: organic matter digestibility (%); ME: metabolizable energy (in MJ per kilogram DM); NE: net energy yield (in MJ per kilogram DM) and SCFA: short chain fatty acid (millimole per g DM).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In agreement with our results, prior studies showed that exposure to Pb affected rumen metabolism by forming lipid soluble organometallic compounds that inhibit the growth and respiration of micro-organisms (Chaudhary *et al.* 2006). Surveys conducted by Phillips *et al.* (2011) have shown that exposure to Pb led to a reduction in the digestion of long particles because of a toxic effect on rumen-micro-organisms. Regarding our study the main finding was a much lower production of total gas and volatile fatty acids (VFA) in the rumen fluid of contaminated with Pb compared to the control and adsorbents supplemented groups. In this regards, Varadyova *et al.* (2006a) observed depressed the gas and methane production, production of VFA and altered the fermentation pattern in ruminal fermentation of sheep grazing in an area polluted. Marounek and Joch (2014), observed decreased molar proportion of acetate with increasing concentrations of lead in rumen cultures. In this study production of VFA in cultures containing Pb, Hg, Cd and As at 50 µg/mL was decreased by 15.9, 40.0, 29.1 and 35.6%, respectively. The elements in increasing order of toxicity based on the inhibition of VFA production at 50 µg/mL were Pb, Cd, As and Hg. The corresponding ranking of trace elements toxicity, based on the inhibition of gas evolution reported by Forsberg (1977) was Pb, As, Cd and Hg. Probably as a consequence of inhibition the microbial activity and the methanogenic population appeared not to be fully able to consume hydrogen produced in bacterial and protozoan communities (Varadyova *et al.* 2006b). In anaerobic digestion medium, Chen *et al.* (2008) demonstrated that the toxic effect of heavy metals is attributed to disruption of enzyme function and structure by binding of the metals with thiol and other groups on protein molecules or by replacing naturally occurring metals in enzyme prosthetic groups.

Change in fermentation pattern (Varadyova *et al.* 2007) and protozoan counts (Hristov *et al.* 2001) by clay sorbent has been documented previously.

In this study addition of different absorbents ameliorated toxic effects of lead and improved ruminal fermentation parameters. With consistent to our results, in study of Varadyova *et al.* (2006a), addition of bentonite to fermentation bottles containing lead tended to enhance production of total and rate of gas production.

Supplementation with 1.5% of dietary DM clay decreased the adverse effects of lead contamination. These results are in agreement with those reported in ruminants by Khalifeh *et al.* (2012) who found that, addition of bentonite led to a significant increase in gas production rate constant and organic matter digestibility. Also, activated carbon has been used as an adsorbent for removal of heavy metal pollutants from wastewater and has proved to be effective (Gueu *et al.* 2007; Okpareke *et al.* 2009).

However, in our study with using different adsorbents, nanosized clay, was more effective for removal of Pb from ruminal medium and has better improvement for fermentation parameters than natural adsorbents. This is partly because of their large surface areas and high activities caused by the size-quantization effect (Hua *et al.* 2012). With agreement with our results, Muhammad and Munawar (2007) mentioned that nano zeolite Y is an effective adsorbent for Pb removal. In another study by Rasouli *et al.* (2012) low silica nano-zeolite X be much suitable adsorbent for the removal of Pb from aqueous solution. Recent studies suggested that many nanosized clay exhibit very favorable sorption to heavy metals in terms of high capacity and selectivity, which would result in deep removal of toxic metals to meet increasingly strict regulations (Deliyanni *et al.* 2009).

CONCLUSION

In conclusion exposure to Pb affected rumen function, reducing rate and total gas production and energy and fatty acids production. Among the different adsorbents, nanosized clay was found to be much suitable adsorbent for the removal of Pb from ruminal medium.

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