Effect of different levels of added selenium without or with arsenic on rumen fermentation parameters in buffaloes under in vitro conditions

CHANDER DATT¹, AJAY KUMAR² and S S KUNDU³

National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

Studies were conducted to find the effect of addition of different levels of selenium (Se) without or with arsenic (As) on rumen fermentation parameters viz., in vitro gas production (IVGP), true organic matter digestibility (TOMD), microbial biomass production (MBP), total volatile fatty acids (TVFA) and individual volatile fatty acids (IVFA) under in vitro conditions. Basal substrate (200 mg) comprising of paddy straw and concentrate mixture (40: 60) along with different levels of Se (0, 2, 4, 8, 10, 12 and 14 μg/200 mg substrate per syringe) was incubated in 100 ml glass syringes. Arsenic, an antagonist of Se in form of sodium arsenite, was used @ 0, 10, 20, 40, 80 and 100 μg levels added to the substrate (200 mg). The inhibitory effect on TOMD, IVGP, MBP and TVFA was observed at 12 μg added Se level. Addition of As at 10 or 20 μg level to the substrate (200 mg) containing 12 μg Se improved rumen fermentation parameters under in vitro system, however, further addition of As (40, 80 or 100 μg) showed negative effects on these parameters.

Key words: Arsenic, Buffalo, Rumen fermentation parameters, Selenium

Selenium (Se) is an essential element and its deficiency results in varied disease conditions in different animal species. On the other hand, chronic seleniumosis in form of Degnala disease has been reported in buffaloes particularly in Punjab, Haryana, western Uttar Pradesh and some parts of Bihar. Selenium supplementation has been reported to affect rumen fermentation in sheep and cattle either under in vitro or in vivo conditions (Mihalikova 2005, Faixova et al. 2007, and Mainville et al. 2009). However, there is very little information available regarding effects of high levels of Se on rumen fermentation in buffaloes. Further, As and Se share many chemical properties, however, both have marked differences in their biological effects (Csanaky and Gregus 2003). Arsenic might decrease the toxicity of Se by combining with it in gastrointestinal tract, thereby, decreasing the absorption of the element (Du Bois et al. 1940). Both Se and As have been reported to increase biliary excretion of each other (Leverend 1977). Antagonism between As and Se, whereby each reduces toxicity of the other, has been well documented in animal models (Pilsner et al. 2011), however, at rumen level, there is negligible information. Therefore, an in vitro experiment was conducted to study the effect of different levels of Se supplementation on rumen fermentation parameters in buffaloes and the efficacy of As to antagonize the negative effects of high level of Se.

MATERIALS AND METHODS

Selenium was added @ 0 (T0), 2 (T1), 4 (T2), 8 (T3), 10 (T4), 12 (T5) and 14 (T6) μg/200 mg substrate and incubated in 100 ml glass syringes as per Menke and Steingass (1988). Sodium selenite was used as Se source. Feed sample (200 mg) comprising of paddy straw and concentrate mixture (40: 60) served as basal substrate. Gas production measurements were done at 24 and 48 h after incubation. Out of different levels (0, 2, 4, 8, 10, 12 and 14 μg) of added Se, the inhibitory effect on TOMD, MBP, IVGP and TVFA was observed at 12 μg added Se level. Addition of As at 10 or 20 μg level to the substrate (200 mg) containing 12 μg Se improved rumen fermentation parameters under in vitro system, however, further addition of As (40, 80 or 100 μg) showed negative effects on these parameters.

The rumen liquor collected from 3 fistulated adult male buffaloes (Body weight, 326.3±5.24 kg) was pooled and used as inoculum source. The animals were fed on diets to meet their nutrient requirements (Ranjhan 1998). Rumen liquor was collected in the morning (8.30 a.m.) before feeding and watering and strained through muslin cloth into a pre-warmed and CO₂ flushed thermos flask. All the incubations were carried out in triplicates. Parameters like in vitro gas...
RESULTS AND DISCUSSION

Basal substrate contained 0.42 ppm Se and 0.23 ppm As. There were significant (P<0.05 or P<0.01) differences among the treatments with regards to TOMD, IVGP, MBP and TVFA (Table 1). Addition of Se up to 10 μg level did not have any significant effect on these rumen fermentation parameters. However, there was significant (P<0.01) reduction in TOMD (P<0.01), IVGP (P<0.01) and TVFA (P<0.05) when compared with 12 or 14 μg Se supplementary level. Microbial production was also inhibited significantly (P<0.01) at this level of additional Se. The effects of addition of either 12 or 14 μg Se were similar. TOMD values decreased from 65.17% (Control) to 59.37% (T5). Martinez and Church (1970) revealed that low Se supplementation (0.01 to 5.00 ppm) slightly reduced cellulose digestion in vitro while higher level of supplementation (7.0 to 20 ppm) caused a significant depression (P<0.05). Bakshi et al. (1986) found significant (P<0.01) depression in the digestibility of nutrients and cell wall constituents as result of feeding paddy straw (2.14 ppm Se) compared to urea treated wheat straw (0.21 ppm Se) in buffaloes. It is possible that rumen microbial protein might have been reduced due to high Se intake/level (Khirwar and Arora 1976, Tekchandani and Arora 1978) which in turn led to depression in digestibility. Se supplementation to Se inadequate diets at lower levels may either not affect digestibility or improve the digestibility of nutrients. For example, digestibility of nutrients and ruminal VFA concentration were not affected due to supplementation of 0.2 ppm Se to the basal diets containing 31.74 ppb Se in sheep (Serra et al., 1994). On the other hand, increase in digestibility and utilization of nutrients was recorded in cows supplemented with 0.2–0.3 ppm of Se to the diets having deficient levels of Se (Vladimirov et al. 2003, Nadarinskaia 2003, and Bukas et al. 2004). IVGP decreased from 29.13 (TC) to 27.45 ml/0.2g substrate (T5).

MBP decreased from 53.68 (TC) to 42.38 mg (T5). Earlier report (Khirwar and Arora 1976) indicated inhibiting effect of inorganic Se on protein synthesis in vitro at 1 ppm level when incubated with buffalo strained rumen liquor and at 5 ppm when incubated with cattle strained rumen liquor. High level of Se in the rumen inhibits microbial protein synthesis (Tekchandani and Arora 1978). Serra et al. (1994), however, found a slight but non-significant decrease in rumen bacterial yield in animals supplemented with inorganic Se at 0.2 ppm level (basal diet contained 31.74 ppb Se) compared to controls. Se content of ruminal fluid was negatively correlated (P<0.05) to rumen bacterial yield. Supplementation of inorganic or organic Se at 0.5 ppm level to the diet containing 0.456 ppm Se did not affect ruminal bacterial and protozoal counts and microbial protein in cattle and buffaloes (Chander Datt and Aruna Chhabra 2008). Dietary supplementation of Se at 0.3 ppm level (basal diet Se= 0.16 ppm) in the diet of buffalo bulls did not show any significant effect on ruminal pH, total-N, TCA precipitable N, NPN, TVFA, their fractions and protozoal number in rumen liquor (Shinde et al. 2008). It is the levels and forms

Table 1. Effect of addition of different levels of Se on rumen fermentation parameters

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IVGP (%)</th>
<th>TOMD** (%)</th>
<th>MBP** (mg)</th>
<th>TVFA* (m mole/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.13 ± 0.31</td>
<td>65.17 ± 0.04</td>
<td>53.68 ± 0.89</td>
<td>12.5 ± 0.15</td>
</tr>
<tr>
<td>T1</td>
<td>28.54 ± 0.31</td>
<td>63.81 ± 0.69</td>
<td>53.38 ± 0.46</td>
<td>12.7 ± 0.12</td>
</tr>
<tr>
<td>T2</td>
<td>28.70 ± 0.43</td>
<td>63.61 ± 0.67</td>
<td>53.60 ± 0.45</td>
<td>12.6 ± 0.12</td>
</tr>
<tr>
<td>T3</td>
<td>29.13 ± 0.42</td>
<td>64.58 ± 0.83</td>
<td>52.60 ± 0.62</td>
<td>12.5 ± 0.17</td>
</tr>
<tr>
<td>T4</td>
<td>28.75 ± 0.43</td>
<td>63.70 ± 0.62</td>
<td>52.15 ± 0.59</td>
<td>12.3 ± 0.11</td>
</tr>
<tr>
<td>T5</td>
<td>27.45 ± 0.52</td>
<td>59.37 ± 0.48</td>
<td>42.38 ± 0.80</td>
<td>10.3 ± 0.13</td>
</tr>
<tr>
<td>T6</td>
<td>27.34 ± 0.35</td>
<td>58.36 ± 0.66</td>
<td>38.40 ± 0.56</td>
<td>10.1 ± 0.09</td>
</tr>
</tbody>
</table>

**Values bearing different superscripts in a column differ significantly (*P<0.05; **P<0.01).
Table 3. Effect of addition of different levels of arsenic along with Se (12 μg) on rumen fermentation parameters

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IVGP (μg)</th>
<th>TOMD** (%)</th>
<th>MBP** (mg)</th>
<th>TVFA* (mmole/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24h *</td>
<td>48h**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>28.63±0.31</td>
<td>40.88±0.43</td>
<td>64.39±0.60</td>
<td>12.6±0.20</td>
</tr>
<tr>
<td>T1</td>
<td>27.25±0.32</td>
<td>34.75±0.60</td>
<td>57.57±0.57</td>
<td>10.4±0.13</td>
</tr>
<tr>
<td>T2</td>
<td>28.63±0.38</td>
<td>40.25±0.39</td>
<td>62.74±0.50</td>
<td>12.2±0.19</td>
</tr>
<tr>
<td>T3</td>
<td>28.50±0.35</td>
<td>39.75±0.60</td>
<td>58.50±0.77</td>
<td>12.1±0.14</td>
</tr>
<tr>
<td>T4</td>
<td>23.25±0.32</td>
<td>32.25±0.32</td>
<td>62.94±0.77</td>
<td>12.1±0.14</td>
</tr>
<tr>
<td>T5</td>
<td>18.38±0.24</td>
<td>26.75±0.48</td>
<td>58.50±0.77</td>
<td>12.1±0.14</td>
</tr>
<tr>
<td>T6</td>
<td>17.50±0.46</td>
<td>25.38±0.55</td>
<td>53.40±0.57</td>
<td>8.5±0.12</td>
</tr>
</tbody>
</table>

a,b,c,d Values bearing different superscripts in a column differ significantly (*P<0.05; **P<0.01).

Out of different levels (0, 2, 4, 8, 10, 12 and 14 μg) of added Se, the inhibitory effect on TOMD, MBP, IVGP and TVFA was observed from 12 μg added Se level. So this level was selected for further in vitro studies. Added As at 10 and 20 μg levels increased TOMD, MBP and IVGP significantly (P<0.01) compared to control and the values were at par with negative control (200 mg substrate; conc.: roughage ratio= 40: 60), however, further addition of As (40 μg onwards) resulted in significant (P<0.01) decrease in the values of these parameters. Same trend was observed for TVFA production. Molar proportion of acetate was not affected by addition of As, however, that of butyrate increased probably at the cost of decreased proportion of propionate resulting in increase in A: P ratio. Forsberg (1978) reported that the rate of fermentation of the rumen microflora was inhibited almost 30% by 5 μg/ml of arsenic added in the form of arsenite, although 304 μg/ml was required to cause 50% inhibition. The rate of fermentation of a separated bacterial fraction was inhibited 37% by 1 μg of arsenite/ml (Table 3). Both arsenate and arsenite inhibited the growth of a number of rumen bacteria in pure culture at concentrations as low as 5 μg As/ml. Forsberg (1978) opined that the concentrations of arsenic causing a significant inhibitory effect on the fermentative activity and growth of some rumen bacteria are less than that reported to be toxic to ruminant animals. Arsenic decreased Se toxicity under most conditions, there is a pronounced synergistic toxicity between arsenic and two methylated selenium metabolites, trimethyl selenonium ion or dimethyl selenide. The consequences of such mechanisms are unexplored (Levender 1977). However, this study clearly showed that addition of excessive levels of Se along with As...
after a particular level had synergistic toxicity effects on rumen fermentation.

Selenium level of more than 12 μg/200mg substrate showed the inhibitory effect on true organic matter digestibility, in vitro gas production, microbial biomass production and total volatile fatty acids. Addition of arsenic at 10 or 20 μg level to the substrate (200 mg) containing 12 μg Se improved rumen fermentation parameters under in vitro system, however, further addition of As (40, 80 or 100 μg) showed negative effects on these parameters.

REFERENCES


