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

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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) alongwith different levels of Se (0, 2, 4, 8, 10, 12 and 14µg/200 mg substrate or 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 selenosis 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 microbial 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 (Levender 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.

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## MATERIALS AND METHODS

Selenium was added @ 0 (Tc), 2 ($T_1$), 4 ($T_2$), 8 ($T_3$), 10 ($T_4$), 12 ($T_5$) and 14 ($T_6$) 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, if any, due to the level of Se. As, an antagonist of Se in form of sodium arsenite, was used as a possible ameliorant at 0, 10, 20, 40, 80 and 100 µg levels added to the substrate (200 mg).

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 $CO_2$ flushed thermos flask. All the incubations were carried out in quadriplicates. Parameters like *in vitro* gas

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

| Treatments | IVGP | | TOMD** (%) | MBP** (mg) | TVFA* (m mole/100ml) |
|---|---|---|---|---|---|
| | 24h * | 48h** | | | |
| Control | 29.13[a] ± 0.31 | 41.75[a]±0.32 | 65.17[a]±0.84 | 53.68[a]±0.89 | 12.5[a]±0.15 |
| $T_1$ | 28.54[a]±0.31 | 41.00[a]±0.46 | 63.81[a]±0.69 | 53.38[a]± 0.46 | 12.7[a]±0.12 |
| $T_2$ | 28.70[a]±0.43 | 41.88[a]±0.43 | 63.61[a]±0.67 | 53.60[a]±0.45 | 12.6[a]±0.12 |
| $T_3$ | 29.13[a]±0.42 | 41.25[a]±0.43 | 64.58[a]±0.83 | 52.60[a]±0.62 | 12.5[a]±0.17 |
| $T_4$ | 28.75[a]±0.32 | 41.00[a]±0.54 | 63.70[a]±0.62 | 52.15[a]±0.59 | 12.3[a]±0.11 |
| $T_5$ | 27.45[ab]± 0.52 | 35.13[b]±0.59 | 59.37[b]±0.48 | 42.38[b]±0.80 | 10.3[b]±0.13 |
| $T_6$ | 27.34[b]±0.35 | 34.50[b]±0.74 | 58.36[b]±0.66 | 38.40[c]±0.56 | 10.1[b]±0.09 |

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

production (IVGP), true OM digestibility (TOMD), microbial biomass production (MBP) were determined. Total volatile fatty acids (TVFA) were analysed (Barnett and Reid 1957). Individual fatty acids (IVFA) viz., acetate, propionate and butyrate were estimated using GLC (NUCON Gas Chromatograph 5700). Analysis of Se and As in basal substrate was done using atomic absorption spectrophotometer equipped with hydride generation (Hitachi Z-5000 Series). Data were analysed statistically (Snedecor and Cochran 2007).

## 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 (TC) to 59.37 (%) ($T_5$). 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 and Hansard 1983) 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, Nadarinskaya 2003, and Bukas *et al*. 2004). IVGP decreased from 29.13 (TC) to 27.45 ml/0.2g substrate ($T_5$).

MBP decreased from 53.68 (TC) to 42.38 mg ($T_5$). 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 rumen 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 2. Effect of supplementation of different levels of Se on molar proportion of IVFA

| Treatments | IVFA (%) | | | A: P ratio |
|---|---|---|---|---|
| | A | P | B* | |
| Control | 69.23 | 19.99 | 10.78[ab] | 3.48 |
| $T_1$ | 68.71 | 20.35 | 10.95[a] | 3.39 |
| $T_2$ | 68.05 | 19.62 | 11.33[a] | 3.52 |
| $T_3$ | 69.42 | 20.51 | 10.08[ab] | 3.39 |
| $T_4$ | 69.12 | 20.78 | 10.10[ab] | 3.33 |
| $T_5$ | 69.15 | 21.31 | 9.54[b] | 3.25 |
| $T_6$ | 69.75 | 20.73 | 9.52[b] | 3.96 |

[a,b,c]Values bearing different superscripts in a column differ significantly (*P<0.05).

Table 3. Effect of addition of different levels of arsenic along with Se (12 µg) on rumen fermentation parameters

| Treatments | IVGP | | TOMD** (%) | MBP** (mg) | TVFA* (mmole/100ml) |
|---|---|---|---|---|---|
| | 24h * | 48h** | | | |
| Control | 28.63$^{a}$±0.31 | 40.88$^{a}$±0.43 | 64.39$^{a}$±0.60 | 52.50$^{a}$±0.42 | 12.6$^{a}$±0.20 |
| $T_1$ | 27.25$^{b}$±0.32 | 34.75$^{b}$±0.60 | 57.57$^{b}$± 0.57 | 41.20$^{c}$±0.69 | 10.4$^{b}$±0.13 |
| $T_2$ | 28.63$^{a}$±0.38 | 40.25$^{a}$±0.43 | 62.74$^{a}$±0.50 | 51.70$^{a}$±0.43 | 12.2$^{a}$±0.19 |
| $T_3$ | 28.50$^{a}$±0.35 | 39.75$^{a}$±0.60 | 62.94$^{a}$±0.77 | 51.15$^{a}$±0.64 | 12.1$^{a}$±0.14 |
| $T_4$ | 23.25$^{c}$±0.32 | 32.25$^{c}$±0.32 | 58.50$^{b}$±0.35 | 44.60$^{b}$±0.38 | 8.8$^{c}$±0.12 |
| $T_5$ | 18.38$^{d}$±0.24 | 26.75$^{d}$±0.48 | 54.80$^{c}$±0.54 | 35.30$^{d}$±0.43 | 8.7$^{c}$±0.11 |
| $T_6$ | 17.50$^{d}$±0.46 | 25.38$^{d}$± 0.55 | 53.40$^{c}$±0.57 | 35.80$^{d}$±0.40 | 8.5$^{c}$±0.12 |

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

of Se which have a bearing on rumen fermentation patterns.

TVFA concentration (Table 2) decreased from 12.5 (Tc) to 10.3 mmole/100ml ($T_5$). Molar proportion of butyrate was found to be reduced in $T_5$, however A: P ratio was not affected due to supplementary Se level. Selenium can also affect the metabolism of rumen microbes as is evident from alteration in VFA content in rumen fluid (Hidiroglou and Lessard 1976) or S incorporation by microbes (Hidiroglou and Zakardas 1976). The concentration of TVFA was not affected by selenite supplementation, however, it increased the relative proportion of butyrate at the expense of acetate (Kim *et al.* 1997). However, sheep showed the opposite effect, with higher acetic and isovaleric acids (Hidiroglou and Lessard 1976) and total VFA concentration, and increased protozoa population, with greater proportion of *Diplodinium* (Naziroglu *et al*. 1997). Supplementation of Se yeast providing 0, 0.15, 0.30 and 0.45 ppm Se in the diet (basal Se level= 0.07 ppm) of cows linearly increased TVFA concentration and altered rumen fermentation pattern from acetate to propionate production as shown by the reduction in ratio of acetate to propionate (Wang *et al*. 2009). Similarly, Naziroglu *et al.* (1997) also reported an increase of total VFA and propionate production by supplementing 0.3 mg/kg Se (sodium selenite). Liu *et al*. (2007) showed reduction in the ratio of acetate to propionate due to an increase in propionate production when steers were fed 300 and 600 mg Se yeast per kg of dietary DM. Se affects rumen microbes and *vice-versa*. e.g. *Butyrivibrio fibrisolvens* and *Bacteroides ruminicola* have been shown to take up selenite but not sulphate or selenate, and both the ruminal bacteria convert selenite to a variety of selenoamino acids (Hudman and Glenn 1985). Protozoa population and their species-wise composition were affected by the level of dietary Se in sheep (Mihalikova *et al.* 2005). These results were undoubtedly influenced by the complex interactions that occur in the rumen fermentation processes.

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 alongwith As

Table 4. Effect of addition of graded levels of arsenic along with Se (12 µg) on molar proportion of IVFA

| Treatments | IVFA (%) | | | A: P* |
|---|---|---|---|---|
| | A | P* | B* | |
| Control | 68.93 | 19.86b | 11.20$^{bc}$ | 3.47$^{ab}$ |
| $T_1$ | 68.99 | 21.11$^{a}$ | 9.90$^{c}$ | 3.28$^{b}$ |
| $T_2$ | 69.13 | 19.35$^{b}$ | 11.52$^{bc}$ | 3.67$^{a}$ |
| $T_3$ | 68.46 | 19.92$^{ab}$ | 11.62$^{bc}$ | 3.58$^{ab}$ |
| $T_4$ | 68.65 | 18.78$^{bc}$ | 12.57$^{ab}$ | 3.66$^{a}$ |
| $T_5$ | 67.81 | 17.95$^{c}$ | 14.24$^{a}$ | 3.77$^{a}$ |
| $T_6$ | 67.71 | 18.00$^{c}$ | 14.29$^{a}$ | 3.76$^{a}$ |

$^{a,b,c}$ Values bearing different superscripts in a column differ significantly (*P<0.05).

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.

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