Effect of inclusion of pentasulphate mixture and arsenic in diets given high level of selenium on feed intake and nutrient utilization in buffaloes

Ajay Kumar¹, Chander Datt², Sonali Prusty³, SS Kundu⁴ and Veena Mani⁵

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Abstract: Sixteen male Murrah buffalo calves were divided into 4 groups with 4 animals in each group based on their body weight and age. The animals were fed on rations to supply 15% less of CP requirements as per NRC (2001). All the animals were fed a basal diet (0.41 ppm Se) comprising of paddy straw, concentrate mixture and green maize (Control, group T₁). The animals in groups T₂, T₃ and T₄ were also supplemented with 10 ppm of Se in the form of sodium selenite. After 60 d of feeding, animals in groups T₃ and T₄ were given supplementary arsenic (40 ppm of diet) in form of sodium arsenite and pentasulphate mixture (9 g/100 kg BW), respectively in addition to Se (10 ppm) being already given. After 85 days of feeding, a metabolism trial was conducted in order to determine DM intake, nutrient digestibility and balances of N and Se. DM Intake was similar in all the groups. Digestibility of nutrients (DM, OM, EE and NDF) and nitrogen retention was also not affected by dietary treatments to any significant extent, however, digestibility of CP and ADF was lower ($P < 0.05$) in group T₂ compared to other groups. Supplementary pentasulphate mixture and As increased excretion of Se which paved the way for its less retention ($P < 0.01$) in the body. Hence, supplementation of either pentasulphate mixture @ 9 g/100 kg BW or arsenic (40 ppm) to the diet already supplied with extra 10 ppm Se improved the digestibility of CP and ADF while reducing the Se load from the body considerably showing the positive effect of arsenic and pentasulphate supplementation in buffalo calves given high level of Se in paddy straw based rations.

Keywords: Arsenic, Buffalo, Nutrient Utilization, Pentasulphate mixture, Selenium

Introduction

Selenium (Se) is an essential trace element for all the categories of livestock and its deficiency results in poor health and production. About 30 selenoproteins have been identified which play important biochemical and physiological functions (Hefnawy and Tórtora-Perez 2010). Nutritional requirement of Se in ruminants is 0.3 ppm (NRC 2001). On the other hand, chronic selenosis in form of *Degnala* disease has been reported in buffaloes. Selenosis in the form of *Degnala* disease affects this species leading to deterioration in health and production status. Cases of *Degnala* disease have been reported in buffaloes fed mainly on paddy straw containing high levels of Se particularly in Punjab, Haryana, Uttar Pradesh (Arora et al. 1975; Bakshi et al. 1986). The maximum tolerable level of Se is 5 ppm (NRC 2005).

The mechanism of action of chronic Se toxicity involves displacing sulphur from S-containing amino acids like methionine and cysteine. Dietary Se level affects feed intake, microbial protein synthesis and nutrient utilization adversely (Khurwar and Arora 1976, Bakshi et al. 1986). Both sulphur (S) and arsenic (As) are antagonist to Se (Sun et al. 1988; Zeng et al. 2005; Gailer et al. 2000; Pilsner et al. 2011) which could be used to alleviate chronic selenosis. The cases of *Degnala* disease in buffaloes have been successfully treated using pentasulphate/Degcure mixture based on antagonistic relationship between Se and S (Arora et al. 1975). Therefore, the effects of supplementation of S (pentasulphate mixture) and As on feed intake and nutrient utilization were studied in male buffalo calves given high level of Se in the diet.

Materials and Methods

Experimental animals and their feeding

Sixteen male Murrah buffalo calves were selected from ICAR-National Dairy Research Institute herd and divided into 4 groups of 4 animals each based on body weight (136.50±36.05, 134.50±24.52, 133.75±33.23 and 133.50±37.45 kg in groups T₁, T₂, T₃ and T₄, respectively) and age (11.14±1.80, 11.11±2.40, 11.08±2.98

¹ Govt. Poultry Farm, Sundervani, Rajouri, Jammu & Kashmir, India

² Animal Nutrition Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India

³ College of Veterinary Science and AH, Chhattisgarh Kamdhenu Vishwavidyalaya-491001, Durg, Chhattisgarh, India

^{4&5} Animal Nutrition Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India

Chander Datt (✉)
Animal Nutrition Division, ICAR-National Dairy Research Institute,
Karnal-132001, Haryana, India
Email: chandatt@gmail.com

and 10.96 ± 2.74 mon.). The animals were fed on rations to supply 15% less of protein and TDN requirements as per NRC (2001). The initial body weights of animals were similar in all the groups. All the animals were fed a basal diet (0.41 ppm Se) comprising of paddy straw, concentrate mixture and green maize. The concentrate mixture comprised (% parts) maize grain 33, groundnut cake 21, mustard cake 12, wheat bran 20, rice bran 11, mineral mixture 2, common salt 1. Chaffed paddy straw, maize fodder and concentrate mixture were fed as total mixed rations in the ratio of 50: 20: 30. The animals in groups T_2 , T_3 and T_4 were supplemented with 10 ppm of Se in form of sodium selenite until blood Se level approached 1.5 ppm which happened at 60 d of feeding. Thereafter, animals were given supplementary arsenic (40 ppm of diet) in form of sodium arsenite and pentasulphate mixture (9 g/100 kg BW) in groups T_3 and T_4 , respectively in addition to Se (10 ppm) being already given. The animals were provided clean and fresh water ad lib. twice a day throughout the study.

Metabolism trial

A metabolism trial was conducted after 85 d of initiation of the experiment to study feed intake, digestibility of nutrients and balances of nitrogen and Se. During the trial, samples of feeds, refusals, water, faeces and urine were collected daily and pooled separately for each animal. The feed, residues and faecal samples were dried (70°C) for 48 h and ground to pass through 1 mm sieve. The retention of Se was determined by subtracting the total amount of mineral excreted in urine and faeces from the amount of mineral ingested while the amount of mineral absorbed was calculated as the difference between the dietary intake and faecal excretion.

Analytical procedures

Proximate analysis of feeds and faeces and N in urine was carried out (AOAC 2005). Fibre fractions were estimated (Van Soest et al. 1991). The levels of Se in feeds, refusals, water, faeces and urine were analysed using atomic absorption spectrophotometer (Hitachi-5000 Z Series) equipped with hydride generation facility (HGAAS) while As and sulphur contents in feeds, refusals and water were estimated using HGAAS and turbidimetric method (Massoumi and Cornfield 1963), respectively.

Statistical analysis

The data were analysed statistically using one-way analysis of variance (ANOVA) as per Snedecor and Cochran (2007). Differences between the mean values of the different groups were considered significant at $P < 0.05$.

Results and Discussion

Feed intake

The chemical composition as well as the Se, As and S contents of the feeds and fodders are provided in Table 1. The chemical composition of the feeds and fodders was similar throughout the experimental period. The dietary concentration of Se in groups T_1 , T_2 , T_3 and T_4 was 0.40, 10.25, 10.32 and 10.43 ppm, respectively with corresponding values of 0.17, 0.16, 40.26 and 0.17 ppm for As and 0.16, 0.16, 0.16 and 0.23% for S. The average DMI in groups T_1 , T_2 , T_3 and T_4 was 2.93 ± 0.09 , 3.02 ± 0.16 , 2.98 ± 0.17 and $2.94 \pm 0.22\%$ BW, respectively. Bakshi et al. (1986) reported significant depression in DM consumption in buffalo calves given urea treated paddy straw containing 2.14 ppm Se as compared to those given urea treated wheat straw (0.21 ppm Se). Such a response could probably be due to differences in the forms of dietary Se. In their study, the intake of Se was mainly through

Table 1 Chemical composition of feeds (% on DM basis)

Parameter	Paddy straw	Maize fodder	Concentrate mixture
Proximate composition			
DM	93.21	22.14	92.14
OM	84.06	92.0	92.02
CP	4.03	6.9	21.66
EE	1.13	1.31	3.73
CF	33.46	29.39	6.92
Total ash	15.94	8.0	7.98
Cell wall constituents			
NDF	66.75	60.51	35.51
ADF	40.21	34.9	12.16
Cellulose	28.40	26.87	7.57
Lignin	4.95	3.15	2.46
Minerals			
S (%)	0.15	0.14	0.2
Se (ppm)	0.50	0.46	0.37
As (ppm)	0.20	0.13	0.21

organic forms (NRC 1983) which might have depressed rumen microbial activity (Tekchandani and Arora 1978; Hansard 1983) while the inorganic Se formed the major part of dietary Se in this study. They also observed decreased digestibility of nutrients which in turn might have affected DM intake. Serra et al. (1994) also did not observe any difference in DM intake on supplementing 0.2 ppm inorganic Se in sheep given basal diet containing 31.74 ppb of Se, however, the overall dietary Se level in their diets was just adequate. Both deficiency and excess of Se in the diet affected feed intake in birds (Bunk and Combs 1980) and excess of Se in the diet reduced DM intake in ruminants (Bakshi et al. 1986). Supplementation of 0.3-0.4 ppm of Se to Se deficient diets increased DM intake and feed efficiency in cattle (Bonomi 2001).

Digestibility of nutrients

The animals in group T₂ showed lower ($P < 0.05$) CP and ADF digestibility as compared to groups T₁, T₃ and T₄ indicating that pentasulphate mixture and arsenic had positive effect on digestibility of these two chemical constituents in the diets of animals given additional 10 ppm Se (Table 2). As per NRC (1980), the maximum tolerable limit of Se for dairy cattle is 2 ppm. Arora et al. (1975) reported occurrence of selenosis in the form of Degnala disease on feeding rice straw containing 0.9 to 6.7 ppm Se. Prasad and Arora (1984) and Bakshi et al. (1986) revealed occurrence of Degnala disease on feeding rice straw with 3.45 ppm and 2.14 ppm Se, respectively. Therefore, the dietary Se levels in groups T₂, T₃ and T₄ were considered high. Selenium tolerance by the animals depends on several factors (NRC 1983; Mc Dowell 1992). Harrison and Conrad (1984) did not find any significant difference in the DM digestibility of Holstein cows where the intake of Se varied from 400-3100 µg/d. Chander Datt and Chhabra (2006)

reported no significant effect on the digestibility of proximate principles in buffalo calves given a supplementary level of 2.7 ppm inorganic Se to the basal diets containing 0.5 ppm Se. The digestibility of DM, CP, OM and NDF was not affected in sheep supplemented with organic or inorganic Se (0.12 mg/head/day) as compared to control group (0.144 ppm Se). However, Serra et al. (1994) reported that supplementing the basal diet (31.74 ppb Se) with 0.2 ppm inorganic Se slightly reduced fibre digestion and tended to increase protein digestion. They opined that alteration in nutrient digestibility could be due to complicated adjustment in rumen because of dietary Se. When given urea treated paddy straw (2.14 ppm Se), Bakshi et al. (1986) found significant reduction in digestibility of nutrients (DM, OM, CP, EE, NFE, NDF, ADF and cellulose). The depressed nutrient digestibility might due to high Se intake which has been reported to depress the rumen microbial activity (Tekchandani and Arora 1978; Hansard 1983, Chander Datt et al. 2013). The discrepancy among the studies might be partly due to differences in chemical forms and levels of dietary Se. An 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). Supplementation of diet with Se-yeast at low level improved rumen fermentation and feed digestion (Wang et al. 2009). The high level of Se in the supplemented group reduced digestibility of CP and ADF (Table 2). In vitro studies of Martinez and Church (1970) showed slight reduction in cellulose digestion when the level of Se in the incubation medium ranged from 0.01 to 5 ppm but significant reduction was observed when the level varied from 7 to 20 ppm. Selenium level of more than 12 µg/200 mg substrate showed the inhibitory effect on true OM digestibility, in vitro gas production, microbial biomass production and total volatile fatty acids. Addition of As at 10 or 20 µg level to the substrate (200 mg)

Table 2 Digestibility of nutrients and nitrogen balance in different groups of buffalo calves

Attribute	Group			
	T ₁	T ₂	T ₃	T ₄
Digestibility coefficient (%)				
DM	62.23±2.08	60.43±2.50	60.65±1.90	61.23±1.60
OM	64.34±1.67	62.67±2.44	59.28±1.90	63.41±2.35
CP	69.17 ^a ±1.08	62.19 ^b ±2.99	65.90 ^a ±1.74	67.64 ^a ±1.83
EE	69.85±3.01	65.49±4.48	65.55±1.16	65.89±2.08
NDF	56.49±2.30	54.88±3.66	56.32±2.43	55.82±2.57
ADF	53.54 ^a ±2.90	48.23 ^b ±2.65	51.32 ^a ±1.42	51.14 ^a ±2.79
Nitrogen balance				
Total N intake (g/d)	68.12±11.95	66.34±9.61	71.10±10.91	67.66±13.66
Faecal N excretion (g/d)	21.0±3.42	23.84±3.52	24.14±4.59	21.89±4.02
N absorbed (g/d)	47.17 ^a ±2.17	42.50 ^b ±1.30	46.86 ^a ±1.53	45.77 ^a ±1.24
N absorbed (% of intake)	69.17 ^a ±1.08	62.19 ^b ±2.99	65.90 ^a ±1.74	67.64 ^a ±1.83
Urinary excretion (g/d)	32.59±2.68	30.75±2.64	33.4±3.28	33.02±1.62
N retained (g/d)	14.58±3.31	13.75±2.98	13.46±2.67	12.75±2.75
N retention (% of intake)	21.39±0.61	18.73±1.05	18.93±0.77	18.84±0.42

^{a,b}Values bearing different superscripts in a row differ significantly ($P < 0.05$)

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. (Chander Datt et al. 2013). 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. Both Se and As have been reported to increase biliary excretion of each other (Levender 1977; Pilsner et al. 2011). Arsenic and Se are antagonistic to each other and form a complex called seleno-bis (*S*-glutathionyl) arsinium ion [(GS₂AsSe)]- which is secreted through bile and finally excreted through faeces (Gailer 2009). Sulphur (S) and Se are antagonist i.e. S either reduces tissue uptake of Se or increases its excretion from the body or both (Arora et al. 1975; Sun et al. 1988).

Nitrogen and Se balance

The average total N intake was similar in four groups (Table 2). The values for N absorbed (g/d) was lower ($P<0.05$) in group T₂

as compared to other groups. Bakshi et al. (1986) reported higher N excretion through faeces and urine in male buffaloes calves resulting in negative N balance. Similar digestibility values were obtained when pentasulphate mixture and arsenic were added to high Se diets.

The dietary Se level in groups T₁, T₂, T₃ and T₄ was found to be 0.40, 10.25, 10.32 and 10.43 ppm, respectively (Table 3). There were significant ($P<0.01$) differences among the groups with regard to Se intake, faecal excretion, apparent absorption, urinary excretion and net retention. The intake of Se by animals in group T₁ was just above the required level of 0.3 ppm (NRC 2001) while the levels in other 3 groups were above maximum tolerable limits of 5 ppm (Mc Dowell 1992; NRC 2001). Chronic selenosis as 'Degnala disease' in buffaloes has been reported on feeding rice straw having 0.9 to 6.7 ppm of Se (Arora et al. 1975) or rice husk with 6.23 ppm of Se (Prasad et al. 1982) or rice straw containing 2.14 ppm of Se (Bakshi et al. 1986). Faecal Se excretion differed significantly ($P<0.01$) among groups. Faecal Se was the lowest (1.11 mg) in group T₁. Supplementation of As (T₃) and pentasulphate mixture (T₄) increased faecal excretion of Se when

Table 3 Absorption and excretory patterns of Se in different groups

Particular	Group			
	T ₁	T ₂	T ₃	T ₄
Total intake (mg)	1.86 ^a ±0.34	47.66 ^b ±1.84	49.43 ^b ±5.89	48.39 ^b ±6.19
Faecal excretion (mg)	1.11 ^a ±0.19	37.94 ^b ±1.5	43.66 ^b ±5.22	42.20 ^b ±5.45
Apparent absorption (mg)	0.75 ^a ±0.14	9.72 ^b ±0.46	5.77 ^c ±0.71	6.19 ^c ±0.76
Apparent absorption (%)	40.34 ^a ±0.79	20.40 ^b ±0.71	11.68 ^c ±0.48	12.81 ^c ±0.40
Urinary excretion (mg)	0.19 ^a ±0.03	3.50 ^b ±0.26	2.57 ^c ±0.46	3.04 ^b ±0.29
Retention (mg)	0.56 ^a ±0.11	6.22 ^b ±0.23	3.20 ^c ±0.32	3.15 ^c ±0.60
Retention (% of intake)	29.91 ^a ±0.72	13.05 ^b ±0.49	6.47 ^c ±0.22	6.50 ^c ±0.58

^{a,b,c} Values bearing different superscripts in a row differ significantly ($P<0.01$)

Table 4 Plane of nutrition of the animals in different treatments

Parameter	Group			
	T ₁	T ₂	T ₃	T ₄
Body weight (kg)	158.61±34.12	159.02±27.78	157.93±36.074	157.5±23.45
DM intake				
kg/d	4.65±0.82	4.79±0.73	4.72±0.84	4.64±0.88
kg/100 kg body weight	2.93±0.09	3.02±0.16	2.98±0.17	2.94±0.22
CP intake				
g/d	426.10±74.75	414.68±60.08	444.39±68.28	422.92±85.41
g/100 kg body weight	268.66±27.22	260.77±24.92	281.38±23.30	268.52±26.15
% of NRC (2001)	81.91±1.35	79.52±2.66	85.81±2.44	81.61±1.94
TDN intake				
kg/d	2.49±0.48	2.28±0.38	2.48±0.36	2.37±0.46
kg/100 kg body weight	1.57±0.06	1.43±0.19	1.57±0.12	1.50±0.15
% of NRC (2001)	98.03±1.35	89.41±2.49	98.03±2.53	93.68±2.67
Nutritive value				
CP % in ration	9.16±0.45	8.66±0.36	9.41±0.39	9.11±0.22
TDN % in ration	53.54±1.49	47.59±0.81	52.54±1.24	51.07±0.70

compared to group T₂. Consequently, apparent absorption (mg/d, % of intake) was more in group T₂ as compared to groups T₃ and T₄. Data on excretion of Se through faeces indicated that the major portion of ingested Se was excreted through faeces (Langlands et al. 1986, Serra et al. 1994, Krishnamurthi et al. 1997). The higher faecal excretion resulting in lower absorption might be due to the fact that rumen microbes convert much of dietary Se to insoluble forms, e.g. elemental Se, metal selenides (Durand and Kawashima 1980, Harrison and Conrad 1984; Hakkarainen 1993) which is not absorbable because it gets adsorbed to indigestible feed particles and then passed out of the gastrointestinal tract along with faeces (Podoll et al. 1992). Rumen also provides a very strong reducing environment which may convert ingested Se to reduced forms which are not available for absorption (Durand and Kawashima 1980; Van Saun 1990) and this type of conversion is more when high concentrate diets are fed (NRC 1983; Gerloff 1992). Organically bound Se is rendered unavailable to a lesser extent as compared to the inorganic Se (NRC 1983; Peter and Whanger 1986). Apparent absorption of Se averaged 40.34, 20.40, 11.68 and 12.81% in groups T₁, T₂, T₃ and T₄, respectively. Apparent absorption ranging from 17 to 64% has been reported in ruminants supplemented with sodium selenite (Harrison and Conrad 1984; Zanetti and Pettinati 1991; Aspila 1991). Another study (Bakshi et al. 1986) showed the apparent absorption of 96 and 57% in male buffaloes given low-Se urea treated wheat straw and high Se (2.14 ppm) urea treated (0.21 ppm) paddy straw, respectively. The apparent absorption of Se in wethers given Timothy hay alone containing 31.74 ppb Se or supplemented with 0.2 ppm inorganic Se was 67.39 and 74.32%, respectively (Serra et al. 1994). Koenig et al. (1991) in studies with dairy cows given 0.19 ppm Se diets, however, observed apparent absorption value of 3% only. The apparent Se absorption rate was significantly higher in the Se-yeast (0.3 ppm) group (62%) than in sodium selenite supplemented (0.3 ppm) lambs (49.6%) or in those fed the unsupplemented basal diet (52.9%, $P < 0.001$). Due to lower absorption rate, the rams given sodium selenite had higher faecal Se excretion than the Se-yeast fed animals whereas no differences between the supplemented groups appeared in urinary Se excretion (Greskova et al. 2013). The differences in absorption of Se from the digestive tract could be due to variations in composition of the rumen microbial population and the diet, chemical form and amount of dietary Se, Se status of animals (Hakkarainen 1993; Koenig et al. 1997) and other interacting factors in feeds like high dietary S, Ca, As etc. reduced the bioavailability of Se (Spears 2003; Chander Datt et al. 2013).

It was clear that supplementation of arsenic and pentasulphate mixture reduced absorption of Se. Interacting factors like S, As and others have been reported to decrease the absorption of Se (Levander 1977; NRC 1980; Peter et al. 1982; Gailer et al. 2000). Urinary excretion of Se (mg/d) increased in Se supplemented groups. At higher level of intake, body tends to excrete the element through all its channels in order to get rid of excess supply to

maintain a homeostatic control and avoid toxicity. Therefore, urinary excretion of Se increased as the intake was increased (Fisher et al. 1980; Harrison and Conrad 1984). It has been demonstrated in rats that Se is excreted mainly in the form of trimethyl selenium (TMSe⁺). Sanz-Alaejos and Diaz-Romero (1993) were of the view that TMSe⁺ though a minor metabolite of Se in urine at low Se intakes occupied a significant role in detoxification of excess Se intake. Ganther and Hsieh (1974) have proposed a scheme of metabolic events involved in the excretion of Se in which glutathione (GSH), glutathione reductase and S-adenosyl methionine (SAM) as methyl group donor are actively involved in its excretion through urine mainly as TMSe⁺ (trimethyl selenide) during chronic selenosis or mainly as dimethyl selenide during acute selenosis conditions. Therefore, deficiency of either GSH or glutathione reductase or SAM or all of these is likely to hamper the detoxification process in conditions of excess Se load. In the present study, the excretion of Se through urine was found to be higher ($P < 0.01$) in high Se groups which could have been facilitated by liver SAM by providing methyl group through methyl transferases and finally its excretion in urine mainly as TMSe⁺. However, Se retention was higher ($P < 0.01$) in high Se groups which indicated that detoxification was not complete and that the rate of entry of Se into liver might have exceeded the rate at which SAM was synthesised by liver cells. Therefore, liver cells might not be able to cope up with the Se methylation process for rapid excretion resulting in higher retention. Additional selenite has been shown to inactivate the methionine-adenosyl transferase enzyme system and this inhibition might have prevented replenishment of SAM. Prasad and Arora (1991) observed significantly lower concentration of SAM in the liver and GSH concentration in erythrocyte in buffalo calves administered either with selenite or selenomethionine compared to the control group.

Retention of Se differed ($P < 0.01$) among the groups. Though the total amount of Se retained increased in groups T₂, T₃ and T₄ when compared to control (T₁) group but when expressed as percent of intake, the retention values were lower in high Se groups indicating that an inverse relationship existed between Se retention and intake of Se. Both retention and intake of Se are related linearly at dietary Se levels from deficient to adequate (Harrison and Conrad 1984) above which relationship becomes inverse (Lopez et al. 1969; Kinkaid et al. 1977). Further, retention of Se by buffaloes was lower in groups T₃ and T₄ compared to that in group T₂ indicating that As and pentasulphate mixture were capable of getting rid of extra Se supply by the animals because of their antagonistic relationship with Se (Whanger 1970; Arora et al. 1975; Levander 1977; NRC 1980; NRC 1983; Peter et al. 1981; McDowell 1992; Gailer et al. 2000).

Plane of nutrition

Mean CP intake was found to be 426.10, 414.68, 444.39 and 422.92 g/d in groups T₁, T₂, T₃ and T₄, respectively (Table 4) The CP

intake was 14.19-20.48% lower when compared to the requirements (NRC 2001) of CP by cattle of similar body weight. This was evident because the animals in all the groups were offered rations containing 15% less CP than their requirements. These differences could be due to higher growth rate (400 g/d) assumed as per recommendation of NRC (2001) whereas under present set of conditions the growth rate was lower (251.4±17.55, 255.2±23.3, 253.3±27.8 and 249.5±24.3 g/d in respective groups) since the animals were not maintained to achieve higher growth rate as paddy straw itself constituted more than 50% of total dietary DM. Likewise, deficit in TDN intake was 7-11% less when compared to the recommendations of NRC (2001).

Conclusions

Supplementation of 10 ppm Se to the control diet (0.41 ppm Se) did not affect feed intake and N retention, however, digestibility of CP and ADF was reduced ($P < 0.05$) in buffalo calves. Supplementation of either pentasulphate mixture @ 9 g/100 kg BW or arsenic (40 ppm) to the diet already supplied with extra 10 ppm Se improved the digestibility of CP and ADF while reducing the Se load from the body to a significant extent.

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