



Response to strategic dietary mineral mixture supplementation in cattle and buffaloes under field condition (Hill region) of Nainital district of Uttarakhand

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ABSTRACT

Calcium, phosphorus, zinc, manganese and copper were found below the critical level in soil, feeds and blood serum of cattle and buffaloes in hill region of Nainital district of Uttarakhand. A study was, therefore conducted to see the effect of dietary supplementation of these minerals as area specific mineral mixture in cattle and buffaloes on reproductive performance, milk yield, their profile in blood serum under field condition in four villages of Nainital district. The calcium, phosphorus, zinc, manganese and copper were supplemented in the form of feed (mineral) mixture which contained ground maize 32.80%, mustard cake 48.69%, di-calcium phosphate 17.85%, copper sulphate 0.12%, manganese sulphate 0.30% and zinc sulphate 0.24%. Fifteen animals (cattle and buffaloes) of different physiological status from each village were selected. Each selected animal was provided 250 g feed mixture daily in order to supply 12 g calcium, 5 g phosphorus, 250 mg zinc, 175 mg manganese and 60 mg copper over and above already present in the ration of animals provided by the farmers for 45 days. The incidence of the anoestrus and repeat breeding cases decreased due to dietary supplementation of area specific mineral mixture. Milk yield was increased and remained persistent in lactating (pregnant) and lactating (non-pregnant) animals. The SGPT, SGOT and ALP activities increased in all categories of animals but were within the normal range. Blood serum calcium and phosphorus concentrations were also increased significantly and reached above their critical levels (8.00 mg/dl and 4.00 mg/dl respectively) in all categories of animals. The serum zinc, manganese and copper concentration also increased but not significantly due to dietary supplementation of these minerals. It was concluded that area specific mineral mixture supplementation in the ration is inevitable in amelioration of reproductive problems and in improving the milk production, reproductive performance and mineral profile in cattle and buffaloes under field condition of Kumaun hill region.

Key words: Area specific mineral mixture, Buffaloes, Cattle, Milk yield, Reproductive performance, Serum mineral profile

In hilly region of Nainital district of Uttarakhand, the most common livestock species are cattle and buffaloes and are mostly reared on crop residues and dry grasses along with locally available green fodder and small amount of concentrate mixture. Generally mineral mixture is not supplemented in the ration. Calcium, phosphorus, copper, zinc, manganese and copper were found low in soil, feeds and blood serum of cattle and buffaloes in hill region of Uttarakhand (AICRP 2006). The present study was, therefore, planned to see the effect of dietary supplementation of these minerals as area specific mineral mixture (ASMM) in different categories of cattle and buffaloes over and above

the existing feeding system/levels, followed by the farmers on milk yield, reproductive performance and certain blood biochemical parameters and mineral status under field condition in Kumaun hills of Uttarakhand.

MATERIALS AND METHODS

Four villages, viz. Songaon, Jangalia, Amia and Gaza basuli, in Nainital (Hill region) district of Uttarakhand were selected for the study. Initially blood samples from 60 animals (cattle and buffaloes) of different physiological status, 15 from each village were collected during the course of investigation and analyzed for calcium, phosphorus, zinc, manganese and copper and also for serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and serum alkaline phosphates (ALP) activities and the information on milk yield and reproductive status of the animals was also collected from the farmers.

Experimental feed (mineral) mixture: To supplement calcium, phosphorus, zinc, manganese and copper as per

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NRC (2001) requirements, a feed (mineral) mixture was prepared in which calcium and phosphorus in the form of di-calcium phosphate (17.85%), zinc in the form of zinc sulphate (0.24%), manganese in the form of manganese sulphate (0.30%) and copper in the form of copper sulphate (0.12%) were used with ground maize (32.80%) and mustard cake (48.69%).

Feeding under field condition: All categories of animals under field condition were fed feeds and fodder as per availability. Fodder tree leaves of *timla*, *dhudhila*, *bhimal*, oak, besides pea, *guria* grass and paddy straw were fed to the animals. Wheat bran, pellet and concentrate mixture were also fed to the animals by some farmers. In addition, each selected animal was provided 250 g of feed (mineral) mixture daily to supply 12 g calcium, 5 g phosphorus, 250 mg zinc, 175 mg manganese, 60 mg copper over and above already present in the ration of animals provided by the farmers. The supplemental feeding trial of area specific mineral mixture to the different categories of animals lasted for 45 days. Thereafter blood samples from the animals were collected to know the calcium, phosphorus, zinc, manganese and copper status and also SGPT, SGOT and ALP activities. The information regarding response on milk yield and reproductive status of animals due to dietary supplementation of area specific mineral mixture was also recorded. The feeds and fodder offered to the animals were analyzed for proximate constituents (AOAC 1995). The SGPT and SGOT activities were determined by the method of Young (1995). The

calcium, zinc, copper and manganese in the blood serum were estimated by atomic absorption spectrophotometer and phosphorus in blood serum was estimated by colorimetric method (Fiske and Subbarow 1925). The data were analyzed statistically as per methods described by Snedecor and Cochran (1994) using 't' test.

RESULTS AND DISCUSSION

The chemical composition and mineral contents of feeds and fodder fed to the animals under field condition are given in Table 1. The chemical composition was in line with those reported by Mudgal *et al.* (2003), Tiwary *et al.* (2007) and Shukla *et al.* (2009).

Mineral contents in feeds and fodder: Calcium content in different types of concentrate and roughages were above the critical level (<0.30%). The phosphorus content in some of the fodders was insufficient to meet the required level and was below than the critical level (<0.25%). Concentrates and roughages offered to the animals have comparatively adequate concentration of zinc and it was above the critical level (<30 ppm). Copper was above the critical level (<8 ppm) in green fodders but most of dry fodders were deficient in Cu content like wheat straw and dry *guria* grass and also some of the tree leaves like *sanjh* and *khainia* leaves in Amia and Gaza basuli villages (Table 1).

Dry matter intake: An average dry matter intake increased significantly ($P < 0.05$) in all categories of animals (Table 2) due to supplementation of area specific mineral mixture

Table 1. Chemical composition and mineral contents of feeds and fodder (on dry-matter basis) of Nainital (Hill region) district of Uttarakhand.

Feeds/Fodder	DM%	CP%	EE%	CF%	Ash%	NFE%	AIA%	Ca%	P%	Zn ppm	Mn ppm	Cu ppm
Timla (<i>Ficus auriculata</i>) leaf	33.52	14.05	2.66	14.09	11.31	58.40	3.31	2.56	0.28	40.67	91.72	10.18
Dhudhila (<i>Lagenaria siceraria</i>) leaf	40.85	10.30	1.86	15.75	19.66	52.44	3.01	2.09	0.21	45.52	87.61	10.21
Bhimal (<i>Grewia optiva</i>) leaf	54.30	13.13	2.73	17.97	7.91	58.25	0.39	2.65	0.26	48.07	89.63	10.33
Oak (<i>Quercus macrocarpa</i>) leaf	77.45	11.03	3.34	32.98	5.25	47.40	0.56	1.65	0.24	48.57	89.12	9.75
Sanjh (<i>Petalonyx nitidus</i>) leaf	42.86	09.66	1.09	18.50	26.52	44.23	0.40	2.28	0.29	46.77	85.1	7.42
Khainia (<i>Ethiopic lchaymat</i>) leaf	61.66	09.73	4.41	20.21	18.55	47.31	6.23	3.12	0.2	42.25	88.5	7.05
Rain (<i>Samanea saman</i>) leaf	49.76	12.31	1.67	13.15	8.95	63.94	3.18	1.87	0.17	38.56	87.03	10.07
Guria (<i>Chrysopogon fulvus</i>) grass	92.24	10.92	1.60	29.13	10.07	48.33	4.91	0.90	0.21	40.61	92.18	12.27
Hathi (<i>Pennisetum purpureum</i>) grass	33.73	7.73	2.21	20.53	19.36	53.20	9.73	1.16	0.27	49.92	92.36	26.12
Paddy straw	89.48	4.88	1.61	39.75	14.17	39.79	9.40	0.86	0.34	40.08	88.18	18.66
Wheat straw	90.00	9.08	1.86	27.05	6.92	55.33	3.80	1.98	0.48	42.63	86.68	10.04
Concentrate pellet	90.61	15.36	2.62	8.36	10.38	63.30	2.77	3.16	0.84	47.72	88.50	10.50
Concentrate mixture	92.35	12.92	4.05	7.27	6.29	69.48	1.77	1.88	0.85	47.37	89.39	18.67
Pea	29.2	17.01	1.63	29.25	7.1	44.92	0.72	2.65	0.26	41.00	85.00	9.00
Green berseem	10.81	16.37	2.91	26.22	10.49	44.02	1.09	4.15	0.27	41.31	89.48	26.29
Green oats	18.14	12.41	2.43	14.64	10.2	60.33	2.26	1.32	0.32	40.56	91.57	14.36
Green oats + berseem	14.44	14.31	2.16	19.91	10.72	53.13	3.26	2.37	0.31	44.12	88.19	21.93
Wheat straw + berseem	45.27	6.71	2.05	33.91	10.06	47.28	4.47	1.32	0.31	41.77	90.11	20.15
Wheat flour	86.30	8.60	3.41	5.65	3.62	78.74	1.95	2.56	0.28	40.67	91.72	10.18
Wheat flour + Paddy flour	90.10	8.00	2.67	6.55	4.40	78.38	1.74	2.09	0.21	45.52	87.61	10.21
[†] Critical level	-	-	-	-	-	-	-	<0.30	<0.25	<30	<40	<8

[†]Prabowo *et al.* (1990).

Table 2. Response to area specific mineral mixture supplementation on dry matter intake (kg/day) in cattle and buffaloes under field condition

Category of animals	Before supplementation	After supplementation
Lactating pregnant cows/buffaloes (n=11)*	5.65 ^b ±0.08	6.33 ^a ±0.06
Lactating non-pregnant cows/buffaloes (n=12)	5.91±0.04	6.02±0.14
Heifers (n=9)*	3.19 ^b ±0.11	3.73 ^a ±0.11
Calves (n=8)*	1.26 ^b ±0.03	1.65 ^a ±0.05
Anoestrus cows/buffaloes (n=11)*	5.07 ^b ±0.04	5.82 ^a ±0.01
Repeat breeding cows/buffaloes (n=9)*	5.14 ^b ±0.12	5.58 ^a ±0.23

Figures in parentheses denote the number of observations. Values bearing different superscripts in a row differ significantly from each other. *P < 0.05.

Table 3. Response to area specific mineral mixture supplementation on milk yield litre/day) in cattle and buffaloes under field condition in Nainital (Hill region) district

Category of animals	Before supplementation	After supplementation
Lactating pregnant cows/buffaloes (n=11)	3.23±0.37	3.68±0.34
Lactating non-pregnant cows/buffaloes (n=12)*	4.97 ^b ±0.46	5.81 ^a ±0.45
Anoestrus cows/buffaloes (n=11)	1.50±0.12	1.74±0.09
Repeat breeding cows/buffaloes (n=9)	0.63±0.16	0.79±0.16

Figures in parentheses denote the number of observations. Values bearing different superscripts in a row differ significantly from each other. *P < 0.05.

Table 5. Response to area specific mineral mixture supplementation on serum enzyme activities (IU/litre) in cattle and buffaloes under field condition

Category of animal ^f (Normal range)	Before supplementation			After supplementation		
	SGPT (14–38 IU/litre)	SGOT (60–118 IU/litre)	ALP (0–488 IU/litre)	SGPT (14–38 IU/litre)	SGOT (60–118 IU/litre)	ALP (0–488 IU/litre)
Lactating pregnant cows/buffaloes (n=11)*	30.42±0.59	74.74 ^b ±5.66	221.70 ^b ±32.52	33.51±1.25	107.24 ^a ±1.70	410.05 ^a ±15.04
Lactating non-pregnant cows/buffaloes (n=12)*	29.28±1.88	68.79 ^b ±8.30	214.48 ^b ±18.44	29.61±1.76	87.41 ^a ±4.46	405.99 ^a ±19.17
Heifers (n=9)*	21.44 ^b ±2.17	49.50 ^b ±7.59	114.29 ^b ±11.01	27.82 ^a ±1.79	71.98 ^a ±4.91	299.96 ^a ±55.54
Calves (n=8)*	17.47 ^b ±2.75	36.35 ^b ±5.61	75.77 ^b ±14.03	25.69 ^a ±2.72	58.82 ^a ±2.28	284.90 ^a ±46.27
Anoestrus cows/buffaloes (n=11)*	14.13±2.05	27.88 ^b ±3.23	69.23 ^b ±5.42	18.61±2.14	53.17 ^a ±2.67	296.75 ^a ±16.48
Repeat breeding cows/buffaloes (n=9)*	10.97 ^b ±1.49	24.15 ^b ±2.80	40.52 ^b ±7.50	14.63 ^a ±1.84	39.15 ^a ±1.02	173.09 ^a ±25.89

Figures in parentheses denotes the number of observations. Values bearing different superscripts in a row for a parameter differ significantly from each other. *P < 0.05.^fSastry *et al.* (1999).

Table 4. Response to area specific mineral mixture supplementation on amelioration of reproductive problems cattle and buffaloes under field condition

Category of animals	Before supplementation	After supplementation
Anoestrus (%) (n=43)*	20.00 ^a	11.67 ^b
Repeat breeding (%) (n=43)*	16.67 ^a	10.00 ^b

Figures in parentheses denote the number of observations. Values bearing different superscripts in a row differ significantly from each other. *P < 0.05.

except in lactating non-pregnant animals of Amia and repeat breeding animal of Gaza basuli village which is due to fact that these animals were not offered required quantity of feed by the farmers. Similar results were also reported by Mahanta *et al.* (2004).

Milk yield: The increase in average milk yield was recorded from 0.32 to 0.50 litre (15.32 to 18.80%) (Table 3). Wu *et al.* (2001), Gimbi *et al.* (2004), Saxena *et al.* (2008) and Sharma *et al.* (2009) also reported increase in milk yield due to supplementation of area specific minerals.

Reproductive performance: Mainly anoestrus and repeat breeding problems were reported by the farmers. The anoestrus problem in cattle and buffaloes were maximum in Gaza basuli village (20.00%) followed by Amia village (16.67%) of low hill region. Whereas the incidence of repeat breeding was maximum in Gaza basuli village (20.00%). The overall anoestrus and repeat breeding cases were 20 and 16.67% respectively (Table 4). After dietary supplementation of area specific minerals, the percentage of reproductive problems as anoestrus and repeat breeding were decreased significantly (P < 0.05) to 11.67 and 10% respectively. Similar findings were reported by Saxena *et al.* (2008) and Sharma *et al.* (2009).

Serum glutamate pyruvate transaminase (SGPT) activity: The overall average serum glutamate pyruvate transaminase (SGPT) activity of animals in all selected villages were within the normal range (14–38 IU/litre) as suggested by Sastry *et al.* (1999) except for repeat breeding cows/buffaloes (10.97 IU/litre) (Table 5), whereas the SGPT activity was lower than normal range in anoestrus and repeat breeding animals which showed the poor nutritional status of animals. After the supplementation of area specific minerals all categories of animals in all villages had normal values of SGPT activity. Similar observations were also reported by Sharma *et al.* (2009) and Saxena *et al.* (2008).

Serum glutamate oxaloacetate transaminase (SGOT) activity: Average serum glutamate pyruvate transaminase (SGOT) activity in animals of selected villages were low in heifers (49.50 IU/litre), calves (36.35 IU/litre), anoestrus animals (27.88 IU/litre) and repeat breeding animals (24.15 IU/litre) (Table 5), which reflected to the poor nutritional status of animals. After the supplementation of area specific minerals, the SGOT activities increased significantly ($P < 0.05$) in all categories of the animals and reached within normal range (60–118 IU/litre) as mentioned by Sastry *et al.* (1999) except in calves (58.82 IU/litre), anoestrus (53.17 IU/litre) and repeat breeding (39.15 IU/litre) animals. The present findings showed that the SGOT activity increased in all animals, however, the lower than normal value of SGOT might be due to poor nutritional status in terms of DM, energy and protein intakes. Similar findings were also reported by Saxena *et al.* (2008) and Sharma *et al.* (2009).

Alkaline phosphatase activity: In all categories of animals the overall average alkaline phosphatase activity were in normal range (0–488 IU/litre) as suggested by Sastry *et al.* (1999). After supplementation of area specific minerals, the ALP activity increased in all categories of the animals (Table 5). Similar values were also reported by Divya *et al.* (2009).

Serum calcium concentration: The overall mean blood serum calcium concentration in calves, anoestrus and repeat breeding animals was below the critical level (< 8.00 mg/dl) as suggested by Miles and Mc Dowell (1983) except lactating pregnant (8.78 mg/dl), lactating non-pregnant (8.67 mg/dl) and heifers (8.61 mg/dl) in all selected villages (Table 6). After the supplementation of area specific minerals all categories of animals showed significant ($P < 0.05$) increase in blood serum calcium concentration but in anoestrus and repeat breeding animals, it was below the critical level (7.52 mg/dl and 7.68 mg/dl, respectively). Our findings corroborated with the observations of Gimbi *et al.* (2004), Wu and Sattar (2000) and Sharma *et al.* (2009).

Serum phosphorus concentration: The overall mean serum phosphorus concentration, prior to supplementation of area specific minerals was above the critical level (< 4.00 mg/dl) in all categories of the animals (Table 6). After supplementation of area specific minerals, all categories of animals in all selected village showed increase in serum

Table 6. Response to area specific mineral mixture supplementation on blood mineral status in cattle and buffaloes under field condition.

Category of animal ^f (Critical value)	Before supplementation				After supplementation			
	Calcium (< 8.00 mg/dl)	Phosphorus (< 4.00 mg/dl)	Zinc (< 0.60 ppm)	Manganese (< 0.20 ppm)	Calcium (< 8.00 mg/dl)	Phosphorus (< 4.00 mg/dl)	Zinc (< 0.60 ppm)	Copper (< 0.65 ppm)
Lactating pregnant cows/buffaloes (n=11)*	8.78 $\pm$ 0.18	5.64 ^b $\pm$ 0.40	1.66 $\pm$ 0.12	0.72 $\pm$ 0.03	9.20 $\pm$ 0.25	6.69 ^a $\pm$ 0.28	2.19 $\pm$ 0.09	0.88 $\pm$ 0.03
Lactating non-pregnant cows/buffaloes (n=12)*	8.67 $\pm$ 0.30	5.80 ^b $\pm$ 0.34	1.28 ^b $\pm$ 0.12	0.62 ^b $\pm$ 0.07	8.91 $\pm$ 0.16	6.51 ^a $\pm$ 0.30	2.36 ^a $\pm$ 0.29	0.84 ^a $\pm$ 0.02
Heifers (n=9)*	8.61 $\pm$ 0.53	5.66 ^b $\pm$ 0.40	1.03 ^b $\pm$ 0.12	0.81 $\pm$ 0.01	9.14 $\pm$ 0.52	6.96 ^a $\pm$ 0.65	2.29 ^a $\pm$ 0.31	0.98 $\pm$ 0.04
Calves (n=8)*	7.55 ^b $\pm$ 0.62	5.48 ^b $\pm$ 0.43	1.63 ^b $\pm$ 0.05	0.52 $\pm$ 0.10	8.43 ^a $\pm$ 0.40	6.61 ^a $\pm$ 0.44	2.22 ^a $\pm$ 0.27	0.84 $\pm$ 0.07
Anoestrus cows/ buffaloes (n=11)*	6.64 ^b $\pm$ 0.40	4.76 ^b $\pm$ 0.28	1.16 $\pm$ 0.10	0.40 ^b $\pm$ 0.04	7.52 ^a $\pm$ 0.39	5.96 ^a $\pm$ 0.44	1.97 $\pm$ 0.27	0.82 ^a $\pm$ 0.07
Repeat breeding cows/buffaloes (n=9)*	5.71 ^b $\pm$ 0.30	4.47 ^b $\pm$ 0.21	1.16 ^b $\pm$ 0.11	0.49 ^b $\pm$ 0.06	7.68 ^a $\pm$ 0.19	5.96 ^a $\pm$ 0.41	1.90 ^a $\pm$ 0.07	0.93 ^a $\pm$ 0.04
								1.18 ^a $\pm$ 0.05

Figures in parenthesis denotes the number of observations. Values bearing different superscripts in a row for a parameter differ significantly from each other. * $P < 0.05$. ^fMiles and Mc Dowell (1983).

phosphorus concentration. Our findings corroborated with the observations of Gimbi *et al.* (2004), Saxena *et al.* (2008) and Sharma *et al.* (2009).

Serum zinc, manganese and copper concentration: The overall average blood serum zinc, manganese and copper concentration in all categories of animals was above the critical level (<0.60 ppm, <0.20 ppm and <0.65 ppm, respectively) as suggested by Miles and Mc Dowell (1983) (Table 6). After the supplementation of area specific minerals all categories of animals further showed improvement in the serum Zn, Mn and Cu concentration and values were above the critical level. Similar results were reported by Sharma *et al.* (2009) in Pithoragarh district of Uttarakhand. The serum Zn, Mn and Cu concentration were also increased in all categories of the animals but these values were not significantly different. Similar observations were also recorded by Sharma *et al.* (2009).

From the present study, it was concluded that dietary calcium, phosphorus, zinc, manganese and copper supplementation as area specific mineral mixture is essential in cattle and buffaloes under existing feeding practices/system in hill region of Nainital district of Uttarakhand for improving milk production, reproductive performance and blood mineral status.

REFERENCES

- AICRP. 2006. Annual Report. *AICRP on Improvement of Feed Resources and Nutrient Utilization in Raising Animal Production*. G.B. Pant University of Agriculture and Technology, Pantnagar.
- AOAC. 1995. *Official Methods of Analysis*. 15th edn. Association of Official Analytical Chemists, Washington, D.C.
- Divya, Tiwari D P and Kumar A. 2009. Effect of different levels of undegradable dietary protein and plane of nutrition on the performance of growing crossbred heifers. *Indian Journal of Animal Sciences* **79**(5): 498–503.
- Fiske C H and Subbarow Y. 1925. The colorimetric determination of phosphorus. *Journal of Biological Chemistry* **66**: 375–78.
- Gimbi A A, Kimambo A E, Kanuya N L, Mtenga L A, Laswai G H and Madsen J. 2004. Effect of concentrate supplementation on plasma minerals and performance of smallholder dairy cattle. *Journal of Animal and Feed Sciences* **13**(1): 487–90.
- Mahanta S K, Khan S A and Bedi S P S. 2004. Effect of supplementation of phosphorus on nutrient utilization in buffaloes. *Indian Journal of Animal Nutrition* **21**(3): 164–68.
- Miles W H and Mc Dowell L R. 1983. Mineral deficiency on the Llanos ranges. *World Animal Review* **46**: 2–10.
- Mudgal V, Mehta M K, Rane A S and Nanavati S. 2003. A survey on feeding practices and nutritional status of dairy animals in Madhya Pradesh. *Indian Journal of Animal Nutrition* **20**(2): 217–20.
- NRC. 2001. *Nutritional Requirements of Dairy Cattle*. 7th Rev. edn. National Research Council, National Academy of Sciences, Washington, D.C.
- Prabowo A, McDowell L R, Wilkinson N S, Wilcox C J and Conrad J H. 1990. Mineral status comparisons between grazing cattle and water buffalo in south sulanesia, Indonesia. *Buffalo Journal* **1**: 17–22.
- Sastry V R B, Karma D N and Pathak N N. 1999. *Laboratory Manual of Animal Nutrition*. Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India.
- Saxena P C, Tiwari D P, Kumar A, Mondal B C and Sharma R J. 2008. Dietary copper and phosphorus supplementation to cattle and buffaloes on production performance under field condition in plain region of Uttarakhand. *Indian Journal of Animal Sciences* **78**(10): 1138–43.
- Sharma J, Kumar A, Tiwari, D P and Mondal B C. 2009. Effect of dietary supplementation of calcium, copper and manganese on production performance of dairy cattle in Pithoragarh district of Uttarakhand. *Indian Journal of Animal Sciences* **79**(7): 686–91.
- Shukla S, Tiwari D P, Kumar A, Mondal B and Upadhyay A K. 2009. Macro and micro mineral profile in soil, feedstuffs and animals in Pithoragarh district of Uttarakhand. *Indian Journal of Animal Sciences* **79** (12): 1258–62.
- Snedecor D W and Cochran W G. 1994. *Statistical Methods*. 8th edn. Iowa University Press, Iowa, USA.
- Tiwari M K, Tiwari, D P, Kumar A and Mondal B C. 2007. Existing feeding practices, nutrient availability and reproductive status of dairy cattle and buffaloes in Haridwar district of Uttarakhand. *Animal Nutrition and Feed Technology Sciences* **7**(2): 177–185.
- Wu Z and Satter L D. 2000. Milk production and reproductive performance of dairy cows fed two concentrations of phosphorus for two years. *Journal of Dairy Science* **83**: 1052–63.
- Wu Z, Satter L D, Blohowiak A J, Stauffacher R H and Wilson J H. 2001. Milk production, estimated phosphorus excretion and bone characteristics of dairy cows fed different amounts of phosphorus for two or three years. *Journal of Dairy Science* **84**: 1738–48.
- Young D. 1995. *Effects of Pre-analytical Variables on Clinical Laboratory Tests*. 2nd edn. AACC Press, Washington, p 489.