



Effect of dietary supplementation of calcium, copper and manganese on nutrient utilization, growth, blood-biochemical and mineral profile in crossbred heifers

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ABSTRACT

Crossbred growing heifers (16) of an average body weight 149 kg and 12–16 month-old were divided into 4 groups of 4 each to study the effect of dietary supplementation of calcium, copper and/ or manganese on nutrients intake, body weight gain, blood biochemical parameters and mineral profile. The heifers of group 1 (control) were supplemented with 60 g feed mixture without calcium, copper and manganese supplementation as top dress, whereas heifers of group 2 were supplemented with 100 g feed (mineral) mixture containing dicalcium phosphate and copper sulphate to supply 11 g Ca and 60 mg Cu /animal / day. The heifers of group 3 were supplemented with 60 g feed (mineral) mixture containing copper sulphate and manganese sulphate to supply 60 mg Cu and 100 mg Mn/animal/day and of group 4 were supplemented with 100 g feed (mineral) mixture containing dicalcium phosphate, copper sulphate and manganese sulphate to supply 11 g Ca, 60 mg Cu and 100 mg Mn/animal/day, respectively. All the heifers were provided concentrate mixture as per requirements along with mixed fodder (green sorghum + green maize + wheat straw). The feeding trial lasted for 100 days. The average daily dry matter and TDN intakes and daily body weight gain were higher in heifers of groups 2 and 4 supplemented with calcium and copper. The digestibility of dry matter, organic matter, crude protein, ether extract and crude fibre was significantly higher in heifers of groups 2, 3 and 4 as compared to control group. The SGPT, SGOT and ALP activities and concentration of total protein, globulin, phosphorus, copper and manganese in blood serum were significantly improved in groups supplemented with minerals but the values were within normal range, whereas cholesterol and glucose concentrations were significantly lower in mineral supplemented groups as compared to control group. The study showed that dietary calcium, phosphorus, copper and manganese supplementation is beneficial for improving the nutrient utilization and weight gain and to maintain the normal blood-biochemical constituents and mineral status in growing crossbred heifers.

Key words: Blood profile, Calcium, Copper, Crossbred heifers, Growth, Manganese, Phosphorus

The essentiality of minerals for growth, health, reproduction as well as normal physiological functions of animal's body is well documented (Chew 2000). In plain area of Uttarakhand, the most common livestock species are cattle and buffaloes, which are mostly reared on crop residues and dry grasses and are used in combination with locally available green fodder and small amount of concentrate mixture. Generally, these animals do not receive mineral mixture supplementation. Shukla *et al.* (2009) showed low profile of some macro and micro minerals especially calcium, copper and manganese in hill region of Uttarakhand, whereas Tiwary *et al.* (2007) reported low profile of phosphorus and copper in soil, feed and blood serum of animals in plain region of Uttarakhand. Calcium deficiency is responsible for

infertility also a disturbed Ca: P ratio has blocking action on the pituitary gland and consequently on ovarian action (Herrick 1977), prolonging the interval of first ovulation. Copper is a component of many enzymes, essential for absorption and transport of iron and necessary for haemoglobin synthesis, besides, needed for maintenance, growth and lactation of animals. Manganese on the other hand influences enzyme systems, bone matrix formation, rigidity of connective tissue, synthesis of estrogen and progesterone and in the formation of prothrombin (Sharma *et al.* 2002). Keeping in view, the present study was conducted to see the effect of dietary supplementation of calcium, copper and/ or manganese on nutrients utilization, body weight gain and certain blood biochemical and mineral status in crossbred heifers.

MATERIALS AND METHODS

Crossbred growing heifers (16) with an average BW 149 kg and 12–16 month-old, selected from the herd of

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instructional dairy farm of the university were divided into 4 groups of 4 each. To supplement calcium, copper and manganese, 4 feed (mineral) mixtures were prepared in which calcium in the form of dicalcium phosphate, copper in the form of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and manganese in form of manganese sulphate ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$) as per NRC (2001) requirement were used with ground maize and mustard cake. The four experimental feed (mineral) mixtures were offered daily as top dress to each heifer were: 60 g (maize 50%, mustard cake 50% without mineral supplementation) in group 1 (control), 100 g (maize 30%, mustard cake 27.46%, dicalcium phosphate 42.3% and copper sulphate 0.24%) in group 2, 60 g (maize 50%, mustard cake 49.10%, copper sulphate 0.39% and manganese sulphate 0.51%) in group 3 and 100 g (maize 30%, mustard cake 27.15%, dicalcium phosphate 42.3%, copper sulphate 0.24% and manganese sulphate 0.31%) in group 4. Thus each animal in group 2 received 11.26 g Ca and 60.69 mg Cu, in group 3 received 60.73 mg Cu and 102.60 mg Mn and in group 4 received 11.25 g Ca, 60.69 mg Cu and 102.40 mg Mn over and above already present in the feed. The feeding trial lasted for 100 days. All the animals were provided concentrate allowance as per NRC (2001) requirement along with *ad lib.* mixed fodder (green sorghum + green maize + wheat straw) to discern the effect of dietary calcium, copper and manganese supplementation on nutrient utilization, body weight gain and blood biochemical parameters and mineral status. Blood samples were collected during mid and end of the course of investigation and analyzed for calcium, phosphorus, copper, manganese level, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), and alkaline phosphatase (ALP) activities and total protein, albumin, globulin and cholesterol concentration in blood serum.

In the middle of the feeding trial, a digestion trial for 6 days was conducted to know the nutrient utilization. The feeds and fodder offered to the animals and faeces voided were analyzed for proximate constituents (AOAC 1995). The SGPT, SGOT and ALP activity and serum cholesterol

concentration (Burtis 1999), total protein, albumin and globulin concentration (Johnson *et al.* 1999) were determined by using standard diagnostic kits. The concentration of copper and manganese in the blood serum and samples of feeds and fodder were estimated by atomic absorption spectrophotometer and calcium in blood serum was estimated by colorimetric method (Ray and Chauhan 1969) whereas phosphorus was estimated by Fiske and Subbarow (1925) method. The data were analyzed statistically (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

The chemical composition and mineral contents of concentrate mixture, experimental feed (mineral) mixtures and fodder fed to the growing crossbred heifers are given in Table 1.

Nutrients intake and digestibility: The average daily dry matter intake was higher in heifers of groups 2 and 4 supplemented with calcium and copper (Table 2). However, no significant difference was found in total dry matter intake, dry matter intake /100 kg body weight and dry matter intake /kg metabolic body weight among different groups of animals. The average daily total digestible nutrients (TDN) intake and TDN intake / 100 kg body weight did not differ significantly among different groups of heifers, however TDN intake / kg metabolic body weight was significantly ($P < 0.05$) higher in groups 2, 3 and 4 than heifers of control group. The average daily digestible crude protein (DCP) intake, DCP intake per 100 kg body weight and per kg metabolic body weight did not differ significantly among different groups. The digestibility of dry matter, organic matter, crude protein, ether extract and crude fibre was significantly ($P < 0.05$) higher in heifers of groups 2, 3 and 4 compared with control group (Table 2). These results are in agreement with the findings of Sawhney (1951) who reported improved DM and CP digestibility in animals supplemented with manganese and Chhabra *et al.* (1986) who reported 10% increase in crude fibre digestibility with trace mineral supplementation in Karan Fries male calves. Mondal *et al.*

Table 1. Chemical composition and minerals content of feeding stuffs (on dry matter basis)

Feeding stuffs	DM (%)	OM (%)	CP (%)	EE (%)	CF (%)	NFE (%)	Ash (%)	Ca (%)	Cu (ppm)	Mn (ppm)
Mixed fodder (green sorghum + green maize + wheat straw)	27.07	92.20	6.58	1.30	31.42	52.90	7.80	0.40	5.27	46.57
Concentrate mixture	88.00	89.30	17.92	2.80	5.70	62.84	10.70	1.01	13.10	65.27
Feed (mineral) mixture 1 (control)	92.89	95.60	25.09	6.90	5.40	58.21	4.40	0.47	12.41	42.83
Feed (mineral) mixture 2 (calcium and copper supplemented)	91.30	58.30	17.69	2.90	3.82	33.89	41.70	11.26	606.90	23.70
Feed (mineral) mixture 3 (copper and manganese supplemented)	93.06	95.30	24.44	6.60	5.28	58.93	4.75	0.46	1012.17	1710.00
Feed (mineral) mixture 4 (calcium, copper and manganese supplemented)	92.00	58.00	17.25	2.70	3.59	35.00	42.00	11.25	606.90	1024.00

(2004) also reported improvement in nutrient digestibility in Black Bengal kids fed diet supplemented with copper.

The intake of copper in heifers of groups 2, 3 and 4 supplemented with copper along with different combinations of calcium and manganese was significantly ($P<0.05$) higher than heifers of group 1, in which copper intake was considerably lower than that recommended in NRC (2001) and ARC (1980) (Table 3). Similarly, calcium intake in heifers

of groups 2 and 4 was significantly ($P<0.05$) higher than groups 1 and 3. Manganese intake was higher in heifers of groups 3 and 4, but calcium and manganese intakes were not lower than those recommended in the heifers of control group. There was difference in total calcium, copper and manganese intakes among different groups due to difference in supplementation of respective minerals.

Body weight gain: The total body weight gain and average

Table 2. Average daily dry matter and nutrients intake and digestibility coefficients of nutrients in heifers fed rations supplemented with calcium, copper and manganese

Particulars	Groups / Treatments			
	1 (Control)	2 (Calcium and copper supplemented)	3 (Copper and manganese supplemented)	4 (Calcium, copper and manganese supplemented)
Average BW (kg)	190.61±16.51	193.93±24.13	192.46±16.36	193.05±17.12
Roughage DMI (kg/d)	3.48±0.19	4.06±0.16	4.05±0.10	4.02±0.13
Concentrate DMI (kg/d)	2.30±0.13	2.34±0.15	2.30±0.12	2.36±0.12
Concentrate: roughage ratio	1:1.51	1:1.74	1:1.76	1:1.70
Total DMI (kg/d)	5.78±0.30	6.39±0.29	6.35±0.16	6.38±0.22
DMI/100 kg BW (kg)	3.06±0.10	3.43±0.36	3.36±0.23	3.36±0.22
DMI/ $W^{0.75}$ kg (g)	113.16±1.68	125.91±8.51	124.17±5.7	124.46±5.17
TDN intake (kg)	3.42±0.13	4.09±0.08	4.02±0.19	4.22±0.11
TDN intake/100 kg BW (kg)	1.81±0.16	2.16±0.36	2.11±0.28	2.23±0.29
TDN intake/ $W^{0.75}$ kg (g)*	66.91 ^b ±4.79	79.76 ^a ±9.75	78.17 ^a ±8.63	82.61 ^a ±8.44
DCP intake (kg)	0.65±0.07	0.69±0.03	0.68±0.05	0.69±0.02
DCP intake/100 kg BW (g)	341.78±10.02	364.05±14.43	359.11±13.28	362.45±12.67
DCP intake/ $W^{0.75}$ kg (g)	12.65±0.14	13.41±2.16	13.31±1.90	13.41±1.33
<i>Digestibility coefficient</i>				
Dry matter*	56.95 ^b ±1.74	63.09 ^a ±1.65	62.60 ^a ±1.80	65.64 ^a ±1.84
Organic matter*	62.86 ^b ±1.93	68.13 ^a ±2.34	67.09 ^a ±2.41	70.71 ^a ±2.38
Crude protein*	82.36 ^b ±1.65	87.65 ^a ±1.17	86.11 ^a ±1.35	88.13 ^a ±1.47
Crude fibre*	43.84 ^b ±2.61	56.69 ^a ±2.76	55.33 ^a ±2.37	58.90 ^a ±2.82
Ether extract*	74.84 ^b ±1.70	87.48 ^a ±2.01	86.02 ^a ±1.85	88.37 ^a ±1.75
Nitrogen-free extract*	65.67 ^b ±2.80	68.25 ^{ab} ±2.67	67.43 ^{ab} ±2.74	71.40 ^a ±2.78

^{a, b}Mean values bearing different superscripts in a row differ significantly; * $P<0.05$.

Table 3. Average daily calcium, copper and manganese intakes in growing crossbred heifers fed ration supplementation of calcium, copper and manganese during feeding trial of 100 days

Particulars	Groups / treatments			
	Control	Calcium and copper supplemented	Copper and manganese supplemented	Calcium, copper and manganese supplemented
Ca intake through roughage (g)	12.16	13.40	12.92	13.76
Ca intake through concentrate (g)	23.73	24.14	23.73	24.34
Ca intake through feed (mineral) mixture (g)	0.28	11.26	0.27	11.25
Total calcium intake (g) *	36.17 ^b	48.80 ^a	36.92 ^b	49.35 ^a
Cu intake through roughage (mg)	16.02	17.65	17.02	18.13
Cu intake through concentrate (mg)	30.78	31.31	30.78	31.57
Cu intake through feed (mineral) mixture (mg)	0.74	60.69	60.73	60.69
Total copper intake (mg)*	47.54 ^b	109.70 ^a	108.50 ^a	116.40 ^a
Mn intake through roughage (mg)	138.30	152.40	147.00	156.50
Mn intake through concentrate (mg)	153.40	156.00	153.40	153.30
Mn intake through feed (mineral) mixture (mg)	2.57	2.37	102.60	102.40
Total manganese intake (mg)*	294.30 ^b	310.80 ^b	402.90 ^a	412.20 ^a

^{a, b}Mean values bearing different superscripts in a row differ significantly; * $P<0.05$.

daily body weight gain were highest in group 2 supplemented with calcium and copper, followed by group 4 supplemented with calcium, copper and manganese (Table 4). However, there was no significant difference in body weight gain among different group of heifers. There was no significant difference in feed conversion efficiency among crossbred heifers of different groups, which is in close agreement with the findings of Attia *et al.* (1987), Tripathi *et al.* (2001) and Saxena (2007).

Blood biochemical parameters: The SGPT and SGOT activities in heifers supplemented with feed (mineral) mixture were significantly ($P<0.05$) higher as compared to control group and were within normal range (Table 5). These results corroborated with the findings of Exton (1980) who reported that in growing lambs the value of AST and ALT were significantly less in the mineral deficient lambs than those supplemented with Ca and P minerals, which may be attributed due to increase in transamination reaction. The serum ALP activity did not differ significantly among different groups, however serum ALP activity was highest

in groups supplemented with manganese (groups 3 and 4). This is in close agreement with the findings of Bhoot *et al.* (1981) who reported that manganese is necessary for growth and ALP activity is required for ossification. The average value of serum glucose was highest in control (group 1), however, the differences were not significant compared with other groups. Similarly, concentration of serum cholesterol was significantly ($P<0.05$) highest in control group. These results are in agreement with the findings of Engle *et al.* (2001) who reported that deficiency of copper stimulates the liver synthesis of cholesterol. The serum total protein, albumin and globulin concentrations were significantly ($P<0.05$) higher in groups 2, 3, and 4 as compared to control group. These results are in agreement with the findings of Mondal *et al.* (2004) who reported increased nitrogen retention because of copper supplementation.

Serum mineral profile: The blood serum calcium concentration in heifers of all groups was within normal range and did not differ significantly among the groups before and after supplementation of feed (mineral) mixture containing

Table 4. Average body weight gain and feed conversion efficiency in growing crossbred heifers fed ration supplementation of calcium, copper and manganese during growth trial of 100 days

Particulars	Groups / Treatments				SEM±
	(Control)	(Calcium and copper supplemented)	(Copper and manganese supplemented)	(Calcium, copper and manganese supplemented)	
Initial body weight (kg)	149.23±14.39	149.38±18.90	149.13±13.65	149.08±14.89	0.07
Final body weight (kg)	200.08±17.17	203.43±17.95	201.83±16.75	203.00±17.58	0.75
Total weight gain (kg)	50.85±3.65	54.05±5.02	52.70±4.84	53.92±3.47	0.74
Body weight gain/ d (g)	508.50±36.47	540.50±48.38	527.00±45.67	539.25±34.73	12.56
Feed conversion efficiency	10.60±1.35	10.60±2.70	10.59±1.03	10.85±1.32	0.22

(Sastry *et al.* 1999), ^ (Miles and McDowell 1983).

Table 5. Average blood biochemical parameters and minerals content in blood serum of crossbred heifers fed ration supplemented with calcium, copper and manganese

Particulars	Groups / Treatments				
	Normal [#] / critical range [^]	Control	Calcium and copper supplemented	Copper and manganese supplemented	Calcium, copper and manganese supplemented
SGPT (IU/litre)	14–38	14.63 ^b ±0.27	18.60 ^a ±1.19	18.05 ^a ±1.40	20.70 ^a ±1.18
SGOT (IU/litre)	78–132	70.60 ^b ±1.11	81.47 ^a ±1.62	80.59 ^a ±0.59	82.10 ^a ±1.79
ALP (IU/litre)	<488	196.76±6.38	209.39±21.06	222.75±11.29	255.34±32.59
Total Protein (g/dl)*	7.56	6.15 ^b ±0.06	6.87 ^a ±0.09	6.76 ^a ±0.08	6.86 ^a ±0.09
Albumin (g/dl)	3.60	3.25±0.04	3.34±0.02	3.34±0.03	3.34±0.05
Globulin (g/dl)*	3.96	2.90 ^b ±0.08	3.54 ^a ±0.08	3.42 ^a ±0.08	3.52 ^a ±0.05
Glucose (mg/dl)*	35–55	61.44 ^a ±0.54	55.27 ^b ±0.64	56.11 ^b ±1.07	54.81 ^b ±0.58
Cholesterol (mg/dl)*	50–230	115.18 ^a ±1.00	102.99 ^b ±1.85	105.51 ^b ±1.06	104.00 ^b ±1.66
Calcium (mg/dl)	9.4–12.2	10.80±0.06	10.86±0.11	11.09±0.29	11.19±0.16
Phosphorus (mg/dl)	4–9	4.30 ^b ±0.11	5.87 ^a ±0.09	5.35 ^a ±0.14	5.51 ^a ±0.36
Copper [^] (ppm)	<0.65	0.48 ^b ±0.03	0.70 ^a ±0.01	0.71 ^a ±0.02	0.72 ^a ±0.01
Manganese [^] (ppm)	<0.2	0.24 ^b ±0.01	0.26 ^b ±0.01	0.32 ^a ±0.00	0.34 ^a ±0.01

^{a,b}mean values in a row with different superscripts differ significantly *($P<0.05$).

Ca, P, Cu and Mn (Table 5). The serum phosphorus concentrations in animals of groups 2, 3 and 4 were significantly ($P < 0.05$) higher as compared to control group but the values were within the normal range. The phosphorus concentration was reduced in animals of groups 3 and 4 supplemented with manganese. It may be due to the fact that manganese increases activity of serum ALP which utilizes P for synthesis of soft tissues and decreases phosphorus concentration and increases calcium concentration in blood so as to maintain Ca: P ratio. These results are in agreement with the findings of Bhoot *et al.* (1981) and Kalita and Sarmah (2006). Serum copper concentration in heifers of groups 2 (0.63 ppm), 3 (0.64 ppm) and 4 (0.66 ppm) were significantly ($P < 0.05$) higher than control group (0.47 ppm) and were close to critical level (< 0.65 ppm) where as copper concentration in control group was below critical level. Low concentration of copper in control group might primarily be due to low copper concentration in feedstuffs fed to animals as well as decreased gut absorption. These results are in agreement with the findings of Kegley and Spears (1994) and Saxena (2007) who reported that dietary copper supplementation increased the copper level in blood serum of cattle. The concentration of serum manganese was significantly ($P < 0.05$) higher in heifers of groups 3 and 4 in which manganese was supplemented along with copper and with calcium and copper, respectively, as compared to control and group 2. However, manganese concentration was above critical level (< 0.20 ppm) in heifers of all groups.

From the findings of the present study it is concluded that dietary calcium, phosphorus, copper and manganese supplementation is beneficial for improving the nutrient digestibility and utilization and to maintain the blood biochemical constituents and mineral status in growing crossbred heifers.

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