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Correlations between dietary intake of different minerals with their concentrations in serum, hair and milk in Murrah buffaloes

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ABSTRACT

The samples of blood, hair and milk along with feeds and fodders consumed by the buffaloes (156 milch, 77 heifers) were collected at random. The samples were analyzed for calcium (Ca), phosphorus (P), zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) contents. The intake of different minerals was calculated from data of intake of different feeds and fodders and their mineral composition. Correlations were drawn between dietary intake of different minerals and their concentrations in serum and hair of heifers and in milk for milch animals. In milch buffaloes, serum P had inverse correlation while Mn concentration in hair had positive correlation with Ca intake. Mn intake had positive correlation with its concentration in hair and also with hair Fe, Zn and P, but negative correlation with serum and milk P. Intake of Fe had negative correlation with hair concentration but positive with its concentration in serum. Dietary intake of Cu did not influence its concentration in serum, hair and milk but reduced hair Fe significantly. Serum Zn concentration increased significantly with its dietary supplies but had negative correlations with serum Cu, serum Fe, and Mn in milk or hair. Positive correlations existed between intakes of Fe and Cu in serum or milk, serum Fe, Mn in serum, milk and hair while negative correlations were observed serum and milk P, and Fe in hair. In heifers, serum and hair Ca has negative correlation with Ca intake. Intake of Zn, Mn or Cu did not influence their concentration in serum or hair. Study concluded that significant correlation exists between dietary intake and level of minerals in hair, serum and milk and also amongst themselves. Therefore, there is a possibility of predicting intake of certain minerals from hair, serum or milk mineral concentration.

Key words: Buffalo, Calcium, Copper, Hair, Iron, Manganese, Milk, Minerals, Phosphorus, Zinc

Farm animals derive their mineral requirement mainly from feeds and fodders offered to them in diet as little or no mineral mixture is supplemented. Though, regional mapping of feeds and fodders is relatively a rapid, reliable and line data on the level of macro and microelements, but under field conditions it is very difficult to assess the intake of minerals and mineral status of animals for optimum health and production due to numerous interactions existing amongst various minerals. A dietary deficiency of a mineral is sooner or later reflected in subnormal concentration of the mineral in certain of the animal's tissues and fluids, and a dietary excess of a mineral is similarly reflected in above-normal concentration (Underwood and Suttle 1999). Blood/serum is commonly used for assessing mineral status. However, under field conditions collection of blood from buffaloes becomes difficult and sentimental too for the farmers due to difficulty in restraining animals and animal welfare issues. In the present study, an attempt was made to assess the interrelationship of serum, hair and milk mineral status of buffaloes with their dietary intake with the objective to assess body mineral status through analysis of hair and milk mineral status. For such study correlations were drawn between intakes of minerals with their concentration in different tissue, and between minerals *inter se* in different biological material.

MATERIALS AND METHODS

In the first phase extensive survey was conducted in native places of Murrah buffaloes from February to April. *Rabi* season was chosen because it is considered as healthy season. Moreover, animals generally get sufficient quantities of greens grown in winter and are kept in similar dietary regimen constituting those greens from December. Therefore, true picture of mineral status can be obtained. Twenty representative villages/sites from 2 districts were selected for this purpose to obtain a data with wide variability in terms of feeding, feeding plane, milk yield etc. From each site a

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minimum of 10 animals were randomly chosen for sampling. Samples of blood (156 from milch buffaloes and 77 from heifers), milk (142) and hair from tail (233) were collected. Simultaneously samples from feeds (292) and fodders (283) offered, were collected for laboratory analysis. The commonly used roughages included wheat straw, *bajra kadbi*, *jowar kadbi*, *bathua*, oats and berseem whereas concentrates used were cotton seed, cotton seed cake, wheat flour/*dalia, bajra* grains, *guar* seeds, feed pellets and mustard cake. The farmers were interviewed for feeds and feeding practices followed during the whole year, history of the disease if any. Samples of drinking water were also collected from common tank/pond from each village.

The milk samples were collected from the same animal whose blood sample was taken. Each animal was milked in a clean cane or bucket. The whole milk was well mixed and 25 ml sample was taken in a labeled double corked plastic bottle. Formalin (40%) was added @ 0.1 ml for 25 ml milk as a preservative and samples were transported to laboratory for analysis. The hair samples were cut and packed in small polythene bags for analysis.

Live weight of the animals was recorded (Nagacenkar 1980). Milk yield of individual buffalo was recorded. Daily offer of concentrates, green fodder and dry roughages were verified with the help of spring balance. The daily residue left was nil and if any residue was found, it was added to the next diet.

The samples were processed and analyzed for calcium (Ca), phosphorus (P), zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) contents following standard procedures. The samples, except that of milk, were digested with nitric and perchloric acid (4: 1) mixture. The hair samples were dusted off for extraneous contamination if any, cut into 1 cm length and washed with acetone again before digestion following standard method. Milk samples were dried in silica crucibles on water bath (100°C) and dried samples were ignited at 450°C. HCl-extract was prepared using 50% hydrochloric acid solution for digestion. For digestion the amount of samples taken was 1 g each for feed and fodders, 10 ml for milk, 2 ml for serum and 0.2 g for hair. The concentrations of Ca, Zn, Cu, Fe and Mn in water, feeds, fodders and biological materials were estimated using atomic absorption spectrophotometer. Phosphorus of serum samples was estimated as per Tausky and Shorr (1953) while that of milk and hair according to Ward and Johnston (1962). AOAC (1984) procedure was followed for the estimation of phosphorus content of feeds and fodders. Intake of different minerals was calculated for each milch buffalo and heifer using the data of dry matter intake from different roughage and concentrate sources and their mineral composition. Correlation between the variables was computed as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Correlation of mineral intake with their content in serum, milk and hair: Correlation of minerals intake with their

Table 1. Correlation of mineral intake with their concentration in serum, milk and hair of milch buffaloes

Tissue/fluid concentration	Dietary concentration/intake					
	Calcium	Phosphorus	Zinc	Copper	Iron	Manganese
Blood serum						
Calcium	–0.123	–.020	–0.051	0.119	0.094	0.033
Phosphorus	–0.243**	0.007	0.199*	0.045	–0.252**	–0.277**
Zinc	0.053	0.042	0.217**	0.085	–0.099	0.069
Copper	–0.090	–0.121	–0.262**	–0.060	0.174*	0.136
Iron	0.143	–0.034	–0.253**	0.081	0.255**	0.019
Manganese	0.047	–0.077	–0.147	–0.093	0.015	–0.019
Milk						
Calcium	–0.042	0.039	–0.124	–0.033	–0.021	–0.079
Phosphorus	0.095	-.040	0.072	–0.066	–0.182*	–0.194*
Zinc	0.057	0.096	–0.095	–0.081	0.054	0.177*
Copper	0.043	–0.074	–0.148	–0.059	0.197*	–0.148
Iron	0.080	0.143	0.098	0.109	0.032	0.077
Manganese	0.043	–0.114	–0.206	–0.083	0.274**	0.059
Hair						
Calcium	0.058	0.101	0.058	0.095	0.056	0.060
Phosphorus	0.100	0.129	–0.012	0.124	0.131	0.261**
Zinc	0.083	0.091	0.092	0.191*	0.240	0.307**
Copper	0.081	0.031	–0.049	–0.003	0.121	0.111
Iron	–0.137	–0.134	0.007	–0.290**	–0.227**	0.441**
Manganese	0.178*	0.016	–0.200*	0.073	0.425**	0.420**

N=156; *P<0.05; **P<0.01.

content in serum milk and hair for milch buffalo is given in Table 1 and heifers in Table 2. Intake Ca influenced negatively (P<0.01) the serum P while a positive correlation (P<0.01) was observed with Ca intake and hair manganese. Intake of P did not influence the concentration of any mineral in blood, hair or milk. A direct relationship (P<0.01) was observed between dietary intake of Zn and its concentration in serum. However, dietary Zn intake, was correlated (P<0.01) negatively with serum Cu and Fe and concentration of Mn in hair (P<0.05) and milk (P<0.01). The intake of Cu was negatively correlated (P<0.01) with hair Fe while it correlated (P<0.05) positively with hair Zn. The intake of Mn was negatively correlated (P<0.01) with serum and milk P while it has positive correlation (P<0.01) with hair P, Zn, Fe and Mn concentration. The intake of Fe was also correlated negatively (P<0.01) with serum P and hair Fe while positively (P<0.01) with serum Fe, hair Zn and Mn, and milk Cu and Mn concentration.

Correlations, drawn in heifers between intake (Table 3) and concentration in serum and hair (Table 4), indicated that the serum and hair Ca concentration were correlated (P<0.01) inversely with its dietary concentration. Similarly the concentration of P in serum was also inversely related (P<0.05) with its intake. However, dietary P concentration improved the concentration of Zn and Mn in hair and Mn in serum as positive correlations (P<0.01) were observed between these parameters. A negative (P<0.05) correlation existed between dietary concentration of Zn and concentration of Ca in hair or serum Fe concentration. Positive correlations (P<0.01) were found between intake of Zn and Zn serum concentration or hair P concentration. With the increase in Cu intake, Fe concentration of hair decreased as a negative correlation (P<0.01) existed. However, dietary Cu concentration has positive correlation (P<0.01) with hair P, hair Zn and hair Mn. Fe concentration in serum and hair decreased with its intake as negative correlation existed between intake and serum Fe (P<0.05) and hair Fe (P<0.01), however, with increased Fe intake the concentration of Mn in hair improved significantly (P<0.01). Dietary concentration of Mn had only negative correlation (P<0.01) with Fe in hair.

Ca intake did not correlate with its content in serum and hair in milch animals but such phenomenon existed in heifers. This may be due to homeostasis mechanism existing in the body as once the blood level is achieved; the absorption of calcium is reduced through less synthesis of calcium binding protein signaled by parathromone and consequent reduction in conversion of vitamin D_3 to its active form. Moreover, the haemostatic or physiological mechanism regulating serum Ca are more effective than those for P or most other minerals (Underwood 1981) and serum Ca is maintained closely at about 10 mg/100 ml by this regulatory mechanism irrespective of dietary intake. Negative correlation between Ca intake and serum P found in milch animals is obvious as a close inverse interrelationship exists between these 2 elements. Moreover, dietary imbalances of Ca and P existed due to variable non-judicious roughage to concentrate ratio in ration. Generally, the animal received berseem with wheat straw or dry roughages alone as sole diet or straw-oats-concentrates (limited quantity) showed deficiency of P. Similarly, the high yielding animals which were fed substantially high quantities of concentrates compared to low yielding animals had good P status. A slow decline in serum calcium associated with hormonal and metabolic changes were seen in pigs in response to dietary deficiency of calcium (Underwood and Suttle 1999). Therefore, feeding pattern and financial constraint of farmers for supplementation of concentrates has a great impact on Ca and P imbalance as

Table 2. Correlation of mineral intake with their concentration in serum and hair of buffalo heifers

Tissue/fluid concentration	Dietary concentration/intake					
	Calcium	Phosphorus	Zinc	Copper	Iron	Manganese
Blood serum						
Calcium	–0.292**	–0.205	0.096	–0.136	–0.181	–0.091
Phosphorus	–0.103	–0.283*	0.186	–0.119	–0.199	–0.007
Zinc	0.115	0.052	0.375**	0.043	–0.219	–0.054
Copper	–0.090	0.150	–0.131	0.186	–0.017	–0.007
Iron	0.150	–0.038	–0.238*	–0.138	–0.241*	–0.010
Manganese	0.156	0.238*	–0.033	0.216	0.123	0.087
Hair						
Calcium	–0.297**	0.027	–0.229	–0.144	0.031	0.179
Phosphorus	0.037	0.208	0.388**	0.320**	0.071	0.004
Zinc	0.124	0.308**	0.098	0.312**	0.122	0.074
Copper	–0.151	0.019	–0.114	0.015	0.016	0.111
Iron	–0.058	–0.242*	0.086	–0.458**	–0.309**	–0.344**
Manganese	0.238	0.334**	–0.177	0.367**	0.311**	0.035

N, 77; *P<0.05; **P<0.01.

the heifers and even many milch buffaloes were mainly raised on roughages with little or no concentrate resulting in P deficiency. Anke (1966) reported that dietary Ca had an antagonistic effect on P content of hair but in present investigation no such correlation existed. A low correlation between Ca and P intake and hair content of these minerals was reported (Wysocki and Klatt 1971). In the present study similar trend existed in P. However, in heifer, Ca intake had negative correlation with hair Ca concentration. No correlation existed between P intake and its level in serum, hair or milk in milch animals while a negative correlation existed in heifers. Cohen (1973) found no correlation between P concentration in pasture and concentrations of P in hair from grazing steers. Early responses to a diet low in P are a fall in serum or plasma inorganic P and a small rise in serum calcium. Supplementary calcium does not invariably decrease P absorption and the lowering of serum inorganic P may be partly due to increase P retention by the skeleton, a common response to calcium supplementation (Braithwaite 1984, Field *et al.* 1985, Rajaratne *et al.* 1994, Sharma *et al.* 2004). Such dietary interactions do not invalidate, however, the use of serum in organic P for diagnosis purposes.

Intake of copper did not correlate with its concentration in serum, hair or milk. There has also been bad reputation of serum copper as a diagnostic aid compared with liver copper in initially hypo-cupraemic animals (Suttle 1994). This is due to interaction of other minerals interfering absorption of Cu. Therefore, the present study also confirmed statement of Underwood and Suttle (1999) that determination of copper in the diet or pasture has no diagnostic value in ruminants unless other elements with which copper interacts are determined.

The negative correlation between Fe intake and serum ($r = -0.252^{**}$) or milk ($r = -0.182^{*}$) P might be due to negative interrelationship between these two elements as excess Fe decreases the utilization of P. Correlation ($r = 0.22$) existed between Fe intake and serum Zn concentration in heifers indicating that high level of Fe may interfere with utilization of Zn. Hair Mn concentration was highly correlated with Fe intake in milch as well as heifers. Similarly milk Mn was positively correlated with Fe intake, which indicated that excess Fe helped in utilization of Mn. Zn intake, improved the Zn concentration of serum in heifer and milch animals. Thus, blood serum Zn concentration may be good indicator of dietary Zn intake in buffaloes. Zn concentration in blood serum or plasma has also been used as most widely used indicator, but low values are usually an early change and measure of deficiency, lacking certainly sensitivity as a diagnostic criterion (Underwood and Suttle 1999). However, a study reported a reduction in growth before any fall in plasma zinc in heifers (Engle *et al.* 1997).

Mn intake was highly correlated with that of its hair concentration of milch buffaloes but not in heifers in the present study. Underwood and Suttle (1999) also reviewed that attempts to use manganese in hair as an index of status had given conflicting results and widely varying individual values, but the manganese concentration in wool and feathers was more useful.

Correlation between minerals concentration of serum and hair or milk: Serum Ca was negatively correlated ($P<0.01$) with its hair concentration but positively with milk concentrations (Table 1). Mn, Cu and Zn in hair had positive correlation ($P<0.01$) with serum Ca. A very high ($r = -0.515$) negative correlation ($P<0.01$) existed between serum P concentration and hair Mn concentration. Similarly highly significant negative correlation ($P<0.01$) existed between serum P and milk Mn and with Cu in milk and hair. It indicated that higher serum P concentration might be decreasing the utilization of these 2 minerals at tissue level. Positive and significant ($P<0.01$) correlation existed between serum Zn and milk P, serum Cu and hair P and milk Cu, serum Fe and Hair or milk Mn and milk Ca, and serum Mn and hair Mn.

The correlation between serum mineral concentration and with hair concentration in heifers is given in Table 2. In heifer also serum Cu had positive relation with hair Cu. Similarly negative correlation existed between serum P and hair Cu. Though, a negative correlation existed between serum Cu and hair Zn in milch as well in heifers but it was significant ($P<0.05$) in heifer only. In this case too very high negative correlation ($P<0.01$) existed between serum P and hair Mn concentration. Serum Fe was negatively correlated ($P<0.05$) with hair P. Scanty information is available indicating correlation amongst mineral concentration of different tissues. These correlations have made complex in identifying or suitable sensitive body part/tissue as an index for assessing mineral status, because the mineral concentration in different tissues/body parts are likely to be influenced by concentration of other minerals beside the dietary intake of a particular mineral in question.

Correlation between mineral concentration of hair and milk: When correlation were drawn between concentration of mineral in hair and milk (Table 1) negative correlations were observed between hair Ca and milk Cu ($P<0.01$), hair Ca and milk Zn or Mn ($P<0.05$), hair Zn and milk P ($P<0.05$) and hair Mn and milk P ($P<0.05$). On the other hand positive correlations also existed between Hair P and milk Fe, Cu ($P<0.01$) or Zn ($P<0.05$), hair Zn and milk Fe ($P<0.01$) hair Cu and Fe ($P<0.01$) or Mn ($P<0.05$) in milk and hair. These correlation may be useful is predicting the mineral status of milk through analysis of hair or *vice-versa*. Gaafar *et al.* (2012) have also reported that significant positive correlations exist between the intake, absorption, retention and the concentration of all minerals in hair.

The significant correlations existed between concentration of minerals in hair, serum, milk and their dietary intake. Similarly, the concentration of minerals in hair, serum and milk correlated with each other. Therefore, there is possibility of predicting intake of certain mineral from hair, serum or milk mineral concentration. However, Gabryszuk *et al.* (2010) have demonstrated that the hair levels of macroelements (Ca, Na, P) did not correlate with their levels in the milk. They further stated that the determination of K, Mg, Al, As, Bo, Co, Fe, Ge, Hg, Pb, Se and Se in hair could be useful for long-term monitoring of the mineral status of cows, and useful for establishing reference values for some elements. Significant co-relations also existed between mineral intake and their status in certain vital organs (Sharma *et al.* 2002).

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