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## Relations between macro and trace elements in the serum of dairy cows in Urmia, Iran

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### Abstract

Serum concentrations of the major nutritive elements, Mn, Mo, Cu, Fe, Mg, Ca, Na, K, P and Cl, and their interrelationships were evaluated in 20 dairy herds in Urmia, northwest of Iran. Values were determined by atomic absorption, auto analyzer spectrophotometry and flame photometer. Mean Mn, Mo, Ca, P and K were less and Fe and Cu were higher than the reference values. Herd effects were present for the majority of elements, but not K, Na, Fe and Cl. The strongest correlations were positive between Cu/Mo and Ca/Mn, with weaker but significant positive correlations between Mn/Ca, Mo/Cu, Fe/K and Fe/Cl. Negative correlations existed between Ca/K, Ca/Mo, Mg/Mo, Mg/Mn, Mg/P, Fe/Mn, and Fe/Mo. Correlations coefficients with Mo and to a lesser extent Mn were higher than for other elements. There were two principle components in the dataset, explaining 12 and 11% of the variation respectively, involving mainly Fe and Mo in the first component and Ca and Mo in the second. It is concluded that Fe, Mo, Mn and Ca concentrations in the serum of dairy cows in this region were of particular importance in explaining variation in the element concentrations in serum of cows in Urmia, Iran.

**Keywords:** blood serum, dairy cows, Iran, macro-elements, trace elements

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### Introduction

Relationships between elements in the serum of dairy cows will ultimately determine element supply for metabolic purposes. Accurate diagnosis of Cu, Se, Fe, I, Zn, Mo and Mn, which have essential functions as metalloenzymes in ruminants, (1) is of particular importance. Macro-elements are also involved in the pathology of milk fever, (2) left and right displacement of abomasum, (3) ketosis(4) and fat cow syndrome(5) and are most commonly abnormal in the periparturient

period and during lactation (6). Deficiencies of the elements Mn (7) and combined deficiencies of Mo and Cu, (8) P and Ca, (9) Mn, Mo and Fe, (10) Mn and P, (11) macro-elements in general, (12) P, Mn and Cu, (13) trace elements in general, (14) and Cu deficiency (15) are associated with weight loss, anemia, bone abnormalities and low production and reproduction performance in dairy cows (1). Therefore, simultaneous study of trace and macro-elements is essential to fully

appreciate effects on production and reproduction efficiency.

The Urmia region in the northwest of Iran contains alkaline, carbonate and sandy soil and is generally accompanied by Cu, Se and I deficiencies (16,17). For this reason large amounts of mineral additives are used to prevent the aforementioned deficiencies, which may be partly to blame for secondary Mn deficiency in ruminants. There is limited evidence of macromineral deficiencies from a single herd, (18) but there have to date been no systematic surveys of deficiencies across herds in the region. In this survey we also included Mn, in addition to the more commonly recognized elemental deficiencies of Cu, Se, Mo etc, because of the possibility of secondary Mn deficiencies.

Mn is recognized as the fifth most abundant metal in the ground and its deficiency is associated with low growth and reproductive disorders (16). Mn is essential for activation of the glycozyltransferase for mucopolysaccharides in bone cartilage; deficiency leads to bone deformity and blood coagulation disorders (16) and through its influence on the corpus luteum and testicles adversely affects bovine reproduction (19). In primary, enzootic Mn deficiency bone deformities occur(20), the metabolism of carbohydrates and lipids is adversely affected and animals remain emaciated (21). A forages concentration of 80 mg/kg Mn is required for normal reproduction in cows; (1) alkaline and sandy soils predispose to primary Mn deficiency and, if forage concentrations are less than 40 mg/kg is reached, growth and reproduction disorders occur in ruminants (14). Mn is poorly absorbed from the bovine gastrointestinal tract, with possible competition from dietary Ca, P, (21) Cu, Fe and Mo (20).

The goals of study were: 1. determination of the serum Mn, Mo, Fe, Cu and macro-elements in dairy cows in Urmia. 2. Comparison of the trace and macro-elements values in dairy herds. 3. Determination of the relationship between trace and macro-elements in cows in this region.

### **Experimental:**

A representative sample of 20 herds from Urmia, northwest Iran, were randomly selected from licensed industrial dairy herds with Holstein-Friesian cows in a 2012 national veterinary register. Herd size varied from 70 to 400, the number of parturitions was from two to four and all cows were milked twice daily. All cows were fed ad libitum four times per day a diet consisting of various mixtures of lucerne, apple pulp, concentrate (14% CP, produced by the Mahabad Concentrate Factory, Mahabad, Iran) and maize silage. The relationship between feed composition and serum and milk elements will be described in a later paper.

Ten lactating cows were selected at random from each herd for sampling, all of which were in good health condition at the time of sampling. For each cow five ml blood was extracted by jugular venipuncture into a test tube without EDTA. These were then separated by centrifuge at 3000 g for five minutes and stored at -20°C before analysis. Serum Mn and Mo were determined by atomic absorption spectrophotometry (Shimatzo Japan) using the relevant lamps. Serum Fe, Cu, Ca, Mg, P and Cl were measured by auto analyzer (RA-1000, USA) using appropriate commercial kits (Pars Azmon. Iran). Serum Na and K were measured by flame photometer (Jenway, Clinical PFP7, UK) using appropriate standards.

### **Statistical analysis:**

The Moods Median test was used to evaluate herd effects for all elements except Mg, as the residuals of an analysis of variance were not normally distributed by the Anderson-Darling test, even after mathematical manipulation of the data. For Mg a general linear model was constructed with the herd effect as the only factor. A dendrogram clustering the elements was created with Single Linkage, Correlation Coefficient Distances. A factor analysis with Varimax rotation was conducted for all elements. Correlation coefficients were determined for ranked data as the element concentrations were not

normally distributed by the Anderson-Darling test was used for analysis. (P<0.01). The statistical package Minitab (version 16)

**Table 1.** Descriptive statistics and herd effects for each element

| Element     | Mean | Median | SE      | Prob.Herd effect    | Reference value                   |
|-------------|------|--------|---------|---------------------|-----------------------------------|
| Mn(ng/dl)   | 15.4 | 13.9   | 0.288   | <0.001 <sup>1</sup> | 21-28 <sup>(6,34)</sup>           |
| Mo(ng/dl)   | 0.59 | 0.58   | 0.00445 | <0.001 <sup>1</sup> | 1.6-3.6 <sup>(10,15)</sup>        |
| Fe (µg/dl)  | 65.6 | 65.5   | 1.27    | 0.21 <sup>1</sup>   | 23.3-44.9 <sup>(24,25)</sup>      |
| Cu (µg/dl)  | 40.2 | 38.0   | 0.683   | <0.001 <sup>1</sup> | 8-23.6 <sup>(24,26)</sup>         |
| Ca (mmol/l) | 1.91 | 2.04   | 0.028   | <0.001 <sup>1</sup> | 2.3- 2.7 <sup>(39,40)</sup>       |
| P (mmol/l)  | 1.26 | 1.33   | 0.016   | 0.01                | 2.43-3.1 <sup>(24,23)</sup>       |
| Mg (mmol/l) | 1.23 | 1.19   | 0.018   | <0.001 <sup>2</sup> | 1.45 - 2.58 <sup>(24,39,40)</sup> |
| Na (mmol/l) | 134  | 134    | 0.55    | 0.32 <sup>1</sup>   | 137-148 <sup>(1)</sup>            |
| K (mmol/l)  | 3.6  | 3.5    | 0.027   | 0.62 <sup>1</sup>   | 5-7 <sup>(24)</sup>               |
| Cl (mmol/l) | 82.4 | 84.0   | 1.08    | 0.06                | 95-108 <sup>(1)</sup>             |

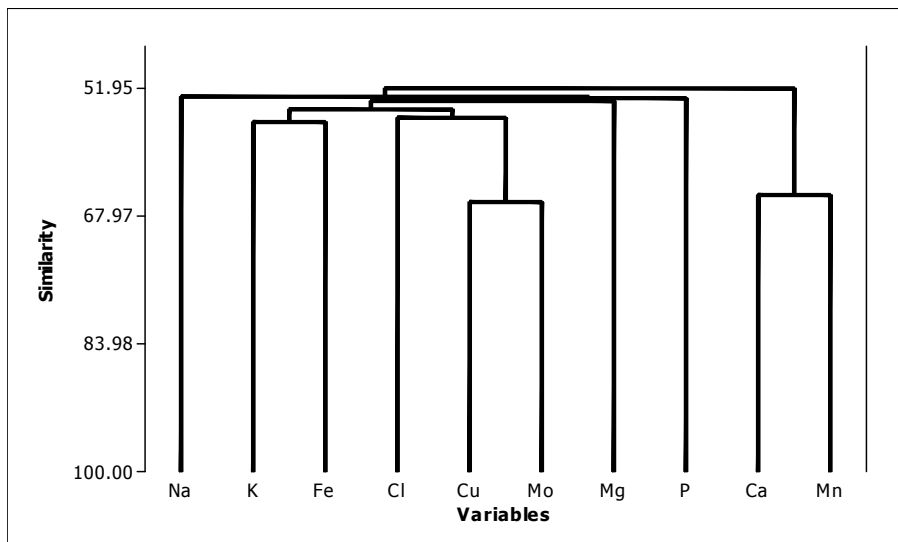
<sup>1</sup> = Moods median test    <sup>2</sup> = General linear model

**Table 2.** Spearman rank correlations of element concentrations in serum. Rank correlation on top, probability below.

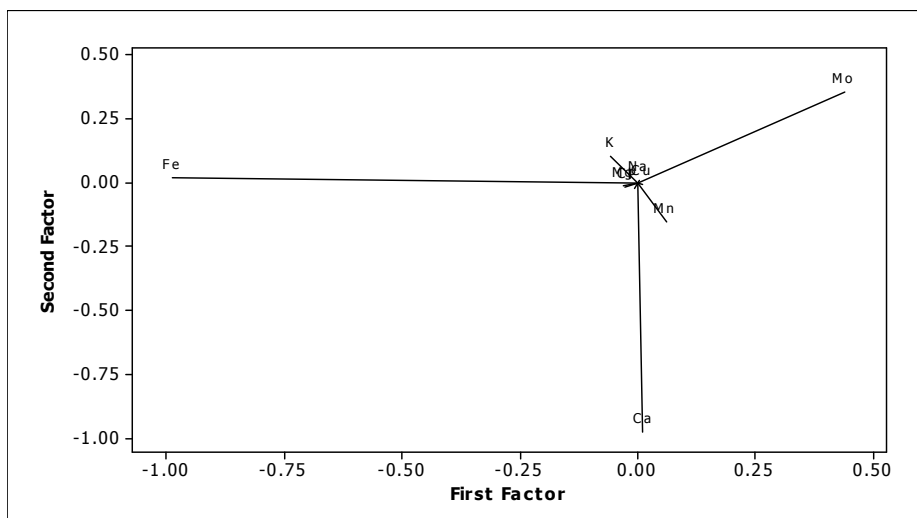
|    | Na            | K              | Mg             | Ca             | P             | Cl            | Cu             | Fe             | Mn             |
|----|---------------|----------------|----------------|----------------|---------------|---------------|----------------|----------------|----------------|
| K  | 0.10<br>0.18  |                |                |                |               |               |                |                |                |
| Mg | -0.02<br>0.82 | -0.03<br>0.64  |                |                |               |               |                |                |                |
| Ca | -0.04<br>0.56 | -0.16<br><0.02 | -0.01<br>0.85  |                |               |               |                |                |                |
| P  | 0.03<br>0.70  | 0.03<br>0.72   | -0.18<br><0.01 | 0.11<br>0.12   |               |               |                |                |                |
| Cl | 0.11<br>0.11  | -0.03<br>0.65  | -0.11<br>0.13  | 0.04<br>0.57   | 0.08<br>0.24  |               |                |                |                |
| Cu | 0.01<br>0.89  | 0.02<br>0.82   | 0.05<br>0.47   | 0.01<br>0.90   | 0.04<br>0.62  | 0.06<br>0.42  |                |                |                |
| Fe | -0.06<br>0.44 | 0.16<br><0.03  | 0.03<br>0.70   | -0.08<br>0.27  | 0.06<br>0.43  | 0.06<br><0.43 | -0.01<br>0.99  |                |                |
| Mn | -0.06<br>0.39 | -0.06<br>0.37  | -0.23<br><0.01 | 0.35<br><0.01  | -0.03<br>0.65 | -0.02<br>0.83 | -0.28<br><0.01 | -0.34<br><0.01 |                |
| Mo | 0.04<br>0.56  | -0.04<br>0.55  | -0.41<br><0.01 | -0.40<br><0.01 | -0.05<br>0.48 | 0.03<br>0.65  | 0.45<br><0.01  | -0.49<br><0.01 | -0.17<br><0.02 |

**Table 3.** Eigen value contributions to two factors with Eigen values more than 1.0

| Element | Factor 1 | Factor 2 |
|---------|----------|----------|
| Na      | -0.004   | -0.006   |
| K       | 0.073    | -0.102   |
| Mg      | -0.061   | -0.050   |
| Ca      | -0.086   | -1.136   |
| P       | 0.012    | 0.033    |
| Cl      | 0.025    | 0.014    |
| Cu      | 0.060    | 0.083    |
| Fe      | -1.129   | -0.090   |
| Mn      | -0.108   | 0.131    |
| Mo      | -0.238   | -0.211   |



**Figure 1.** Dendrogram clustering element concentrations in serum



**Figure 2.** Leverage of the elements in a Factor analysis with varimax rotation

## Results

Herd effects were present in all elements ( $P < 0.01$ ) except K, Na and Fe, with a trend for a herd effect for Cl (Table 1). There were significant positive correlations between Fe and K, Mn and Ca, and Mo and Cu (Table 2). There were significant negative correlations between Ca and K/Mo, Mg and Mn/Mo/P, and Fe with Mn/Mo. The correlations coefficients for elements linked to Mo and to a lesser extent Mn were particularly high.

The dendrogram (Figure 1) demonstrated strongest correlations between Cu and Mo, and Ca and Mn. The factor analysis found two variables with Eigen values more than 1.0, which contained the Eigen contributions listed in Table 3. These explained 12 and 11% of the variation, respectively. In the first factor, Fe and to a lesser extent Mo were particularly influential, and in the second factor, Ca had the greatest influence, with Mo of secondary importance (Table 3). The leverage graph suggested that the first factor was primarily influenced by variation in Fe and Mo, with the second factor primarily influenced by Ca and to a lesser extent, Mo (Figure 2).

## Discussion

Serum Ca, P and K concentrations in the serum of Urmia dairy cows were lower than recommended, (1,21) but other elements were present in satisfactory concentrations. Serum Mo in this study was less than reported by Randhawa and Randhawa (15) and Jokubauskiene et al (10). An increase in Mo is mostly related to secondary Cu deficiency; primary Mo deficiency is not common. The close connection between the two elements is confirmed in the dendrogram primary cluster of Mo and Cu. A concentration of 3 mg/kg Mo in forages is sufficient to provide the preliminary requirements of the animals. (21) Soils with high Mo, the forages growing from these soils, and Mo supplementations are the main reasons for secondary Cu deficiency, (22) but the adequate level of

serum Cu confirms that Mo was not critical in these herds. There are no recommendations for the Mo content of forages, but the consumption of forages with 44 mg Mo for a period of several weeks does not cause secondary Cu deficiency, (10,16) although it has been observed to reduce the estrus cycle and pregnancy rate. (22) Serum Mo has been reported to be reduced in high production cows compared to low producers, reduced from pregnancy to parturition, but not influenced by different seasons (10).

Cu and Fe are agonists in the diet and metabolic function of dairy cows (23). Serum Cu and Fe values are normally recommended to be at least 23.6 and 44.9  $\mu\text{g/dl}$  (12,24, 25, 26) and in this study were 40.2 and 65.6  $\mu\text{g/dl}$ , respectively, which is more than reported by others authors (15,25). The coordination of Fe and Cu in the hematopoietic system (27) and their competition with Mn and Mo absorption reveals the primary role of these four elements. Indeed Fe was the primary antagonist to Mo in the principle components analysis in our study, demonstrating the primary role of Fe in the relationship between Mo and Cu. Competitive effects of Fe and Cu with Mn and Mo in dairy cows depend on sulfur concentrations in the forages, (28,29) however, such a connection has not been reported in dairy calves. (30)

Study on Mn status in animals is based on the evaluation of the Mn in soil, forages, blood, milk and body tissues, with the priority being blood, (21) as selected in this study. The essentiality of Mn in ruminants is less than that of Cu, Fe, Se, I and Zn. (1,24) Gut absorption capacity of Mn in ruminants is weak (typically only up to 1% of dietary Mn), (8) meanwhile different factors influence Mn absorption, and therefore provides the condition for Mn deficiency in dairy cows. Reports of Mn absorption and its deficiency are limited, because of the lack of evidence of deficiencies. Based on the literature, the occurrence of clinical hypocalcaemia in cows is 15-fold more than Mn deficiency, thus, Ca and P continuously administered in ruminant feed which

will probably result in reduction in Mn absorption (31). The normal value for serum Mn is occasionally less than 20 ng/ml, (32) in agreement with this study, but less than that reported by other authors, (7,33, 34, 35,36) who report concentrations up to 37 ng/ml. It is therefore possible that production and reproduction are constrained in Urmian dairy cows by Mn deficiency (14).

Mn is affected by age, (20,37,38) species, gender, (21) season, (10) parturition(10) and dietary Mn concentration. (10,20,22,37) Mn deficiency is widely diagnosed by calf congenital chondrodystrophia, (1) feeding on forages from alkaline soils, (21) using maize silages during the first pregnancy and calving (20). The possibilities of competitive or synergistic effects of Mn with Ca, P and Fe are unresolved (9,12). The presence of bone abnormalities in some Urmian calves, on sandy and alkaline soils in industrial herds using mostly maize silage are most likely responsible for low serum Mn. However, it has been proposed that abundant use of Ca supplementation for the prevention and treatment of hypocalcaemia in Urmia cows may also be responsible (35,36). However, this study provided evidence of a positive relationship between Mn and Ca (Table 2. Figure 1), in contrast to the work of Lopez et al (12) and Suttle (21) who report a competitive relation between them. Hansen (20) also found a negative correlation between Fe and Mn in the serum of dairy cattle.

### Conclusions

This study emphasizes that the concentrations of nutritive elements in the serum of dairy cows are closely related, therefore attention should be paid to the balance of elements rather than absolute values. Fe, Mo, Mn and Ca concentrations in the serum of dairy cows were of particular importance in relation to other elements.

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