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Comparison of Mineral Levels in Bone and Blood Serum of Cattle in Northwestern Turkey

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Abstract: In order for the mineral levels in the body to be accurately determined, it is essential to determine the mineral content of bones together with that of blood serum. In this study, blood serum and bone samples were collected from cattle in and around the provinces of Istanbul, Tekirdag, Kirklareli and Edirne and it was attempted to determine reference values. In each region, blood and bone samples were collected from 100 heads of cattle and Phosphorus (P), Calcium (Ca) and Magnesium (Mg) proportions were calculated. According to the results, it was found that the levels of P, Ca and Mg in bone samples of the cattle in Northwest of Turkey were 132.76±17.84, 333.23±163.02 and 2.38±0.32 mg g^{-1}, respectively, while mineral levels in blood serum were 7.88±1.05, 10.80±0.71 and 2.37±0.68 mg dL^{-1}, in the same order. It was thought that these values might constitute a reference point for serial measurements that would be made for keeping the cattle in our region under control against metabolic diseases.

Key words: Cattle, blood serum, bone, phosphorus, calcium, magnesium

INTRODUCTION

In domestic animals, trace elements play an important role in growth, reproduction and their livestock productivity. Despite this fact, mineral deficiency or imbalances are still frequently encountered phenomena almost anywhere in the world that cause serious economic loss (Dakka and Abdel-All, 1992; Gerloff and Swenson, 1996; Uysal, 1998). In cattle, diseases such as parturient paresis (hypocalcemia), grass tetany (hypomagnesemia) and left-side and right-side displacement of the abomasum (alkalemia, hypochloremia and hypocalcemia) are characterised by electrolyte deficiencies and generally become obvious in the first phase of lactation. Their negative economic impact is considerable (Mosel *et al*., 1990; Dakka and Abdel-All, 1992; Fleming *et al*., 1992; Bjorkman, 1994).

For this reason, elements such as calcium and phosphorus-containing minerals are of importance for cattle both during their growth and after they have reached maturity (Shupe *et al*., 1988; Uysal, 1998). Their exists also, a well-known relationship between the level of phosphorus deposits in the body and diseases such as botulism. This connection has prompted scientists to investigate the mineral levels in body tissue and body fluids (Beighle *et al*., 1994). However, in quite a few cases, electrolyte imbalances are difficult to detect because the animals affected show no clinical symptoms (Dakka and Abdel-All, 1992).

Consulting organisations recommended by the Working Groups of the Agricultural Research Commission (ARC) have also stated the necessity to determine the major minerals in cattle breeding as a matter of priority (Shupe *et al*., 1988). In case the increased demand for electrolytes during pregnancy, birth and other physiologically demanding events cannot be met by the animals' ration, the skeletal system serves as an important source for the plasma calcium demand in order to preserve its concentration (Van De Braak *et al*., 1987; Shupe *et al*., 1988; Mosel *et al*., 1990, 1993, 1994). Because of this protective mechanism, the mineral concentration in blood is no good indicator for level of mineral concentrations in

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animals (Beighle *et al*., 1994). For a number of reasons, the blood samples taken for the determination of mineral concentrations do not provide reliable results. A variety of factors such as exercise, excitation during blood sampling, the hour of the day, the feeding hours, the season, the animal's age and the point the blood is drawn from have an influence on the results (Beighle *et al*., 1994; Uysal, 1998). Mineral deficiency observed in the blood serum is of doubtful value as an indicator of correct calcium and phosphorus levels in an animal due to the fact that they are replenished from mineral depots in the bones (Beighle *et al*., 1994). For this reason, researchers emphasize the importance of measuring both the mineral levels in blood serum and in the bones in order to obtain a correct picture of the mineral concentrations of the entire body. This guideline to mineral content measurements of the body has led to a demand for new researches on this subject (Shupe *et al*., 1988; Mosel *et al*., 1990, 1994; Beighle *et al*., 1994). The ensuing investigations have yielded significant results for the clinical evaluation of metabolic diseases (Beighle *et al*., 1994).

Only a limited number of studies have so far been carried out in Turkey on the effects of trace elements in animals (Sezer and Ozdemir, 2002). The objective of this study, is the determination of the level of minerals in both blood serum and bones of cattle under different physiological conditions in Northwestern Turkey. With this research, reference values for cattle in the region would be obtained, which are necessary for the use of bone tissue mineral concentrations in routine clinical diagnostics. The lack of such reference values currently poses the biggest obstacle to the application of such a method. Evaluation of bone biopsy samples has become an important method for the detection of a number of metabolic diseases in animals before they become acute and offers the opportunity of continuous monitoring of pregnant, lactating or growing animals (Beighle *et al*., 1994). The establishment of this method as a diagnostic tool in veterinary medicine would help in obtaining additional disease-related information on the levels of Ca, P and Mg in bones, which would support the development of successful treatments in cases of parturient paresis, grass tetany, phosphorus deficiency and a number of other diseases where classical methods prove inconclusive.

MATERIALS AND METHODS

Cattle in and around the provinces of Istanbul, Tekirdag, Kirklareli and Edirne served as material of this study. From each region >24 months female animals of holstein breed were analysed. They were from different herds with different feeding regimes. From each region clinically healthy 100 heads of cattle delivered to the abattoir were examined. Blood samples were taken from the life animals via juguler vena puncture and bone samples from the same animals in accordance with approved techniques, after slaughtering (Williams *et al*., 1991a, b; Beighle *et al*., 1994; Gerloff and Swenson, 1996). In total 400 samples each of blood serum and bone were collected. The Ca and Mg levels in dry bone samples were determined with the aid of a Shimadzu atomic absorption spectrometer, model AA-680, while the concentration of dry bone P was measured spectrophotometrically with the molybdenum blue method (Shupe *et al*., 1988; Mosel *et al*., 1990; Williams *et al*., 1991a, b; Beighle *et al*., 1994; Mosel *et al*., 1994). The minerals in the blood serum samples were determined with a Ciba Corning autoanalyser, model Express Plus. When statistically evaluating the results obtained, the mean values and standard deviations were calculated for every province with t-test, the various provinces were compared and the statistically significant differences with respect to mineral concentrations in bones and blood serum examined with one-way anova using KyPlot package program.

RESULTS

The concentrations of minerals in blood serum and bone samples taken from cattle in Northwest of Turkey are given in Table 1 and 2.

Mineral concentrations in the examined bone samples were found to be higher for calcium and phosphorus (333.23±163.02 and 132.76±17.84 mg g^{-1}, respectively), while, the values for magnesium, 2.38±0.32 mg g^{-1}, were lower. Comparison between provinces showed statistically significant differences in the levels for Ca and Mg in particular in the regions of Kirklareli and Edirne ($p<0.001$). For phosphorus between provinces, however, no statistically significant difference could be established.

Examination of mineral concentrations in blood revealed calcium concentrations in all regions were in the normal reference value. Istanbul and Edirne provinces were statistically significantly higher ($p<0.001$) than Tekirdag province. The Mg concentrations were established to be higher in Istanbul, Kirklareli and Edirne provinces than in Tekirdag province with the levels in Istanbul and Edirne higher than the reference values and those in Tekirdag and Kirklareli provinces within the reference range. Differences between all regions were found to be statistically significant ($p<0.001$). P levels, though higher than the reference value range showed no statistically significant difference.

Table 1: Mean value, standard deviation and statistical significance of mineral concentrations in dry bone samples of cattle in Northwest Turkey

Province	N = 100	P (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})
Istanbul	X±Sx	130.26±20.17	310.8±140.7^{c}	2.6±0.15^{c}
Tekirdag	X±Sx	140.21±20.12	330.64±170.4^{bc}	2.27±0.52^{c}
Kirklareli	X±Sx	130.28±10.92	420.86±220.46^{b}	2.28±0.29^{b}
Edirne	X±Sx	130.3±20.18	270.63±120.52^{a}	2.38±0.32^{a}
Mean	X±Sx	132.76±17.84	333.23±163.02	2.38±0.32

Table 2: Mean value, standard deviation and statistical significance of mineral concentrations in blood serum samples of cattle in Northwest Turkey

Province	N = 100	P (mg dL^{-1})	Ca (mg dL^{-1})	Mg (mg dL^{-1})
Istanbul	X±Sx	7.95±1.16	11.75±0.5^{a}	2.54±0.71^{d}
Tekirdag	X±Sx	7.9±1	9.17±0.5^{b}	1.97±0.8^{c}
Kirklareli	X±Sx	7.7±1.05	10.25±1.32^{ab}	2.2±0.4^{b}
Edirne	X±Sx	8±1	12.05±0.52^{a}	2.79±0.83^{a}
Mean	X±Sx	7.88±1.05	10.80±0.71	2.37±0.68

In each group significant statistical differences were established between provinces designated with different letters (for Ca and Mg: $p<0.001$)

DISCUSSION

The bones impart mechanical strength to the muscle-skeleton system and provide functional capabilities, they also serve as mineral storage and are of vital importance for a range of metabolic activities such as growth, reproduction and livestock productivity (Van De Braak *et al*., 1987; Shupe *et al*., 1988; Gerloff and Swenson, 1996; Uysal, 1998). The blood serum data are insufficient for the determination of the mineral concentrations in animal bodies. It has been reported that for a correct determination of the mineral concentrations in the body, bone samples from the ribs, the hip or the feet could be used (Shupe *et al*., 1988; Jones *et al*., 1990; Williams *et al*., 1991b; Beighle *et al*., 1994; Mosel *et al*., 1994). In this study, the minerals concentrations in cattle were investigated by measuring both blood serum and bone samples with the objective of determining reference values in Northwest of Turkey. The study thus, served the purpose of establishing the criteria necessary to identify metabolic diseases at an early stage and to permit continuous control of the level of livestock productivity of the animals.

The application of bone biopsy techniques has become very simple and samples can be taken swiftly without causing any discomfort to the animals (Shupe *et al*., 1988; Jones *et al*., 1990; Williams *et al*., 1991b; Beighle *et al*., 1994; Mosel *et al*., 1994; Gerloff and Swenson, 1996). If this method would be used routinely in veterinary medicine, a number of metabolic diseases seen frequently in animals during pregnancy, lactation and growth could be detected early and the significant economic losses they cause could be prevented while, their productivity could be increased at the same time (Beighle *et al*., 1994). Researchers in this field also stress the necessity of a more extensive publication of reference values, in order to permit a routine usage of the method, which has not yet been achieved despite the fact that reference values for mineral concentrations in bones do exist in literature (Beighle *et al*., 1994; Gerloff and Swenson, 1996).

In this study, the level of minerals in the blood and bones of animals have been determined and compared and at the same time reference values for mineral concentrations in bones established. This is the first time this has been done in Turkey for cattle. A correlation of those levels with livestock productivity will then permit the calculation of the economic loss suffered from mineral deficiency.

It is known that the phosphorus content in the animal ration effects bone development to a large extent (Williams *et al*., 1991a). It is also known, however, that besides chemical and physical properties, bone development is influenced by range of other factors such as age, ration, hormones and diseases (Shupe *et al*., 1988; Williams *et al*., 1991a). Beighle *et al*. (1994) reported important differences in the values of P, Ca and Mg of ashed bone samples taken from bones of animals of different age, sex and breed. In the dry bone samples analysed in this study an important influence of those factors was found in particular for Mg and P. Gerloff and Swenson (1996) had stressed in their study that for the determination of body phosphorus depots from bone phosphorus measurements, dry or defatted dry samples should be used and they had advised against the use of ashed samples. For this reason, in this study dry bone samples taken from over 2 years-old female animals of culture breed were analysed. With this approach it was attempted to reduce the above-mentioned variations and to obtain more reliable information about metabolic diseases observed in Turkey. This restriction in terms of age, sex and breed was motivated by the fact that economically important metabolic diseases usually appear in over 2 years-old animals of high-yielding culture breeds (Van De Braak *et al*., 1987; Beighle *et al*., 1994). Comparison of the results of this study with those reported by Beighle *et al*. (1994), who worked on animals of similar characteristics. Mineral concentrations in the examined bone samples were found to be higher for calcium and phosphorus than those reported in literature (203.68±1.0 and 108.25±0.41 mg g^{-1}, respectively), while, the values for magnesium were lower than previously reported values (5.51±0.05 mg g^{-1}) (Bjorkman, 1994).

Examination of mineral concentrations in blood revealed calcium concentrations in all regions were in the normal reference value of 9.7-12.4 mg dL^{-1} (Carlson, 1990). The Mg concentrations in Istanbul and Edirne were established higher than the reference values and those in Tekirdag and Kirklareli provinces within the reference range of 1.8-2.3 mg dL^{-1} (Carlson, 1990). P levels, though higher than the reference value range (5.5-6.5 mg dL^{-1}) (Carlson, 1990) showed no statistically significant differences.

With respect to the mineral concentrations in blood serum samples, it was found that the P level was rather high while, the Ca and Mg concentration were within the normal limits in cattle in Northwestern Turkey. This was interpreted as a sign for ossification related to high P level in the blood serum resulting in high Ca and P levels in the bones. This in turn points to a mineral rich region or to high-dose mineral additives in the animal feed and a lower probability of the emergence of metabolic diseases.

As has been pointed out by Beighle *et al.* (1994), bone mineral concentrations may serve as a powerful tool for prognosis of the development of metabolic diseases. The ease with, which bone samples can be taken and the availability of serial sample collection techniques make it possible to keep animals under observation during certain phases of their development (pregnancy, lactation, growth) and help to avoid unnecessary culling.

CONCLUSION

As a result of this study on the level of minerals in bones of healthy cattle in Northwestern Turkey, reference values were determined, which may, however, exhibit certain regional differences. On the basis of those reference values, the animals may be monitored for metabolic diseases in various phases of their lives with serial measurements of bone samples. An approach that will help to reduce economic losses to a minimum.

The difference between the results found in this investigation and those reported by other researchers as well as the dearth of publications on this subject require more extensive research including other regions in order to be able to establish reliable reference parameters for mineral concentrations in bone samples.

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