Research Article

Trace Elements Detection in Cow Urine by Atomic Absorption Spectrophotometer

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Abstract

This study determined the concentrations of some trace elements (Cu, Ca, Zn and Fe) in urine samples of healthy normal cycling cow. Early morning mid-stream urine samples were collected for analysis. The samples were digested using micro-wave assisted digestion method and analyzed with atomic absorption spectrophotometer. The results showed the concentrations of Cu (0.001–0.059 mg L^{-1}), Ca (0.001–0.0871 mg L^{-1}), Zn (0.07–0.365 mg L^{-1}), and Fe (0.008–0.351 mg L^{-1}) in urine samples. The study concluded the presence of trace elements in normal cow urine.

Keywords: Trace elements, Urine, Atomic Absorption Spectroscopy, Cow

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Introduction

Trace mineral intake and absorption is required for a variety of metabolic functions including immune response, reproduction and growth [1]. Trace minerals have important roles in immune function and may affect health in transition dairy cows [2]. An optimum concentration range of each particular element is required for performing the vitally important functions of the body in different environments. Although, trace elements are required in little quantities (less than 100 mg/kg dry matter) but it plays a critical role in immune function, oxidative metabolism and energy metabolism in ruminants which are directly or indirectly involved in growth, production and reproduction [3]. The results of the analysis of biological fluids such as blood, urine, blood plasma, and serum adequately reflect the concentration levels of the elements in the body [4]. Their importance in metabolic processes necessitates their determination in the body fluids and tissues [5]. Different analytical methods have been employed for estimation of trace elements in biological samples. Atomic Absorption Spectrophotometer (AAS) is one of them. It is used for determination of almost all metals, metalloids and some non-metals (B, Si, P) in plasma, blood, urine, and milk due to rapid analysis, very good precision (repeatability), low limit of detection, no or moderate interferences, easy automation, low cost [6], [7]. In the present study we have used flame AAS for the determination of Cu, Ca, Zn and Fe in the urine of normal cycling healthy cows.

Materials and Methods

Collection of urine

Midstream early morning urine samples were collected from indigenous cattle of Instructional Livestock Farm Complex (ILFC), DUVASU, Mathura. Total 20 samples were collected in wide mouth plastic bottles. Due care was taken to avoid contamination in the sample. Therefore, the bottles were washed with 1% nitric acid. Samples were stored at -20^{0}C until analyzed.

Reagents Used

All reagents used for this work were of analytical grade and they include: nitric acid (HNO_3), perchloric acid ($HClO_4$) and sulphuric acid (H_2SO_4). Solutions were prepared using doubly-distilled deionised water.

Preparation of reagents

Triple acid mixture

Concentrated nitric acid (HNO_3), perchloric acid ($HClO_4$) and sulphuric acid (H_2SO_4) were mixed in the proportion of 3:3:1.

Standard solution

Sl. No.	Trace minerals	Standard solution
1.	Zn	0.5, 0.75, 1.0 (ppm; mg/l)
2.	Cu	0.5, 1.0, 1.5 (ppm; mg/l)
3.	Ca	1.0, 2.0, 3.0 (ppm; mg/l)
4.	Fe	1.0, 2.0, 3.0 (ppm; mg/l)

Estimation of trace elements

Estimation of trace elements (Cu, Ca, Zn and Fe) in urine samples of cow was done using flame mode of Atomic Absorption Spectrophotometer (Model No. AAS 400; Perkin Elmer, USA).

Procedure

For analysis, samples were brought to normal temperature. Samples were digested by tri-acid mixer (HNO_3:H_2SO_4: $HClO_3$ = 3:1:1). 20 ml sample was mixed with 15 ml tri-acid mixer. They were digested at 250^0C until a clear digested solution was obtained. The final volume was made to 40 ml and filtered through Whatman filter paper 42 to remove silica and other insoluble residues from digested samples.

A series of working standards were run in AAS for the preparation of the standard curve. Finally, the samples were run and the readings were noted. Concentrations of minerals were estimated by multiplying AAS value with dilution factor.

Blank Determination

Blank determination was carried out following the same procedure described above for urine samples. 0.1 M HNO_3 in the final solution maintained the acidic environment and avoided formation of insoluble hydroxides before analysis. The digested blank samples were analysed for Cu, Ca, Zn and Fe.

Results and Discussion

Concentration (mg L^{-1}) of trace elements in urine samples

Table 1 presents the trace elements concentration in the urine. The concentrations of Zn (0.273±0.076 mg L^{-1}) and Fe (0.176±0.128 mg L^{-1}) were higher compared with other elements analysed in urine samples. Calcium concentration in urinary sample varied from 0.001 to 0.0871 mg L^{-1}. Whereas, zinc and copper concentrations were found to be in the range of 0.07 to 0.365 mg/L and 0.001 to 0.059 mg/L, respectively.

Table 1 Concentration (mg/L) of trace elements in urine control samples

Elements	Mean ±SD
Cu	0.001±0.0017
Ca	0.035±0.03
Zn	0.273±0.076
Fe	0.176±0.128

The analysis of urine is less informative than the analysis of blood because urine is an excretion material and the association between the state of the body and the concentration of the elements in urine is less evident than blood [8]. Biological fluids are very challenging materials because of their complex matrix composition and also due to very small concentrations of the most of the trace elements. Hence, the determination of trace elements requires a strict method. In the atomic absorption analysis of biological fluids, urine is a more complex material than blood due to lower analyte concentrations in urine and the presence of significant concentrations of salts [9]. In a study conducted at Junagadh Agriculture University in the year 2016, the analysis of urine samples of 400 Gir cows using gas

chromatography-mass spectrometry (GC-MS) method showed traces of gold ranging from 3-10 mg from one litre urine in ionic form along with silver, iron, boron and calcium [10].

Conclusion

Although, we have been able to determine levels of Cu, Ca, Zn and Fe in the urine samples of healthy normal cycling cows in this study but the urine results showed more variation. This is significant because trace minerals have important roles in immune function and may affect health in dairy cows.

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