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# Determination of Trace Elements in Cow Placenta by Tungsten Coil Atomic Emission Spectrometry

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**Abstract** Tungsten coil atomic emission spectrometry (WCAES) is used to determine trace levels of Mn (403.1 nm) and Cr (425.5 nm) in cow placenta. All samples were collected in Ilha Solteira, SP, Brazil. The instrumental setup is based on a tungsten filament extracted from 150 W, 15 V microscope light bulbs, a solid state power supply, fused silica lens, crossed Czerny-Turner spectrograph, and a thermoelectrically cooled charge-coupled device detector. The limits of detection (LOD) and quantification (LOQ) for Cr are 2 and 8 μg $L^{-1}$, and 20 and 60 μg $L^{-1}$ for Mn, respectively. Recoveries for 0.30 mg $L^{-1}$ spikes of each analyte were in the range 93.0–103.0%, and relative standard deviation (RSD) was between 6.50 and 7.20% for both elements. Placenta samples were microwave-assisted digested with diluted $HNO_3$ and $H_2O_2$ and analyzed by WCAES. The results for Cr and Mn were compared with values obtained by tandem inductively coupled plasma mass spectrometry (ICP-MS/MS). No statistically significant difference was observed between the different methods by applying a paired *t* test at a 95% confidence level. The average concentrations of Cr and Mn in the placentas evaluated were 0.95 ± 0.22 and 2.64 ± 0.39 μg $g^{-1}$, respectively. By using a short integration time, LODs for Cr and Mn were lower than values reported by recent works using a similar WCAES system.

**Keywords** Placentome · Cow embryo · Micronutrients · Tungsten atomizer · Trace analysis

## Introduction

The requirements of minerals in cattle (e.g., Na, Mg, K, Co, Cr, Mo, Mn, S, and Se) vary according to the type and level of production, the age, the concentration and the chemical form of the mineral in feeds, and its relations with the other nutrients in the diet during the gestation period, lactation, and confinement [1, 2]. The cow placenta, as in several animal species, is responsible for respiratory exchanges, storage, and supply of essential nutrients from the mother to the embryo during pregnancy [2–6]. The exchange of nutrients is accomplished by the placentome [7], which is described in the literature as the largest area of contact between the fetus and the mother, with nutrient transport taking place via blood vessels [8].

The essential nutrients responsible for the development of cow embryos are classified in macronutrients (e.g., Na, Cl, Mg, S, and K) and micronutrients (e.g., Co, Cu, Mo, Mn, Cr, and Se), both of which supplied through the food ingested by the mother [9, 10]. Although present in small amounts, micronutrients are fundamental to the development of important features such as bone structure, and the reproduction and central nervous systems. They are also essential to the function of many important enzymes [11]. Manganese, for example, is essential to the development of the bone structure, liver, pancreas, and many enzymatic processes [12]. The main effects of Mn deficiency on the fetus during pregnancy are

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retarded growth, abnormality of the skeleton, reproductive degeneration, abortions, and abnormalities in the newborn [13]. Chromium is known to have two main functions in cattle: it is involved in glucose tolerance factor (GTF) and as an antistress agent. GTF is the means by which the Cr has an improving effect on insulin binding and increases the number of insulin receptors on the cell surface and sensitivity of β cells present in the pancreas. The antistress factor promoted by chromium supplementation reduces the negative effects of stress, which are linked to low immunity. This is mainly due to the increase of cortisol with greater action of the cellular defenses [13, 14]. This micronutrient is present in natural forage, bran, and grain. However, Cr content in these food sources is frequently not high enough to meet nutritional requirements, especially considering the elevated metabolism level of these animals in stress conditions such as lactation and periods of pre- and postpartum [14].

Determining the concentrations of micronutrients in placenta may be one of the most effective strategies to better understand the effects of these elements on pregnant cows, their embryos, and the potential long-lasting consequences for the future calf. Despite their importance, limited information can be found in the literature on analytical procedures specific to trace element analysis of bovine placenta. The lack of works describing such applications may be related to the low analyte concentrations and the sample matrix complexity, with potentially severe effects on precision and accuracy [15–17].

The present study seeks to quantify Cr and Mn in placenta samples of cow by tungsten coil atomic emission spectrometry (WCAES), and demonstrate the efficiency and simplicity of operation of the WCAES method for complex samples. The first descriptions of the WCAES system were published in 2005 and 2006 [18, 19]. Since then, this simple, potentially portable, and sensitive atomic spectrometric method has been successfully applied to a variety of samples and analytes [20–27]. WCAES may represent an interesting alternative to complex and expensive methods such as inductively coupled plasma with optical or mass spectrometry detection (ICP OES and ICP-MS) for applications in trace element analysis of complex samples such as bovine placenta [28]. In this work, six samples of placenta, from cows at different stages of pregnancy, were analyzed by WCAES. The results for Cr and Mn were then compared to values determined by tandem ICP-MS (ICP-MS/MS) to evaluate the method's accuracy.

## Materials and Methods

### Instrumentation

Figure 1 shows a schematic representation of the WCAES instrumental setup. A 150 W, 15 V tungsten filament obtained from a microscope light bulb (Osram XENOPHOT 64633 HXL, Pullach, Germany) is used as atomizer. The bulb's glass cover is removed, and its base is kept intact so it can be attached to a conventional two-pronged power socket. The atomizer is kept in a T-shaped borosilicate glass cell (Ace Glass, produto No. D131703, Vineland, Nova Jersey, EUA) to prevent its oxidation during the atomization step, and a gas mixture of 10% $H_2$ and 90% Ar flowing at 1.0 L $min^{-1}$ is used as protecting gas [29]. Additional details on the atomization cell configuration can be found in the literature [18–20]. The radiation emitted during atomization was focused through a 25 mm diameter, 75 mm focal length, fused silica lens onto the entrance slit of the spectrometer. The 1:1 tungsten atomizer image was projected approximately 1 mn off axis of the slit to improve the signal-to-background ratio [19, 20]. The spectrometer is composed of a 25-μm entrance slit Czerny-Turner monochromator (MonSpec 18, Scientific

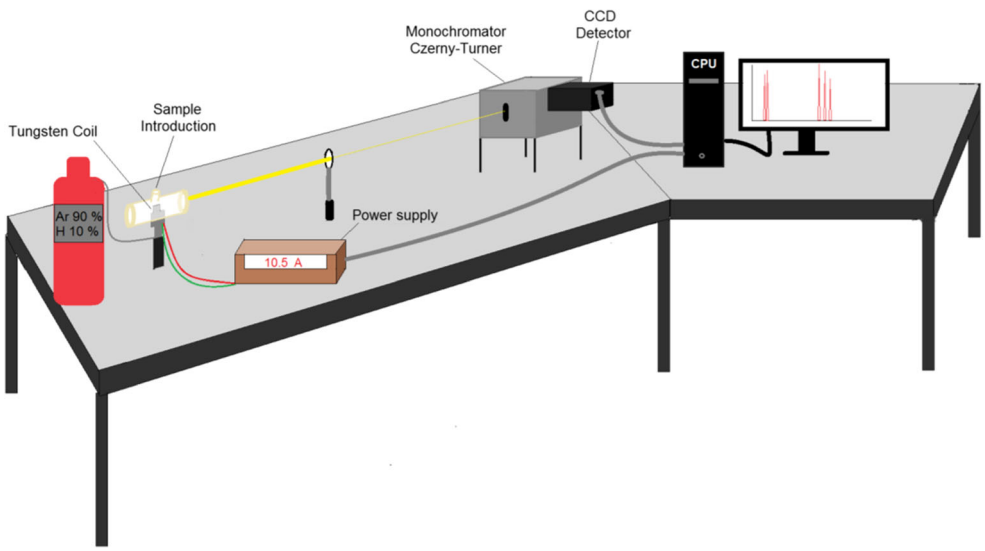


**Fig. 1** Schematic diagram of the WCAES instrumental setup

**Table 1** Heating program used for digesting the placenta samples

| Step | Power (W) | Temperature (K) | Time (min) | Pressure (bar) |
|---|---|---|---|---|
| 1 | 100 | 393 | 5 | 75 |
| 2 | 600 | 413 | 5 | 75 |
| 3 | 1000 | 463 | 10 | 75 |
| 4 | 0 | 298 | 15 | 75 |

Mea-surement Systems Inc., Grand Junction, CO, USA) with a linear dispersion of approximately 2 nm/mm at 400 nm, and a charge-coupled device (CCD) detector (Spec-10, Princeton Instruments, Roper Scientific Trenton, NJ, USA). More details on the energy supply, atomizing cell, monochromator, and detector used in this work can be found elsewhere [30].

A tandem ICP-MS (ICP-MS/MS, 8800 triple quadrupole ICP-MS, Agilent Technologies, Japan) equipped with two quadrupoles (Q1 and Q2) and a third generation octopole reaction system ($ORS^3$) positioned between Q1 and Q2 were used to evaluate the accuracy of the WCAES method. The $ORS^3$ was pressurized with pure oxygen (≥99.999%, Air Products, São Paulo, Brazil) to react with the analytes and promote the formation of oxide species that were monitored at interference-free mass-to-charge ratios (m/z) by Q2 [31]. The MS/MS mode was used in these determinations, and the main instrument operating conditions were as follows: radio frequency (RF) applied power 1550 W, plasma gas flow rate 18 L $min^{-1}$, auxiliary gas flow 1.8 L $min^{-1}$, carrier gas flow rate 1.09 L $min^{-1}$, sampling depth 8 mm, integration time 3 s, stabilization time 20s, spray chamber temperature 2 °C, $O_2$ flow rate in the $ORS^3$ 0.5 mL $min^{-1}$, Q1 set to monitor m/z 52 and 55, and Q2 set to monitor m/z 68 and 71 for Cr and Mn, respectively.

The preservation of the samples and preparation for digestion was carried with a freeze-dryer LD 3000 (Terroni Equipamentos Científicos Ltda., São Carlos, SP, Brazil) and a cryogenic mill (SPEX 68000 Freezer/Mil, Metuchen, NJ, USA). Microwave-assisted digestion (MAD) system were carried out employing Anton Paar Multiwave microwave oven (Graz, Austria) equipped with 25 mL quartz vessels and an Anton Paar Multiwave 3000 microwave oven (Graz, Austria) equipped with 15 mL PTFE vessels.

**Table 2** Heating cycle during the stages of drying, pyrolysis, and atomization for measurements using WCAES

| Step | Temperature (K) | Time (s) | Process | Signal acquisition |
|---|---|---|---|---|
| 1 | 373 | 85 | Drying | No |
| 2 | 1472 | 25 | Pyrolysis | No |
| 3 | 298 | 10 | Preatomization | No |
| 4 | 3360 | 3 | Atomization | Yes |

**Table 3** Evaluation of the analytical signal integration time ($n = 20$) for Mn and Cr using a solution with 0.30 mg $L^{-1}$ of each analyte

| Integration time (ms) | n° of spectra | Element | S/N ratio | RSD |
|---|---|---|---|---|
| 10 | 50 | Mn | 28 | 4 |
| | | Cr | 24 | 4 |
| 30 | 35 | Mn | 36 | 3 |
| | | Cr | 25 | 4 |
| 50 | 20 | Mn | 18 | 6 |
| | | Cr | 16 | 5 |
| 100 | 15 | Mn | 14 | 7 |
| | | Cr | 13 | 8 |

## Reagents and Analytical Solutions

All solutions were prepared using trace metal grade nitric acid (Fisher, Pittsburgh, PA, USA) and distilled-deionized water (18 M Ω cm, Milli-Q®, Millipore, Bedford, MA, USA). Single-element stock solutions of Cr and Mn (1000 mg $L^{-1}$, SPEX CertPrep, Metuchen, NJ, USA) were used to prepare the standard reference solutions used for calibration and in spike experiments. The external standard calibration method was used in all determinations. Hydrogen peroxide 30% $v\ v^{-1}$ (Acros, Morris Plains, NJ, USA) and trace metal grade nitric acid were used to digest the samples. All the placenta samples were collected in the city of Ilha Solteira, SP, Brazil, under the supervision of a veterinarian. Animals from different regions were chosen to minimize biases due to differences in nutritional conditions.

## Sample Preparation

Placentome samples were drawn of premature placenta from six different cows at different stages of pregnancy. The collection was performed with a scalpel n° 2, and the placentomes were stored in polypropylene tubes numbered A1–A6. After collection, samples were kept in a domestic freezer at 268 K. Freeze drying was carried out at 223 K with hermetic compressor, during 48 h in a freeze-dryer built in stainless steel AISI 304, with polished sanitary mirrored, forced ventilation, thermal protection, and a drainage system based on ball valve attached to the vacuum pump. In order to ensure the homogeneity of samples and efficiency of the digestion step, the sample was cryogenically ground using a cryogenic. The grinding program was carried out in 2 cycles with 2 min of freezing and 3 min of grinding. Between each cycle, a standby of 2 min was employed.

The powder samples were then subjected to digestion using a MAD system [32, 33]. Sample aliquots of approximately 0.25 g were digested using 2.5 mL of $HNO_3$, 1.5 mL of $H_2O$, and 1.0 mL $H_2O_2$ in a closed vessel MAD system equipped with a 6-position rotor. The heating program used

**Table 4** Analytical features for WCAES

| | Mn | Cr |
|---|---|---|
| Linear equation | $I = 1093.9C + 5.433$ | $I = 5289.4C + 41.193$ |
| Linear dynamic range (mg $L^{-1}$) | 0.1–1.0 | 0.1–1.0 |
| $R^2$ | 0.9978 | 0.9957 |
| LOD (mg $L^{-1}$) | 0.020 | 0.002 |

for digestion is described in Table 1. The digests were diluted to 25 ml for quantification.

### Tungsten Coil Atomic Emission Spectrometry Determinations

Reference solutions and sample aliquots of 25 μL were placed directly on the tungsten atomizer using an automatic micropipette (Eppendorf 5–50 μL, Brinkman, Westbury, NY, USA). All solutions were prepared in 1% $HNO_3$. A solution containing both analytes (1.0 mg $L^{-1}$ each) was used to determine the most efficient WCAES heating program (Table 2). More details on the WCAES heating cycle and the determination of the coil surface temperature can be found in the literature [34, 35]. Different integration times were evaluated to improve sensitivity for Mn and Cr. Atomic emission signals for Cr and Mn were monitored at 425.5 and 403.1 nm, respectively.

## Results and Discussion

### Evaluation of Analytical Signal Integration Time

The main challenges for Mn quantification in WCAES are the relatively low sensitivity and the potential spectral interferences in complex matrix determinations. Strategies such as the application of chemical modifiers to increase signal intensity and minimize spectral interference have been described [26, 36, 37]. A simple alternative, which requires no chemical modification, is based on the optimization of the analytical signal integration time to enable temporal separation between the analyte and potential matrix interfering species. For a 500 ms integration time, limits of detection (LOD) for Cr and Mn were calculated as 0.06 and 0.9 mg $L^{-1}$, respectively, with a linear dynamic range between 1.00–50.0 mg $L^{-1}$. These values are relatively high when compared with other methods such as GFAAS, MIP OES, ICP OES, and ICP-MS, which can provide LODs at the low μg $L^{-1}$ level [32, 38–40]. To improve sensitivity, we have evaluated four different integration times, i.e., 10, 30, 50, and 100 ms, using a solution with 0.30 mg $L^{-1}$ of each Cr and Mn and the heating cycle shown in Table 2. Signal-to-noise ratio (S/N) was used as the evaluating parameter [41], and the results ($n = 20$) are presented in Table 3. As it can be seen, the best S/N were observed for a 30 ms integration time, with values of 25 and 36, and relative standard deviations (RSD) of 4 and 3% for Cr and Mn, respectively.

**Table 5** Summary of the limits of detection obtained in WCAES by several authors

| Element | Conditions | LOD (μg $L^{-1}$) | Ref. |
|---|---|---|---|
| Cr | | 70 | 23 |
| Cr | Cobalt as chemical modifier | 70 | 38 |
| Cr | Three emission lines | 4 | 30 |
| Cr | IL-DLLME | 3 | 27 |
| Cr | | 2 | This work |
| Mn | | 900 | 37 |
| Mn | Three emission lines | 500 | 28 |
| Mn | Magnesium as chemical modifier | 50 | 37 |
| Mn | Portable WCAES | 50 | 22 |
| Mn | | 20 | This work |

### Analytical Figures of Merit

The LOD for Cr and Mn determined by WCAES were calculated according to IUPAC's recommendations as three times the standard deviation of the blank signal ($n = 15$) divided by the calibration curve slope [42]. The blank is a solution of 1% $HNO_3$ $v\,v^{-1}$. Similarly, the limits of quantification (LOQ) were calculated as 10 times the standard deviation of the blank signal ($n = 15$) divided by the calibration curve slope. The results are presented in Table 4. As it can be seen for the specific instrument configuration used in this work, the use of a shorter integration time and the optimization of the heating cycle have resulted in a superior analytical performance when compared to recent publications (Table 5). The LODs are adequate for trace element applications using this simple and a potentially portable system.

**Table 6** Recovery tests for Cr and Mn in premature placenta determined by WCAES (all concentrations are in mg $L^{-1}$ and $n = 3$)

| Element | Sample | Added | Mean | RSD | Recovery % |
|---|---|---|---|---|---|
| Mn | A1 | 0.30 | 0.29 ± 0.02 | 7.4 | 97 |
| | A2 | 0.30 | 0.28 ± 0.02 | 6.9 | 93 |
| Cr | A1 | 0.30 | 0.31 ± 0.02 | 6.5 | 103 |
| | A2 | 0.30 | 0.28 ± 0.02 | 7.2 | 93 |

**Table 7** Comparison of Mn and Cr determination by WCAES and ICP-MS/MS (all concentrations are in mg $L^{-1}$ and $n = 3$)

| Sample | WCAES | ICP-MS/MS | WCAES | ICP-MS/MS |
|---|---|---|---|---|
| Premature Placenta | Mn | | Cr | |
| A1 | 2.72 ± 0.34 | 2.62 ± 0.07 | 1.31 ± 0.48 | 1.34 ± 0.14 |
| A2 | 2.39 ± 0.23 | 2.33 ± 0.06 | 0.61 ± 0.16 | 0.77 ± 0.01 |
| A3 | 3.14 ± 0.19 | 2.87 ± 0.07 | 0.73 ± 0.10 | 0.69 ± 0.01 |
| A4 | 3.00 ± 0.63 | 2.90 ± 0.09 | 0.99 ± 0.23 | 1.22 ± 0.05 |
| A5 | 2.52 ± 0.55 | 2.36 ± 0.16 | 1.10 ± 0.20 | 1.12 ± 0.13 |
| A6 | 3.09 ± 0.50 | 3.10 ± 0.03 | 0.94 ± 0.14 | 1.10 ± 0.03 |

### Accuracy and Precision

Spike experiments using two digested samples of placenta (A1 and A2) were carried out to evaluate the method's accuracy. Recoveries for the 1:1 diluted samples spiked with 0.30 mg $L^{-1}$ of each Cr and Mn were in the 93.0–103.0% range (Table 6). Precisions, represented as % RSD, were between 6.5 and 7.2% for both elements.

The WCAES method was applied to all placenta samples, and the results were compared to values obtained by ICP-MS/MS. The results are shown in Table 7. As it can be seen, no statistically significant difference is observed between the different methods by applying a paired *t* test at a 95% confidence level [43].

The dynamic exchange of Mn between gestating cows and embryos, which is essential to the development of the fetus, is well described in the literature [8]. In this study [8], the mother's liver was used as a bioindicator of manganese transfer to the embryo. The results showed that 3.20 ± 0.19 μg $g^{-1}$ of Mn present in the mother can result in 0.88 μg $g^{-1}$ of this element available for an adequate formation of the embryo using liver as a bioindicator of manganese. This observation indicates an efficient transport of nutrients via the bloodstream, since the liver has a great effect on the distribution of red blood cells. However, there is no information on Mn levels in cow placenta. The results presented in the present work show that Mn levels in placenta (an average of 2.64 ± 0.39 μg $g^{-1}$) are similar to values found in the liver of gestating cows [8]. Considering the Mn levels required for the development of a healthy embryo [8], WCAES may represent a simple and effective alternative for the quantification of this element in placenta, with a LOD 44-fold lower than the recommended values.

It has been shown that supplementing cows with 6.25 mg $day^{-1}$ of Cr in the first 28 days of the mating season can increase both the percentage of successful pregnancies and milk production [44]. Chromium is involved in many metabolic functions. It activates certain enzymes, stabilizes amino acids and nucleic acids, and may also alter protein synthesis; although, the mechanisms underlying this process are not completely understood. On the other hand, the effect of Cr on insulin sensitivity has been clearly demonstrated in cattle [45]. Despite its importance, especially in stressful conditions, limited information is available on Cr levels in pregnant cows. As demonstrated here, an average of 0.95 ± 0.22 μg $g^{-1}$ Cr is found in cow placentas.

## Conclusions

Chromium and manganese concentrations in cow placenta are important biomarkers of both cow and embryo health. Determining these elements in such a complex matrix is challenging, and the procedure described here represents a green alternative to more complex techniques. WCAES' low power requirements, as well as low consumption of samples, reagents and gases, are important advantages for the analysis of delicate biological tissue samples such as placenta.

WCAES is an effective method to determine Cr and Mn in cow placentas. No chemical modification or preconcentration procedures are required, and a simple external calibration method is sufficient to achieve adequate accuracies and precisions. Matrix effects may be minimized by temporal separation of analytes and interfering concomitants using short integration times. This is a simple, inexpensive, and sensitive method with potential applications in the field.

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**Compliance with Ethical Standards**

**Conflict of Interest** The authors declare that they have no conflict of interest.

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