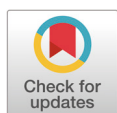


Comparison of sample preparation procedures of inductively coupled plasma to measure elements in dog's hair

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Abstract

The pre-treatment condition affects on the element analysis of inductively coupled plasma (ICP). In this study the pre-treatment condition of ICP has been studied to quantify elements in dog's hair. The hair samples were collected from twelve female Beagles by clipping them into 1 or 2 cm at the back neck. The qualitative and quantitative analysis of elements in hairs were performed by using ICP. By ICP nine elements were qualitatively detected and quantitatively analyzed (Ca, Cu, Fe, K, Mg, Na, P, Se, Zn). The measured amounts of elements were compared between 3 step and 2 step procedures which were with and without the acetone based washing step. The quantitative analysis showed that the concentrations of K, Na, P, and Se were significantly decreased in hair samples with acetone-based washing ($p < 0.005$ or 0.001) unlike those without the acetone-based washing. It implied that some minerals are lost by the acetone based washing during the sample preparation step. Therefore, the acetone based washing process is not suitable for quantifying elements in dog's hair. In addition, the results of qualitative and quantitative analysis were compared. Although there was a difference in absolute values of elemental contents in hair, the results of qualitative and quantitative analysis were significantly correlated each other. This finding suggested that the results of qualitative analysis can be used to monitor elemental contents in dog's hair.

Keywords: Canine, Elements, Hair, Inductively coupled plasma, Sample preparation

INTRODUCTION

Inductively coupled plasma (ICP) is one of a plasma source which uses an energy provided by electrical current from electromagnetic induction. ICP has been applied in an analytic technique to quantify element concentrations. Emission spectrometers using ICP is an important tool for analyzing multi-element determinants and often used to analyze trace elements in biological specimen [1-3]. Plasma is an ionized gas and a variety of samples can be introduced into plasma. For that, samples should be prepared according to the physical property [4,5]. Aqueous samples need no additional process to be introduced into a plasma and only ensured to be in solution by nitric acid (HNO₃). Solid samples are

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Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions

Conceptualization: Chun JL, Kim KH.
 Data curation: Bang HT.
 Formal analysis: Kim M, Lee YK.
 Methodology: Jeong JY, Kim B.
 Validation: Ji SY.
 Writing – original draft: Chun JL, Kim KH.
 Writing – review & editing: Reddy KE, Lee SD.

Ethics approval and consent to participate

The animal study design was approved by IACUC review board (Approval Number: NIAS20191665). We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violates any of these. This manuscript has not been published and is not under consideration by another journal.

not able to be introduced into the plasma directly. Typically, acid digestion is used to dissolve solid samples. Dissolution of solid samples is usually performed by hot acid digestion. Solid biological samples like hairs need to be digested by chemicals as strong acid(s) or alkali to dissolve it [5].

Nutritional elements *in vivo* has been focused to investigate the relationship with the physiological functions in the body [6,7]. Nutritional elements are accumulated in blood, bones, muscles and skins and can be measured [8–10]. For example, blood and urine tests have been used to detect minerals which are considered as a parameter for nutritional status or heavy metal contamination [11–14]. However, blood can be easily affected by daily diets and it is difficult to trace the progress of a certain contamination. In addition, urine test detects only excreted minerals that it is not possible to estimate minerals *in vivo*. Hair can be the biological source to investigate hazardous or essential nutrient elements which are accumulated in hairs [15]. In particular, hairs are relatively stable and not easily altered. Moreover, hairs contain high concentration of elements compared to blood and urine, besides the easiness to obtain hair samples [7,16].

Minerals are essential components to maintain various physiological functions in the body. Calcium, phosphorus, magnesium, sodium, chloride, potassium and sulfur are required in a large amount and iron, zinc, copper, chromium, iodine, selenium, manganese and fluorine are needed in a small amount. It is very important to keep the balance of essential minerals. Minerals are absorbed through foods and used to body functions [8,9,17]. "Left-over" minerals after used are excreted by urine or accumulated in tissues like hairs and nails. Particularly, hair contains a history of minerals uptaken through foods in a long term that elements in hair have been considered as useful biological indicators of health [15].

The analysis of element compositions in hair is able to monitor an individual's nutritional status. ICP has been used to detect element compositions in hair [2]. Unlike human, the study of minerals in dogs has not been attracted. Regarding the importance of minerals dog's hair would provide various data to monitor health condition using ICP. Unfortunately, the eligibility of the hair sample preparation in ICP has not been tested. It is important to establish a proper method for the preparation of hair samples because it affects on a reliable outcome after ICP analysis. For sample introduction hair samples are required to be dissolved in liquid. Prior to the sample decomposition external contaminations are removed during washing process. In general, acetone-based washing is commonly used. Here we investigated whether the acetone based washing process during dog's hair sample preparation could affect on ICP analysis. The establishment of an effective protocol to analyze minerals in hair will facilitate the understanding the sequential health status in dogs.

MATERIALS AND METHODS

Animals

Animal experiments were approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science, South Korea (Approval number: NIAS20191665). All animals were monitored by veterinarians if needed and experiments were performed according to the approved protocols. In total twelve Beagles were enrolled in this study. Dogs were maintained in individual and separated kennels with a temperature controlled system. Dogs were fed commercial diets twice a day and water were provided *ad libitum*.

Hair samples

Dog's hairs were cut from 1–2 cm above the skin of back neck and collected in glass tubes. 100 mg of hairs were used to analyze elements for each experiment. Collected hairs from an individual dog were divided into 50 mg. Each 50 mg of hairs were used for the control group with the ace-

tone-based washing step and for the experimental group without the washing process.

Hair washing

5 mL of acetone was added into 50 mg of dog's hairs in a glass tube. Hairs in acetone was sonicated for 2 min. After discarding acetone, 5 mL of 0.01% triton solution was added to hairs and sonicated for 2 min. After repeating these processes twice deionized water was added prior to do another sonicate for 2 min. At the end of the washing step hairs were dried in desiccator with silica gel.

Hair digestion

For mineralization 0.1 g of dried hairs were placed in a glass tube. 2.5 mL of nitric acid (70% HNO₃) were added to each sample and incubated at 80 °C heating block for 24–48 hours. When it became transparent deionized water were added up-to 50 mL in total volume. As the final process digested hairs were filtered through 0.45 µm of polyvinylidene fluoride (PVDF) syringe filter (HM, Korea).

ICP analysis

The measurement of all elements was performed by ICPS-7510 (Shimadzu, Japan). The plasma gas flow was 1.0 L/min and RF power was 0.8 kW. Eighteen minerals were targeted to analyze qualitatively, i. e., arsenic (As), boron (B), calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), iodine (I), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorus (P), selenium (Se), silicon (Si), vanadium (V), zinc (Zn). Among them, nine elements were detected; Ca, Cu, Fe, K, Mg, Na, P, Se, Zn. With them we performed qualitative and quantitative analysis.

Statistical analysis

Differences between the control group and the treatment group were evaluated by Student's *t*-test. Correlation coefficients between the qualitative measurements and the quantitative measurements was also analyzed by Pearson correlation analysis. Statistical significance was considered to be $p < 0.05$.

RESULTS AND DISCUSSION

In dogs, minerals are involved in a variety of physiological functions. However, there is less experiment about minerals or chemicals in dog's hairs. Hair can be an effective biological sample to study elements including minerals because it is easy to obtain without any pain or surgical process [15,17]. Moreover, hair grows that it is possible to obtain accumulated information of elements. ICP system has been used to analyze elements in various biological samples including hairs [10]. To collect reliable results, it is required to process a proper pre-treatment step which prevents from any exogenous contamination [5,18]. On the other hand, it is important to keep endogenous elements without losing that allows to measure the actual amount of elements accumulated in samples.

Here, we tested whether the pre-treatment process affect on the measurement of elements in dog's hair samples. To measure minerals there were 3 steps: washing, sample digestion and ICP analysis. Dog's hair samples were processed through the acetone-based washing step which has been used as a common method in ICP analysis. There were three steps as a usual method for the control group and two steps as the test for the treatment group without washing. We targeted eighteen elements and Ca, Cu, Fe, K, Mg, Na, P, Se, and Zn were detected after standardized in the qualitative analysis (Table 1). Ca, K, Mg, Na, and P are classified in macro elements which are usually expressed in terms of milligrams per 1,000 kcal. Ca and P are essential for internal skeleton. Ca,

Table 1. The effect of washing step on the qualitative analysis of elements in dog's hairs

	Ca	Cu	Fe	K	Mg	Na	P	Se	Zn
2 step (µg/g)	1,382,142.5	351.2	310.4	1,146	540.8	2,478.6	866.6	56.3	1,715.2
3 step (µg/g)	1,311,745.1	373.9	254.9	436.9	543.3	917.3	673.6	56.8	1,894.1
Difference (%)	-5.1	6.4	-17.9	-61.9	5	-63	-22.3	9	10.4
Correlation	0.9	0.9	0.6	0.4	0.9	0.4	0.7	0.9	0.6
t-test	0.745	0.158	0.242	0.012	0.98	0.002	0.096	0.952	0.026

Values are mean.

Table 2. The effect of washing step on the quantitative analysis of elements in dog's hairs

	Ca	Cu	Fe	K	Mg	Na	P	Se	Zn
2 steps (µg/g)	712.1	0	0	1,326.5	63.7	2,968	428.7	4.1	46.8
3 steps (µg/g)	627.7	0	0	122.6	62.1	369.1	249.5	2.3	59.3
Difference (%)	-11.8	0	0	-90.8	-2.5	-87.6	-41.8	-43.7	26.6
Correlation	0.7	0	0	0	0.9	0.1	0.7	0.9	0.1
t-test	0.602	0	0	< .000	0.948	< .000	0.004	< .000	< .000

Values are mean.

Mg, K, and Na have important roles in nervous impulse, muscle contraction and cell signaling. Fe, Zn, Cu, Mn, I, and Se are considered to be trace elements which are needed in a smaller amount in pet foods. Usually, trace elements are involved in enzymatic reactions. Fe, Zn, and I are important in protein and hormone structures.

In qualitative analysis, Ca, Fe, K, Na, and P are detected 5.1, 17.9, 61.9, 63, and 22.3 less after 3 step process than those after 2 step process (Table 1). The amount of Zn is detected 10.4% more in 3 step process than those in 2 step process (Table 1). Especially, K and Na were significantly decreased. In quantitative analysis, Cu and Fe were not detected (Table 2). Ca, K, Mg, Na, P, and Se were decreased 11.8%, 90.8%, 2.5%, 87.6%, 41.8%, and 43.7% in 3 step process compared to those in 2 step process (Table 2). Moreover, K, Na, P, and Se were significantly decreased. It implied that the acetone based washing process which has been considered to be a standard method would result in losing some endogenous minerals during pre-treatment process. In addition, we compared the results from qualitative and quantitative analysis (Fig. 1). The values of qualitative analysis had linearized functional relationship with those of quantitative analysis. It supported that the qualitative analysis would be used to quantify elements in hairs as the semi-quantitative analysis.

Hair contains a sequential and various information including nutritional and toxic elements [7]. The content of elements in hair has been used as a marker of nutritional and physiological status of animals. However, in dogs, hair has not been used as much as its value as a biological parameter. In addition, the comprehensive analysis of untargeted elements has been developed and applied using metallomic analysis [19]. At the same time, it is important to establish the analytical condition of minerals because the comprehensive analysis is affected by the sample preparation process [18,20,21]. This study emphasized the need for the establishment of the hair sample preparation for ICP analysis. Meanwhile we also showed that the qualitative analysis would be another method to quantify minerals in hair by using ICP. The results would contribute to develop an effective method to analyze minerals in dog's hair.

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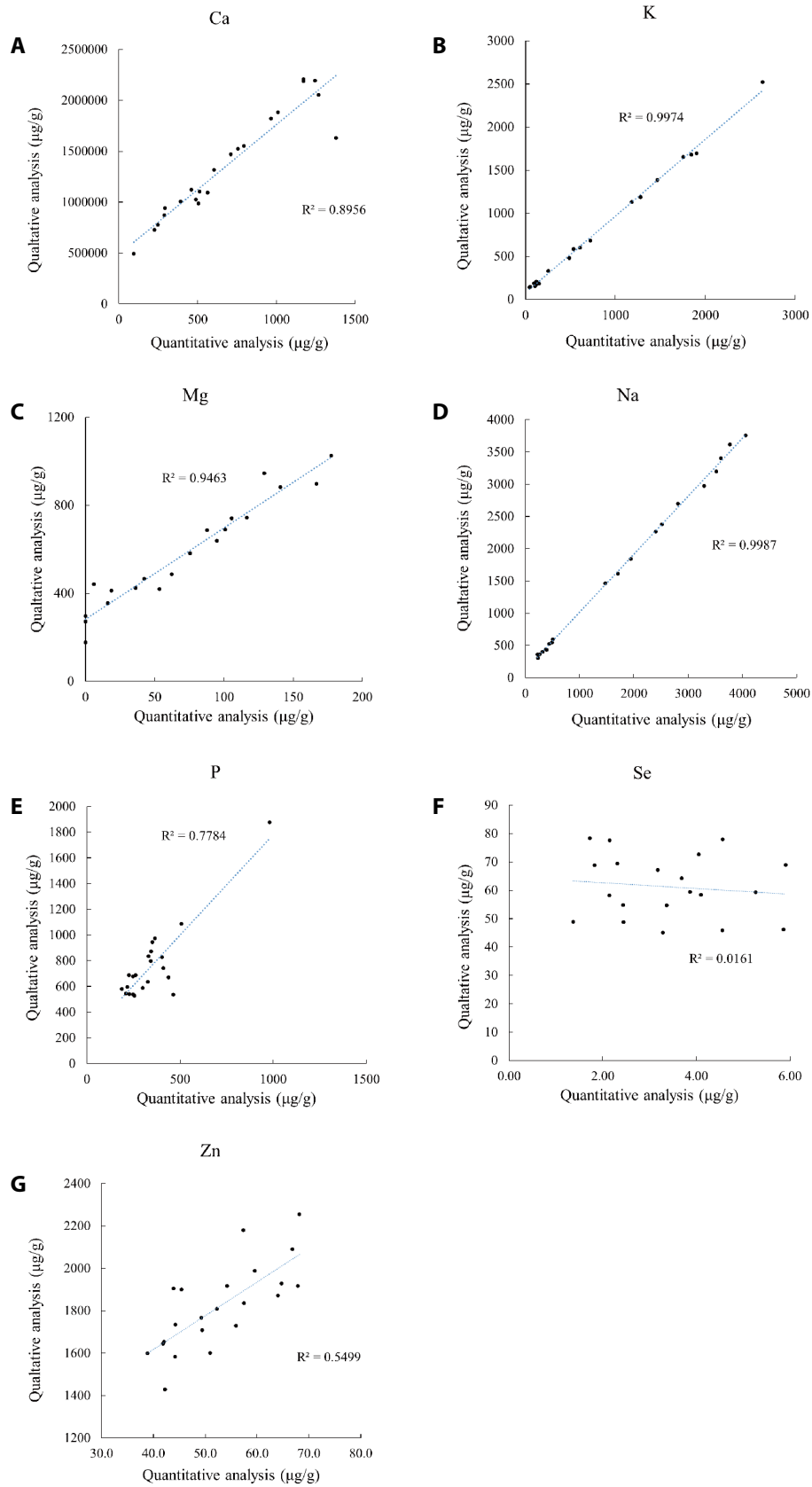


Fig. 1. Correlation of quantitative and qualitative analysis in ICP. The correlation between quantitative and qualitative data of (A) calcium, (B) potassium, (C) magnesium, (D) sodium, (E) phosphorus, (F) selenium, (G) zinc. ICP, inductively coupled plasma.

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