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ARTICLE

Evaluation of Closed-Vessel Conductively-Heated Digestion System with Diluted Acid for Analysis of Animal Feed by ICP-MS

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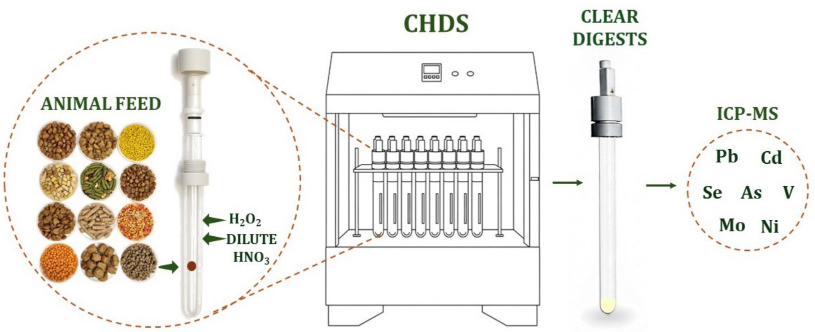

A conductively-heated digestion system (CHDS) with closed-vessel and diluted acid was evaluated for the preparation of animal feed for subsequent determination of As, Cd, Mo, Ni, Pb, Se and V by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The analytical performance of the digestor was checked by analyzing fish tissue, tuna and corn bran certified reference materials (CRMs) using 1 mL of 30% (w w^{-1}) hydrogen peroxide plus 2 mL of nitric acid at 3.5, 7 and 14 mol L^{-1} HNO_3. The recoveries of analytes in CRMs digested at different acid concentrations varied from 80 to 116% (3.5 mol L^{-1} HNO_3), 86 to 118% (7 mol L^{-1} HNO_3), and 80 to 123% (14 mol L^{-1} HNO_3). The method was applied to real animal feed samples, and the CHDS results were like those of comparative microwave digestion (MWD). Quantification limits of analytes found by CHDS and MWD were also similar, concerning different acid concentrations. However, the use of diluted nitric acid in the preparation of animal feed samples proved to be a viable and efficient approach, allowing lower residual acidity, reduction in reagent consumption and acid waste generation, in accordance with the principles of Green Chemistry.

Keywords: animal feed, nitric acid, sample preparation, conductively-heated digestion system, ICP-MS

INTRODUCTION

Animal feed is the mainstream option for animal nutrition due to its practicality, easiness of access. By adequately meet the nutritional and energy requirements of animals, a wide variety of feed formulations is available on the market and can be selected according to the breed, size, age, and health predispositions

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of the animals.[1-3] The composition of these feeds can directly influence health, so accurately identifying certain elements is important since their presence at adequate concentrations can significantly contribute to preventing health problems. However, high levels of certain elements can lead to toxic effects on the body.[4] In this context, it is crucial to monitor the concentration of such elements like arsenic (As), cadmium (Cd), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), and vanadium (V). Although these elements are generally present at low concentrations, they can be toxic when accumulated at excessive levels. Controlling these elements is essential to ensuring the safety and well-being of animals.[4,5] Inductively coupled plasma mass spectrometry (ICP-MS) is a highly sensitive technique that enables the precise determination of trace and ultra trace elements. This technique is frequently used to identify potentially toxic elements in various samples. However, this technique is subject to polyatomic interference, which is caused by the combination of matrix ions and argon gas during ionization. This interference can compromise the accuracy of the results. One way to minimize this effect is to promote the complete decomposition of the matrix by employing an efficient digestion method.[6-8]

In 2014, Miranda, Pereira Filho, and Gomes Neto[9] developed a new conductively-heated digestion system (CHDS). The CHDS was successfully applied to various matrices, including plants,[9,10] feed,[11] meat,[12] agricultural materials,[13] and dog feed.[14] The instrument was commercially released two years ago with the launch of the Vert Simplify digestor, an evolution of that system. The performance of the Simplify model was evaluated by analyzing different matrices, including plant materials,[15] oil sludge,[16] biomass and biochar,[17] and animal feed samples.[18] The CHDS has demonstrated high efficiency in decomposing various types of samples. Using closed vials minimizes analyte loss and prevents contamination during digestion. Conductive heating via a thermal block ensures uniform and controlled temperature distribution. Additionally, the CHDS system is versatile, effectively decomposing different matrices with high reproducibility. Furthermore, the system's low operating and maintenance costs offer an economical and practical alternative for sample preparation.

Additionally, Costa et al. (2025) demonstrated that digesting animal feed samples with CHDS and diluted nitric acid efficiently decomposes the matrix, allowing for the subsequent determination of macronutrients and micronutrients via inductively coupled plasma optical emission Spectrometry (ICP OES).[18] Considering the sensitivity limitations of that work for determining analytes at trace and ultra-trace levels, the importance of animal feed quality control and the growing demand for more sustainable analytical methods, this study evaluated the performance of the Vert Simplify digestor to prepare bird, dog, horse, rabbit, cat and fish feed samples using diluted nitric acid for subsequent determinations of As, Cd, Mo, Ni, Pb, Se and V by ICP-MS.

MATERIALS AND METHODS

Reagents, analytical solutions and sample

Twelve feed samples, including two of each for birds, cats, dogs, fish, horses and rabbits, were purchased from a pet store in Araraquara, SP, Brazil. The samples were previously ground in a Spex 6750 cryogenic mill (Metuchen, NJ, USA) using a program with two grinding cycles, each lasting 3 minutes, and a 10-minute cooling ramp. This procedure yielded particles with an average diameter of less than 50 µm. The samples were then dried in an air-circulating oven (TE-394/2 Tecnal, Piracicaba, SP, Brazil) at 75 °C until constant mass. After drying, the samples were placed in appropriate containers and stored in a desiccator.

The performance of the CHDS digestion method was evaluated by digesting certified reference materials (CRMs) of Durum Wheat Flour (DUWF-1 8436) and Corn Bran (BRAN-1 8433) (National Research Council Canada),[19] and reference material (RM) of Tuna Fish (1101) (University of São Paulo, Brazil).[20] The particle sizes of CRMs and RM are approximately 50 µm.[19,20] The aqueous solutions were daily prepared by using deionized water (18.2 MΩ cm), produced by a reverse osmosis purification system (Gehaka Master System MS2000, São Paulo, Brazil). The digestion procedure used nitric acid (HNO_3, 69%, J.T. Baker, Deventer, Netherlands), which was previously purified by distillation in a boiling system, as well as hydrogen peroxide (H_2O_2, 30%, Merck, Darmstadt, Germany). The vessels were previously decontaminated by immersing them in a 10% (v/v) HNO_3 solution for 24 h. After the acid bath, they were thoroughly cleaned with deionized water. The multi-element working standard solutions were prepared by appropriate dilution of 1000 mg L^{-1} single-element stock standards (SpecSol®, QUIMLAB, São Paulo, Brazil) with 5% HNO_3 (v v^{-1}) medium. The concentration range used in calibration curves for As, Cd, Se, Mo, Ni, Pb, and V was 0.01 – 500 µg L^{-1}.

Instrumentation

CHDS digestor (Simplify, Vert Technologies, São Paulo, Brazil) was used to digest animal feed samples, CRMs, and RM. The CHDS contains an aluminum heating block with 24 slot positions and a heating element of 1600 W, which enables temperatures up to 350 °C. The vessels are made of quartz, sealed with polytetrafluoroethylene (PTFE) lids, and tolerate a maximum pressure of 28 bar.[15] For comparison purposes, all samples were also digested using an Anton Paar model Multiwave 3000 microwave digestor (Graz, Austria). Dissolved organic carbon was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Thermo Scientific ICAP 7400, Waltham, USA). An 8900 Triple Quadrupole ICP-MS/MS (Agilent Technologies, Japan) inductively coupled plasma mass spectrometer (ICP-MS) was used to determine As, Cd, Mo, Ni, Pb, Se, and V in the samples. The instrumental parameters optimized for ICP-MS determinations are presented in Table I.

Table I. Instrumental operating parameters used for ICP MS data acquisition

Instrumental parameters	Operating conditions
RF Power	1500 W
Plasma gas-flow rate	15 L min^{-1}
Auxiliary gas-flow rate	0.68 L min^{-1}
Nebulizer gas-flow rate	0.5 L min^{-1}
Spray chamber	Double-pass Scott type
Nebulizer	Concentric
Operation mode	Single quad (He 5.5 mL min^{-1})
Isotope (*m/z*)	As (75), Cd (114), Mo (98), Ni (60), Pb (208), Se (78), and V (51)

Digestion procedures

For the CHDS digestions (n = 3), 200 mg of each sample was weighed into the digestion vessels. Then, 2.0 mL of nitric acid at 3.5, 7.0, or 14.0 mol L^{-1} and 1.0 mL of hydrogen peroxide were added, resulting in a total volume of 3.0 mL. Considering the dilution effect due to the addition of hydrogen peroxide, the final acid concentrations were 2.3, 4.7, and 9.3 mol L^{-1} HNO_3, respectively. The digestion procedure was performed using the following temperature program: (a) a temperature ramp of 10 °C min^{-1} from room temperature to 250 °C, (b) 20 min at 250 °C, and (c) a 25-min cooling period. Note that 250 °C corresponds to the temperature of the aluminum heating block while the internal temperature of the reaction medium was approximately 200 °C.[15] After digestion, the solutions were transferred to polypropylene tubes and diluted to a final volume of 25 mL with ultrapure water.

For microwave-assisted digestion (MW, n = 3), 200 mg of each sample was weighed into PTFE vessels. Then, 4.0 mL of nitric acid (3.5, 7, or 14 mol L^{-1}) and 2.0 mL of hydrogen peroxide were added. The microwave heating program consisted of three stages: (a) a 25-min ramp to 1200 W, reaching 200 °C; (b) a 15-min hold at 1200 W (200 °C); and (c) a 20-min cooling/venting period at 0 W. The final digests were transferred to polypropylene tubes and diluted to 25 mL with ultrapure water.

RESULTS AND DISCUSSION

To evaluate digestion efficiency using CHDS, the mass fraction (µg kg^{-1}) of As, Cd, Mo, Ni, Pb, Se, and V were determined by ICP-MS in CRMs and RMs. The CRMs and RM were selected based on their similarity to the animal feed samples to ensure comparable results. Digestions performed in closed vessels using HNO_3 and H_2O_2 allow the regeneration of nitric acid because when HNO_3 is used for oxidation of organic matter, the main product is $NO_{2(g)}$, which reacts with $O_{2(g)}$ (from H_2O_2) and $H_2O_{(g)}$, producing HNO_3 according to the following reactions:[6,13]

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_{2(g)}$$

$$4NO_{2(g)} + 2\ H_2O_{(g)} + O_{2(g)} \rightarrow 4\ HNO_{3(aq)}$$

This regeneration is useful to reduce the amount of acid in the process of digestion, resulting in lower residual acidity.[6,13] Additionally, this method offers several advantages, including lower analytical blank values, reduced costs, and greater compliance with green chemistry principles.[6,13] Tables II show the results obtained for As, Cd, Mo, Ni, Pb, Se, and V by ICP-MS after digestion in CHDS with different HNO_3 concentrations.

The analyte recovery for the CRM and RM digested under different concentrations of nitric acid were 80 – 116% (3.5 mol L^{-1} HNO_3), 86 – 118% (7 mol L^{-1} HNO_3) and 80 – 123% (14 mol L^{-1} HNO_3). The digestion method using CHDS showed good trueness for all analytes, with recovery rates exceeding 80% under all evaluated conditions. It should be noted that the use of diluted HNO_3 (3.5 mol L^{-1}) was satisfactory to ensure accurate results, demonstrating the feasibility of using low concentrations of nitric acid in the digestion process of animal feed without compromising analytical performance. Precision (as relative standard deviation, RSD) were between 0.5 – 24% for 3.5 mol L^{-1} HNO_3, 0.1 – 29% for 7 mol L^{-1} HNO_3, and 0.1 – 29% for 14 mol L^{-1} HNO_3.

Statistical significance was evaluated by means of an unpaired *t*-test at 95% confidence level. In general, most of the results obtained with the CHDS digestion method using 3.5, 7, and 14 mol L^{-1} HNO_3 were not statistically different from the certified values. Notably, in some cases, significant differences were observed. However, trueness was close to 100% and this discrepancy can be attributed to small standard deviations resulting in shorter confidence intervals that do not overlap at a 95% confidence level.

Evaluating the influence of HNO_3 concentration variation in CRM and RM digests using the CHDS system revealed that each sample and analyte responded differently to changes in acid concentration. However, the trueness data did not show a clear trend in association with the HNO_3 concentration (3.5, 7, or 14 mol L^{-1}), suggesting that variation is random and the acidity does not significantly affect the results. Therefore, diluted nitric acid can be used for digesting these samples in CHDS without affecting trueness.

Table II. Results (mean ± standard deviation, n = 3) and recoveries (%) for trace and ultra-trace elements determined in CRMs and RM digested by CHDS using different nitric acid concentrations

CRM	HNO_3 (mol L^{-1})	Mass Fraction (μg kg^{-1})													
		As	%	Cd	%	Mo	%	Ni	%	Pb	%	Se	%	V	%
Tuna Fish (1101)	Certified	4680**		34**		*		20**		48**		5500**		*	
	3.5	4682 ± 51	100	33 ± 1	96	116 ± 1	-	18 ± 4	92	51 ± 3	106	5869 ± 80	107	78 ± 1	-
	7	5005 ± 280	107	39 ± 3	114	161 ± 1	-	23 ± 3	113	51 ± 7	106	5202 ± 800	95	75 ± 0	-
	14	5003 ± 4	107	40 ± 2	119	129 ± 1	-	16 ± 2	80	46 ± 3	95	5095 ± 89	93	70 ± 8	-
DUWF-1 (8436)	Certified	30*		110 ± 50		700 ± 12		170 ± 12		23 ± 6				21 ± 6	
	3.5	26 ± 1	88	107 ± 12	97	710 ± 86	101	170 ± 16	100	23 ± 3	101	1128 ± 37	-	23 ± 5	109
	7	26 ± 1	88	111 ± 1	101	751 ± 1	107	178 ± 9	105	20 ± 1	88	1388 ± 37	-	23 ± 2	110
	14	32 ± 1	107	129 ± 10	117	659 ± 60	94	203 ± 12	120	28 ± 2	123	1498 ± 94	-	22 ± 4	105
BRAN-1 (8433)	Certified	2*		12 ± 5		252 ± 39		160*		140 ± 34		45 ± 8		5*	
	3.5	1.7 ± 0.4	83	13 ± 1	105	286 ± 6	114	161 ± 6	101	141 ± 15	101	47 ± 7	105	4 ± 1	80
	7	1.7 ± 0.5	86	12 ± 1	97	293 ± 3	116	183 ± 2	114	161 ± 3	115	53 ± 1	118	5 ± 1	109
	14	2.1 ± 0.6	103	12 ± 3	102	300 ± 13	119	182 ± 11	114	138 ± 13	98	50 ± 11	111	6 ± 1	117

*not available; **non-certified values.

Arsenic, Cd, Mo, Ni, Pb, Se, and V were determined by ICP-MS in bird, cat, dog, fish, horse and rabbit feed samples. The samples were digested using the CHDS and MWD methods with HNO_3 concentrations of 3.5, 7, and 14 mol L^{-1}. All final digests were clear, indicating the complete decomposition of organic matter. To ensure digestion efficiency, the residual carbon content (RCC) was determined. The RCC values in general ranged from 0.09 to 0.17% (3.5 mol L^{-1}), 0.06 to 0.14% (7 mol L^{-1}), and 0.01 to 0.13% (14 mol L^{-1} HNO_3), suitable for analysis by ICP-OES and ICP-MS,[21] Considering the high presence of protein and fat in the samples, which are rich in carbon, the residual carbon levels were consistently low, demonstrating the effectiveness of the CHDS digestion method.

Shown in Table III are results (in µg kg^{-1}) for As, Cd, Mo, Ni, Pb, Se and V obtained by ICP-MS for animal feed samples prepared in CHDS and MWD using 1 mL of H_2O_2 and 2 mL of HNO_3 3.5 mol L^{-1}. The data related to HNO_3 concentrations of 7 and 14 mol L^{-1} are provided in Tables SI and SII of the Supplementary Material, respectively. Significant variations in analyte mass fractions were observed in animal feed. The highest and lowest levels (µg kg^{-1}) of V, Ni, As, Se, Mo, Cd and Pb were respectively 2,920 (horse) and 247 (dog), 5,020 (fish) and 1,203 (dog), 824 (fish) and 205 (bird), 700 (fish) and 196 (rabbit), 1580 (bird) and 413 (fish), 331 (fish) and 114 (cat), 928 (fish) and 275 (cat). These variations may be related to the composition of the ingredients used in each type of animal feed, the specific raw materials used, or possible environmental contamination associated with the production process. Identifying higher levels is particularly relevant for assessing toxicological risks, especially with potentially toxic elements such as arsenic, cadmium, and lead. In general, all analyte mass fractions found in animal feed were consistently below the established toxicity and tolerance limits.[4,22,23]

The mass fractions (µg kg^{-1}) of As, Cd, Mo, Ni, Pb, Se and V found in all samples digested by the proposed and comparative methods were similar. Figure 1 illustrates correlation plots of the mass fractions of analytes in animal feed samples determined by ICP-MS after digestion in CHDS and MWD using different concentrations of nitric acid. Typical linear correlations were ≥ 0.997 (3.5 mol L^{-1}), ≥ 0.996 (7 mol L^{-1}) and ≥ 0.996 (14 mol L^{-1}). Statistical tests were performed on all results to assess precision and accuracy by *F*-test and paired *t*-test, respectively. A comparison between CHDS and MWD results indicated that 99%, 93%, and 94% of the *F*-test results and 89%, 88% and 93% of the paired *t*-test results for 3.5, 7 and 14 mol L^{-1} HNO_3, respectively, exhibited no significant difference at a 95% confidence level.

The analyte determinations using CHDS showed relative standard deviations (RSDs) found to range from 0.2 to 18.3% at 3.5 mol L^{-1}, 0.1 to 18.9% at 7 mol L^{-1}, and 0.1 to 18.3% at 14 mol L^{-1}. The RSDs obtained using MWD were 0.1 – 17.7%, 0.1 – 13.2%, and 0.1 – 16.3% for the same samples.

Table III. Results (mean ± standard deviation, n = 3) for trace and ultra-trace element determined (μg kg^{-1}) in feed samples after digestion in CHDS and MWD using diluted nitric acid (3.5 mol L^{-1})

Sample	Digestion method	Mass Fraction (μg kg^{-1})						
		V	Ni	As	Se	Mo	Cd	Pb
Bird 1	CHDS	2007 ± 12	1797 ± 134	226 ± 1	501 ± 46	1580 ± 25	167 ± 0	390 ± 35
	MWD	2043 ± 31	1754 ± 56	247 ± 3	551 ± 41	1568 ± 34	165 ± 2	355 ± 54
	t-value	1.9	0.5	10.5	1.4	0.5	1.5	0.9
Bird 2	CHDS	274 ± 6	1219 ± 114	205 ± 1	195 ± 9	1261 ± 21	142 ± 1	297 ± 19
	MWD	300 ± 6	1294 ± 68	217 ± 1	210 ± 22	1279 ± 11	142 ± 0	294 ± 14
	t-value	5.2	1.0	12.2	1.2	1.3	0.5	0.2
Dog 1	CHDS	253 ± 6	1203 ± 65	212 ± 4	349 ± 9	1012 ± 27	134 ± 0	397 ± 3
	MWD	247 ± 3	1196 ± 21	222 ± 2	342 ± 4	1053 ± 29	138 ± 1	387 ± 8
	t-value	1.6	0.2	3.7	1.3	1.8	9.0	1.9
Dog 2	CHDS	327 ± 12	1290 ± 39	257 ± 3	407 ± 14	608 ± 4	157 ± 3	384 ± 9
	MWD	318 ± 6	1275 ± 94	270 ± 5	404 ± 9	620 ± 7	157 ± 10	388 ± 3
	t-value	1.1	0.3	3.7	0.3	2.5	0.1	0.6
Horse 1	CHDS	2023 ± 308	4615 ± 41	778 ± 16	324 ± 59	846 ± 44	132 ± 18	636 ± 48
	MWD	2120 ± 22	4316 ± 133	732 ± 24	332 ± 59	843 ± 97	146 ± 2	639 ± 49
	t-value	0.5	3.7	2.7	0.2	0.1	1.4	0.1
Horse 2	CHDS	2920 ± 14	4425 ± 42	716 ± 8	353 ± 11	1053 ± 12	163 ± 10	707 ± 129
	MWD	3482 ± 26	4550 ± 86	729 ± 2	508 ± 28	1073 ± 26	151 ± 3	705 ± 51
	t-value	33.2	2.3	2.8	9.0	1.2	2.0	0.02
Cat 1	CHDS	928 ± 24	2118 ± 97	430 ± 25	451 ± 11	1384 ± 27	114 ± 4	614 ± 37
	MWD	952 ± 61	2078 ± 64	444 ± 14	461 ± 35	1313 ± 87	115 ± 1	641 ± 12
	t-value	0.6	0.6	0.8	0.5	1.4	0.5	1.2

(continued on next page)

Table III-contd. Results (mean ± standard deviation, n = 3) for trace and ultra-trace element determined (µg kg^{-1}) in feed samples after digestion in CHDS and MWD using diluted nitric acid (3.5 mol L^{-1})

Sample	**Digestion method**	**Mass Fraction (µg kg^{-1})**						
		V	**Ni**	**As**	**Se**	**Mo**	**Cd**	**Pb**
Cat 2	CHDS	389 ± 30	1208 ± 58	251 ± 4	663 ± 22	567 ± 35	127 ± 6	275 ± 31
	MWD	397 ± 43	1296 ± 60	263 ± 5	654 ± 21	564 ± 14	122 ± 3	260 ± 4
	t-value	0.3	1.8	3.2	0.5	0.2	1.3	0.8
Fish 1	CHDS	1597 ± 14	5020 ± 998	728 ± 10	700 ± 41	1038 ± 54	119 ± 1	928 ± 14
	MWD	1514 ± 10	5384 ± 87	771 ± 8	690 ± 45	1135 ± 40	117 ± 0	951 ± 17
	t-value	8.4	0.6	6.0	0.3	2.5	4.3	1.9
Fish 2	CHDS	427 ± 11	1263 ± 41	824 ± 27	554 ± 24	413 ± 19	331 ± 17	307 ± 37
	MWD	419 ± 18	1253 ± 82	875 ± 30	566 ± 56	408 ± 10	327 ± 11	349 ± 15
	t-value	0.7	0.2	2.2	0.3	0.4	0.4	1.8
Rabbit 1	CHDS	953 ± 32	2591 ± 100	263 ± 7	196 ± 5	1075 ± 49	153 ± 3	522 ± 80
	MWD	1007 ± 23	2514 ± 136	282 ± 4	195 ± 15	1025 ± 48	154 ± 2	548 ± 8
	t-value	2.4	0.8	4.1	0.1	1.2	0.7	0.6
Rabbit 2	CHDS	380 ± 14	1599 ± 120	354 ± 12	439 ± 59	962 ± 38	138 ± 3	321 ± 13
	MWD	394 ± 48	1565 ± 79	349 ± 8	445 ± 23	946 ± 30	128 ± 0	340 ± 12
	t-value	0.5	0.4	0.6	0.2	0.6	6.6	1.8

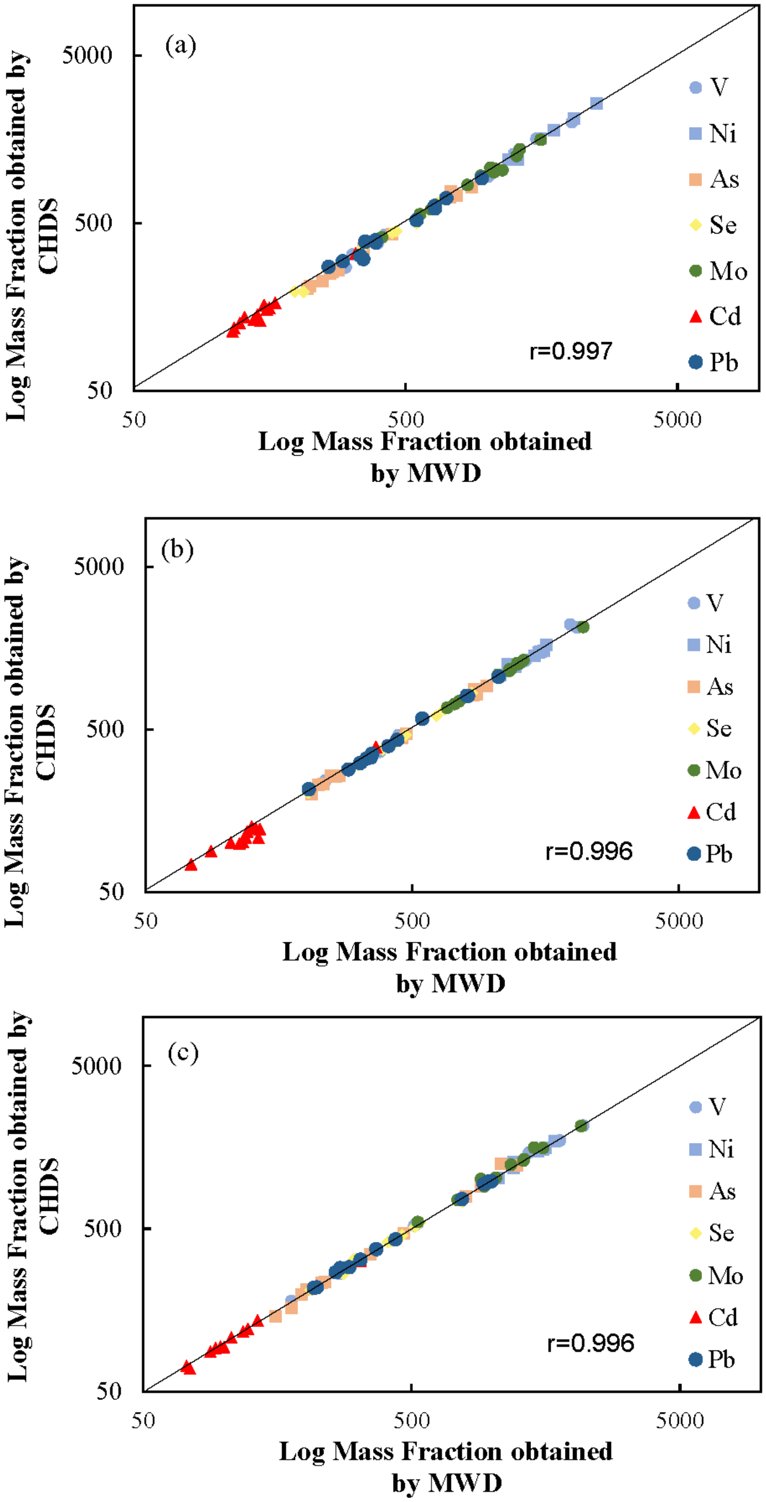

Figure 1. Correlation plot of the log of the Mass fraction (μg kg^{-1}) of trace and ultra trace elements in animal feed samples, as determined by ICP-MS after digestion in CHDS (y-axis) and MWD (x-axis), with the use of HNO_3 at concentrations of (a) 3.5 mol L^{-1}, (b) 7 mol L^{-1}, and (c) 14 mol L^{-1}.

The limits of quantification (LOQ) were calculated according to IUPAC recommendations.[24] Table IV presents data for As, Cd, Mo, Ni, Pb, Se and V obtained using CHDS and MWD. In general, the LOQ observed for CHDS and MWD using HNO_3 at 3.5, 7 and 14 mol L^{-1} were similar. These data demonstrate the effectiveness of the CHDS system to prepare animal feed for determining trace and ultra-trace elements using ICP-MS. Furthermore, variation in HNO_3 concentration showed no observable trend and did not influence the LOQ results. This demonstrates that the use of diluted acid is feasible for preparing animal feed samples.

Table IV. Limits of quantification (in µg kg^{-1}) for analytes determined by ICP-MS after digestion in CHDS and MWD methods using different nitric acid concentrations

Element	Method	Nitric acid, mol L^{-1}		
		3.5	7	14
V	CHDS	31	36	33
	MWD	48	25	19
Ni	CHDS	286	211	186
	MWD	245	164	122
As	CHDS	52	55	51
	MWD	44	59	38
Se	CHDS	66	47	51
	MWD	55	59	76
Mo	CHDS	186	214	398
	MWD	156	221	361
Cd	CHDS	5	13	15
	MWD	9	10	18
Pb	CHDS	52	61	86
	MWD	75	77	59

CONCLUSIONS

The application of diluted nitric acid in sample preparation using CHDS has shown promise for the digestion of animal feed for different species, including birds, cats, dogs, horses, fish, and rabbits. Determining As, Cd, Mo, Ni, Pb, Se, and V in CRMs and RMs demonstrated the high accuracy of the proposed method even using diluted nitric acid. The results showed that CHDS performs similarly to MWD digestion. Additionally, the limits of quantification obtained with CHDS were similar to those of MWD, independent of the HNO_3 concentration used, which contributes to reduced waste generation. Furthermore, the present study demonstrated not only the efficiency of the diluted acid digestion process, but also its potential to promote a more sustainable and environmentally responsible approach to animal feed quality control.

Conflicts of interest

There are no conflicts of interest to declare.

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REFERENCES

(1) Costa, S. S. L.; Pereira, A. C. L.; Passos, E. A.; Alves, J. P. H.; Garcia, C. A. B.; Araujo, R. G. O. Evaluation of the Chemical Composition of Dry Feeds for Dogs and Cats. *J. Braz. Chem. Soc*. **2018**, *23* (12), 2616–2625. https://doi.org/10.21577/0103-5053.20180142

(2) Zicker, S. C. Evaluating Pet Feeds: How Confident Are You When You Recommend a Commercial Pet Feed? *Topics in Companion Animal Medicine* **2008**, *23* (3), 121–126. https://doi.org/10.1053/j.tcam.2008.04.003

(3) Costa, S. S. L.; Pereira, A. C. L.; Passos, E. A.; Alves, J. P. H.; Garcia, C. A. B.; Araujo, R. G. O. Multivariate Optimization of an Analytical Method for the Analysis of Dog and Cat Feeds by ICP-OES. *Talanta* **2013**, *108*, 157–164. https://doi.org/10.1016/j.talanta.2013.03.002

(4) National Research Council. *Mineral Tolerance of Animals* (2nd Ed.). The National Academies Press: Washington, DC, 2005.

(5) Association of Official Agricultural Chemists (AOAC). Official Methods of Analysis: *Guidelines for Standard Method Performance Requirements*. AOAC International: Gaithersburg, MD, 2016.

(6) Krug, F. J.; Rocha, F. R. P. *Métodos de Preparo de Amostras para Análise Elementar*. (2nd Ed.). EditSBQ: São Paulo, SP, 2019.

(7) de la Rocha, S. R.; Sánchez-Muniz, F. J.; Gómez-Juaristi, M.; Marín, M. T. L. Trace Elements Determination in Edible Seaweeds by an Optimized and Validated ICP-MS Method. *J. Feed Compos. Anal*. **2009**, *22* (4), 330–336. https://doi.org/10.1016/j.jfca.2008.10.021

(8) Evans, E. H.; Giglio, J. J. Interferences in inductively coupled plasma mass spectrometry - A Review. *J. Anal. At. Spectrom*. 1993, *8*, 1–18. https://doi.org/10.1039/JA9930800001

(9) Miranda, K.; Pereira-Filho, E. R.; Gomes Neto, J. A. A New Closed-Vessel Conductively Heated Digestion System: Fostering Plant Analysis by Inductively Coupled Plasma Optical Emission Spectroscopy. *J. Anal. At. Spectrom*. **2014**, *9*, 825–831. https://doi.org/10.1039/C3JA50369K

(10) Miranda, K.; Vieira, A. L.; Gomes Neto, J. A. High-Throughput Sugarcane Leaf Analysis Using a Low Cost Closed-Vessel Conductively Heated Digestion System and Inductively Coupled Plasma Optical Emission Spectroscopy. *Anal. Methods* **2014**, *6*, 9503–9508. https://doi.org/10.1039/C4AY01841A

(11) Miranda, K.; Vieira, A. L.; Bechilin, M. A.; Fortunato, F. M.; Virgilio, A.; Ferreira, E. C.; Gomes Neto, J. A. Determination of Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Se, and Zn in Feedstuffs by Atomic Spectrometry After Sample Preparation Using a Low-Cost Closed-Vessel Conductively Heated Digestion System. *Feed Anal. Methods* **2015**, *9*, 1887–1894. https://doi.org/10.1007/s12161-015-0371-8

(12) Vieira, A. L.; Miranda, K.; Virgilio, A.; Ferreira, E. C.; Gomes Neto, J. A. Evaluation of an Improved Closed-Vessel Conductively Heated Digestion System for the Analysis of Raw Meat Samples by ICP Techniques. *J. Anal. At. Spectrom*. **2018**, *33*, 1354–1362. https://doi.org/10.1039/C8JA00121A

(13) Traversa, L. C.; Santiago, J. V. B.; Oliveira, E. M.; Ferreira, E. C.; Virgilio, A.; Gomes Neto, J. A. Closed-Vessel Conductively Heated Digestion System for the Elemental Analysis of Agricultural Materials by High-Resolution Continuum Source Flame Atomic Absorption Spectrometry (HR-CS FAAS). *Anal. Lett*. **2023**, *56*, 2443–2456. https://doi.org/10.1080/00032719.2023.2174133

(14) Costa, R. C.; Santiago, J. V. B.; Ferreira, E. C.; Virgilio, A.; Gomes Neto, J. A. Closed-Vessel Conductively Heated Digestion of Dry Dog Feed for Spectrometric Determination of Essential Nutrients. *Braz. J. Anal. Chem*. **2023**, *11*, 41–54. https://doi.org/10.30744/brjac.2179-3425.ar-4-2023

(15) Vieira, A. L.; Carvalho, G. G. A.; Gomes Neto, J. A.; Oliveira, P. V.; Kamogawa, M. Y.; Virgilio, A. A Convective Heated Digestion System with Closed Vessels: A New Digestor for Elemental Inorganic Analysis. *J. Anal. At. Spectrom*. **2024**, *39*, 356–363. https://doi.org/10.1039/D3JA00328K

(16) Pinto, A. L. M.; Souza, A. L.; Araujo, L. G.; Marumo, J. T.; Cotrim, M. E. B.; Guilhen, S. N. Innovative Sample Preparation Method Using a Conductively Heated Digestion System for Trace Element Analysis of Radioactive Oil Sludge by ICP-OES. *J. Anal. At. Spectrom*. **2026**, *296* (1), 128488. https://doi.org/10.1039/D5JA00042D

(17) Oliveira, E. M.; Ferreira, E. C.; Gomes Neto, J. A.; Virgilio, A. Closed-Vessel Conductively Heated Digestion System with Diluted Nitric Acid for the Preparation of Biomass and Biochar Samples. *Talanta* **2026**, *296* (1), 128488. https://doi.org/10.1016/j.talanta.2025.128488

(18) Costa, R. C. V.; Ferreira, E. C.; Gomes Neto, J. A.; Virgilio, A. Evaluation of a Cost-Effective and Environmentally Friendly Digestion Method for Preparing Animal Feed Samples for Elemental Analysis. *Anim. Feed Sci. Technol*. **2025**, *329*, 116511. https://doi.org/10.1016/j.anifeedsci.2025.116511

(19) National Research Council Canada (NRCC). Certified Reference Materials: *Wheat Flour (8436) and BRAN-1 (8433)*. Ottawa, Canada, 2022. Available at: https://nrc-digital-repository.canada.ca/eng/view/object/?id=d092223f-4000-49df-93c1-b6e901f33fbe (accessed 10/2025).

(20) Carioni, V. M. O.; Chelegão, R.; Naozuka, J.; Nomura, C. S. Feasibility Study for the Preparation of a Tuna Fish Candidate Reference Material for Total As Determination. *Accredit. Qual. Assur*. **2011**, *16*, 453–458. https://doi.org/10.1007/s00769-011-0796-8

(21) Bizzi, C. A.; Flores, E. L. M.; Nóbrega, J. A.; Oliveira, J. S. S.; Schmidt, L.; Mortari, S. R. Evaluation of a Digestion Procedure Based on the Use of Diluted Nitric Acid Solutions and H_2O_2 for the Multielement Determination of Whole Milk Powder and Bovine Liver by ICP-OES Based Techniques. *J. Anal. At. Spectrom*. **2014**, *29*, 332–338. https://doi.org/10.1039/c3ja50330e

(22) National Research Council. *Nutrient Requirements of Dogs and Cats*. The National Academies Press: Washington, DC, 2006. https://doi.org/10.17226/10668

(23) Dzanis, D. A. The Association of American Feed Control Officials Dog and Cat Feed Nutrient Profiles: Substantiation of Nutritional Adequacy of Complete and Balanced Pet Feeds in the United States. *The Journal of Nutrition* **1994**, *124* (suppl 12), 2535S–2539S. https://doi.org/10.1093/jn/124.suppl_12.2535S

(24) Currie, L. A. Nomenclature in Evaluation of Analytical Methods Including Detection and Quantification Capabilities (IUPAC Recommendations 1995). *Anal. Chim. Acta* **1999**, *391*, 105–126. https://doi.org/10.1016/S0003-2670(99)00104-X.

SUPPLEMENTARY MATERIAL

Table SI. Results (mean ± standard deviation, n = 3) for trace and ultra-trace element determined (μg kg^{-1}) in animal feed samples digested by CHDS and MW using HNO_3 7 mol L^{-1}

Sample	Digestion method	Mass Fraction (μg kg-1)						
		V	Ni	As	Se	Mo	Cd	Pb
Bird 1	CHDS	2221 ± 1	1525 ± 32	233 ± 12	385 ± 23	2138 ± 28	122 ± 1	332 ± 11
	MWD	1954 ± 24	1551 ± 5	232 ± 2	392 ± 27	2186 ± 15	134 ± 7	336 ± 10
	t-value	19.3	1.4	0.1	0.3	2.6	2.8	0.5
Bird 2	CHDS	244 ± 3	1643 ± 154	201 ± 1	205 ± 5	1262 ± 9	102 ± 0	338 ± 10
	MWD	236 ± 10	1111 ± 45	209 ± 0	203 ± 10	1235 ± 33	116 ± 0	349 ± 4
	t-value	1.3	5.7	9.0	0.3	1.4	253.0	1.8
Dog 1	CHDS	288 ± 20	1218 ± 49	229 ± 1	406 ± 12	1148 ± 12	101 ± 4	285 ± 46
	MWD	283 ± 35	1216 ± 31	222 ± 4	424 ± 37	1153 ± 31	104 ± 1	288 ± 2
	t-value	0.2	0.04	2.9	0.8	0.3	1.4	0.1
Dog 2	CHDS	365 ± 21	1082 ± 13	258 ± 1	378 ± 19	748 ± 15	100 ± 2	355 ± 22
	MWD	377 ± 28	1069 ± 44	258 ± 1	387 ± 6	748 ± 5	112 ± 0	352 ± 23
	t-value	0.6	0.5	0.5	0.8	0.03	12.6	0.1
Horse 1	CHDS	2910 ± 101	4723 ± 70	885 ± 9	306 ± 4	1085 ± 17	89 ± 0	806 ± 21
	MWD	2909 ± 125	4746 ± 32	854 ± 45	311 ± 8	1048 ± 9	88 ± 11	808 ± 62
	t-value	0.02	0.5	1.2	1.0	3.3	0.2	0.1
Horse 2	CHDS	3018 ± 239	4788 ± 10	824 ± 12	483 ± 77	1278 ± 14	109 ± 5	808 ± 2
	MWD	3008 ± 12	4765 ± 22	870 ± 39	489 ± 25	1263 ± 8	132 ± 1	801 ± 25
	t-value	0.1	1.7	1.9	0.1	1.6	7.5	0.5
Cat 1	CHDS	1321 ± 113	1658 ± 43	443 ± 25	464 ± 13	1336 ± 40	75 ± 1	586 ± 10
	MWD	1316 ± 127	1593 ± 68	457 ± 24	472 ± 39	1305 ± 103	74 ± 9	545 ± 31
	t-value	0.05	1.4	0.7	0.4	0.5	0.1	2.2

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Table SI-contd. Results (mean ± standard deviation, n = 3) for trace and ultra-trace element determined (µg kg^{-1}) in animal feed samples digested by CHDS and MW using HNO_3 7 mol L^{-1}

Sample	Digestion method	Mass Fraction (µg kg-1)						
		V	Ni	As	Se	Mo	Cd	Pb
Cat 2	CHDS	464 ± 18	1258 ± 108	263 ± 4	664 ± 14	683 ± 12	124 ± 5	311 ± 22
	MWD	445 ± 2	1139 ± 16	247 ± 4	661 ± 87	674 ± 12	130 ± 2	318 ± 20
	t-value	1.8	1.9	4.4	0.1	0.9	1.9	0.4
Fish 1	CHDS	2127 ± 45	5210 ± 444	934 ± 6	841 ± 16	1286 ± 20	109 ± 2	1060 ± 37
	MWD	2079 ± 95	5255 ± 35	948 ± 7	835 ± 47	1240 ± 2	118 ± 1	1052 ± 55
	t-value	0.8	0.2	2.5	0.2	3.9	7.9	0.2
Fish 2	CHDS	445 ± 17	1503 ± 91	819 ± 2	607 ± 13	717 ± 7	392 ± 9	433 ± 6
	MWD	443 ± 14	1506 ± 75	849 ± 15	615 ± 19	720 ± 8	365 ± 4	439 ± 3
	t-value	0.2	0.1	3.5	0.6	0.5	4.9	1.5
Rabbit 1	CHDS	1345 ± 101	1487 ± 17	262 ± 1	303 ± 57	1171 ± 12	126 ± 2	415 ± 27
	MWD	1335 ± 25	1486 ± 14	267 ± 2	314 ± 15	1169 ± 2	125 ± 1	404 ± 9
	t-value	0.2	0.1	3.0	0.3	0.3	1.2	0.6
Rabbit 2	CHDS	438 ± 13	1430 ± 95	470 ± 11	427 ± 11	1174 ± 3	118 ± 2	397 ± 2
	MWD	437 ± 10	1440 ± 32	474 ± 12	429 ± 3	1157 ± 60	120 ± 0	406 ± 1
	t-value	0.03	0.2	0.4	0.3	0.5	2.1	7.3

Table SII. Results (mean ± standard deviation, n = 3) for trace and ultra-trace element determined (µg kg^{-1}) in animal feed samples digested by CHDS and MW using HNO_3 14 mol L^{-1}

Sample	Digestion method	Mass Fraction (µg kg^{-1})						
		V	Ni	As	Se	Mo	Cd	Pb
Bird 1	CHDS	2148 ± 105	1563 ± 245	163 ± 15	419 ± 8	2133 ± 71	121 ± 5	217 ± 2
	MWD	2180 ± 31	1577 ± 34	178 ± 0	419 ± 16	2140 ± 105	123 ± 3	216 ± 4
	t-value	0.5	0.1	1.7	0.02	0.1	0.4	0.1
Bird 2	CHDS	180 ± 8	1360 ± 91	144 ± 4	211 ± 6	1314 ± 91	88 ± 1	270 ± 9
	MWD	178 ± 4	1358 ± 29	156 ± 1	210 ± 13	1304 ± 48	89 ± 2	261 ± 11
	t-value	0.5	0.03	5.4	0.1	0.2	0.8	1.2
Dog 1	CHDS	364 ± 39	1177 ± 16	197 ± 3	415 ± 51	1571 ± 104	107 ± 11	291 ± 9
	MWD	250 ± 19	1197 ± 2	195 ± 1	409 ± 26	1429 ± 30	106 ± 0	292 ± 10
	t-value	4.6	2.2	1.5	0.2	2.3	0.2	0.2
Dog 2	CHDS	265 ± 9	1220 ± 103	212 ± 5	546 ± 9	752 ± 40	116 ± 9	286 ± 17
	MWD	269 ± 5	1184 ± 21	203 ± 1	538 ± 31	742 ± 4	117 ± 1	270 ± 30
	t-value	0.5	0.6	3.0	0.4	0.4	0.2	0.8
Horse 1	CHDS	3637 ± 138	4520 ± 1196	1250 ± 36	443 ± 26	987 ± 77	93 ± 6	762 ± 27
	MWD	3644 ± 73	4322 ± 388	1078 ± 3	436 ± 29	971 ± 63	93 ± 2	769 ± 53
	t-value	0.1	0.3	8.3	0.3	0.3	0.1	0.2
Horse 2	CHDS	4534 ± 282	5081 ± 278	1224 ± 48	322 ± 9	1570 ± 44	136 ± 3	979 ± 76
	MWD	4521 ± 30	5814 ± 77	1236 ± 147	346 ± 34	1544 ± 29	133 ± 3	991 ± 59
	t-value	0.1	4.4	0.1	1.2	0.9	1.2	0.2
Cat 1	CHDS	1464 ± 89	1714 ± 148	346 ± 7	325 ± 18	1334 ± 98	72 ± 10	672 ± 13
	MWD	1366 ± 28	1708 ± 61	352 ± 7	306 ± 34	1312 ± 53	72 ± 4	661 ± 72
	t-value	1.8	0.1	0.9	0.9	0.4	0.1	0.2
Cat 2	CHDS	523 ± 83	1027 ± 115	234 ± 34	732 ± 53	1011 ± 138	136 ± 1	218 ± 0
	MWD	511 ± 83	1057 ± 20	238 ± 9	771 ± 57	905 ± 14	133 ± 4	221 ± 2
	t-value	0.2	0.4	0.2	0.9	1.3	1.2	2.1
Fish 1	CHDS	1614 ± 59	5876 ± 182	784 ± 10	757 ± 20	1239 ± 22	69 ± 1	936 ± 57
	MWD	1653 ± 109	5203 ± 37	799 ± 6	755 ± 31	1172 ± 99	74 ± 1	928 ± 7
	t-value	0.5	6.3	2.2	0.1	1.1	7.5	0.2

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Table SII-contd. Results (mean ± standard deviation, n = 3) for trace and ultra-trace element determined (µg kg^{-1}) in animal feed samples digested by CHDS and MW using HNO_3 14 mol L^{-1}

Sample	Digestion method	Mass Fraction (µg kg^{-1})						
		V	Ni	As	Se	Mo	Cd	Pb
Fish 2	CHDS	358 ± 14	1499 ± 20	909 ± 28	515 ± 13	551 ± 7	318 ± 4	323 ± 10
	MWD	359 ± 7	1476 ± 1	908 ± 24	513 ± 7	527 ± 23	322 ± 3	322 ± 10
	t-value	0.1	1.9	0.02	0.2	1.7	1.5	0.1
Rabbit 1	CHDS	1747 ± 136	1284 ± 188	233 ± 14	265 ± 25	914 ± 119	93 ± 8	430 ± 42
	MWD	1780 ± 117	1198 ± 105	230 ± 6	278 ± 3	932 ± 34	100 ± 1	435 ± 19
	t-value	0.3	0.7	0.3	0.9	0.3	1.4	0.2
Rabbit 2	CHDS	542 ± 40	1533 ± 73	469 ± 37	456 ± 9	1026 ± 10	94 ± 6	375 ± 34
	MWD	527 ± 27	1545 ± 9	467 ± 6	460 ± 20	1029 ± 18	97 ± 1	368 ± 22
	t-value	0.5	0.3	0.1	0.3	0.3	0.7	0.3