Chapter 7

Nuclear Analytical Techniques in Animal Sciences: New Approaches and Outcomes

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1. Introduction

"Interest in problems relating to the food and nutrition of man is already widespread and sincere (...) The time is not distant when it will be generally recognized that man should pay at least much attention to problems relating to his own food as to the study of the food of domestic animals". [1]

Those statements above were made by the US Secretary of Agriculture, J. Sterling Morton in the name of the USDA (United States Department of Agriculture) in 1896 to introduce the publication '*The Chemical Composition of American Food Materials*' of Atwater and Woods [1].

In his Letter of Transmittal, 116 years ago, Sterling Morton pointed-out the relevance of the knowledge of nutritive values of national food materials, since there were available in North American only results made in German products [1].

Since 1896, sensitive analytical techniques have become available which allow measurement of essential and toxic elements in food, feed and animal products. Several improvements have taken place in the last decades contributing to great improvements in the analytical quality of results produces. Substantial progress was also achieved by providing standardized equipment based in semiconductor detectors.

Chemical analyses have been improved and laboratories are largely widespread in the five continents, but Sterling's concerns are contemporary and vivid as never before.

In 1936, Hevesy and Levi first utilized a neutron source to analyze dysprosium in Y_2O_3 inaugurating an era of great development in studies involving multi-element determination

in many and varied areas of Sciences, including: material engineering, chemistry, agronomy, animal sciences and nutrition. Later, the Hevesy and Levi's technique were identified as Neutron Activation Analysis [2].

The Instrumental Neutron Activation Analysis (NAA) has a great advantage to other (humid) techniques due to the absence of effects of chemical binding to the trueness of results since the NAA is the only technique for quantitative element determination based on phenomena occurring in the atomic nucleus. The total dissolution may not be guaranteed for the entire sample in humid techniques [3].

Other outstanding characteristics include: element specificity, multi-element determination capacity, and sensitivity [2,3].

Potential interferences, sources of error and contributions to uncertainty of measurement are well known and quantifiable [3].

Some authors define NAA as "Mature, completed in development'', a stage in which the initial problems have been overcome. However, there are various analytical challenges in the many applications for which INAA may be the preferred technique to obtain information on elements and their concentrations [3].

Oppositely, applying others analytical techniques to study solid samples by flame atomic absorption spectroscopy, graphite furnace absorption spectroscopy, inductively coupled plasma spectroscopy, or inductively coupled plasma mass spectrometry, the sample must first be digested to get the analyte metals in solution. Digestion dissolves only those fractions of metals that can be put into solution under relatively extreme conditions and therefore enables measurement of available metals. Sample digestion by humid procedures generally uses highly corrosive reagents that are strong acids and strong oxidants and demand expert personnel using the proper equipment, including fume hoods and adequate personnel protection. It is expected new developments of high-powered microwave digestions systems coupled to these "open-sample dependent" techniques [4].

2. Neutron activation analysis in Brazil in the new millennium

Brazil has developed technology based on nuclear research reactors, two (IPEN and CDTN) work on neutron activation analysis. At IPEN, São Paulo, there is one research reactor (a 5 MW pool type) and a cyclotron both involved with radioisotopes production. At the CDTN, Belo Horizonte, there is a very active Triga research (IPR-R1) reactor [5]. Two unique duties in both of these institutes are promoting of basic nuclear teaching for students and workers in nuclear industry and embracing the applied research in many fields like: medicine, nutrition, animal sciences, geology, environmental sciences among others [5].

There is an interesting study of lunch meals served at the Cafeteria of the School of Public Health used by students and workers in the University of Sao Paulo (USP). That study could be considered an turning-point in Nuclear Analytical Applications in Brazil, giving pace for a

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new generation of studies in Life Sciences. The aim of that study from 2000 was to assessing the nutritional adequacy of diets served to University students related to essential elements and also monitoring for some toxic elements [6].

Since 2000, our research group has been dedicated to apply Neutron Activation Analysis in Animal Sciences. Starting from acquiring data for minerals in bovine tissues of the Brazilian cattle; at that point we could not find any study involving such tissues in Brazil in the nutrition field [7].

In that first study [7], our objective was focused to assess the elemental composition of animal tissues to support health and nutritional studies. Since cattle were and still are the most prevalent source of protein among Brazilian families, including meat, milk and dairy products. Determining the elemental concentration in cattle tissues is especially important because these materials are used for multipurpose objectives such as the assessment of animal health, the quality of human foods consumed, and as a potential environmental biomonitor. Chromium, copper, sodium, potassium, iron, and zinc levels were determined in bovine tissues—kidney, liver and muscle—from cattle bred and raised in a potentially metal contaminated region because of mineral activities.

We verified the essential element concentration and possible contamination by toxic elements in cattle tissues that can affect human nutrition. There was a good agreement the between values reported international organizations such USDA and FAO and the Brazilian analytical results obtained; the required data quality was also achieved. The higher iron, chromium, and copper concentrations could reflect the influence of the fate of environmental contaminants depositing in the animal tissues by biochemical processes. The pollutants in the environment reaching the livestock through water and forage may have caused it. In this first study it is not possible to affirm that the higher iron, chromium, and copper concentrations mean that these elements are playing the role of toxic elements because it was a preliminary sampling. However, the presence of such elements that is not reported elsewhere should be verified in detail during other studies. It is important to analyze animal tissues since this matrix can be used an efficient biomonitor to assess the animal's health, and the quality of human foods as well. The application of k0-instrumental neutron activation analysis was considered an effective multi-elemental method used to determine mineral concentration of biological material.

It is well established that (tropical) Brazilian soils are phosphorus deficient or/and have low phosphorus bioavailability as long most of the phosphate molecules are biding in insoluble composts [8]. This phosphorus deficiency affects both crops and livestock produced in Brazil. To avoid it, it has been applied phosphorus sources in soil, plant and animal nutrition. The most popular sources of phosphorus are rock phosphates, dicalcic phosphate, and in some cases, bone meal.

Rock phosphates are a source of many elements considered as contaminants in animal nutrition and to the environment as well. These product are plenty used in Animal Nutrition.

3. A Case of nuclear analytical application in animal sciences: Uranium in rock phosphates and rabbit muscles from animals receiving uranium determined by neutron activation analysis

Nuclear applications in Animal Sciences are not a novelty. It could be considered as the two first studies from 1949 using rabbits to assess the biological consequences of the uranium ingestion: Decreased body weight was reported for rabbits exposed to 11 mg U/m^3 as uranium tetrachloride dust for 35–40 days [9]. Rabbits lost 22% of their body weight during a 30 days exposure to 0.9 mg U/m^3, dogs and cats lost approximately 25% of their body weight during a similar exposure to 9.5 mg U/m^3. Similar effects were observed with uranium tetrafluoride [10].

Introduction: Phosphorus (P) deficiency in crops is an important constraining factor n plant and animal yields, especially in hot humid tropics where soils are predominantly acidic and often extremely P deficient with high P fixation capacities [11,12].

Phosphorus combines with oxygen forming oxides called phosphates. Phosphates are defined as compounds, which contain phosphorus-oxygen (P-O) linkages. The P-O bond has a length of 1.62Å with bond angles of 130° at the oxygen atoms and 102° at the phosphorus atoms at the pentoxide P_2O_5, the only oxide of phosphorus that is produced commercially [13].

Phosphate rock denotes the product obtained from the mining and subsequent metallurgical processing of phosphorus bearing ores. By flotation of phosphate rock it is formed apatite concentrates. These phosphate products are the major phosphorus sources in soil nutrition, also plenty used in animal formulations by industries [14].

…'On their way up the chimney the gases go through four separate treatments. P_2O_5 used to go right out of circulation every time they cremated some one. Now they recover over ninety-eight per cent of it. More than a kilo and a half per adult corpse. Which makes the best part of four hundred tons of phosphorus every year from England alone." Henry spoke with a happy pride, rejoicing whole-heartedly in the achievement, as though it had been his own. "Fine to think we can go on being socially useful even after we're dead. Making plants grow.'…} Brave New World, Aldous Leonard Huxley [15].

This text above was extracted from the 1932 fictional worldwide bestseller *Brave New World* [15]. It could sound so fantasist back when it was firstly published. Maybe it will sound reality in a future not far ahead. Currently, a reduced and shortening number of phosphate sites are mined around the world. Based on current phosphate extraction rates and economic trends in the 1990s, more than half of main phosphate producer countries will have exceeded the life of their reserves up to 2024 [16].

Phosphate ranks second (coal and hydrocarbons excluded) in terms of gross tonnage and volume of international trade [16].

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Sedimentary ore deposits have provided about 80%–90% of world production of phosphate [6]. Oppositely, in Brazil igneous deposits represent 80% of the national reserves of phosphate rocks. The major Brazilian phosphate rock reserves are concentrated in the states of Minas Gerais, Goiás and São Paulo [17].

In 2009, the Brazilian phosphate-related products market was shared as phosphate fertilizers (89.12%), animal feed – mainly dicalcium phosphate (6.91%), soil amendment (1.01%), and the remaining was not informed (2.96%) [17].

Ingredient (tons.year^{-1})	***2005***	***2011***	***2012***$^{\nabla}$
Dicalcium Phosphate	216.400	404.761	560.274
Bone Meal	225.400	n.i.	n.i.
Limestone	634.000	1.247.016	1.276.388

n.i., not informed., $^{\nabla}$ forecasted in 2012, May for the entire year

Table 1. Phosphorus and calcium sources used in the Brazilian Feed Industry [8]

Products	**Quantity (tons)**	**FOB x U$1,000.00**
Raw material	915.449	84.040
Industrialized	2.861.719	945.170
Total	3.777.168	1.029.210

Table 2. Brazilian imports of phosphates, raw and industrialized products [17]

Country	**Raw Material (%)**	**Industrialized Material (%)**	**Total (%)**
United States	n.a.	33.31	30.59
Algeria	20.90	n.a.	n.a.
Morocco	63.80	24.54	27.74
Israel	10.30	8.97	9.08
Tunisia	4.71	7.95	7.68
Russia	n.a.	6.74	6.19
Spain	0.13	n.a.	n.a.
Others	0.16	18.49	18.71

Table 3. Brazilian imports of phosphates by countries, raw and industrialized products. Total pondered by the price FOB, DNPM [17]

Data in table 1 demonstrate the economic relevance of the products under consideration in this study for the Brazilian feed industry. Foreign products are also of concern, as long Brazilian crops and animal yields rely on a great amount of imported phosphate products (tables 2 and 3).

Indeed, any phosphate mined worldwide may contain accessory-gangue minerals and impurities that can be hazardous to man and animal such: Cd, Hg, Pb and V [16].

The United States Agency for Toxic Substances and Disease Registry [19] publishes the CERCLA Priority List of Hazardous Substances that includes substances, which have been determined to be of the greatest public health concern [19]. Uranium is the 97th substance ranked in the list (table 4).

Rank	Substance	Total Points*
1	Arsenic	*1665*
5	Polychlorinated Biphenyls	*1344*
97	**Uranium**	***832***

*The ranking of hazardous substances on the CERCLA Priority List is based on three criteria (i,ii,iii). They form altogether the Total Score = Σ (i) Frequency of Occurrence + (ii) Toxicity + (iii) Potential for Human Exposure = Σ (i) up to 1.800 Points + (ii) up to 600 points + (iii) up to [300 concentration points + 300 exposure points] [20]

Table 4. Compilation of some hazardous substances (including uranium) in the CERCLA List [19, 20]

This study deals with the uranium, since to face the main constraints low inherent P in soil and plants, rock phosphates are likely to be more extensively disseminated in the agriculture and these phosphorus sources carry uranium from their structures to the environment and human food chain as well [21].

Uranium is the heaviest natural element in the nature; it is hazardous element in man and animal health, not just it presents radioactivity but also it presents metallotoxicity once it is a heavy metal. Furthermore, uranium presents many radionuclides with high radioactivity and energy [22].

Health implications of human exposure to uranium are well documented: cancer, liver and kidney diseases and reproduction impairment [21,22].

Uranium in nature is more plentiful than silver (Ag) and about as abundant as arsenic (As). It is found in very small amounts in the form of minerals, especially in rocks, soil, water, air, plants and animal tissues that could be consumed as food containing varying amounts of uranium [23].

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Practically every rock phosphate contains uranium in its structure [16,24]. The amounts of this and others hazardous substances vary widely among phosphates sources and it may vary even in the same deposit. Thus, mining, milling, industrializing and using phosphate products in soil and animal nutrition are anthropogenic activities increasing the potential for human exposure to uranium [24,25].

German studies in the 1970´s pointed-out the evidence for a raising uranium presence in rivers and groundwater in regions with intensive use of phosphate fertilizers in agriculture (figure 1). Uranium derived from phosphate fertilizers is likely to be adsorbed on the uppermost soil layers and its content on the water is correlated to the HCO_3^- content in the river [25].

Uranium content can be determined by the nuclear method of the Neutron Activation Analysis. This is a precise, fast (short turn-around), sensitive and non-destructive method [2].

4. Material and procedures

Phosphate sample preparation: phosphates were acquired in the local market of Minas Gerais. Aliquots of 100 grams were randomly taken for each product pack to be grounded to obtain a particle size of 200 Tyler mesh (75 μm) establishing similar conditions for all samples (99% of conformity of the particle size of each product). Aliquot of 1000 mg of each grounded product was weighted and sealed in small polystyrene capsules.

Animal breeding: Two groups of twelve (6 females and 6 males) New Zealand white rabbits (30 days of life) were selected and separated in two groups housed individually receiving a different phosphorus source (dicalcium phosphate and bovine bone meal).

Two rabbit feeds were designed to allow the introduction of the P source and to offer sufficient nutrient intake to meet rabbit nutritive requirements. Each one of the feeds had the same 98 percent (dry basis) of fiber, energy, and amino acids. Both formulations were based in raw materials as %, dry basis: Alfalfa meal 34.63, Soy oil 1.00, Sugar cane 2.00, Salt 0.50, Lysine 0.25, Methionine 0.04, Limestone 1.0, Premix 0.40, Maize 6.05, Wheat straw 25.0, Soybean meal 12.13, Maize by products 15.00 and the remaining 2.0 from the selected P source – dicalcium phosphate and bone meal, both materials analyzed in first part of the experiment.

Feeds were processed in order to turn the mixed products into a compact mixture. After that, the meal was conditioned by mixing it on dry steam in a conditioner; this conditioned product was pressed by rolls to pass through the holes of the pelleting die which shapes the meal into the final pellet shape of 3.00 mm to permit a good balance between pellet quality and good intestinal motility.

Animals were housed in stainless steel cages with a fenestrated floor to allow feces to drop through into a pan. Absorbent material was placed in the pan to collect urine and minimize ammonia release due to the bacterial breakdown of urea.

Good quality water was provided through a nipple-drinking system that provides water at all times. Food was provided by a J-hopper attached to the front of the cage. J-hopper

prevents the rabbit from defecating in their food. Animals were fed *ad libitum* from 30 to 72 days - age considered as ideal slaughtering that allows rabbit to reach its commercial live weight, i.e. 2.0 kilograms.

The 24 rabbits were slaughtered via humanitarian euthanasia and their *longissimus dorsi* muscles were extracted and prepared by freeze-dry process in order to be irradiated. Approximately 100 g of each specimen was frozen at –70°C and lyophilized. Each freeze-dried sample was powdered and homogenized and around 1000 mg was taken into polyethylene irradiation vials.

Irradiation: Each one of the full-filled capsules with samples was placed in a polyethylene container for the pneumatic transporting system. Individually, samples and standards in the vials were transported into the neutron flux using the pneumatic transport system of the reactor IPR-R1 in the CDTN/CNEN (Centre of the Nuclear Technology Development) in Belo Horizonte, Brazil. The reactor was operated at 100 kW-thermal power under a neutron flux of $6.6x10^{11}$ neutrons.cm^2.s^{-1}. Additionally, phosphates were studied by the well-established Colorimetric Method to assess their phosphorus - P_2O_5 content, at the EC-4 Sector of the Nuclear Technology Development Centre, institute from the Brazilian Nuclear Energy Commission (CDTN/CNEN).

5. Analytical technique applied on elemental determination

The neutron activation technique (NA) is based on nuclear properties of the nucleus of the atom, radio- activity, and the interaction of radiation with matter. The simplest description of the technique says that when one natural element is submitted to a neu- tron flux, the reaction (n,γ) occurs. The radionuclide formed emits gamma radiation, which can be meas- ured by suitable equipment. About 70% of the ele- ments have nuclides possessing properties suitable for neutron activation analysis. At the Nuclear Technology Development Centre (CDTN), there is a nuclear reac- tor TRIGA MARK I IPR-R1 that allows the application of this technique [7].

The k0-instrumental neutron activation analysis (k0-INAA,) a variation of NA in which the sample is irradiated without previous chemical preparation was used in this study. This specific method is based on nuclear constants—the k0 factors and some reactor parameters.

Rabbit tissues were irradiated in the reactor TRIGA MARK I IPR-R1. At 150 kW the thermal neutron flux is 6.6×10^{11} neutrons cm^2 s^{-1}. The samples were irradiated simultaneously with standards of gold and sodium as comparators, and the reference materials. The elements were determined through three schemes of irradiation: 5 minutes to detect the short half-life radionuclides; 4 hours to detect the medium, and 20 hours, the long half-life radionuclides.

After suitable decay time, the gamma spectroscopy was performed in a HPGe detector, 10% of efficiency, FWHM 1.85 keV and ^{60}Co, 1332 keV, connected to a multichannel analyzer. The calculations were based on the reactor parameters: k0 constants using the Solcoy Sofware ©.

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6. Results

Phosphate	Total U [µg.g^{-1}]	Ratio P:U
Amm. polyphosphate, 45% P_2O_5 , *Brazil*	37 ± 4[a]	5580:1
Super-simple phosphate, 17% P_2O_5 , *Brazil*	49 ± 5[a]	1485:1
Dicalcium phosphate, 45%, P_2O_5 , *Brazil*	187 ± 9[a]	1050:1
Monoamm. phosphate, 51% P_2O_5 , *Brazil*	183 ± 9[a]	1215:1
Super-triple phosphate, 45% P_2O_5 , *Brazil*	34 ± 4[a]	3975:1
Israeli rock phosphate, 31% P_2O_5 , *Israel*	145 ± 7[a]	935:1
Rock phosphate, 31% P_2O_5, *Florida, USA*	59[b]	2300:1
Rock phosphate, 28% P_2O_5, *Tanzania*	390[b]	303:1
Rock phosphate, 29% P_2O_5, *Mali*	123[b]	1030:1
Bovine Bone Meal	1.0 ± 0.8[a]	27650:1

[a] U experimental results by Delayed Neutrons Technique, P by Colorimetric Method

[b] U and P data extracted from FAO/IAEA [16]

Table 5. U content and the ratio [phosphorus: uranium] in the tested phosphates

Phosphorus Source	U Concentration [µg.g^{-1}]
Dicalcium Phosphate	1.25 ± 0.45[a]
Bovine Bone Meal	0.91 ± 0.29[a]

[a] The results were evaluated ($p \leq 0.05$) by t-test using Microsoft Excel 2000 software [26]. Means with the same letter ([a]) are not significantly different.

Table 6. Uranium content in the *longissimus dorsi* muscle from rabbits fed with two different sources (as 2% in the feed) of phosphorus, n=12. The data are presented as mean ± SD, raw basis.

7. Discussion

Concentrations of uranium are quite variable in the phosphate products. The average ratio of phosphorus to uranium in phosphates appeared not to be only related to their origin since all tested Brazilian phosphates are from igneous deposits (ratio varying from 1000-4000 atoms of phosphorus to 1 atom of uranium), instead the foreign data are related exclusively to sedimentary rocks (ratio varying from 300-2400 atoms of phosphorus to 1 atom of Uranium). Differences amongst tested products are the region of exploitation of phosphate ores that implies different ages of mineralization, deposit types and accessory minerals associated that may vary in phosphates of the same origin, and finally they are separated by the routes of production of each final product for those are industrialized.

No significant difference was observed in uranium presence in the muscle tissues from rabbits (tab. 6) receiving dicalcium phosphate and those animals receiving bovine bone meal: average

uranium content are 1.25 ± 0.45 $\mu g.g^{-1}$ for dicalcium phosphate fed group and 0.91 ± 0.29 $\mu g.g^{-1}$ for the bovine bone meal fed group. Possibly, *longissimus dorsi* muscle is not a target tissue for uranium in mammals. Indeed, uranium is often associated to replace calcium in the bone hydroxyapatites; both are considered mutual inorganic cation exchangers in apatite [21].

8. Conclusions

In conclusion, Nuclear analytical techniques have been helping to improve the human welfare in terms of health acting as outstanding tools working around the triade: diagnosis, prevention and treatment. In many analyses, Nuclear techniques are not merely an option, but are the only feasible solution to address some assessments.

Following the '*Precautionary Principle*' [27] what states that if an action might theoretically or logically cause harm, then those who wish to undertake this action (of using these substances in this case) have a greater moral responsibility to demonstrate good evidence that the use does not cause any harm, not now not in the long run.

Nuclear Analytical Techniques have a long and useful history in Life Sciences. These applications in Agriculture are a warranty for mutual benefits for both arenas, as long Nuclear Power generation is currently in a moratorium after Fukushima-Daichi 2011 disaster, and Agriculture for food and energy production is boosting and evolving.

Disclaimer

Data and statements expressed in this paper are those from the authors, and do not necessarily reflect organisations with which the authors are affiliated. The authors do not endorse any equipment or material cited herein.

Acronyms and abbreviations

AOAC Association of Official Analytical Chemists

ASTDR Agency for Toxic Substances and Disease Registry

CERCLA Comprehensive Environmental Response, Compensation, and Liability Act

CDTN *Centro de Desenvolvimento da Tecnologia Nuclear*, Nuclear Technology Development Centre, Belo Horizonte, Brazil

CNEN *Comissão Nacional de Energia Nuclear* Nuclear Energy (Brazilian) National Commission

CRM Certified Reference Material

DNPM *Departamento Nacional de Produção Mineral*, National Department for Mineral Production, Brazil

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IAEA International Atomic Energy Agency

IPEN *Instituto de Pesquisas Energéticas Nucleares* Nuclear Energy Research Institute, São Paulo, Brazil

USDA United States Department of Agriculture

USEPA United State Environment Protection Agency

USP University of Sao Paulo

pg picogram

pmol picomole

PHS Public Health Service

PMR proportionate mortality ratio

ppb parts per billion

ppm parts per million

ppt parts per trillion

yr year

> greater than

= equal to

< less than

% percent

α alpha

β beta γ gamma

δ delta

μm micrometer

μg microgram

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