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STABLE ISOTOPES APPLIED IN LIFE SCIENCES IN UNESP – BOTUCATU CAMPUS

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ABSTRACT

Universities and research centers that use stable isotopes follow a worldwide trend in owning laboratories isotope ratio that serves multiple users, as well as minimize maintenance costs, operation and staff training. For this purpose it was created in 1998, the Center Stable Isotopes "*Centro de Isótopos Estáveis*" - CIE, which is an auxiliary unit linked to the Institute of Biosciences (IB) of the São Paulo State University "*Universidade Estadual Paulista*" - UNESP Botucatu Campus, Brazil. The best way to CIE grow and achieve excellence are already disclose the methodologies employed and practice areas consolidated to find new partnerships with different applications, and also seek new methods of analysis that can expand areas. The aim of this study is to disseminate methodologies for analysis of isotope ratio and areas of the CIE. The CIE analyzes the isotopic ratios $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$ and $^{18}O/^{16}O$ (CHON), and for the carbon samples can be solid, liquid and gas (in the form of CO_2) for the other elements is only possible to analyze in solid or liquid form. All samples are always analyzed separately by the destructive method. The CIE addition to performing scientific partnerships with all university, complementary and auxiliaries units of UNESP Botucatu campus also have partnerships in other academic units on campus of UNESP Jaboticabal and Dracena and São Paulo University in Piracicaba campus and is open to new partnerships in other research units.

1. INTRODUCTION

Although the ratios of stable isotopes are very small in nature, they have great importance to scientific research, it can identify a natural variation or relative enrichment in organic and inorganic matter serving as a marker and also characterizing the material. This property of stable isotopes has been studied, refined and applied over the past years since the discovery of isotopes at the beginning of the last century.

Today stable isotopes are used as a powerful tool for scientific research in large areas of Life Sciences and Earth and Exact Sciences throughout the world. Stand out the Agricultural Sciences, Physiological, Health, Medical Physics and Geosciences with existing studies using the ratio of stable isotopes, mainly of light elements such as $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$ (CHONS).

Analytical techniques for determining the stable isotopes ratio or isotope ratio are summarized in the use of isotope ratio mass spectrometry - IRMS, but there are other less widely used techniques. The process of determining the isotopic ratio by IRMS consists of the following steps: sample preparation, system input samples in the gas phase in IRMS; separation of ions in IRMS; detection of ions of different mass, and production of electrical signal [1-3].

Sample preparation depends on the type of input system in IRMS, element analysis and physical state (solid, liquid or gas) of the sample, but does not require great complexity, only the quantification, drying and grinding. The separation and detection of ions and production of electrical signal known who possess a technology has been perfected over the years. The technological challenge is in the sample entry system in the IRMS, where separates the other components leaving only the sample gas is ionized (CO_2, N_2, H_2, CO, or SO). In this process the liquid or solid samples are transformed into gases after being separated in accordance with the isotope to be analyzed [1].

Universities and research centers that use stable isotopes follow a world trend in owning analysis laboratories isotope ratio that serves multiple users, as well as minimize maintenance costs, operation and staff training. For this purpose it was created in 1998, the Center Stable Isotopes *"Centro de Isótopos Estáveis"* - CIE, which is an auxiliary unit linked to the Institute of Biosciences (IB) of the São Paulo State University *"Universidade Estadual Paulista"* – UNESP, Botucatu Campus, Brazil.

The initiative of creating the CIE left nearly thirty researchers from the four units of the College of UNESP Botucatu campus (Institute of Biosciences, College of Agricultural Sciences, College of Veterinary Medicine and Animal Science and College of Medicine) who joined forces to bring to campus one isotope ratio mass spectrometer (IRMS) that could perform stable isotope analyzes and assist in the various lines of existing research on campus, aiming to conduct analysis of isotopic ratios of elements "CHONS" [4].

Since its inception, the CIE is growing and is gaining prominence in the scientific community through its building area of about 220m^2 to 480m^2, received 14 national and international awards, provided more than 300 technical reports, participated in over 280 publications including articles and abstracts congress, obtained two ministerial decrees published in the Official Gazette accrediting Center to conduct official reports of food and beverages, the park extended its major equipment in operation from 1 to 5 IRMS. Being awarded Thematic Project in the area of Animal Science with funding from FAPESP, and Project in the area of food and beverages, with financial support from CNPq / MAPA [4].

The best way to CIE grow and achieve excellence are already disclose the methodologies employed and practice areas consolidated to find new partnerships with different applications, and also seek new methods of analysis that can expand areas. The aim of this study is to disseminate methodologies for analysis of isotope ratio and areas of the CIE.

2. METHODOLOGY USED IN CIE

The CIE analyzes the isotopic ratios $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$ and $^{18}O/^{16}O$, and for the carbon samples can be solid, liquid and gas (in the form of CO_2), to the other elements is possible

only the analysis in solid or liquid form. All samples are always analyzed separately by the destructive method.

In the preparation of solid samples are used between 30 to 60μg sample of dried and crushed capsules that are placed on tin or silver. The drying and crushing are required for homogeneity of the material analyzed. In the preparation of liquid samples is performed by centrifugal separation or distillation in the preparation of gas samples exclusively of carbon breath test or analysis of CO_2 is not required any previous separation and the sample is stored in glass tubes for specific 12mL after being introduced into the IRMS according to table 1.

The CIE has five IRMS four of German origin, two for C, H, O, N and S (solid and liquid sample) acquired in 1994 and 2010, one for C, H, O and N (liquid sample) acquired in in 2010, one for H and O (solid sample) acquired in 2010 and last one IRMS of English origin to C (sample gas) acquired in 2011. To assist in the preparation of the samples CIE also has a nitrogen liquefier for production of liquid nitrogen with a capacity of 8L/hour fueling Botucatu campus community and the region near the town.

Table 1 Sample preparation and input system of the samples IRMS in the CIE.

Sample preparation (physical state)	**Isotope ratio**	**Input system of the samples IRMS**	**Gas analysis in IRMS**
(solid) drying, grinding and weighing	($^{2}H/^{1}H$),($^{18}O/^{16}O$)	silver capsule for pyrolysis system	H_2, CO
	($^{13}C/^{12}C$),($^{15}N/^{14}N$)	tin capsule for combustion and reduction sistem	CO_2, N_2
(liquid) distillation or centrifugation	($^{2}H/^{1}H$),($^{18}O/^{16}O$)	silver capsule and eppendorf for pyrolysis system	H_2, CO
(gas) CO_2 in 12mL tube	($^{13}C/^{12}C$)	chromatography	CO_2

3. APPLICATION OF STABLE ISOTOPES IN CIE

In Animal Sciences the CIE assist scientific research that investigates the feeding preference, time of weaning, free choice and nutrition of various creations of animals for the production of eggs, meat, milk and dairy products. These studies facilitate the management and production of animals which use the natural variation of the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in plants of the C_3 and C_4 photosynthetic cycle [5-7].

Each tissue or biochemical fraction may present "isotopic memory itself," according to the isotopic content of the food, and the renewal fee biochemistry. Isotope turnover means as the continuous renewal of the chemical elements and consequently of isotopes that constitute the body tissue or organism as a whole. This can occur through turnover of tissue renewal resulting from the process of synthesis and degradation in adult tissues and / or their growth in tissue formation (isotope dilution).

In agribusiness, several years ago, animal byproducts are being used to feed pigs, poultry and fish. However, the use of flour, fish, bovine meat and bone meal, blood and feathers, poultry

offal, blood plasma and milk by-products have been widely discussed and even banned the feed in some cases, reflecting the consumer uncertainty after the BSE (bovine spongiform encephalopathy - a disease of "mad cow disease") and problems of contamination of animal products by *Salmonella* and *Escherichia coli*. When the consumer demonstrates restrictions on certain categories of food, occurs pressure on producers to production practices are replaced by other methods.

The line of research in aviculture in CIE (Thematic Project of PAPESP) aims to track the inclusion of ingredients of animal origin in poultry diets by the technique of stable isotopes of carbon and nitrogen. The use of stable isotope technique is presented as a potential alternative to the certification process of broilers and eggs, when these birds are raised without the use of animal ingredients in food [8].

Technology in food and beverage the CIE (Project CNPq / MAPA) is also active in research on adulteration and can identify origins and purity of the ingredients in the final product. For example: if the honey is pure; if the beer has only fraction of malt or unmalted cereals; if the juices are pure or have added sugars; if the wine is produced only from the grapes; or carbon dioxide beverage is natural or artificial [9].

In agronomy the CIE has conducted studies where it was possible to distinguish chemical organic herbicide and studies related to cultures water deficit [10]. In botany, it was possible to study from isotope ratios, the carbon cycle in plants subjected to water stress situation. The CIE also conducted studies in zoology with digestibility of fiber in guinea pigs and eating habits and preferences of crab.

All these applications of stable isotopes in CIE were performed based on natural variation in isotopic ratio derived from plants C_3 and C_4 photosynthetic cycle. The CIE also has application to isotopic enrichment of ^{13}C in amino acids.

New applications have emerged in CIE using enriched stable isotopes of carbon, hydrogen and oxygen in Medicine and Nutrition. Medicine in the CIE analyzes isotope ratio $^{13}C/^{12}C$ of CO_2 in expired breath of patients before and after oral ingestion of ^{13}C-labeled substrate for detection of *Helicobacter pylori* infection.

In human nutrition research the CIE is conducting analyzes of the enriched isotope ratio $^{2}H/^{1}H$ and $^{18}O/^{16}O$ in patients submitted to bariatric surgery to determine the energy expenditure from doubly labeled water ingestion.

Again using natural variation of carbon and nitrogen the CIE also has new applications in progress in Forensic Science with the possibility of identifying the geographical origin of planting drugs narcotics.

4. FINAL CONSIDERATIONS

The CIE addition to performing scientific partnerships with all university, complementary and auxiliaries units of UNESP Botucatu campus also have partnerships in other academic units on campus of UNESP Jaboticabal and Dracena and São Paulo University in Piracicaba campus and is open to new partnerships in other research units.

In addition to supporting scientific research the CIE also serves on the education offering annual discipline in the 1st semester entitled "Environmental Stable Isotopes" by the program graduate in Animal Science FMVZ / UNESP. All 16 researchers who collaborate with the CIE orient undergraduate students, completion of course work and postgraduate.

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