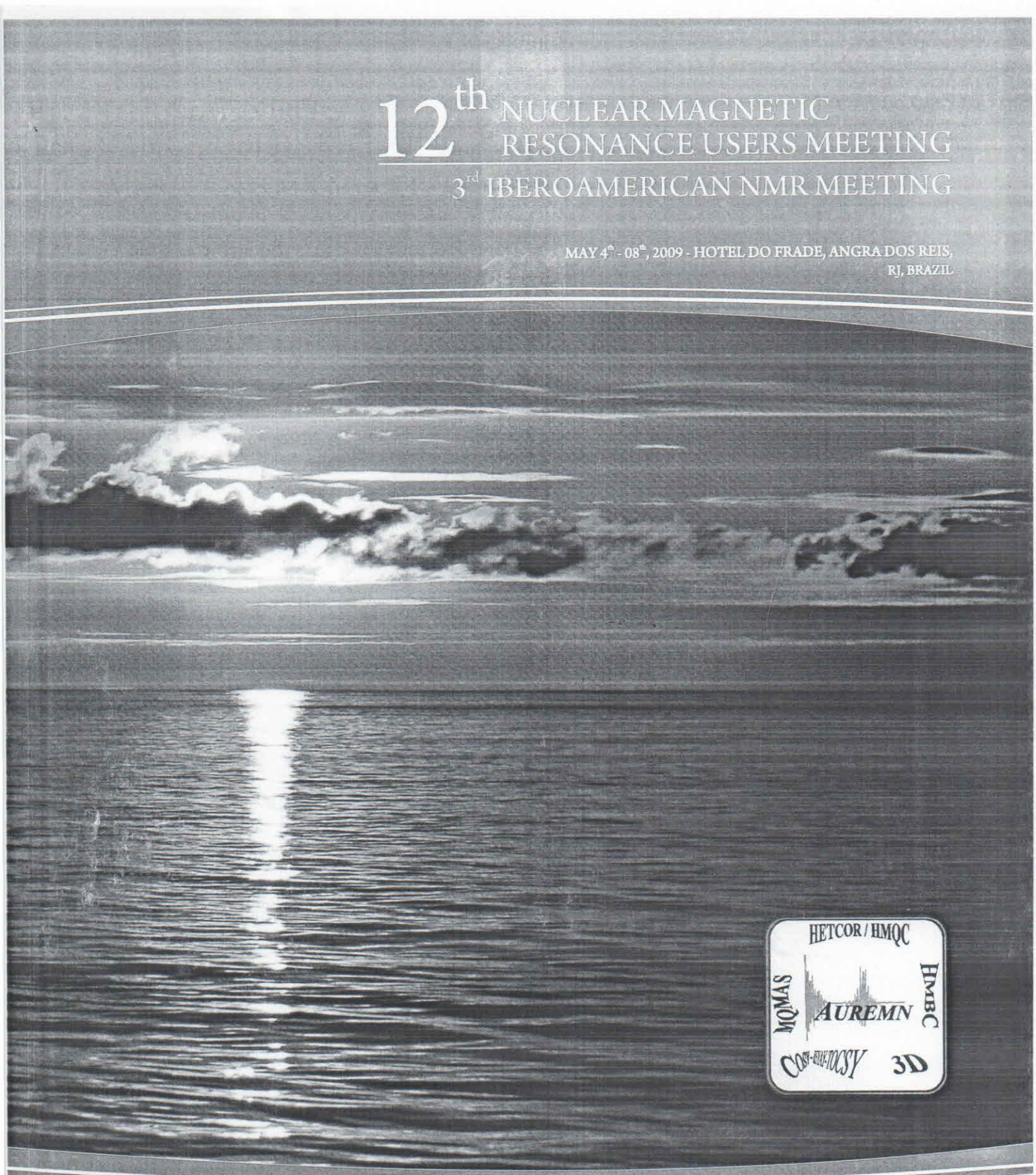
12th NUCLEAR MAGNETIC RESONANCE USERS MEETING

3rd IBEROAMERICAN NMR MEETING

MAY 4th - 08th, 2009 - HOTEL DO FRADE, ANGRA DOS REIS, RJ, BRAZIL



EXTENDED ABSTRACTS BOOK

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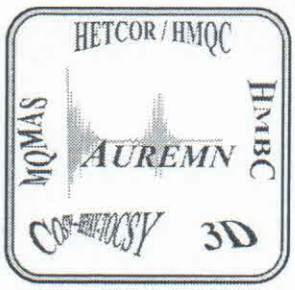

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BOVINE METABOLOMICS: A NMR APPROACH

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Food safety is, in today's society and economy, a constant concern for the inspection organs, mainly after some serious diseases outbreaks, like bovine spongiform encephalopathy (mad cow disease), foot-and-mouth disease and some helminthiasis. These diseases may cause severe human health problems, even death in mad cow disease case, or just represent great losses for cattle production, as foot-and-mouth disease does. Parasitic diseases represent a big economic problem, once they can reduce animal growing and development, as well as milk production, and in some advanced cases, death. Ivermectin is a wide spectrum anthelminthic drug employed on bovines, for worm diseases control, and its structure is shown in Figure 1. Its misadministration, where doses higher than allowed are applied into animals, can be harmful for humans, who will ingest contaminated milk, milk products like cheese, yogurt and meat. At high levels, Ivermectin can cause fever, joint or muscle pain and CNS depression, and hence must be avoided.

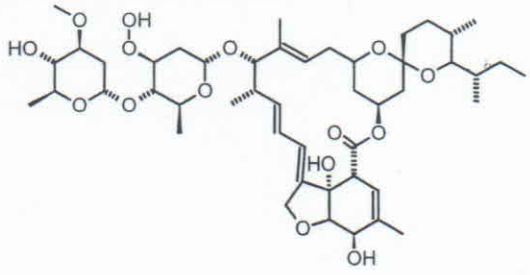

Figure 1 – Ivermectin molecular structure.

Molecules such ivermectin disturb animal natural metabolism. Thus, the major focus of this work is not the investigation of drug traces on biofluids, but the metabolic alterations caused by the antihelmintic treatment. This is the point of Metabolomics and Metabonomics. While the first one is responsible for establishing the natural metabolites levels for an organism, like a biological fingerprint, the second one studies the response of this levels to external stimuli, like stress, bad feeding or drugs. Thus, the metabolites analysis becomes a powerful, innovating tool for detecting irregular drug administration for those animals. Furthermore, it can be applied for several animal and human diseases, as cancer, diabetes and gout, which are characterized for metabolites levels disturbs.

In this work we present the initial studies on bovine metabolomics, where blood plasma and urine were analyzed, in order to verify metabolic variation after ivermectin administration, employing high-resolution NMR as analytical technique. For these studies, samples of blood plasma and urine were provided by Embrapa Southeast Livestock, from both male and female adult oxen. Samples were lyophilized, and water was replaced by D_2O, to provide the lock signal, followed by addition of 100 mM of phosphate buffer, at pH = 7.4. Urine samples have been filtrated in porous membrane (average pore diameter = 0.45 nm) and added with 10 mM sodium azide (NaN_3), to avoid bacterial contamination.[1] Both urine and plasma were analyzed in a NMR spectrometer, Varian INOVA 400, with magnetic field of 9.4 T, which provides a frequency of 400 MHz for ^{1}H. A standard pulse sequence for ^{1}H was employed, with 256 scans.

In order to obtain a good visualization of the interest peaks, HDO signal must be suppressed. For this, we employed the Jump-and-Return technique,[2] which is composed by two 90° pulses (with phases x and –x, respectively), spaced by a period τ. A scheme of Jump-and-Return sequence and its effect over magnetization vectors is shown in Figure 2.

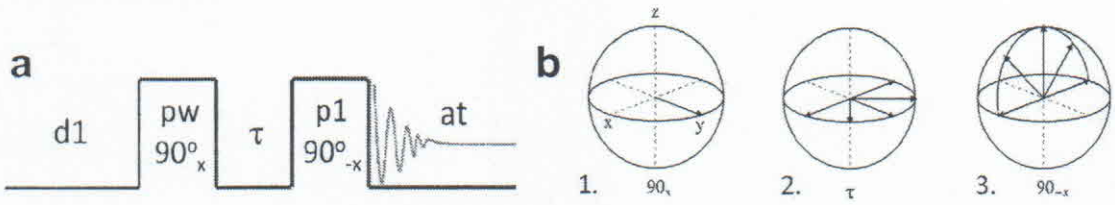

Figure 2 – Jump-and-Return sequence (a) and its suppression mechanism (b): A x-phased pulse drop the magnetization to *x'y'* plan (b-1). The time spacer τ allows the vectors to spread around the center frequency (HDO, in offset)(b-2). The –x-phased pulse send the magnetization to the *x'z'* plan (b-3). Once in offset, HDO signal coincide with *z* axis resulting in no *xy* component, eliminating the corresponding peak.

As result, we have a drastic reduction on HDO signal, what allows the observation of many peaks corresponding to metabolites. Figure 3 shows urine and blood plasma NMR spectra after suppression of HDO signal.

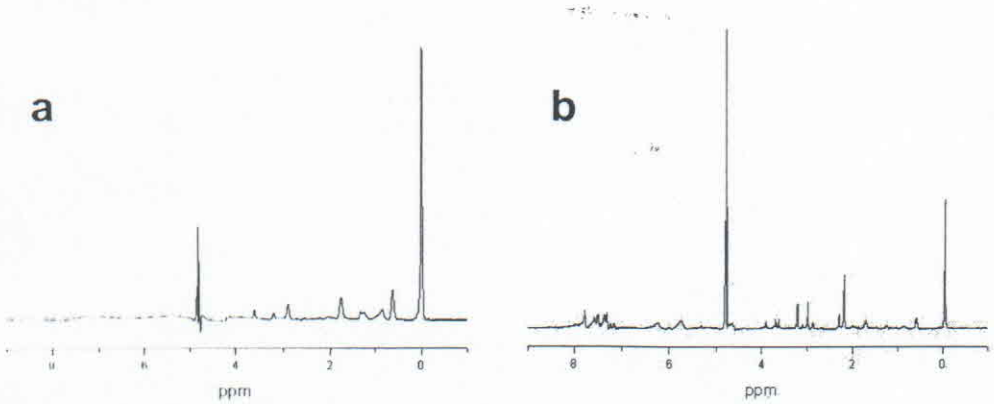

Figure 3 – NMR spectra for bovine urine (a) and blood plasma (b).

The large amount of peaks as shown needs a potent tool for metabolites identification, and so we employed both Human Metabolome Database (HMDB)[3] and Metabolomics Database[4] which allows identifying taurine, creatine, creatinine, hippurate, citrate and urea as main metabolites. These bases represent an essential feature in this work, once the metabolite recognizing is fundamental for the next steps, where chemometrics analysis and neural networks will be conducted, intended for the development of a novel, powerful and safe diagnostic method for both animal and human diseases.

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