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Metabolic Studies of Bovine Urine and Blood Plasma using ^{1}H and ^{13}C-SSPF High Resolution NMR after Treatment with Ivermectin

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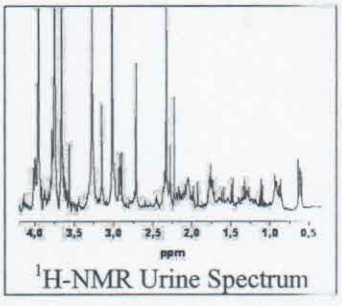
Bovine Metabolomics represents a novel tool for inspecting meat and dairy products quality and helping to detect animal diseases or disorders. In our studies, 24 Dutch Holstein female calves received intravenous Ivermectin 4% (dosage of 1 mL/50 Kg), from which urine and blood plasma were analysed before (blank), and 3 and 6 hours after treatment. Samples were analysed by using ^{1}H NMR PRESAT sequence, and ^{13}C SSFP (Steady-State Free Precession) sequence. The ^{13}C SSFP sequence leads to an average 3-fold increasing in the signal-to-noise ratio, when compared to standard ^{13}C sequences, avoiding the need for isotope labelling, and thus representing a pioneer technique for metabolic analysis. Metabolites were identified by using online databases, and chemometric studies were carried out to highlight the variations in both urine and plasma samples (both ^{1}H and ^{13}C-SSPF spectra). Several changes were observed in post-treatment samples, such as a considerable increasing in concentration of aminoacids and histamine (what clearly denotes an allergic reaction) and a decreasing of citrate levels. The information from these studies are the very first step for establishing a chemical fingerprint for ivermectin misuse and/or the non-observance of the drug withdrawal term and consequent contamination of milk and meat.



^{1}H-NMR Urine Spectrum

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Towards Understanding the Folding Mechanism of a Four-Helix-Protein, ACBP the Cooperative Formation of Specific Tertiary Contacts in the Unfolded State

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In studies of the ensembles of unfolded structures of a four helix bundle protein ACBP –acyl coenzyme A binding protein -we have detected the presence of potential precursors of native tertiary structures. These observations were based on the perturbation of NMR chemical shifts of the protein backbone atoms by single site mutations. Some mutations change the chemical shifts of residues remote from the site of mutation indicating the presence of an interaction between the mutated and the remote residues. This suggests that the formation of helix segments and helix-helix interactions is cooperative. We can begin to track down the folding mechanism of this protein using only experimental data by combining the information available for the rate limiting structure formation during the folding process with measurements of the site specific hydrogen bond formation in the burst phase, and with the existence prior to the folding reaction of tertiary structures in the ensemble of otherwise unfolded structures observed in the present study. We envisage that the detection of long range interactions in ensembles of unfolded proteins is not only restricted to ACBP. For many proteins it has been noticed that after the unfolding transition residual structure prevails which disappears only upon increase of the denaturant concentration as observed either by CD and/or NMR or other techniques. It is the ensemble of transient residual structure in the unfolded state, which has been characterized in case of ACBP, however, it seems most likely that many other unfolded proteins could be candidates for a similar study. The method is therefore seen to have a potential to become an important experimental tool for the advancement of our understanding of the protein folding problem.