Metabolomics for animal production; an introduction

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ABSTRACT

Metabolomics will be one of the most important tools in the exploitation of the bovine genome sequencing project. The results of metabolomics will play important roles in many facets of animal production. This paper will give an introduction of metabolomics and its application in the field of animal production. It serves as the start of the metabolomics session at the 2006 conference of New Zealand Society of Animal Production covering the origin, analytical chemistry and ongoing research in metabolomics with a special focus on this research within New Zealand.

Keywords: metabolomics, mass spectrometry, NMR, introduction

INTRODUCTION

Metabolism is the key to animal production. Efficient utilisation and conversion of feed into meat, milk or wool is all dependent on metabolism, even animal diseases and toxicosis like facial eczema are the result of complex metabolic processes. Every metabolic process is controlled and regulated by one or more genes, and finding the linkage between those genes and metabolic processes is called metabolomics. Metabolomics will be one of the most important tools in the exploitation of the bovine genome sequencing project and its results will help in many facets of animal production.

In twenty years time, farming will be a high tech business, where the farmer can rely on nano-scale analytical technology, which can tell if the animal is performing well, if food intake is sufficient, diagnose early stages of disease (like TB or toxicosis) and pregnancy. As an example, it will record the key processes in the rumen for fibre degradation and methanogenesis. These types of technologies will assist the farmer in decisions on grazing regimes, because the same technology will be able to show which pasture has the optimal composition for which type of animal. These results will not only be based on empiric findings, but on an understanding of how the genetics of each animal affects all these processes.

We are still a long way from this, but we can not ignore the possibilities. If it is going to happen New Zealand will be one of first places where it can happen. The research has started and this session will show that metabolomics based research is already well underway in New Zealand.

Metabolomics

Metabolomics is a logical follow on from the functional genomics wave, led by the multitude of whole genome sequences now in the public domain (Brown *et al.*, 2005). Genes are transcribed and translated into proteins, of which many are involved in metabolic processes (e.g. enzymes etc.). Genome sequencing programs yield information on many genes, with many unknown function, which hampers the utilisation of this knowledge and annotating of function to genes is therefore the next step (Goodacre, 2005). Studying the effect of genes on metabolism is known as metabolomics, and is now mentioned in the same breath with genomics and proteomics (Corella & Ordovas, 2005).

Genomics defines on the genome. Likewise, metabolomics assumes that there is something called the metabolome, which can be defined as all the metabolites in a biological sample (Sumner, *et al.*, 2003). However the metabolome, in contrast to the genome, is never static, and therefore demands time course experiments to capture that dynamic (Fell, 2005). Fluctuation is not the only factor which makes metabolomics difficult, a specific metabolite has a specific function at a specific place and time in the cell, and that function in that cell can change depending on a number of variables (Fiehn, *et al.*, Weckwerth, 2003). Therefore, the metabolome or metabolic profile will always obscure information on the actual function of a metabolite, by neglecting spatial and time factors. Even with these shortcomings, metabolomics has proven to be a powerful tool and has helped to annotate function to genes and discover new metabolic pathways (Goodacre, 2005).

However metabolic profiling does not provide direct evidence of the dynamics behind the cell metabolism. Another subset of metabolomics is metabolic flux analysis or tracer-based metabolomics (Lee & Go 2005). It consists of measuring the flux of metabolites and their turnover through the metabolic pathways by examining the distribution of stable isotope atoms from a labelled substrate in metabolite pools (Lee & Go). Metabolic flux analysis is a technically demanding approach with lower throughput and higher costs than metabolic profiling, especially for larger animals, but it provides valuable information about the state of different metabolic pathways active in the organism (Lee & Go). Metabolic profiling and metabolic flux analysis are both pivotal for characterising a phenotype.

ANALYTICAL CHEMISTRY

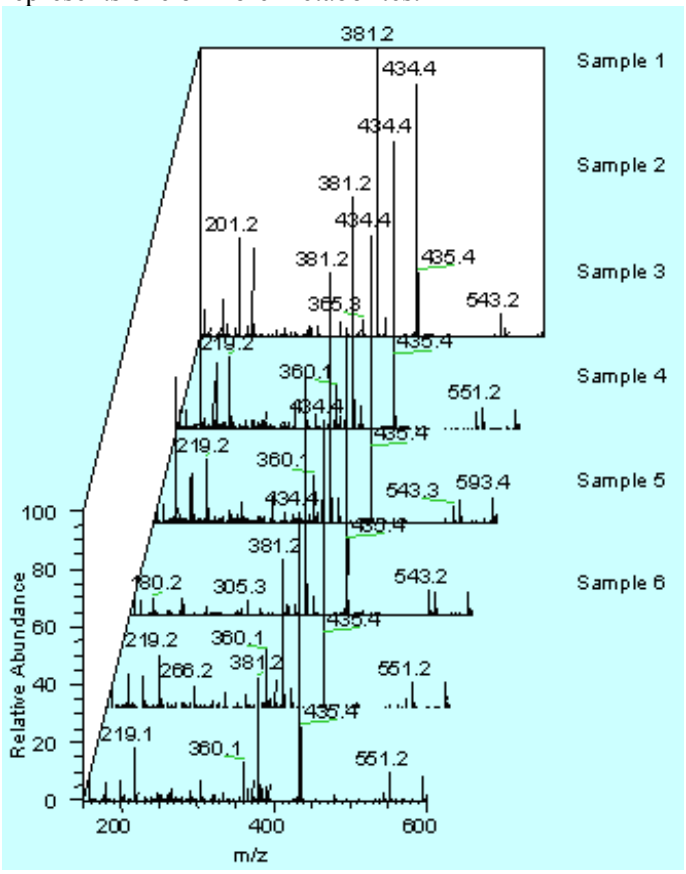
Any attempt to capture the metabolome will depend on analytical chemistry, but this concept is new. Analytical chemistry has always dealt mainly with the measurement of specific known compounds, metabolomics demands analytical methodology that measures all compounds within a sample, even those metabolites that are unknowns (Dunn, *et al*., Johnson, 2005). This is technically difficult, but has driven analytical chemistry in a new direction and yielded some new methodologies, each focussing on different aspects of the metabolome. This demands a careful selection of analytical methodology in each metabolomics experiment. At the same time, the newly developed methodology can be applied to other scientific questions.

The main two technical platforms in metabolomics are nuclear magnetic resonance (NMR) and mass spectrometry (MS). There is no doubt that NMR is the method of choice to analyse the main metabolites in a sample. NMR is highly quantitative and only biased towards the presence of protons. Its response is linear towards all protons in a sample and therefore independent of standards, which is important when measuring unknown metabolites or metabolites with unknown function (Pauli *et al.*, 2005). The limitation of even today's most sensitive high field NMR machines is their inability to measure low abundant and often also mid-abundant compounds (Ratcliffe & Shachar-Hill, 2005). This range of metabolites is much easier to analyse with MS, either coupled to liquid (LCMS) or gas chromatography (GCMS). GCMS has been widely applied in the analysis of primary metabolites in plants (Fiehn & Weckwerth, 2003). Direct infusion MS (see Fig. 1) is a rapid method for metabolic profiling and therefore used in high-through-put experiments, but is less suitable for quantification (Smedsgaard & Frisvad, 1996). The main disadvantage of MS is that the actual measurements happen in the gasphase on ions, which means that the compounds measured, must be able to carry charge and be volatile. These properties differ between compounds and impede the quantification, which makes the methodology dependent on standards. This means that absolute quantification of unknowns is impossible. The use of chromatography increases the resolution of the profiling, by adding the extra dimension of separation over time. However the deconvolution of all the analytes present in a sample and the alignment of multiple chromatograms is still a challenge (Jonsson *et al.*, 2005).

DATA

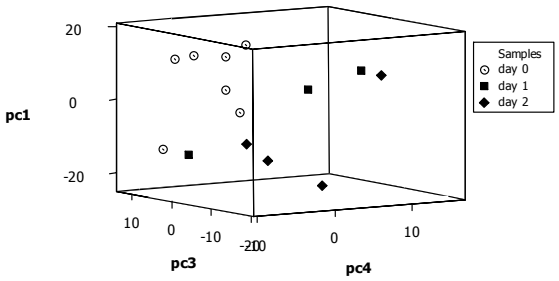
The fluctuations in the metabolome make it necessary to use sufficient numbers of samples in one experiment to either capture the fluxes or to be able to rule-out unwanted fluctuations. Each sample will yield a vast number of data points leading to huge datasets (Figure 1).

FIGURE 1: The mass spectra of raw extracts of 6 clonal plants (*Lolium perenne*) obtained through direct infusion mass spectrometry. It shows the variations in between samples and the complexity of the data. Every bar in the mass spectrum represents one or more metabolites.



In an unbiased approach there is no redundant data and the analysis of the data is dependent on multivariate statistics (Figure 2). This topic is further discussed in the next chapter.

FIGURE 2: A principle component analysis (based on correlation) based on the mass spectra of sheep urine. Day 0 was the first collection of urine before the animals were dosed with water, day 1 and 2 is urine from the same animals, either 1 or 2 days after dosage (courtesy of K. Fraser and G. Lane, AgResearch).



ONGOING RESEARCH

Metabolomics is rapidly developing into a very diverse research area and is being pursued by different research groups in New Zealand. The Foundation for Research Science and Technology has invested together with AgResearch Limited and HortResearch in the establishment of plant metabolomics platform for New Zealand based in Palmerston North. This has resulted in the generation of a large range of metabolomics capabilities that facilitate metabolomics research from the gene to the metabolite and back. These capabilities are also employed by animal research and the efforts in this area are described in this session. The different papers in this session highlight the different perspectives and strategies being used metabolomics research.

FROM THE GENE TO METABOLITE FUNCTION

Many genes in any genome are involved in metabolism. By studying the genetic differences with metabolomics it becomes possible to attribute these differences to fluctuations in specific metabolites. This approach is an important step in the understanding of gene function that can be later exploited at different levels. In this session an example will be given of how this strategy has been applied to in the study of fibre degradation by rumen microbes, and will demonstrate how this strategy has yielded an improved understanding of gene function.

FROM FUNCTION TO METABOLITE TO GENE

There can be different reasons to follow function back to the metabolites responsibles for that function. When there is known function or activity, a metabolomics approach can be used to trace that activity to the metabolites responsible for that function. These metabolites can then be used as nutriceutical or pharmaceutical directly (as described in the third paper) or can be used as biomarkers diagnosis of diseae, health status etc. This type of research is also known as metabonomics (Dunn *et al.*, 2005).

METABOLOMICS

When animals are exposed to different treatments their metabolism will respond differently. This very open statement can be applied in many types of research. But it can be very difficult to predict how and to what extend an animal will respond to a treatment. The metabolic profile of an animal can change in response to a dose of water (Figure 2). This sounds strange, but something as simple as animal handling affects the animal, and thus its metabolism. Such findings should both caution and reassure metabolomics scientists. It is possible to makes distinctions between sample sets, even very subtle ones, but observed differences might have been caused by many different (unwanted) things. The metabolomics approach can help to determine biomarkers of specific treatments or states (like food intake, toxicoses etc.) (Nicholson *et al.*, 2002; Robertson, 2005). In the fourth paper we will see an example of such an approach, where the metabolic effects of different diets are studied.

METABOLOMICS, NEW ZEALAND AND LIVESTOCK

The research area called metabolomics is still in its infancy but has been established in New Zealand and is certainly on a worldwide scale. However, several areas within metabolomics are dominated by high profile Institutes in Europe, Japan and the US, especially concerning model species like yeast, Arabidopsis, rats and humans. The application of metabolomics to the area of animal production has been established in New Zealand, especially AgResearch Limtied has been instrumental in this. Metabolomics projects are invariably dependent on multi-disciplinary science

team and are therefore dependent on larger teams of scientists (molecular biologists, analytical chemists, statisticians, animal physiologists), and this kind of approach to science favours large organisations like AgResearch Limited.

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