
Preface

Plant secondary metabolites are a diverse group of molecules that are involved in the adaptation of plants to their environment but are not part of the primary biochemical pathways of cell growth and reproduction. In general, the terms *plant secondary compounds*, *phytochemicals*, *antinutritional factors*, and *plant xenobiotics* have been used in the literature to refer to this group of compounds. There are well over 24,000 structures, including many compounds that have antinutritional and toxic effects on mammals. This number does not include the oligomeric polyphenolic compounds (proanthocyanidins and hydrolyzable tannins) that are just now being more accurately described and will increase the number by several thousand. Some major plant secondary metabolites or phytochemicals that occur in plants include protease inhibitors, lectins, alkaloids, nonprotein amino acids, cyanogenic glycosides, saponins, and tannins. These compounds are involved in defense against herbivores and pathogens, regulation of symbiosis, control of seed germination, and chemical inhibition of competing plant species (allelopathy), and therefore are an integral part of the interactions of species in plant and animal communities and the adaptation of plants to their environment.

Much of the research on plant secondary metabolites has concentrated on their toxic and antinutritional effects on livestock. Toxic plant secondary metabolites are present in plants at low concentrations (generally less than 2% of the dry matter) and have negative physiological effects when absorbed, such as neurological problems, reproductive failure, goiter, gangrene, and death. Examples are alkaloids, cyanogenic glycosides, toxic amino acids, saponins, and many others. Nontoxic phytochemicals lower digestibility of nutrients and affect palatability. Higher concentrations (>2% of dry matter) of these compounds are required for eliciting negative effects, and the primary site of activity is in the digestive tract or the sensory organs associated with feeding behavior. These plant secondary metabolites include tannins, protease, and amylase inhibitors. Compounds that have a structural role in the plant (e.g., lignin, biogenic silica, and cutin) lower the extent of microbial digestion of cell wall polysaccharides.

This division between groups of plant secondary metabolites is not exclusive. For instance, hydrolyzable tannins are potentially toxic to ruminants. The major lesions are hemorrhagic gastroenteritis, necrosis of the liver, and kidney damage with proximal tubular necrosis. Excessive and fast consumption of oaks and

other tree species that contain more than 5% hydrolyzable tannins results in high mortality and morbidity in cattle and sheep.

In addition, plant secondary metabolites are also associated with improved nutritive value and may have beneficial effects on animal health. Proanthocyanidins, more commonly called condensed tannins in the animal nutrition literature [present in forage legumes such as sainfoin (*Onobrychis viciaefolia*), bird's-foot trefoil (*Lotus corniculatus*), and *Lotus pedunculatus*], are associated with improved protein digestion and metabolism in ruminants and in protecting ruminants against legume bloat. Tannins may also protect ruminants against helminthiasis. Growing interest in the potential health-promoting effects of plant secondary metabolites in human foods has prompted research on their potential to prevent or treat cancer, circulatory disease, and viral infection. The mechanisms by which these substances have beneficial effects on health may also be related to their toxic effects, and the difference between toxicity and beneficial effects may be dose- and structure-dependent. However, mechanisms of toxicity and health-promoting effects of most of the plant secondary metabolites in human and animal diets are not well established.

Interest in plant secondary metabolites has risen dramatically in recent years among plant molecular biologists and plant breeders because of their diverse effects, which, in addition to those mentioned above, include antioxidant, antiviral, antibacterial, and anticancer effects. To name few recent developments, molecular biologists have made genetic modifications in proanthocyanidin biosynthesis in forage plants with the aim of eliminating bloat, improving the efficiency of conversion of plant protein into animal protein (increase rumen undegradable protein and thus increase protein availability postruminally), reduce greenhouse gases, and reduce gastrointestinal parasites; and plant breeders have developed and commercialized rapeseeds (canola) with low levels of glucosinolates and erucic acid, and cottonseed with low gossypol. Genetically modified rice, which expressed insecticidal cowpea trypsin inhibitor, has also been produced. The emerging molecular genetic approaches have tremendous potential to unravel the regulatory genes that control plant secondary metabolite biosynthesis. This information, together with increased knowledge of the enzymes specific for the pathway, could facilitate the genetic engineering of plants.

Most of the plant resources, especially in the tropical regions, are rich in plant secondary metabolites, and the lack of information on the appropriate methods for their determination has been the main bottleneck in better understanding the enzymes and biochemical pathways in their synthesis, the genes responsible for controlling major biochemical processes, and the physiological significance of plant secondary metabolites, and in exploiting the beneficial effects of these phytochemicals.

A number of methods are available in the literature for quantification of plant secondary metabolites. This manual does not present all the available methods; rather, based on our over-a-decade experience on quantification of plant secondary metabolites, it contains the methods for analysis of some important plant secondary metabolites that have worked well in our hands and that can be conducted in laboratories equipped with basic facilities. The methods have been written in a recipe-like format designed for direct practical use in the laboratory. The chemical nature of plant secondary metabolites, their known physiological effects, and their mechanism of action are also briefly presented. This work has originated as a result of numerous requests, especially from scientists from developing countries, for appropriate methods to quantify plant secondary metabolites.

It is hoped that the reliable assays presented in this manual will contribute to the safe and efficient use of locally available feed resources, and better equip the researchers to meet the unprecedented challenge presented by the huge demand for feed, which is driven by the increasing demand for animal protein in developing countries. This manual will also enable better understanding of plant–animal interactions, which is of importance not only for animal agriculture but also for games. The methodologies given in the manual could also be used for determination of plant secondary metabolites in human food and in studying the implications of their consumption on human health and welfare. The manual will also further the interests of molecular geneticists in genetically engineering the plants for introduction of value-added nutraceuticals and food and forage quality traits.

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