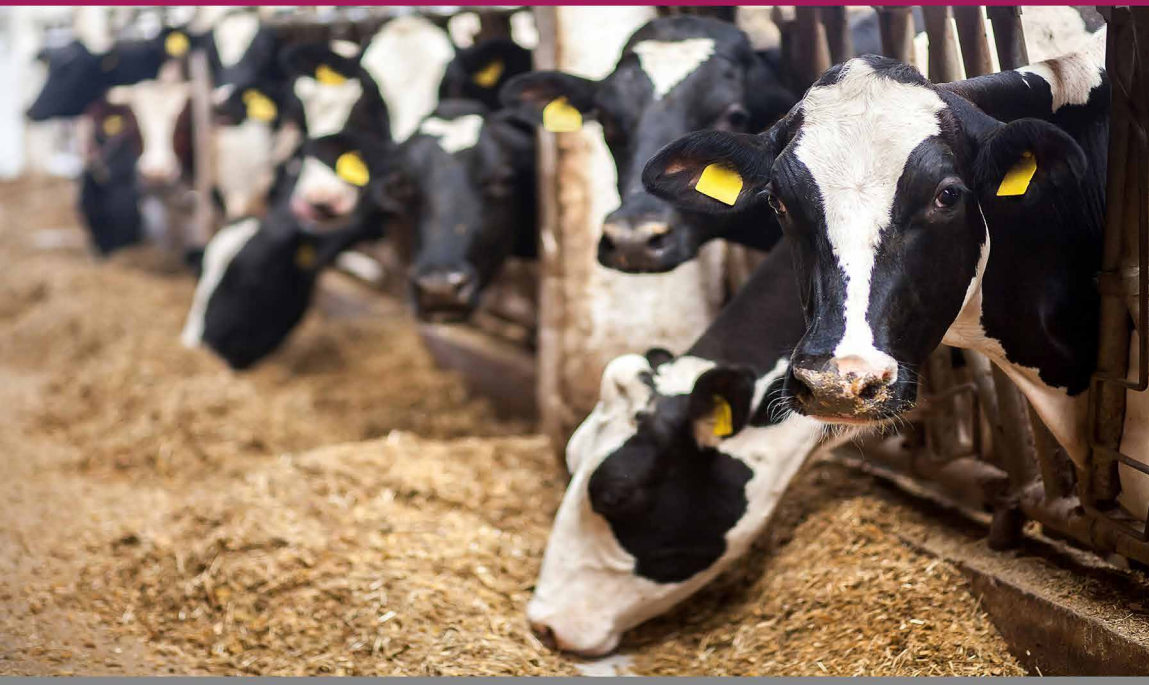
BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Developing animal feed products

Edited by Dr Navaratnam Partheeban, formerly Royal Agricultural University, UK



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Introduction

The animal feed sector faces increasingly complex challenges. It needs to improve feed digestibility/efficiency whilst also promoting growth and enhancing both product quality and safety. At the same time, it has an increasingly important role in helping to promote animal health, welfare and the sustainability of farming.

Part 1 of this volume reviews advances in optimising the key stages in developing successful new animal feed products, from assessing feed ingredients, product development and processing to maintaining quality and safety. Chapters also feature authoritative discussions by leading experts on the relationship between nutrition and animal health. Part 2 examines quality and safety assurance by demonstrating how best to implement risk management systems for the prevention and control of contaminants present in animal feed.

Part 1 Developing animal products

Part 1 opens with a chapter focused on techniques for identifying new animal feed ingredients and additives. The identification of potential novel feed ingredients requires quick and effective analytical techniques to assess their composition and functionality. Chapter 1 provides a practical guide to routine and standard methods to detect the main traits of interest in animal feed ingredients and additives. The chapter discusses techniques to evaluate feed nutritional value, including chemical composition and nutrient digestibility. It then reviews different *in vitro* methods for feed evaluation systems as well as assessment of nutrient molecular structure. The chapter also discusses analysis of feed bioactive compounds and their functionality. The chapter concludes with a case study on seaweed as a potential novel feed for livestock.

The next chapter reviews the effect of processing techniques on the quality of animal feed. Chapter 2 begins by examining how extrusion cooking can directly influence the nutritional value of a given formulation component, such as starch, in a dry animal feed formulation. It then goes on to discuss the processing of cereals for food and feed, focusing specifically on the range of cereal processing conditions currently used in the industry. A case study on developing animal feed products from starch is also provided, primarily focusing on dry cat food. The chapter then summarises the importance of extrusion cooking technology in the manufacturing of food and feed products and the need to achieve the correct level of water solubility in these products.

Expanding on topics previously discussed in Chapter 2, Chapter 3 focuses on the processing techniques that are used to optimise digestibility and the

nutritional value of animal feed. It discusses the two most popular methods of particle size reduction, hammermills and roller mills. The chapter then moves on to review the effects of particle size reduction on swine and broiler feed production and performance, which is then followed by an analysis of the effects of pelleting on swine and broiler feed performance. The chapter concludes by highlighting the importance of feed processing research.

The final chapter of Part 1 addresses trends in analytical techniques for testing animal feed. The challenges for the feed analysis sector are to ensure feed quality and safety. Innovative strategies need to be implemented to authenticate feed and feed ingredients and to check that they fulfil all labelling obligations. Chapter 4 focuses on the authentication of feed by different but complementary analytical techniques, such as microscopy, vibrational spectroscopy, genomics and proteomics. The chapter explores ways techniques can be combined to find solutions for complex testing challenges, as well as developments in data science to achieve faster, real-time analysis.

Part 2 Quality and safety assurance

The first chapter of Part 2 focuses on the developments in techniques to test the efficacy of animal feed products. Animal feed products affect both healthy growth of livestock, their environmental impact (e.g. through emissions) and the safety and quality of livestock food products. All these areas are regulated by agencies such as the European Food Safety Authority (EFSA). Chapter 5 first discusses EFSA guidance on how to compile dossiers for feed additives. The chapter then discusses key issues and steps in demonstrating the efficacy of new animal feed products in key areas such as: reduction of nitrogen (N) excretion, reduction of feed contamination of by mycotoxins and, finally, reduction of methane emissions with feed additives. Key steps, such as the use of *in vitro* and *in vivo* studies, to test the effects of feed additives are discussed in detail.

Chapter 6 examines advances in understanding key contamination risks in animal feed, specifically mycotoxins. Mycotoxins accumulate in corn, cereals, soybeans, sorghum, peanuts, and other food and feed crops, directly in the field or during transportation, processing or storage. Consumption of mycotoxin-contaminated food or feed can lead acute or chronic toxicity in humans and animals, as well as crop losses. The chapter reviews the toxicity of the six mycotoxins, the foods they commonly contaminate, and the current methods used to detect and control these mycotoxins.

Chapter 7 considers risk management systems for prevention and control of contaminants in animal feed. Feed safety remains an issue for both the health of livestock and as a prerequisite for food safety. Risk assessment plays a role in identifying and determining potential risks of contaminants. When

contaminants limits are defined, risk management systems can be designed for prevention and control. This chapter focuses on management systems used to prevent and control contaminants in animal feed. A brief introduction to feed contaminants and new feed ingredients is given, followed by a description of the main risk management systems applied in Europe and the US.

The final chapter of the book addresses developing effective product dossiers for regulatory approval of new animal feed products. Chapter 8 describes the key steps in compiling product dossiers required to gain regulatory approval for new animal feed products. As an example, the chapter describes the data needed for a feed additive dossier in the European Union (EU) as mandated by the EFSA.

Chapter 1

Techniques for identifying new animal feed ingredients and additives

Jordi Ortuño, Institute of Global Food Security – Queen's University Belfast, UK; Alexandros Ch. Stratakos, Centre for Research in Biosciences – University of the West of England, UK; and Katerina Theodoridou, Institute of Global Food Security – Queen's University Belfast, UK

1 Introduction

Food supply in many regions has never been so secure yet, at the same time, faced greater challenges. Global warming, the exponential rise of urban populations and globalisation (with the risk of crises such as COVID-19) are promoting competition between production of food, feed and fuel that will require innovative solutions to ensure enough safe food in the future. Animal food production is at the forefront of this challenge, reflected in the transition towards a more sustainable model of production. The global demand for improved food safety and animal welfare, together with reduced air-, water- and soil-derived contamination, is incompatible with many current production systems. As an example, the European Union, the major global producer of dairy products and second in production of pig meat, is only able to produce 20% of the protein requirement in livestock feed. This deficiency is made up

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by imports of vast amounts of soy from other countries, often with a huge environmental impact derived from changes in land use, such as deforestation of vast regions of the Amazon rain forest in Brazil.

The search for new alternative feed resources must avoid direct or indirect competition with human food resources. Beyond that, ideal novel ingredients and additives should not only provide similar or better production performance than conventional ingredients, but also offer extra added value in addressing challenges such as improving animal health and welfare (e.g. in promoting gut health) or reducing contamination risks. A number of examples illustrate this.

One example is marine algae (micro and macro) which meet nutritional requirements for ruminants and, at the same time, do not compete for arable land and are less vulnerable to weather fluctuations related to climate change. They also possess bioactive compounds (bromoform, phlorotannins, saponins) capable of reducing ruminal methane production by up to 98%. A second example is insects, which can upcycle agri-food wastes into feed ingredients, potentially reducing reliance on cultivated soy. In addition, the presence of chitin in insect exoskeletons has prebiotic effects that promote beneficial bacteria in the gut, competitively excluding pathogens and promoting healthy gut function. Another example is silvopastoralism, a multifunctional system that combines animal production with pasture, shrubs and trees, thus promoting environmental protection and soil conservation. Most of the tree species employed in such systems are abundant in condensed tannins, which are able to reduce ammonia emissions, a key factor in soil and water acidification, eutrophication and global warming.

The identification of these potential novel ingredient sources requires rapid and effective analytical techniques capable of assessing composition and functionality, and screening for the effects of intrinsic variability (e.g. resulting from species or cultivars) and extrinsic variability (e.g. seasons, weather conditions, substrate composition). The present chapter aims to provide a practical guide to routine and standard methods employed to detect the main traits of interest in different sources of animal feed ingredients and additives.

2 Chemical analysis to determine protein, fibre, fatty acid content and digestibility

Assessing the nutritional value and quality of a new animal feed ingredient or additive for livestock diets requires determining chemical composition, intake, palatability, acceptability and digestibility. This section looks at ways of analysing the chemical composition of an ingredient or additive.

2.1 Protein content and digestibility

Crude protein content can be estimated by multiplying the nitrogen content by 6.25 to provide a quantitative indicator of feed protein content. This conversion factor has been used since the 19th century and assumes the nitrogen content of proteins to be 16% (Mariotti et al., 2008). However, this approach can be inaccurate in the case of novel ingredients (e.g. seaweed or insects), often resulting in an overestimation of protein content. Specific nitrogen-to-protein conversion factors, calculated from amino acid composition and total nitrogen, provide more reliable protein quantification.

Different methods are commonly used to determine feed protein content. The first is the classical Kjeldahl method for the determination of feed nitrogen (AOAC, 1990, method 977.02). The Dumas method is also widely used to assess crude protein content, is fast and requires no corrosive chemicals in contrast to the Kjeldahl method. The Dumas method measures all forms of nitrogen however provides a slight overestimation of total protein nitrogen content compared to the Kjeldahl method (Mæhre et al., 2018).

Identification of the protein value of a new feed requires assessment of not only protein quantity but also its quality. Protein quality can be determined partly by the quantity of essential and non-essential amino acids (AAs), and partly by their digestibility which measures the amount that can be absorbed from the gut and utilized. The 'traditional' method for the analysis of amino acids is based on ion-exchange chromatography with spectrophotometric detection (IEC-Vis) in which AAs are separated in the chromatographic column and subjected to post-column derivation with ninhydrin. IEC-Vis is recommended as a technique for official feed control in numerous countries.

The amino acid tryptophan can be determined by the high-performance liquid chromatography (HPLC) method with fluorometric detection (Szkudzińska et al., 2017). However, the analysis of AAs is complicated by the lack of a simple and reliable protein hydrolysis method. A simple, accurate and reliable method for the analysis of AA composition is Reversed-Phase High-Performance Liquid Chromatography with Diode-Array Detection (RP-HPLC-DAD), making analysis simple and accessible to any routine analytical laboratory (Liyanaarachchi et al., 2020).

Rapid and reproducible methods have been developed for the estimation of *in vitro* feed digestibility in ruminants and monogastric animals. There are several *in vitro* methods for assaying protein digestibility in monogastric animals which work by simulating the digestive process of the studied animal. The pepsin digestibility assay is one of the most widely used assays to evaluate the quality of feed and protein ingredients in poultry (Bryan and Classen, 2020).

In vivo animal trials are among the most accurate ways to measure protein digestibility in nutritional evaluation of feeds but such trials are time consuming

and expensive. *In vivo* estimation of protein quality of a feed ingredient is normally achieved by feeding the ingredient to the intended animal while assessing the extent to which nutrients are absorbed by the terminal intestine.

In vitro methods have been developed to estimate protein digestibility of ingredients in pigs, simulating protein digestion along the gastrointestinal tract (GIT) by incubating protein sources with pepsin and pancreatin sequentially, using incubation conditions matching those in the GIT. One problem is that protein sources with a similar *in vitro* protein digestibility, based on determination of nitrogen solubility, can differ in the extent of release of free amino acids or di- and tri-peptides. Chen et al. (2018b) have developed a method for the prediction of *in vivo* protein digestion kinetics.

2.2 Fibre content

Fibre is characterised as hydrolytically indigestible and partially fermentable and is a complex and highly variable component of plant-based feedstuffs (NRC, 2007). The most ways to estimate fibre content in feed ingredients include (Kerr and Shurson, 2013):

- crude fibre (CF),
- acid detergent fibre (ADF),
- neutral detergent fibre (NDF),
- soluble and insoluble fractions of total dietary fibre (TDF), and
- non-starch polysaccharide.

The method for the determination of crude fibre is designed to divide carbohydrates into digestible and indigestible fractions. It is an indicator of the energy value of the feed: when crude fibre values are high, feed energy is low because crude fibre is considered indigestible. Crude fibre accounts for most of the cellulose and a portion of the hemicellulose and lignin but no ash and, as a result, underestimates true fibre. CF is not a good indicator of digestibility in ruminant animals, and the use of this assay in feeds for ruminants has declined significantly (Fahey et al., 2018).

To measure detergent fibres (ADF and NDF) the Van Soest 1963 detergent fibre analysis system is used. Plant cell substances can be divided into less digestible cell walls (hemicelluloses, cellulose and lignin) and highly digestible cell contents (starch and sugars). The acid detergent fibre (ADF) represents the least digestible fibre portion in the animal feed. The highly digestible cell contents are separated from the cell walls by using two different detergent systems:

- NDF = Hemicelluloses + Cellulose + Lignin + Ash, and
- ADF = Cellulose + Lignin + Ash.

ADF is the residue remaining after boiling a test material in an acid detergent solution. The neutral detergent fibre (NDF) is the residue or insoluble fraction left after boiling a feed material in a neutral detergent solution and contains insoluble plant cell wall components.

Non-starch polysaccharides (NSP), together with lignin, have been defined as the total dietary fibre (TDF) in feedstuffs. In the last few decades, there has been a great interest in gut microbiota and their effect on the host's metabolic regulation. A beneficial shift in the microbial ecosystem of animals can be promoted by various dietary and non-dietary interventions. Diet is ranked as one of the most important and effective regulators of gut microbiota composition, specifically dietary fibre. Studies with pigs and poultry have shown that fermentation characteristics of fibres and their beneficial effects on gut health vary widely, based on type, form and physicochemical properties of the TDF. It is important, therefore, to have information on the different TDF types in a new feed additive or ingredient (Jha et al., 2019). TDF is primarily analysed by enzymatic-gravimetric methods which solubilise the different fibres fractions with enzymes and solvents and measure the weight of residues after these treatments (Gidenne, 2014).

2.3 Fatty acid content

Fatty acids are carboxylic acids with either saturated or unsaturated aliphatic chains. They can be divided according to chain length into (Baltić et al., 2017):

- short-chain fatty acids (<C6),
- medium-chain fatty acids (C6-C12),
- long-chain fatty acids (C13-C21), and
- very-long-chain fatty acids (≥C22)

Fatty acids can be present in their free fatty acid (FFA) forms. However, they most often exist in bound forms such as cholesterol and phospholipids (PL). In feeds, lipids are mainly found in the form of triacylglycerols (triacylglycerides – TAG), which make up to 99% of lipids of plant and animal origin (Fennema, 1996).

The diet of animals influences the fatty acid composition of animal products (i.e. meat, milk and eggs). This relationship is stronger in monogastrics than in ruminants where dietary fatty acids are hydrogenated in the rumen (Chilliard et al., 2008). There is an increasing recognition of the health benefits of polyunsaturated fatty acids (PUFA) which are divided into omega-3 and omega-6 fatty acids as well as docosahexaenoic acid (DHA). These are seen as having nutraceutical properties e.g. in the development of the nervous system and the maintenance of cognitive function (Butler, 2014; Balić et al.,

2020). The inclusion of fats in ruminant diets is also considered a promising way to manipulate the rumen microbial community to reduce methane emissions (Vargas et al., 2020). It is therefore important for human and animal health as well as the environment to determine the fatty acid content of a potential new animal feed ingredient or additive before use in animal nutrition.

Total lipid content of feedstuffs is generally estimated by the Soxhlet method or by cold extraction methods using organic solvents (Bligh and Dyer, 1959 and Folch et al., 1957 techniques). Both methods rely on chloroform and methanol to form a monophasic solvent system to extract and dissolve the lipids. The Folch method is regarded as the most reliable for complete recovery of total lipids, but the Bligh and Dyer method is more widely known and used. After transmethylation the fatty acid composition of the extracted oil is determined by analytical techniques such as gas-liquid chromatography, mass spectrometry (GC-MS), gas chromatography with flame ionization detection (GC-FID), and liquid chromatography-mass spectrometry (LC-MS) (Leiva and Granados-Chinchilla, 2020; Chiu and Kuo, 2020).

3 Techniques to determine nutritive value and digestibility: rumen *in vitro* fermentation techniques, protein evaluation systems and models

As discussed in the last section, the nutrient composition of feed is commonly determined in the lab with chemical analysis. However, this is not sufficient to determine its nutritive value after digestion. As noted, *in vivo* animal trials to determine the nutritive value and digestibility of feeds are time consuming and expensive and require large quantities of feed. For these reasons *in vitro* systems have been developed and are very useful for the screening of novel animal feed ingredients and additives.

The basis of all *in vitro* rumen screening methods is anaerobic fermentation of a feed sample (substrate) with a buffer solution, simulating ruminant saliva, to produce a filtered rumen liquor which can be analysed. From these *in vitro* tests, it is possible to collect information on fermentation patterns, total gas and methane production, ammonia-nitrogen content, volatile fatty acids, nutrient digestibility and degradability, as well as microbial community composition (Getachew et al., 2004; Smith et al., 2020).

3.1 RUSITEC

The RUSITEC continuous *in vitro* fermentation system (Picture 1) simulates the rumen environment in a standardised, reproducible way, which avoids the inherent variability in individual animals. By combining high-throughput sequencing with a targeted quantitative metabolomics approach, it is able

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