Plant cell wall chemistry: implications for ruminant utilisation

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REVIEW ARTICLE

RUMINANTS

Abstract

Ruminants have adapted to cope with bulky, fibrous forage diets by accommodating a large, diverse microbial population in the reticulo-rumen. Ruminants are dependent on forages as their main sources of energy and other nutrients. Forages are comprised of a complex matrix of cellulose, hemicellulose, protein, minerals and phenolic compounds (including lignin and tannins) with various linkages; many of which are poorly defined. The composition and characteristics of polysaccharides vary greatly among forages and plant cell walls. Plant cell walls are linked and packed together in tight configurations to resist degradation, and hence their nutritional value to animals varies considerably, depending on composition, structure and degradability. An understanding of the inter-relationship between the chemical composition and the degradation of plant cell walls by rumen microorganisms is of major economic importance to ruminant production. Increasing the efficiency of fibre degradation in the rumen has been the subject of extensive research for many decades. This review summarises current knowledge of forage chemistry in order to develop strategies to increase efficiency of forage utilisation by ruminants.

Keywords: ruminant, cellulose, hemicellulose, pectin, lignin, tannins

1. Introduction

Ruminants have played an important role in human society and agriculture since domestication some 10,000 years ago and provide substantial quantities of food, both meat and milk, in addition to leather and wool (Clutton-Brock, 1999). Globally, plants, including herbage, forage, and agricultural by-products that are mainly comprised of cell walls, can feed approximately 1.5 billion cattle, 1.2 billion sheep, 1 billion goats and 0.2 billion buffalo (Lee, 2018). Importantly, these food sources, readily used by ruminants, cannot be digested by monogastric animals. As the global human population is predicted to reach approximately 9.8 billion by 2050 compared to 7.8 billion today (Lutz and Samir, 2010), the demand for milk and meat will increase dramatically. Ruminants can meet this challenge as they have evolved a unique and complex digestive system, which is characterised by both anatomical and functional adaptations that accommodates the large microbial population required to degrade plant cell walls (Annison and Bryden, 1998).

Microbial fermentation of feedstuffs provides ruminant animals with about 70% of their metabolic energy, and the resulting microbial protein accounts for as much as 90% of the amino acids reaching the small intestine (Bergman, 1990). Therefore, ruminants can survive on fibrous, low protein feeds that are poorly utilised by monogastric livestock (Burns, 2008; Weimer and Odt, 1995). The rumen microbiome is a dynamic system that changes with the feedstuff consumed (Henderson *et al.*, 2015). Rumen digestion of fibrous plant materials is limited primarily by the complex structure of plant cell walls, particularly the physical protection provided by lignin, covalent linkages between lignin and phenolic compounds and cell wall polysaccharides (Yu *et al.*, 2005). There are other factors, such as tannins, proteins, structural carbohydrates and minerals, in herbage and forage that affect rumen breakdown and utilisation of these materials.

Improved understanding of the chemical and the physical characteristics of forage allows plant breeders to improve

feeding quality and animal nutritionists to formulate diets to achieve maximal efficiency of ruminant production. This paper provides an overview of the current knowledge of plant chemical composition and physical characteristics, and how this relates to rumen digestion. It is an oversimplification to view forage cell walls primarily as a substrate for microorganisms, rather than feed for the ruminant. Meeting the challenge of increasing the efficient utilisation of cell walls by ruminants can increase the supply of human food.

2. Chemistry and digestion of plant cell walls

Before reviewing cell wall chemistry, it is important to have an appreciation of the general structure of cell walls as this has a significant influence on composition and digestion. Cell walls are multi-layered with an outer (middle lamella) and an inner layer (primary cell wall). Some plants have an additional inner layer or secondary cell wall. The middle lamella is mainly composed of pectin polysaccharides, while the primary cell wall is composed of cellulose in a matrix of hemicellulose and pectin polysaccharides. This provides the plant with the strength and flexibility to grow and develop. After cessation of growth in some plants, the primary cell wall thickens into the secondary cell wall, further strengthening the plant. In addition to cellulose and hemicellulose, secondary cell walls are often lignified, which has decreased digestibility. The interested reader is referred to Burton *et al.* (2010) for a detailed account of the structure and function of cell walls.

3. Carbohydrates

Since herbage carbohydrates are the main source of dietary energy for ruminants, herbage composition has long been of interest as a factor in determining nutritive quality. The carbohydrates in ruminant diets can be divided into two groups: the 'available' or intracellular carbohydrates, comprising simple sugars and α -linked storage saccharides (starch or fructans), which are readily digested, and partially available carbohydrates, i.e. structural polysaccharides that make up plant cell walls, described as non-starch polysaccharides (Englyst, 1989).

Carbohydrates are compounds composed of polyhydroxy aldehydes (aldoses), polyhydroxy ketones (ketoses), polyhydroxy alcohols (sugar alcohols), polyhydroxy acids (including uronic acids) and their simple derivatives (Leegwater, 1987). Monosaccharide units are interconnected by hemiacetal linkages (the 'glycosidic bonds') that result in the elimination of one molecule of water at each linkage, to produce di-, tri- or polysaccharides containing two, three or more monosaccharide units or residues. Polymers with 11 or more monosaccharide units are defined as polysaccharides (Theander *et al.*, 1993). The hydroxyl group is linked to the first carbon atom of a sugar molecule

in the ring formation, forming two different glycosidic bonds; alpha (α) and beta (β). These bonds can produce quite different substances, although the components may be identical. For example, two glucose molecules linked by α bond form the disaccharide maltose, whilst many glucose molecules linked in the same way produce starch. Similarly, a β bond between two glucose molecules produces the disaccharide cellobiose and cellulose is a β -linked glucose polymer. Both starch and cellulose are glucans. Starch is a α glucan and cellulose is a β glucan. Starch and cellulose have the same chemical composition, but their properties are different. In particular, starch and most other α -linked glucose polymers are hydrolysed by amylase, which is secreted into the alimentary tract of most animals. In contrast, the digestion of β -linked polymers, such as cellulose, is achieved solely by microbial activity.

The cell walls of higher plants are complexes of cellulosic microfibrils embedded in a matrix of interwoven hemicellulosic polymers, pectins (Lampugnani *et al.*, 2018), insoluble proteins (Flint and Forsberg, 1995), silicon (Lam *et al.*, 1990), phenolic compounds and other anti-nutritive factors. By virtue of their role as an envelope, cell walls show resistance to chemical and microbial attack (Vanholme *et al.*, 2010). Cellulose, hemicellulose and pectins make up 90% of the dry matter (DM) of cell walls (Albersheim *et al.*, 1996). Cellulose and hemicellulose contents of some feedstuffs are shown in Table 1. In general, plant cell wall concentration is negatively associated with DM intake, DM digestibility and animal performance (Dehority and Johnson, 1961). Plant cell walls contain three types of structural polysaccharides; cellulose, pectin rich in galacturonic acid residue and hemicellulose rich in xylose (Van Soest, 1994). Legume cell walls have more pectin and less cellulose and hemicellulose than grass cell walls (Morrison, 1979).

4. Cellulose

Cellulose, a crystalline microfibril polysaccharide found in all land plants (Buranov and Mazza 2008), is the most abundant organic polymer on the planet (Ragauskas *et al.*, 2006). It makes up the greatest proportion of the cell wall component of most ruminant diets and consists entirely of β -1,4-glucan (Delmer and Amor, 1995). The glucan chain is linear and contains up to 14,000 anhydroglucose residues (Coughlan, 1985). A number of glucan chains in cellulose are held together by hydrogen bonds, which are formed between an oxygen atom on one sugar unit, and the hydrogen atom of a hydroxyl group on and between adjacent chains. The conformation of cellulose favours the formation of these bonds (Khazraji and Robert, 2013). Therefore, many such bonds can occur between the glucan chains of cellulose. Although a single hydrogen bond is weak, a number of them together make cellulose molecules

Table 1. Cellulose and hemicellulose content of feedstuffs (% DM).

Feedstuff	Cellulose	Hemicellulose	Reference
Barley straw	37.5		Holtzapfel, 2003
Coastal bermuda grass	25	35	Saha, 2003
Forage sorghum	24-38	12-22	Corredor <i>et al.</i> , 2009
Grasses	25-40	35-50	Kumar <i>et al.</i> , 2009
Lucerne and grass mixture ¹	28-30	11-12	Vasileva and Naydenova, 2017
Maize cobs	45	35	Kumar <i>et al.</i> , 2009; Saha, 2003
Maize stover	40	25	Saha, 2003
<i>Medicago murex</i> cv. Zodiac	37.2	15.9	Li, 1992
Rice straw	35	25	Saha, 2003
Sorghum straw	32.5		Holtzapfel, 2003
Sugarcane bagasse	40	24	Saha, 2003
Switchgrass	45	30	Saha, 2003
<i>Trifolium resupinatum</i> cv. Kyambra	28.7	11.5	Li, 1992
<i>Trifolium subterraneum</i> cv. Junea	29.4	11.4	Li, 1992
Wheat straw	30	50	Kumar <i>et al.</i> , 2009; Saha, 2003

¹ Lucerne: tall fescue (1:1) or lucerne : cocksfoot (1:1).

pack tightly together in parallel to form a stable multi-chain fibril which is an extremely cohesive structure.

Only a single reaction (hydrolysis of β 1-4 glucosidic bonds) is required to convert cellulose to soluble products, but both biological degradation and acid hydrolysis are made difficult by the insolubility of the substrate and the inaccessibility of the glucosidic bonds, especially in the crystalline regions (Warren, 1996). When cellulose is treated chemically, the covalent glycosidic bonds usually break before the chains can be separated. Even enzymes specific for the glucan chains are not very effective in degrading the intact cellulose fibre. Cellulose can be broken down by enzymes, but the degradation process is always slow (Albersheim, 1975) with obvious implications for rumen digestion. When cellulose forms complex structures with lignin or links to hydroxyproline-rich glycoproteins *via* phenolic cross-linkages, digestibility is further reduced (Lodish *et al.*, 2000).

The accessibility of cellulose to hydrolysis can be increased by steaming or alkali treatment to make the cellulose less crystalline and less protected by associated components (such as lignin or silica), or milling to increase surface area (Chenost and Kayouli, 1997), which increases cellulose digestibility (Morrison, 1974). Unprotected and lignin-free cellulose and hemicellulose can be degraded completely (McLeod and Minson, 1974).

Cellulose digestion

The principle cellulose degrading components are a mixture of endoglucanases and cellobiohydrolases which work in combination with β -glucosidase (Wood and Wilson, 1995). Weimer and Odt (1995) divided the ruminal cellulosic bacteria into two groups according to their function, i.e. those that adhere to plant fibres and those that do not. Williams and Strachan (1984a,b) found that the enzymes involved in the degradation of plant structural

polysaccharides were most active in the adherent particle-associated microorganisms, whereas, non-structural soluble saccharides were metabolised by the liquid phase, non-adherent population when these authors separated rumen digesta into liquid and particulate phases and examined the level of activity of polysaccharide-degrading enzymes in the microbial population in both phases. *Fibrobacter succinogenes* is known to possess an extracellular cellulase which is secreted and diffuses in the environment. Studies with *Ruminococcus albus* have indicated that this cellulase is a complex of several enzymes with specific functions in the stepwise degradation of cellulose to glucose (Yu and Hungate, 1979). Most of the predominant cellulolytic *Ruminococcus* spp. degrade and utilise hemicellulose (Table 2). The activities of the enzymes are regulated, but the exact mechanism is still unclear.

Rumen fungi exhibit greater capability for the degradation of lignified plant tissues (Akin and Benner 1988). Presently six anaerobic genera have identified; *Neocallimastix*, *Piromyces*, *Caecomyces*, *Orpinomyces*, *Anaeromyces* and *Cyllamyce* (Puniya *et al.*, 2014). They produce a broad range of hydrolases, including cellulases (Paul *et al.*, 2004), hemicellulases (Mountfort and Asher, 1989), proteases (Michel *et al.*, 1993), amylases and amyloglycosidases (Paul *et al.*, 2004), feruloyl and p-coumaroyl esterases (Paul *et al.*, 2004), various disaccharidases (Chen *et al.*, 1994), pectinases (Gordon and Phillips, 1992), exonucleases or avicelases (Cabe, 1998).

Fungal cellulases have been classified into three major groups: (1) cellobiohydrolases (1,4- β -D-glucan cellobiohydrolase, EC 3.2.1.91), which attack cellulose molecules stepwise from the nonreducing end, liberating cellobiose; (2) endoglucanases (1,4- β -D-glucan 4 glucanohydrolase, EC 3.2.1.4) which randomly cleave β -glucosidic bonds in the middle of cellulose chains; (3) β -glucosidases or cellobiase (EC 3.2.1.21), which hydrolyse

Table 2. Major rumen bacterial species grouped according to substrate fermented (compiled from Yokoyama and Johnson (1988) with species names updated (Krause and Russell, 1996)).

Cellulolytic species	<i>Fibrobacter succinogenes</i> <i>Ruminococcus flavefaciens</i> <i>Ruminococcus albus</i> <i>Butyrivibrio fibrisolvens</i>
Hemicellulolytic species	<i>Bacteroides ruminicola</i> <i>Butyrivibrio fibrisolvens</i> <i>Ruminococcus</i> sp.
Pectinolytic species	<i>Butyrivibrio fibrisolvens</i> <i>Bacteroides ruminicola</i> <i>Lachnospira multiparus</i> <i>Succinivibrio dextrinosolvens</i> <i>Treponema bryantii</i> <i>Streptococcus bovis</i>
Amylolytic species	<i>Ruminobacter amylophilus</i> <i>Streptococcus bovis</i> <i>Succinimonas amylolytica</i> <i>Bacteroides ruminicola</i>
Ureolytic species	<i>Succinivibrio dextrinosolvens</i> <i>Selenomonas</i> sp. <i>Bacteroides ruminicola</i> <i>Ruminococcus bromii</i> <i>Butyrivibrio</i> sp. <i>Treponema</i> sp.
Methane-producing species	<i>Methanobrevibacter ruminantium</i> <i>Methanobacterium formicicum</i> <i>Methanomicrobium mobile</i>
Sugar-utilising species	<i>Treponema bryantii</i> <i>Lactobacillus vitulinus</i> <i>Lactobacillus ruminus</i>
Acid-utilising species	<i>Megasphaera elsdenii</i> <i>Selenomonas ruminantium</i>
Proteolytic species	<i>Ruminobacter amylophilus</i> <i>Bacteroides ruminicola</i> <i>Butyrivibrio fibrisolvens</i> <i>Streptococcus bovis</i>
Ammonia-producing species	<i>Bacteroides ruminicola</i> <i>Megasphaera elsdenii</i> <i>Selenomonas ruminantium</i>
Lipid-utilising species	<i>Anaerovibrio lipolytica</i> <i>Butyrivibrio fibrisolvens</i> <i>Treponema bryantii</i> <i>Eubacterium</i> sp. <i>Fusocillus</i> sp. <i>Micrococcus</i> sp.

glycosidic bonds to terminal non-reducing residues in β -D-glucosides and low molecular weight oligosaccharides into glucose. These enzymes work in unison (Eriksson *et al.*, 2002, Jalak *et al.*, 2012) and it has been proposed (Lynd *et al.*, 2002) that endoglucanases randomly attack the amorphous regions of cellulose, thereby creating sites for cellobiohydrolases, which then proceed into the crystalline regions of the fibre. With β -glucosidases performing the last hydrolysis step, thus preventing the build-up of cellobiose, which inhibits cellobiohydrolases. However, the interpretation of this mechanism is still debated (Jalak *et al.*, 2012).

5. Pectins

Pectins are a group of amorphous galacturonic acid-rich polysaccharides. Galacturonic acid comprises approximately 70% of pectin (Mohnen, 2008). Pectin is an important component of the plant cell wall and is typically included with the non-structural carbohydrates. Pectin is interconnected by α 1-4 linkages, leading to non-linearity and coiling of the polygalacturonans (rhamnogalacturonans) main chain with L-rhamnose inserts (Thakur *et al.*, 1997). Some of the rhamnose units are branched with side chains of L-arabinose (arabinan) and D-galactose (galacans) or both (arabinogalactans), and some galacturonic acid units may be present as methyl esters or as acetates. The variation in polysaccharide content of the pectic fraction depends on the method of isolation, plant developmental stage, tissues extracted and species. The complicated structure of pectin has been demonstrated in the review by Caffall and Mohnen (2009). In general, the pectin content of legumes is higher than grasses (Mohnen, 2008). The amounts of extractable pectic polysaccharides, on a cell wall basis, range from 1.0-10% in grasses and 20 to 35% in dicotyledons (Mohnen, 2008).

The distinction between pectin and hemicellulose is by no means clear. Chemical classification, according to solubility, characterises pectins as soluble in hot neutral solutions of ammonium oxalate or EDTA, whereas hemicellulose requires acid or alkali treatment. But there is an overlap between pectic and hemicellulosic groups. Some hemicellulose in delignified tissues is hot-water soluble, while some galacturonic acid is not accessible to hot EDTA and is trapped in cell walls (Hatfield, 1989). Covalent crosslinks have been detected between pectins and hemicellulose (Broxterman and Schols, 2018). Pectins bind calcium to the same extent as organic acids. Pectins can bind with iron and zinc (Gawkowska *et al.*, 2018), but there is no significant binding to magnesium (Gawkowska *et al.*, 2018).

As a true fibre, pectin is resistant to mammalian enzymes and must be digested by microorganisms in the gastro-intestinal tract. The major pectinolytic bacteria in the rumen are listed in Table 2. *Butyrivibrio fibrisolvens* possesses an extracellular pectinolytic enzyme, an exopectate lyase which breaks the pectin chain at the terminal end. Other pectinolytic rumen bacteria possess endopectate lyase, which cleaves randomly along the pectin chain (Wojciechowicz *et al.*, 1982). Pectin is first hydrolysed to pectic acid and methanol by pectinesterase. The pectic acid is then cleaved by polygalacturonidases to galacturonic acids, which, in turn, yields xylose (McDonald *et al.*, 1973). The pectin-fermenting protozoa are the genus *Isotricha* (Table 3). The end-products are VFAs, lactic acid, lipids and hydrogen. Pectin appears to be a readily degradable source of energy in the rumen, but does not lower rumen

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Table 3. The major genera of ciliate protozoa and substrates fermented in the rumen (data of Williams and Coleman (1985) and Yokoyama and Johnson (1988)).

Genera	Substrates fermented
<i>Isotricha</i>	
<i>Intestinalis</i>	starch, sucrose, glucose and pectin
<i>Prostoma</i>	starch, sucrose, glucose and pectin
<i>Dasytricha</i>	
<i>Ruminantium</i>	starch, maltose, cellobiose and glucose
<i>Entodinium</i>	
<i>Bursa</i>	starch and hemicellulose
<i>Caudatum</i>	starch, cellobiose, glucose, maltose and sucrose
<i>Furca bilobum</i>	
<i>Simplex</i>	starch
<i>Diplodinium</i>	
<i>Polyplastron</i>	cellulose, glucose, starch and sucrose
<i>Dipodinium</i>	cellulose, hemicellulose and starch
<i>Diploplastron</i>	cellulose, hemicellulose and starch
<i>Eudiplodinium</i>	cellulose, hemicellulose and starch
<i>Ostracodinium</i>	cellulose, hemicellulose and starch
<i>Eremoplastron</i>	cellulose, hemicellulose and starch
<i>Epidinium</i>	
<i>Ecaudatum caudatum</i>	cellulose, hemicellulose, starch, sucrose and maltose
<i>Ophryoscolex</i>	
<i>Caudatus</i>	cellulose, hemicellulose and starch

pH from lactate production, which occurs with other non-structural carbohydrates (Ben-Ghedalia *et al.*, 1989; Strobel and Russell, 1986). Pectin is unusual among rumen fermentable energy sources because it possesses rapid and extensive degradation characteristics of other non-structural carbohydrates without the propensity to lower rumen pH or contribute to acidosis. The fermentation of pectin *in vitro* increased acetate but not lactate production in cattle (Strobel and Russell, 1986), and similar results were reported in sheep (Ben-Ghedalia *et al.*, 1989). Chesson and Monro (1982) showed that legume pectic substances were degraded at a faster rate and to a greater extent than other polysaccharides, but a portion was resistant to repeated ammonium oxalate extraction and is not completely degraded in the rumen. They suggested that the pectic fraction resistant to microbial degradation may represent a portion cross-linked by phenolics or covalently bound to lignin. This was supported by visual evidence from electron microscopy (Wilson, 1994).

Pectin differs from other non-structural carbohydrate due to its fate during ensiling. Most of the non-structural carbohydrates and some of the starches are fermented, whereas pectins remain 90% unfermented after 90 days ensiling in lucerne (Ben-Ghedalia *et al.*, 1991). The apparent digestibility of pectin in sheep was much lower than hemicellulose and cellulose at all levels of pasture maturity (Waite *et al.*, 1964). Moreover, pectins disappeared from the reticulo-rumen of cattle at a rate between that seen for simple sugars and hemicellulose or cellulose, and hence may by-pass the rumen as undigested particles (Bailey, 1967).

6. Hemicellulose

Hemicellulose is the most complex of the plant polysaccharides and the most difficult to characterise, as it is a mixture of polysaccharides and includes xylans, β -glucans (gum), xyloglucan, and mannans. Recent research has shown that hemicellulose, in its different compositional configurations, has an important role in facilitating compression and tension (stretchiness), especially in the secondary plant cell wall (Berglund *et al.*, 2020); providing the plant with biomechanical flexibility.

Xylans

Xylans form the bulk of the hemicellulose fraction and appear to be based on linear chains of β 1-4 linked D-xylopyranose units carrying short side chains to a varying extent (Haltrich *et al.*, 1995). The chemical composition of the substitute side chain groups and the frequency of their occurrence depend on the origin of the xylan, as well as on the extraction procedures used for its isolation. Hatfield (1989) divided xylans into three main groups according to the type of substitutions on the main xylan chain: (1) arabinose producing arabinoxylans, (2) glucuronic acid and 4-O-methyl glucuronic acid forming glucuronoxylans and (3) combinations of arabinose and glucuronic acid giving glucuronoarabinoxylans.

Arabinoxylans are widespread in the cell walls of all flowering plants, but less abundant in non-gramineous species (Carpita, 1996). Arabinose residues are furanosidically attached to xylose units mainly at the 3- or at the 2- and 3-positions in the same xylose unit in wheat and rye (Theander *et al.*, 1993). In barley, many xylose residues are substituted by arabinose units only on the 2-position (Vieter *et al.*, 1992). The extent of substitution on the xylan backbone by arabinose varies from one in every two to eight xylose residues. Occasionally arabinose substitution may appear as short side chains of two to three residues containing arabinose, xylose and galactose (Wilkie, 1979). Wheat arabinoxylan contains 65.8% xylose, 33.5% arabinose, 0.1% mannose, 0.1% galactose, and 0.3% glucose (Gruppen *et al.*, 1992). Monosaccharide concentrations in some feedstuffs are listed in Table 4.

The glucuronic acid may be 1-2, 1-3 or 1-4 linked to xylose (Van Soest, 1994). The extent of substitution on the xylan backbone by glucuronic acid varies in legumes from one in every five to fifteen xylose residues. Grass xylans are generally considered to be less frequently substituted with glucuronic acid residues (Mazumder *et al.*, 2002). The predominant xylans are the glucuronoarabinoxylan type. Common structural features for this acidic type of polysaccharide are a main chain of β 1-4 linked xylose units to which single units of L-arabinose and D-glucuronic acid or its 4-O-methylether are attached. The degree of

Table 4. Monosaccharide composition of feedstuffs (% DM).

Feedstuff	Arabinose	Galactose	Mannose	Xylose	Glucose	Reference
Barley ¹	8.5-9.0	2.5-3.0	1.5-2.0	10	77.0	Bader Ul Ain <i>et al.</i> , 2019
Sorghum ¹	2.0	0.1-0.2	0.19-0.3	2.5	0.8-0.9	Bader Ul Ain <i>et al.</i> , 2019
Wheat ¹	33-34	2.0-3.0	6.0-7.0	45-50	1.5-2.0	Bader Ul Ain <i>et al.</i> , 2019
Barley straw	4.0	1.71	1.26	15		Holtzapple, 2003
Cotton gin trash	2.0	0.1	1.9	4		Holtzapple, 2003
Maize stover	2.8	0.75	0.25	13		Holtzapple, 2003
<i>Medicago murex</i> cv. Zodiac	2.67	2.17	1.04	8.12		Li, 1992
Rice hulls	2.6	0.1	2.7	12.3		Holtzapple, 2003
Rice straw	4.0	0.4	1.6	13		Holtzapple, 2003
Sorghum straw	3.0	0.2	0.8	15		Holtzapple, 2003
<i>Trifolium resupinatum</i> cv. Kyambra	3.09	3.22	1.93	5.05		Li, 1992
<i>Trifolium subterraneum</i> cv. Junea	3.14	2.17	0.8	4.99		Li, 1992
Wheat straw	2.1	2.16	0.72	16.9		Holtzapple, 2003

¹ (%) in endospermic cell wall.

polymerisation of xylans is much lower (50-200 residues) than that of cellulose (up to 10,000 units). The presence of side sugar units and substitutions may prevent tight association between xylan chains (Theander and Aman, 1984). The percent of xylose and the xylose to arabinose ratio increase with plant maturity in grasses (Brice and Morrison, 1982; Burritt *et al.*, 1984).

Xylans and their associated glucuronic acid from lucerne cell walls were the least digestible cell wall carbohydrates when fed to sheep (Ben-Ghedalia and Miron, 1984). Burritt *et al.* (1984) found the correlation between percent xylose and *in vitro* DM digestibility was -0.85 ($P<0.01$) and this correlation was significantly increased in maturing grass cell walls ($r=-0.94$, $P<0.01$). This implied that more of the xylan chain was linked to lignin *via* glucuronic acid or directly to lignin, resulting in reduced digestibility in mature grass (Ben-Ghedalia and Miron, 1984). Nordkvist and Aman (1986) found that xylans played a dominant role in lucerne digestibility and there was a very strong relationship between xylose content and *in vitro* degradability ($r^2=0.96$, $P<0.01$). They stated that it was possible to get good prediction of nutritive value of lucerne from analysis of the xylose content. However, monosaccharide composition and cellulose to hemicellulose ratios have not proven to be generally useful for determining the chemical nature of indigestibility. As isolated polysaccharides, both cellulose and hemicellulose are fully digestible (Wilson, 1994).

Structural features of xylans play a role in the extent of degradation. The xylose to arabinose ratio is indicative of the degree of arabinose branching from the main xylose backbone i.e. the linearity or branching of hemicellulose (Albrecht *et al.*, 1987). McNeil *et al.* (1975) suggested that a lower degree of arabinose branching may increase the formation of noncovalent bonds between the xylose backbone and cellulose, which would increase the rigidity of cell walls when they mature. The effect of xylan-cellulose binding on carbohydrate degradation in the rumen is unknown (Albrecht *et al.*, 1987). Advances in genetic and

biochemical research may provide knowledge and insights for manipulation of digestion in the gastrointestinal tract and understanding of cell wall digestion (Hespell and Whitehead, 1990).

The effects of the extent of arabinose-substitution of the xylan chain on degradability are controversial. Brice and Morrison (1982) reported that the extent of purified mature ryegrass hemicellulose degradation was directly related to the xylose to arabinose ratio. Xylans with a high degree of arabinose substitution (xylose:arabinose, 3:1) were less readily degraded by hemicellulases isolated from the rumen than less frequently substituted xylans (xylose:arabinose, 8:1; Brice and Morrison, 1982) because arabinose side chains must be removed before the xylose backbone can be hydrolysed. However, their study utilised only one specific enzyme complex that may not have contained all the activities required to degrade branched xylans. It is not clear if this relationship exists *in vivo* because there are the synergistic activities of multiple forms of hydrolases in the rumen. Wedig *et al.* (1987) inoculated lucerne and orchard grass hays with rumen fluid and stated that a high xylose to arabinose ratio indicated a high degree of polymerisation with little bonding with other monosaccharides and low digestibility. In addition, a low xylose to arabinose ratio indicated a short chain polymer with a large amount of branching with other monosaccharides and high digestibility. In digestibility trials, arabinose side chains linked to a xylose backbone were more readily degraded than in-chain xylose units in wheat straw, barley straw, white clover, ryegrass (Gordon *et al.*, 1983) and lucerne (Nordkvist and Aman, 1986).

Beta-glucans and xyloglucans

Beta-glucans consist of mixed β 1-3 and β 1-4 linked D-glucose in linear chains. Approximately 50-70% of the molecule is made up of glucose residues linked β 1-4 and the remaining 30% as linked β 1-3 (Carpita, 1996). Bailey and Gaillard (1965) found that β -glucanase could cleave

both β 1-3 and β 1-4 linked glucose disaccharides from ryegrass and red clover hemicellulose.

Xyloglucans are the most abundant glucans in primary cell walls of legumes and some cereals. All xyloglucans consist of a backbone of β 1-4 linked glucopyranose units, and the side units are xylopyranose attached by α 1-6 linkages to xylose units. In addition, galactose and fucose may attach to xylose units (Hatfield, 1989). Xyloglucans and hydroxyproline-rich glycoprotein (extensin) with side chains of L-arabinose and D-galactose form a highly branched structure that is resistant to digestion (Theander *et al.*, 1993). Xyloglucans and β -glucans have similar properties, and stain blue-black with iodine, but have no relationship to starch (Yuguchi *et al.*, 2005).

Mannans

Mannans are non-cellulosic hexosans with linear chains consisting of β 1-4 linked D-glucopyranose and D-mannopyranose. Mannans occur in a pure form that are free of other sugars or glucomannans, galactomannans and galactoglucomannans, containing different amounts of α -linked glucose or galactose as side chains along the main chain (Ibrahim *et al.*, 2009). Mannan molecules are packed together, but not as tightly as cellulose molecules and are associated with plant cell walls, but to a lesser extent than cellulose (Warren, 1996). Galactoglucomannans are extractable with strong alkali (15-24%), whereas glucomannans require 24-4% alkali-borate (Bailey, 1973).

Hemicellulose is more closely related to lignin than other polysaccharide fractions because ester bonding occurs between components of lignin and xylose, arabinose and uronic acid (Barton *et al.*, 1982). Van Soest (1994) noted that only arabinoxylans and glucuronides may be lignified, but the gum-like mannans and xyloglucans cannot be lignified. The content of non-cellulosic polysaccharides in grasses (including temperate and tropical species) is much higher than in legumes (Butler and Bailey, 1973; Lowry *et al.*, 1992).

Hemicellulose digestion

Digestion of hemicellulose is complex, as it is a mixture of various sugars and glucosidic linkages. Moreover, the characteristics of hemicellulose vary greatly in different herbages and plant cell wall types (Chesson, 1990). Hemicellulases are produced by some rumen bacteria, ciliate protozoa (Table 2 and 3) and fungi. Morrison (1979) reported that the hemicellulases isolated from cell free rumen fluid can only hydrolyse the bond between two unsubstituted xylose residues in the main chain of isolated xylans from grasses. The products are oligosaccharide fragments (not xylose) which are small enough to be utilised by bacteria. Hemicellulolytic enzymes present in filtered rumen fluid are the endo type, which means they are

capable of cleaving a variety of linkages randomly. Enzymes hydrolysing side chains include α -D-glucosiduronidase and α -L-arabinofuranosidases, whereby the former attack the α -D-(1-2) linkage in glucuronoxylans and the latter the 1-3 linkage branch points in arabinoxylan. Xylanase efficiently degrades linear xylan chains, and slowly or incompletely degrades branched molecules. The activities of the enzymes produced by polysaccharide-degrading rumen bacteria, protozoa and fungi are influenced by carbohydrate substrate, with polysaccharide substrates producing higher more polysaccharidase (Williams, 1988).

Ruminants digest more hemicellulose than cellulose when fed grass diets and more cellulose than hemicellulose when fed legumes (Wedig *et al.*, 1987). Digestibility of hemicellulose is closely related to that of cellulose and negatively related to lignification. The main factors controlling organic matter digestibility are the concentrations of both hemicellulose and cellulose and the extent to which they are lignified (Minson, 1971). Feruloyl and p-coumaroyl esterases from fungi breakdown the ester linkages between hemicellulose and lignin in plant cell walls, permitting separation of hemicelluloses and cellulose from lignin (Borneman and Akin, 1990), thus increasing the rate of digestion by cellulases and hemicellulases.

Arabinose, mannose and galactose are degraded more rapidly in the rumen than glucose and xylose (Li *et al.*, 1992) and xylose is the least digestible among cell wall neutral monosaccharides (Li, 1992). In ruminants, higher concentrations of xylose and mannose appear to decrease digestibility of forages (Li, 1992; Li *et al.*, 2012). Galacto-mannans reduced the degradable DM fraction which indicated that the complex may inhibit microbial fermentation (Li *et al.*, 1992). Addition of pentoses (L-arabinose, D-ribose and D-xylose at a concentration equivalent to 25% hay DM) reduced fermentation of hay by rumen microorganisms and increased propionate and valerate concentrations (Gascoyne *et al.*, 1988).

Although the microbes listed in Table 2 and 3 can digest different components of feed, they are individually incapable of producing the variety of enzymes that are required to efficiently digest an entire ration. Polysaccharide breakdown in the rumen depends on the synergetic activities of multiple forms of hydrolases from a complex microbial population (Chesson, 1990; Jalak *et al.*, 2012; Williams, 1988).

7. Phenolic compounds

The term 'phenolic compounds' embraces a wide range of substances which possess an aromatic ring bearing a hydroxyl substituent, including their functional derivatives. These compounds, with more than 8,000 currently identified structures (Dai and Mumper, 2010), are widely distributed in leaves, stems, roots, flowers, fruits and seeds and are

almost universally present in animal feeds derived from plants (Singleton and Kratzer, 1969). In this review the focus was on the most abundant natural phenolic compounds in animal feeds, i.e. lignins and tannins. Both of these are either wholly or partially derived from the products of the shikimic acid pathway (Knaggs, 2001) and are present in plant tissues as polymers, but not in a free state nor in simple combinations (as glycosides or esters). Tannins are distinguished from lignin by their relative solubility and ability to combine with protein to form leather-like precipitates, which are resistant to attack by proteases or microbes (Van Soest, 1994). Moreover, most tannins have a molecular weight of 300-3,000 daltons, whereas lignin exceeds 5,000 daltons (Swain, 1979). Despite their molecular differences, lignin and tannins have anti-nutritive characteristics and are equally effective in preventing over-grazing by herbivores (Kara and Sürmen, 2019).

8. Lignins

Lignins are the second most abundant organic polymer in the world after cellulose (Zeng *et al.*, 2013). They provide mechanical support and give strength and rigidity to plant cell walls and therefore plants can tolerate with abiotic and biotic stresses (Frei, 2013). Lignin is important during plant growth and development, as it facilitates water conduction through xylem vessels. Lignin from plant residues has a significant impact on soil organic carbon cycling, as a carbon pool influencing nutrient mineralisation and in carbon sequestration (Frei, 2013).

The structure of lignins varies between plant species, types of cell and parts of the cell wall within the same plant, but the complete structure and subcomponents are not clearly defined (Whetten *et al.*, 1998). Unlike polysaccharides, which can be hydrolysed to give simple, well-defined products, it is a challenge to depolymerise lignin by hydrolysis with chemical agents or enzymes because the

polymer possesses an ordered structure which involves strong carbon-carbon or ether linkages interlinking its constituents. Therefore, it is difficult to do structural analysis. Lignin content and composition vary substantially between plant species and analytical methods (Table 5).

High temperature and/or drought increases lignin content in *Lolium perenne* L. and *Medicago lupulina* L. (Erice *et al.*, 2006; Farfan-Vignolo and Asard, 2012). Lignins are generally more condensed and potentially less reactive in legumes than those isolated from grasses. As plants mature, lignin concentrations increase (Dixon and Summer, 2003; Jung *et al.*, 1997). In animal nutrition, lignins play a crucial role in limiting the availability of plant cell wall material to herbivorous animals and anaerobic digestion systems (Grabber, 2005; Hatfield and Fukushima, 2005; Javaid *et al.*, 2018). Lignins are a significant anti-nutritive factor in ruminants and other herbivores, although lignified dietary fibre plays an important role in maintaining gastrointestinal function and health in humans (Ferguson *et al.*, 2001). Several investigators (Buxton and Russel, 1988; Hartley, 1972; Lapierre, 1993) have divided the lignin of forages into non-core and core components.

Non-core lignins

Non-core lignins, which act as precursors for core lignin synthesis, are monomers composed primarily of ester-linked *p*-coumaric acid and ferulic acid residues (Theander and Aman, 1984). Non-core lignins commonly have only one covalent linkage of a phenolic compound (usually cinnamic acid) to either core lignin or hemicellulose. Ferulic acid is mainly associated with hemicellulose whereas *p*-coumaric acid is associated mainly with core lignin (Figure 1) (Ralph and Helm, 1993).

Core lignin

Core lignin is comprised of highly condensed, high molecular weight polymers of cinnamyl alcohols in a three-dimensional structure (Ganewatta *et al.*, 2019). Core lignin usually has two or more covalent bonds between monomers within its molecule. The most important precursors in its biosynthesis are three closely related phenylpropanoid monomers (C6-C3), which differ in methoxylation at the 3- and 5-carbon positions of the aromatic ring, i.e. *p*-coumaryl (no methoxylation) alcohol (I), coniferyl (monomethoxylation) alcohol (II), and sinapyl (dimethoxylation) alcohol (III) (Theander and Aman, 1984). In polymers, these precursors are present as the corresponding structural components: *p*-hydroxyl benzaldehyde, guaiacyl (vanillin) and syringaldehyde, respectively (Grabber *et al.*, 1997; Vanholme *et al.*, 2010). More monomers have been identified as being involved in lignin polymerisation, including hydroxycinnamic acids

Table 5. Lignin composition of forages (% DM) (Jung *et al.*, 1997).

Forage species	Acid detergent lignin	Klason lignin
Legumes		
Lucerne	7.7-9.4	10.4-13.5
Birdsfoot trefoil	8.8-9.4	12.2-14.3
Ladino clover	4.9-5.2	9.5-10.3
Red clover	6.3-7.9	13.4-13.7
C3 grasses		
Barley	2.1-2.9	6.6-8.7
Oats	2.9-4.1	9.5-10.7
Orchardgrass	2.7-3.9	11.6-12.5
Reed canarygrass	1.6-2.2	9.6-10.1
Perennial ryegrass	2.8-3.4	8.4-11.7
Smooth brome grass	5.9-6.1	12-12.8
Tall fescue	1.7-2.5	6.4-8.4
Timothy	3.3-4.8	9.6-10.1
C4 grasses		
Big bluestem	3.9-4.8	7.2-10.3
Sorghum sudangrass	2.6-3.3	8.5-9.0

Figure 1. Linkage of p-coumaric and ferulic acids to other cell wall components (Jung, 1989).

and aldehydes, as well as coniferyl and sinapyl acetates or coumarates (Grabber *et al.*, 2010).

Barton *et al.* (1982) using C^{13} nuclear magnetic resonance (NMR), found the presence of lignin-carbohydrate complexes. Lignins have been found to bind to cellulose (Jung and Fahey, 1983a), xylose, arabinose, mannose and uronic acids of heteroxylans of hemicellulose (Lawoko *et al.*, 2005; Mueller-Harvey *et al.*, 1986) (Figure 2, Iiyama *et al.*, 1994). The amount of binding increases with plant maturity (Bailey, 1973). Characterising the lignin-carbohydrate complex has been the subject of research for many decades in ruminant nutrition (Moreira *et al.*, 2013; Ralph and Helm, 1993) as well as biofuel production (Balakshin *et al.*, 2011; Zeng *et al.*, 2013).

Lignins associate with cell wall proteins rich in hydroxyproline and glycine to form complexes which are nutritionally unavailable (Iiyama *et al.*, 1993). The lignin and protein cross-linkages reduce the solubility of the cell wall polysaccharide components, strengthen cell structure and are resistant to fermentation (Buxton and Redfearn, 1997). Cell walls of parenchymatous tissues in dicotyledonous plants contain polyphenolic compounds (5-10%) in the form of polysaccharides-protein-polyphenol complexes (Selvendran, 1983). Morrison (1980) found the concentrations of lignin and hemicellulose to be highly correlated ($r=0.87$) in ten varieties of temperate grasses.

Lignins are the main factor limiting digestibility in forages (Fukushima *et al.*, 2015). Kondo *et al.* (1987) found a significant negative relationship between *in vivo* DM digestibility and lignin (measured as acid detergent fibre) content $r=-0.98$ ($P<0.001$) for Italian ryegrass $r=-0.97$ ($P<0.001$) for orchard grass, $r=-0.97$ ($P<0.01$) for perennial ryegrass, and $r=-0.98$ ($P<0.05$) for lucerne. Buxton and Russel (1988) reported that the correlation coefficients of lignin concentration and cell wall digestibility in grass and legume stems were -0.96 and -0.92, respectively. This suggested that cell walls would be completely digested if no lignin existed. Saponification of the lignin-hemicellulose linkages following alkali treatment increases digestibility of the cellulosic matrix of grasses (Hartley, 1978). In legumes and other dicotyledonous plants, however, the lignin-carbohydrate linkages are much less susceptible to hydrolysis by alkali and any increase in digestibility is restricted by the extent of cleavage of carbohydrate from the lignified matrix (Van Soest, 1981). Destruction of lignin-linked p-coumarates, ferulates and hemicellulose by alkali improved enzymatic access to polysaccharide and enhanced the hydrolysis of xylan (Martínez *et al.*, 2016).

Lignin has been reported to protect between 1.4 (Van Soest, 1981) and two times (Grabber, 2005) its own weight of cell wall carbohydrates from microbial fermentation. This is dependent upon covalent linkages between carbohydrates and lignin. The concentration of cell wall material is able to moderate the effect of lignification. Lucerne contains

Figure 2. Linkages between lignin and polysaccharides (Iiyama *et al.*, 1994).

less cell wall and a higher lignin content compared to a grass with the same digestibility (Van Soest, 2015). The cross-linkage of matrix components may affect cell wall degradability to a greater extent than lignin concentration alone (Jung and Casler, 1991). However, any reduction in lignin concentration, hydrophobicity and cross-linking can improve enzymatic hydrolysis and utilisation of structural polysaccharides for nutritional and industrial purposes (Grabber, 2005). Lignin limits cell wall digestion to a greater extent than total forage DM digestibility. Although hemicellulose is covalently linked to lignin, its digestibility is not more affected by lignin than cellulose digestion *in vitro* (Jung and Vogel, 1986).

Lignin reduces cell wall digestibility by combining with cell wall components (Jung, 1989; Morrison, 1974) and by direct inhibitory effects of phenolic acids on rumen microorganisms (Grabber *et al.*, 1997; Jung and Fahey, 1983a,b). P-coumaric and ferulic acids depress cellulolytic activities of *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* (Chesson *et al.*, 1982). P-coumaric acid inhibits the growth of rumen xylan-utilising bacteria and decreases the motility of the rumen protozoa with *Entodiniomorphs* spp. being more susceptible than *Holotrichs* spp. (Akin, 1982). P-coumaric, ferulic and sinapic acids inhibit the ability of rumen fungi to colonise and degrade lucerne and bermudagrass fibres, although these microbes have the ability to attack certain lignified

tissues (Akin and Rigsby, 1985). Supplementation with *p*-coumaric, ferulic, salicylic and vanillin linearly depressed *in vitro* cellulose and starch digestibilities (Jung and Fahey, 1983b). *Trans*-*P*-coumaric acid inhibited apparent DM digestibility, reduced VFA accumulation, and changed the molar ratio of acetate/propionate/butyrate (Theodorou *et al.*, 1987). Methyl- and butyl-esters of *p*-coumaric acid were more toxic than free *p*-coumaric acid (Hartley and Akin 1989), whereas *p*-coumaric acid was more toxic than any other phenolic acid to rumen microorganisms (Akin, 1986; Jung, 1989; Varel and Jung, 1986). *P*-coumaric acid (Buxton and Russel, 1988), ferulic acid (Hartley, 1972), vanillin (Cymbaluk *et al.*, 1973), *p*-hydroxybenzaldehyde (Cymbaluk *et al.*, 1973; Reeves, 1985), syringaldehyde (Reeves, 1985) and the *p*-coumaric acid to ferulic acid ratio (Burritt *et al.*, 1984) were negatively associated with cell wall and DM digestibility. The presence of phenolics depresses mammalian enzyme activities in *in vitro*, and activities of pancreatic RNase have been shown to be inhibited by caffeic, ferulic, gallic and syringic acids, and pancreatic trypsin, lipase and amylase were inhibited by phenolics in studies conducted by Jung and Fahey (1983a). Varel and Jung (1986) suggested that phenolic monomers inhibited digestibility of cellulose and xylan, possibly by influencing the attachment of fibrolytic microorganisms to fibre particles.

Lignin has often been stated to be indigestible (Arora and Sharma, 2009; Falcon *et al.*, 1995; Kara and Sürmen, 2019; Li *et al.*, 1992) as there is no known ruminal anaerobic fermentation (Van Kuijk *et al.*, 2015) or mammalian enzymatic systems for its degradation. In contrast, Fahey *et al.* (1979) and Jung *et al.* (1983) found that lignin is partially digestible, mainly in the stomach, with only minor changes taking place in the intestines. White rot fungi (Rahman *et al.*, 2011) and termites (Azizi-Shotorkhoft *et al.*, 2016) are able to degrade lignin. White rot fungi produce an array of multienzyme complexes including lignin peroxidases, manganese peroxidases, versatile peroxidases, and laccases (Wong, 2009). These enzymes synergistically and efficiently degrade lignin. Such organisms do not live in the digestive tract, but may hold the future for ligninolytic enzyme production and shed light for biodegradation and open new perspectives in peroxidation chemistry and biotechnology.

The variation in the reports on digestibility and the recovery of lignin may be attributed to various factors (Van Soest, 1994): (1) the incomplete knowledge of lignin structure limits the specificity of all analytical methods used for lignin analysis (Hatfield and Fukushima, 2005); (2) feed and faecal lignin distribution differs relative to particle size; feed lignin tends to be associated with coarse material, whereas faecal lignin is associated with fine material, and very fine faecal lignin particles may be lost during filtration; (3) methods for drying and heating during sample preparation may generate Maillard products; (4) lignin isolated from immature plants

of low lignin content tends to contain digestible non-lignin components which would account for the higher apparent digestibility of lignin in low-lignin materials; (5) lignin in immature grasses are polymerised to a lesser degree, and these low-molecular-weight fragments are absorbed and excreted in urine, thus leading to increased apparent digestibility. Ruminant urine is high in phenols. Ferulic acid in legumes is more digestible than that in the grasses, and is more digestible than *p*-coumaric acid in both grasses and legumes (Jung *et al.*, 1983).

Lignin has long been considered an antinutritional factor as it cannot be readily fermented by rumen microorganisms and inhibits microbial fermentation of the cell wall. Scientists have attempted to reduce lignin content of plants by traditional plant breeding, natural and induced mutation and insertion of transgenes. However, a significant reduction in lignin content caused significantly negative impacts on plant DM yield and long-term survival of some perennial species (Pedersen *et al.*, 2005). Further study is required to incorporate lignin-reducing genes into other genetic backgrounds to achieve moderate lignin reduction without compromising plant health, survival and function.

9. Tannins

Tannins, like lignin, are chemically not well defined, but are rather a group with common properties (Smeriglio *et al.*, 2017). Tannins are any phenolic compound of high molecular weight that contain a large number of phenolic hydroxyls enabling the formation of strong complexes with proteins and other macromolecules, such as cellulose, hemicellulose, pectin, starch and alkaloids (Haslam, 1979). Tannin concentration decreases as plants mature due to the declining activity of enzymes involved in tannin synthesis. Plants grown at high temperatures and in low-fertility, acid soils produce more tannins (McMahon *et al.*, 2000). Research has identified detrimental effects of tannins at high concentrations in feed, but there are beneficial effects at low concentrations in ruminants. Although the classification of tannins presents many problems, it is usual to divide them into two groups: hydrolysable and condensed tannins (Barbehenn and Constabel, 2011).

Hydrolysable tannins

Hydrolysable tannins have a core of polyhydric alcohol, usually glucose, whose -OH groups are esterified, either partly or wholly, to one or more phenolic carboxylic acid such as gallic acid or *m*-digallic acid (gallotannins) or hexahydroxydiphenic acid (ellagitannins) (Jansman, 1993) with molecular weights of 500-3,000 Da (McSweeney and Mackie, 1997). Tannic acid is a well-known gallotannin and contains 8-10 moles of gallic acid per mole of glucose (Freudenberg and Weinges, 1962). The polyester type of structure of these tannins is easily hydrolysed by acids,

bases or tannases derived from fungi such as *Aspergillus* and *Penicillium* spp. (Mangan, 1988; Pierpoint, 1983). Hydrolysable tannins are hydrolysed in the intestine of non-ruminants, although tannases have not been detected in animal tissues or in intestinal bacteria. These hydrolysis products can be absorbed by the gut and the sugars are metabolised, whereas the gallic acids or other phenols are excreted after hydroxylation, conjugation (Pierpoint, 1983), or methylation using dietary methionine and choline as a source of methyl groups (Potter and Fuller, 1968). Partial hydrolysis often occurs spontaneously during extraction and purification (Ribereau-Gayon, 1972). Hydrolysable tannin binds strongly to proteins at pH 3–4, but to a continuously decreasing extent above pH 5 (Barry and Blaney, 1987).

Condensed tannins

The condensed tannins, called proanthocyanidins (Rauf *et al.*, 2019), are mainly derivatives of flavanols and consist of oligomers of the flavan-3-ols (catechins) and flavan-3,4-diols and have a molecular weight of 1000–20,000 Da (McSweeney and Mackie, 1997). These are classified as leucoanthocyanidins as they polymerise upon heating in acid solutions into phlobaphene-like products (tannin-red) as flavan-3-ols do and to anthocyanidin (Ribereau-Gayon, 1972). Both catechins and leucoanthocyanidins are easily converted, by dehydrogenating enzymes or in dilute mineral acids at room temperature, into flavonoid tannins (McLeod, 1974). These are often called catechin tannins, flavolans or procyanidins (Jansman, 1993). Unlike the hydrolysable tannins, condensed tannins do not have a central carbohydrate, but occur as a polymer (Smeriglio *et al.*, 2017). Like lignin, condensed tannins are resistant to hydrolytic degradation due to continuous carbon to carbon linkages

Tannins have a greater affinity for protein than any other substance. Thus tannin-protein binding can occur during mastication and in salivation (Burritt *et al.*, 1987), along with binding to polysaccharides and nucleic acids (Swain, 1979). Condensed tannins have been shown to be highly selective in their binding, whereby large, conformationally loose, proline-rich proteins are bound several orders of magnitude more strongly than small, compact proteins (Hagerman and Butler, 1981). Increasing dietary reactive condensed tannin concentrations in the range of 0–11% of DM linearly increased the duodenal flow of non-ammonium nitrogen per unit of total nitrogen consumed in sheep fed fresh lotus-based diets (Barry and Manley, 1984). Moderate concentrations of tannins (2–5%) can have beneficial effects in ruminants (Getachew *et al.*, 2008; Min *et al.*, 2003; Pawelek *et al.*, 2008) by decreasing the loss (30%) of dietary nitrogen across the rumen, which occurs in sheep and cattle fed fresh diets containing ryegrass and white clover (Barry and Blaney, 1987). Moreover, the passage of protein-tannin complexes through the rumen facilitates protein digestion

in the abomasum and the small, resulting in an increased supply of amino acids and ruminal protein to the animal (Min *et al.*, 2003).

It has been suggested that tannin-containing legumes in pastures can help to control bloat (McLeod, 1974; McMahon *et al.*, 2000). Bloat in ruminants is caused by legumes and has been related to the formation of stable protein foam in the rumen, and tannins appear to inhibit foam production. There is renewed interest in using condensed tannins to prevent bloat, but further study is warranted to fully understand the interactions of these compounds with ruminal microorganisms and to determine their impact on the lower intestinal tract. In general, legumes such as sainfoin (*Onobrychis viciifolia*) and birdsfoot trefoil (*Lotus corniculatus*) containing condensed tannin more than 2–4% of DM are non-bloating (Barbehenn and Constabel, 2011; Rochfort *et al.*, 2008).

Condensed tannins have long been considered as antinutritive factors. They have astringent and inhibitory characteristics (Barbehenn and Constabel, 2011; McAllister *et al.*, 1994; Susmel and Stefanon, 1993) and precipitate the protein and glycoprotein in saliva, reducing its lubricant properties (Ribereau-Gayon, 1972). Several authors have found that high concentrations of condensed tannin (6–9% of DM) can depress cell wall digestibility, voluntary intake of digestible organic matter, metabolisable energy and liveweight gain in sheep (Barahona *et al.*, 2003; Barry and Manley, 1986) and cattle (Burns, 1985). Tannins decrease the digestibility of protein and carbohydrates (McAllister *et al.*, 1994; Susmel and Stefanon, 1993). They can lower rumen VFA, microbial DNA and RND production, low milk yield, low availability of sulphur (Kumar and Singh, 1984) and cause poor utilisation of phosphate by rumen microbes (Sadanandan and Arora, 1979). Tannins interfere with the absorption of iron (Liener, 1990) and cause degenerative changes in the intestines, liver, spleen and kidneys, which can result in mucus appearing in the urine and fatal constipation (Kumar and Singh, 1984). Tannins are able to bind with bacterial and fungal cell walls and affect their normal functions. Tannins sequester nucleic acids, proteins and polysaccharides in plant tissues and hence prevent the breakdown of these polymers by fungal and bacterial extracellular hydrolases (Swain, 1979).

The reduced digestibility of tannin-rich feeds may be due to its interaction with protein and carbohydrates to form insoluble and indigestible enzyme-resistant complexes, and interactions with enzymes *per se* may lead to interference with the digestibility of these substrates. Tannins can significantly inhibit digestive enzymes such as trypsin (Griffith and Mosley, 1980), α -amylase (Griffith and Moseley, 1980), cellulase (Smart *et al.*, 1961), lipase (Griffith, 1979) and enterokinase, which plays a crucial role in the activation of digestive proteases (Oh and Hoff, 1986) by

either forming insoluble protein-phenolic complexes and reducing the solubility of the enzyme protein or by forming a soluble but inactive enzyme-inhibitor complex (Loomis and Battaile, 1966). Most microorganisms are sensitive, to some extent, to the detrimental effects of tannins, even though some can tolerate high concentrations and even degrade and utilise the carbon of tannins for growth. Condensed tannins have a greater inhibitory effect on both enzymes and microorganisms than hydrolysable tannins and other phenols with low molecular weight. Polyphenols with ortho- and para-hydroxyls are more toxic to microorganisms than those with meta-hydroxyls, reflecting their higher potential for oxidation (McLeod, 1974). Tannins increase the excretion of endogenous nitrogen from intestinal mucus resulting in increased faecal nitrogen in rat (Elkin *et al.*, 1996), presumably reflecting increased mucus cell turnover in the small intestine (Sandaradura and Bender, 1984).

Barry *et al.* (1986) reported a positive and linear relationship between dietary reactive condensed tannin concentration and plasma growth hormone, and a negative relationship with 3,5,3'-tri-iodothyronine. Dietary condensed tannin concentrations had no effect on plasma thyroxine, insulin-like growth factors, prolactin, somatostatin, insulin and glucagon. The presence of some phenolic acids or catechin (condensed tannin monomer) *in vitro*, reduced Na⁺-dependent D-glucose uptake (Welsch *et al.*, 1989a) and inhibited intestinal sucrase (EC3.2.148) activity in brush border membrane vesicles from rat intestines (Welsch *et al.*, 1989b).

Scientists have made great efforts to investigate methods of feed detanninification (Gamble *et al.*, 1996; Silanikove *et al.*, 1996; Yu *et al.*, 1996). Polyethylene glycol can bind with condensed tannin and prevent reactions with protein or displace condensed tannin from its protein complex (Getachew *et al.*, 2000). Polyethylene glycol forms much stronger chemical bonds with condensed tannin than plant proteins, and does not release free condensed tannins (Barry and Manley, 1986). The complex of protein and tannins from sainfoin appears to contain simple hydrogen bonding which is reversible by polyethylene glycol and is most stable in the pH range 4-7. Outside this range, the complex readily dissociates. Therefore, protein-tannin complexes which escape rumen fermentation (at about pH 6.5) are readily degraded by gastric (about pH 2.5) and pancreatic (about pH 8-9) secretions (Mangan, 1988). Barry and Duncan (1984) sprayed high-tannin lotus with polyethylene glycol (molecular weight 3350) to combine with the condensed tannins, and reduced the total reactive tannin concentration from 6.3 to 0.7% of DM. This resulted in significant increases in voluntary intake of dry organic matter, metabolisable energy and total digestible nitrogen ($P<0.01$). Apparent digestibilities of organic matter, cellulose ($P<0.05$), hemicellulose ($P<0.01$) and total nitrogen ($P<0.001$) were significantly increased.

Many enzyme activities are inhibited by tannins and can be increased to a great extent by removal of the tannins with polyvinylpyrrolidone. This removal increases cellulose and protein digestibilities in lucerne (Jung and Fahey, 1981). More methods of removal or inactivation of tannins in ruminant feed have been described by Kumar and Singh (1984).

Genetic modification of plants may provide new insight to regulate tannin biosynthesis. However, tannins and lignin share the common biosynthetic pathway. There is positive relationship between tannin and lignin concentrations. Selection for moderate tannin content can be applied to avoid jeopardising plant normal growth and function by substantially varying lignin concentration (McMahon *et al.*, 2000).

10. Proteins

Proteins represent 5 to 10% of cell wall mass (Cassab and Varner, 1988) and are embedded in a complex network of polysaccharides. In general, leguminous plants have a higher crude protein content (15-25%) than temperate grasses (10-20%) and tropical grasses (5-10%), and crude protein content falls off as plants mature (Lyttleton, 1973). Proteins exist as either enzymes or structural components. Structural proteins mainly include the hydroxyproline-rich glycoproteins or extensins, the proline-rich proteins and the glycine-rich proteins.

Extensins are glycoproteins in the cell walls of plants. Extensins are rich in hydroxyproline and serine and combinations of valine, tyrosine, lysine and histidine in dicots, whereas, in monocots, extensins are rich in threonine-hydroxyproline and histidine-hydroxyproline and combinations of lysine and serine (Showalter, 1993). Hydroxyproline concentrations increase in cell walls with plant maturity (Lyttleton, 1973), contributing up to 20% of the amino acid content (Nordkvist, 1987). Most hydroxyproline residues are glycosylated with one to four arabinose residues (Kieliszewski *et al.*, 2011) and serine residues are glycosylated with a single galactose residue (Saito *et al.*, 2014). Extensin has a modifying effect on the properties of the cell wall by forming a framework with cellulose, which increases the strength of the wall. Extensins are widely distributed in dicots and less common in graminaceous monocots (Cassab, 1998). The difference in extensin distribution may be associated with the fact that the chemical structure of the primary cell wall of grasses and their progenitors differs from those of all other flowering plant species (Carpita, 1996). Extensin plays an important role in plant development, wound healing and plant defence (Showalter, 1993). Immunochemical studies have found that extensins accumulate in cell walls close to the area where microbial growth is restricted by the plant. Extensin may act as an impenetrable physical

barrier or may immobilise the pathogens by binding to their surfaces. Those with side chains of L-arabinose and D-galactose in the cell wall is resistant to human digestion. Protein containing hydroxyproline binds to lignin to form lignin-protein complexes which are not readily hydrolysed by enzymes from rumen microorganisms and prevent other cell wall components from degradation and utilisation (Meng, 2002).

Proline-rich glycoproteins contain high concentrations of proline, lysine and hydroxyproline and monosaccharides such as galactose and arabinose, and consist of 25% protein and 75% carbohydrate. The possible role of proline-rich proteins and hydroxyproline-rich proteins in the process of lignification has been proposed in the light of the observed affinity of phenolic substrates for prolyl residues in protein structures (Luck *et al.*, 1994). These glycoproteins may provide sites for selective complexation with its phenolic precursors (p-hydroxycinnamyl alcohols) and, therefore, points of growth for the lignin polymer (Iiyama *et al.*, 1994). Proline-rich glycoproteins have a high affinity for polyphenols (Butler, 1989). Herbivorous mammals can produce unique proline-rich proteins in the salivary glands (up to 45% proline) to provide the first line of defence against the presence of polyphenols in the digestive tract; dietary polyphenols which, in nature, repel predators and act as general feeding deterrents or antifeedant factors in herbivores (Cheeke, 1998). Proline-containing peptides degrade at a slower rate than other peptides in rumen fluid, and incorporating methionine and lysine into proline-containing peptides may protect both amino acids from microbial degradation in the rumen (Yang and Russell, 1992).

Glycine-rich protein consists of 68% glycine and 12% serine and is slightly glycosylated. Some glycine-rich protein contains tyrosine residues and, along with cysteine residues in cell wall proteins, may form cross-links with hydroxycinnamic acids esterified to wall polysaccharides through dehydrogenative dimerisation (Bacic *et al.*, 1988). Tyrosine residues enhance cross-linking of synthetic proteins (polylysine/tyrosine) into lignin-like dehydrogenation products (Cassab, 1998). Isodityrosine, a derivative of tyrosine (formed between two tyrosine residues from different extensin molecules), may serve as a cross-linking agent between different cell wall proteins (Showalter, 1993). Lignin-associated protein may play an important role in its polymerisation (Iiyama *et al.*, 1994). Some plant proteins are more resistant to rumen degradation than others. Hancock *et al.* (1994) found that sulphur-rich protein resist rumen degradation and are hydrolysed rapidly by intestinal proteases.

Kafirin and zein are the main storage proteins in sorghum and maize, respectively and account for approximately 50% of the protein in these grains (Duodu *et al.*, 2001). They

are classified as prolamins which are hydrophobic proteins and insoluble in water (Duodu *et al.*, 2003). Their high hydrophobicity makes them less accessible to enzymatic function in aqueous environments. Kafirin and zein have microbial resistance properties (Lawton, 2002). Moreover, kafirin protein and starch granules are associated within the sorghum endosperm and this interaction reduces starch digestibility.

11. Minerals

Minerals, which comprise the ash component of plant material, constitute about 10% of forage DM. Within this component are minerals that are essential nutrients for animals, (Jones and Wilson, 1987; Underwood and Suttle, 1999). Minerals can have important roles in interacting with other plant constituents, and thereby affect digestion. Importantly, mineral concentrations in plants can vary widely depending on plant species (legumes are higher in calcium, phosphorus, magnesium, copper and zinc contents than grasses), plant growth and maturity (calcium and phosphorus contents usually decline with growth and maturity; Hengmichai, 1987) and soil conditions (Underwood and Suttle, 1999). When potassium is deficient in the soil, grasses are able to extract the element from the soil more effectively than legumes (clovers in particular) (McNaught and Dorofaeff, 1968).

Calcium in plants can be found complexed to carboxyl groups on polysaccharides and to carboxyl or hydroxyl groups on phenolic acid or lignin monomers (Jones, 1978). Pectic rhamnogalacturonans may form stabilising chelated calcium bridges between adjacent chains (Rees, 1972).

Boron is required for lignin synthesis and therefore mainly occurs in the plant cell wall. Boron links with the rhamnogalacturonan fraction of pectins and these borate ester complexes may be responsible for crossing-linking cell wall polymers and providing cell wall stability (O'Neill *et al.*, 2004). Grasses have a lower boron requirement than all other flowering species. Boron deficiency in plants has been associated with inefficient lignification.

Silicon (Si) in the form amorphous silica gel ($\text{SiO}_2 \cdot \text{H}_2\text{O}$) is irreversibly deposited in cells, intercellular spaces and walls of plants (Lam *et al.*, 1990) at concentrations ranging from 0.1 to 10% of dry weight (Currie and Perry, 2007). Silicon acts as a structural element, complementing lignin to strengthen and rigidify cell walls. It is the only nutrient which does not have a detrimental impact on plants when absorbed in excess (Epstein, 1999). Tropical grasses tend to accumulate more silica than legumes. Several *in vitro* studies have demonstrated that silica reduces the digestibility of cell walls (Hartley, 1981; Shimojo and Goto, 1989). Van Soest and Jones (1968) proposed that silica protected cell walls from microbial degradation in a similar

manner to lignin. Shimojo and Goto (1989) suggested that this effect is associated with decreasing the activity of the enzymes involved in forage digestion and not with any innate protection of the cell wall *per se*. In addition, silica may reduce digestion rate by lowering the availability of essential trace minerals (Van Soest, 1981). The nature of the observed depression in digestibility remains puzzling, since others have not found similar results (Balasta *et al.*, 1987). Minson (1971) found that silica did not affect either the digestibility of forage cell walls (*Panicum* spp.), organic matter or structural carbohydrate fractions. Perhaps silica has a close relationship with these polysaccharides as bridging structures, such as R'-O-Si-O-R" and R'-O-Si-O-Si-O-R" (Schwartz, 1973), including cross-linkages of pectic polysaccharides and amylopectin starches (Khalil and Duncan, 1981). The nature of silicon's association with other cell wall components such as cellulose, hemicelluloses, lignins and proteins, is not fully understood (Perry and Lu, 1992).

Phytic acid, *myo*-inositol-1,2,3,4,5,6-hexakiphosphate, is the main storage form of P in many plants. This molecule is highly charged with six phosphate groups extending from the central inositol ring structure, which makes it an excellent chelator of mineral ions (Liener, 1990). Phytic acid is present in a wide variety of plant feedstuffs consumed by ruminants and monogastric animals. Nearly all the phytic acid found in mature seeds is bound to minerals to form a mixed cation salt, known as phytate (Lott *et al.*, 2000). Most cereals, legumes and oilseeds contain phytate up to 5% by weight (De Boland *et al.*, 1976). Maize, grain sorghum, wheat, barley, and oats contain from 56 to 68% of the total P as phytate, whereas 61 to 70% of the P in soybean meal and cottonseed meal is in this form (Nelson *et al.*, 1968). Phytate must be hydrolysed to free inorganic phosphorus in order to be absorbed from the gut.

Monogastric animals lack sufficient endogenous phytase activity to hydrolyse phytates, which has detrimental effects the utilisation of other nutrients in animals (Bryden *et al.*, 2007; Li *et al.*, 2016, 2017). Phytate P is better utilised by ruminants as the rumen contains microorganisms that produce phytase (Guyton *et al.*, 2003). Raun *et al.* (1956) found that the availability of phytate-P was nearly 100% due to the activity of rumen microbes. Approximately 90% of phytate-P is hydrolysed in the rumen when phytate-P content is less than 50% of total levels (Clark *et al.*, 1986; Park *et al.*, 1999). Ellis and Tillman (1961) reported low true digestibility (25.5%) of total P in wheat bran with 91% of P as phytate was fed to sheep, demonstrating that a significant amount of phytate in wheat bran bypassed the rumen. Park *et al.* (2002) found that up to 35% of dietary phytate escaped digestion in the rumen, with no significant breakdown in the abomasum and small intestine and partial degradation in the large intestine. Incomplete degradation of phytate in dairy cows was reported by Kincaid *et al.*

(2005). Phytate degradation in the large intestine is unlikely to have nutritional significance for ruminants (Matsui, 2002; Park *et al.*, 2002) because the absorption of P occurs in the small intestine (Care, 1994). In a study with buffalo, Lakke Gowda *et al.* (1955) found that hydrolysis of phytate occurred in the intestines, not in the rumen.

Phytate has been found to have a negative impact on ruminant nutrition. Studies have shown that diets containing cereal grains, lucerne, various grasses and hays (Martz *et al.*, 1990) have low calcium bioavailability, presumably due to binding by phytate. Reduced liver Cu storage occurs in heifers consuming diets with high phytate (Bowman *et al.*, 2005). Phytate thus serves to increase the mineral requirement of the animal. With regard to protein nutrition, it is expected that, under acid conditions, ionic binding between the basic groups of phytic acid and protonised amino acid residues (arginyl, lysyl and histidyl) occurs (Sigh and Krikorian, 1982) which decreases the solubility of proteins (Liu and Cowieson, 2010). It has been shown that calcium ions interact with protein and phytate to further decrease the solubility of proteins (Saio *et al.*, 1967). Therefore, it is not surprising that phytate inhibits a number of digestive enzymes, such as pepsin, pancreatin, and amylase, leading to a negative effect on digestibility of proteins and carbohydrates (Selle *et al.*, 2000). Inhibition of digestion may result from the chelation of calcium ions, which are essential for the activity of trypsin and amylase. Complexing phytate with proteins could render them more resistant to enzymatic attack (Liener, 1990).

Using exogenous phytase in monogastric diets has increased the availability of phosphorus, calcium, zinc, iron and amino acids (Bryden *et al.*, 2007; Li *et al.*, 2016, 2017). However, little work has been done to examine the impact of exogenous phytase on the availability of dietary P in ruminants and the results vary (Hankins-Herr *et al.*, 2009; Long, 2016). Dietary feed type (Leytem *et al.*, 2007), intrinsic phytase of feed ingredients, feed processing (Lakke Gowda *et al.*, 1955; Park *et al.* 2000), passage rate (Kincaid *et al.*, 2005) and forage:concentrate ratios (Bravo *et al.*, 2003) may alter phytate-P hydrolysis in ruminants. Phytase concentration in the rumen was lower in cattle fed grain than those fed forages (Turner and Leytem, 2004). Phytase supplementation of dairy cow diets increased phytate hydrolysis and P flow from the rumen to the lower digestive tract and increased faecal excretion of P (Brask-Pedersen *et al.*, 2013). Inorganic P released from phytate may inhibit the activity of phytase due to the presence of excessive substrate, as demonstrated for plant phytases (Gibson and Ullah, 1988; Hubel and Beck, 1996), phytases from *Escherichia coli* (Greiner *et al.*, 1993), *Aspergillus ficuum* (Irving and Cosgrove, 1974) and most bacteria (Leytem *et al.*, 2007; Raun *et al.*, 1956). The exception is phytase from anaerobic rumen bacteria, which can tolerate high levels of phosphate without any

negative impact on phytase production. This unique ability facilitates efficient phytate hydrolysis in the rumen, even when there are high substrate levels in the rumen fluid of animals fed concentrated feed. Therefore, optimal feed and management to ensure maximal microbial phytase production in the rumen and ideal rumen conditions for exogenous phytase will allow the efficient utilisation of phytate P. This is important as there is increasing use of cereal grains (up to 50% of DM intake), oil seeds and their by-products in ruminant diets, especially dairy and feedlot cattle that ingest particularly high concentrations of phytate (Humer and Zebeli, 2015). Phytase may allow reductions in total P intake and excretion, as seen in monogastrics.

12. Plant cell wall degradation in the rumen

Forage plants play a vital role in maintaining herbivores. The cell wall comprises 23 to 90% of plant mass (Lee, 2018) and is a major component influencing forage quality, whereby lignin is the primary factor that limits cell wall digestion (Jung and Casler, 1991). In general, cell wall fibre levels are negatively associated with DM intake, DM digestibility and animal performance (Dehority and Johnson, 1961). The degradation of complex chemical and structural components of plant cell walls, as described above, is a major challenge for herbivorous animals. To meet this challenge, ruminants have evolved a complex and intergraded rumen microbiota (Hungate, 1966; Van Soest, 1994).

The ruminant 'stomach' consists of four compartments, the rumen, reticulum, omasum and abomasum (the true enzymatic stomach). This arrangement allows ruminants to integrate the large population of microorganisms in the reticulo-rumen into a symbiotic relationship with the host animal (Indugu *et al.*, 2016; Leng, 2018). The efficiency of ruminants to utilise feedstuffs depends on synergism with a highly diversified rumen microbial ecosystem. This typically consists of more than 50 genera of anaerobic bacteria (10^{10} – 10^{11} cells/ml rumen fluid; Stewart *et al.*, 1997), 40 ciliate protozoa (10^4 – 10^6 /ml; Williams and Coleman, 1997), six anaerobic fungal species (10^3 – 10^5 zoospores/ml) (Nagpal *et al.*, 2009) and bacteriophages (10^8 – 10^9 /ml; Kamra, 2005). These rumen microbes do not require specific energy yielding nutrients and can form biofilms (Leng, 2014). They degrade fibrous plant materials, mainly cellulose and other recalcitrant carbohydrates and proteins to short-term intermediates, such as oligosaccharides, sugars and amino acids. The intermediates become substrates for the production of volatile or short chain fatty acids (VFAs), microbial protein and vitamins to nourish the host animal.

Despite the rumen microbiota and its host evolving together over millennia, the breakdown of lignocellulosic polymers of plant cell walls is an inefficient process, as these polymers are refractory to degradation. This is caused by

lack of enzyme specificity and access to substrate due to the structural complexity of plant cell walls. The situation is made more difficult as the chemical composition and structure of herbage, forage and agricultural by-products varies widely, as an intrinsic feature of a plant species and due to climate, crop management and processing, such as silage production. This diversity, coupled with the complexity of composition and structure of plant cell walls, has made it difficult to accurately predict feeding value for ruminants. However, advances in biotechnology should improve this situation.

13. Biotechnology and future prospects

Improved ruminant utilisation of forages requires greater understanding of the factors that determine the degradability of plant cell walls, and this is a significant research challenge. The past two decades have seen major advances in molecular biology which have implications for meeting this challenge. Areas, such as chemical characterisation of plant cell walls, breeding, microbial identification, a greater understanding of rumen microbial ecology and development of feed enzymes will benefit from the development of molecular tools and advances in biotechnology.

Defining composition and characterising the structure of plant cell walls is pivotal to improving animal utilisation, therefore development of appropriate analytical methodology is required. It is evident from Popper (2020) and Cvrčková and Žárský (2019) that a multidisciplinary approach will be required to achieve this. However, the adoption of more sophisticated methods in the short term is problematical due to the reliance on feed formulation data sets established using older methods that have been built up over many years (Jung, 1997).

Manipulation through plant breeding is a long-term solution to improve digestibility and gene modification can speed up the moderation of lignin content. The challenge is how much lignin and other cell wall components of forage can be reduced to improve digestibility without any negative impact on plant growth and survival. Lignin is essential for normal function and its content increases in line with temperature. Global climate change can have a significant effect on chemical composition of plants, whereby lignin and other plant cell contents are expected to increase and nutritive value will decrease. Breeding new plant species or selection of plants that can tolerate extreme environmental conditions and maintain high nutritional value are necessary to meet demand from human consumers (Flachowsky and Meyer, 2015).

Lignin contains more gross energy than other cell wall components (Frei, 2013), but unfortunately it cannot be utilised by animals. Commercial lignin-modifying

enzymes are oxidative and not hydrolytic, and, thus, are not suitable for animal use, but rather for the paper and pulp industries (Gonçalves, 1996) or for biofuel production and waste management (Deng *et al.*, 2018; Welker *et al.*, 2015). Advances in biotechnology should make it possible to develop lignin-degrading enzymes for feed applications. This will improve the rumen microbial ecosystem to improve feed efficiency and reduce nutrient excretion and the threat of environmental pollution. It may even reduce methane emissions. Isolation and identification of new microorganisms that can break down lignocellulosic chemical bonds will allow the development of new feeding strategies and more efficient use of forage materials by ruminants. This will be greatly assisted by research delineating the complete enzyme hydrolysis of cell wall polysaccharides (Arantes and Saddler, 2010). Within this context, further research is required to provide fundamental information on the interactions among cellulolytic and non-cellulolytic organisms and between rumen microbes and plant cell wall constituents.

Exogenous feed enzymes technologies are becoming available that permit the incorporation of feed enzymes into lick-blocks for grazing ruminants (Ainscough *et al.*, 2019). Cellulase, xylanases, phytase and other enzymes are readily incorporated into monogastric animal diets. However, enzyme supplementation of ruminant diets is yet to be established as a practical solution, as responses to enzymes have been shown to be inconsistent (Beauchemin *et al.*, 2003; Sujani and Seresinhe, 2015), although positive responses to supplemental cellulase and xylanase enzymes have been reported in dairy cows (Arriola *et al.*, 2011; Mohamed *et al.*, 2013; Rode *et al.*, 1999) and in beef steers (Feng *et al.*, 1996). Ortiz-Rodea *et al.* (2013) undertook a meta-analysis of data collected from 29 experiments, which included 52 experimental doses, nine enzymes (cellulase, xylanase, endoxylanase, amylase, protease, hemicellulase, exoglucanase, endoglucanase and glucanase) and 1187 dairy cattle and found that the addition of the enzymes had no effect on milk production, or milk fat, lactose or protein level. The carbohydrases broke linkages joining sugar molecules of cellulose and xylans in plant cell wall components (Beauchemin *et al.*, 2003), but did not hydrolyse bonds between phenolic components and carbohydrates (Yu *et al.*, 2005).

Without this partnership between rumen microbes and cattle, humans would be denied a major source of protein and energy. With the growing global demand for food and transition to greater meat consumption (Gouel and Guimbard, 2018; Tilman *et al.*, 2011), it is important that every effort be made to improve plant cell wall utilisation by ruminants. In this way the growing human population will derive benefit from the vast global forage resources.

Conflict of interest

The author declares no conflicts of interest.

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