Concept Paper

Purified lignin and its impacts on farm animals

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Received: 5.11.2018/Accepted: 23.12.2018

ABSRACT

Lignin is a polyphenolic compound and second most abundant natural compound after cellulose. Lignin is strongly intermeshed with cellulose and hemicelluloses; does not exist in pure form. Purified lignin can be produced by several techniques like Alcell process and its chemical structure is different from native lignin. Purified lignin is mostly used for industrial and construction purposes. But it has a great function on animal performance as an antimicrobial substance and prebiotic. Therefore purified lignin or Alcell lignin can be used in the animal feed industry.

Keywords: Lignin; purified lignin; animal performance; antimicrobial effects; prebiotic

INTRODUCTION

After cellulose the lignin is the second most abundant natural compound on earth and represents up to 33 per cent of plant cell walls. It refers to a group of phenolic polymers that provide strength and rigidity to the woody cell walls of plants. In humans polyphenols exert several health benefits such as inhibit oxidation of low-density lipoproteins thereby decreasing risks of heart disease, have anti-inflammatory and anti-carcinogenic properties and are effective antioxidants for food lipids. Lignin content of fodders in animal feeding is mostly regarded as a barrier to nutrient digestibility. Lignin as lignosulphonate is a useful feed pellet binder to feed manufacturers. In its purified form lignin contains several low molecular weight phenolic monomers that have biological effects.

Lignin is synthesized by an enzyme which initiates dehydrogenative polymerization of three monomeric aromatic alcohols viz coumaryl, coniferyl and synapyl alcohols. Differences in the proportions of these alcohols affect lignin concentrations among the plant species. The strong carbon-carbon and ether linkages in lignin make it resistant to degradation. For this reason lignin is generally accepted as the primary entity responsible for limiting digestion of forages thereby reducing its nutritional value. Monogastric animals cannot digest lignin but the rumen bacterial flora facilitates degradation of benzyl ether linkages of lignin polymers.

Sources and chemical structure

Lignin is a complex poly-phenolic, high-molecular weight polymer and naturally present in the cell walls of plants and trees. It gives mechanical support to leaf blades and stems and strength and rigidity to plant walls. It also acts as a physical barrier against pests and diseases in plants.

Lignin content varies among plant species. For example legume fiber is more lignified than grasses but legumes are typically more digestible because they contain less fiber and lignin is concentrated mostly in the stems leaving the leaf fiber relatively lignin-free. Lignin is thought to interfere with microbial degradation of fiber polysaccharides by acting as a physical barrier and by cross-linkages to polysaccharides by ferulate bridges. Several grass cell types become lignified during maturation whereas xylem and tracheary cells are the only major lignified tissues in legumes.

Negative impact of high lignin substance of forages

Lignin is considered as an anti-quality component in forages due to its negative impact on the nutritional availability of plant fiber. Several mechanisms have been suggested for how lignin may inhibit cell wall digestion. However it is now known that lignin simply acts as a physical barrier to the microbial enzymes reaching their target polysaccharides. Lignin content reduces the digestible energy content of forages and dry matter intake of animals. It binds the cell wall protein along with cellulose and further reduces its availability to microbes. Some essential macro- and micro-minerals also bind with lignocellulosic network and pass through faeces without absorption.

Purified lignin

In wood lignin is strongly intermeshed with cellulose and hemicellulose and does not exist in a pure form. In the paper-making industry purified lignin is recovered as a by-product of cellulose production during wood-pulping. However differences in pulping treatments yield different lignin fragments. In the sulfite process sulphuric acid is used to convert lignin into lignosulphonates. The Kraft process which uses sodium hydroxide and sodium sulphide to extract lignin from cellulose in the wood fibers is more efficient and yields stronger fiber. In contrast the Alcell process disrupts the chemical integrity of native lignin thereby yielding purified lignin fragments as a co-product. This process involves aqueous ethanol as the cooking liquor at temperatures between 185 and 195ºC. In contrast to Kraft and sulphite lignin, Alcell lignin consists of low molecular weight phenolic fragments with enhanced hydrophobicity. Hence lignin in its purified form possesses chemical structures that differ from native lignin. For this reason purified lignin may possess biological properties not characteristic of native lignin. Purified lignin is mostly used for industrial and construction purposes. Different lignin production technologies and comparison of different isolated polymeric lignins have been presented in Tables 1 and 2 respectively.

Purified lignin role in animal performance

Differences in the form, concentration of lignin as well as animal species may affect the response of purified lignin supplementation in animals.12.5 g/kg DM of Alcell lignin in Holstein's calves is sufficient to improve body weight gain (Phillip et al 2000). Indulin (40 and 80 g/kg of DM), a purified Kraft lignin from the paper industry has the positive effect on growth performance and feed efficiency in broiler chickens (Ricke et al 1982).

Antimicrobial effects of purified lignin

The antimicrobial properties of the phenolic fragments of lignin are well recognized. The phenolic components of lignin have been reported to inhibit the growth of microorganisms such as *Escherichia coli*, *Saccharomyces cerevisiae*, *Bacillus licheniformis* and *Aspergillus* spp. The side chain structure and nature of the functional groups of the phenolic compounds are major determinants of the antimicrobial effects of lignin. However the minimum inhibitory concentrations have variability in the antimicrobial activity of different phenolic fragments (Table 3).

Purified lignin can alter the fermentation pattern of the chicken intestinal tract by inhibiting the growth of certain bacteria eg indulin (40 and 100 g/kg of DM) has the potential to reduce volatile fatty acid concentrations in the ceca and large intestine of chickens. Ruminant responses to dietary lignin are variable. Alcell lignin has no effect on rumen

Table 1. Lignin production technologies

Process	Typical conditions	Lignin recovery method
Dilute acid lignin	Dilute acid (H_2SO_4, HCl), 160- 200ºC	Lignin recovery after enzymatic hydrolysis of insoluble fraction
Alkali lignin	Alkali (NaOH, NH_3), above 130-160ºC	Lignin recovery after precipitation of soluble fraction by acid
Steam explosion lignin	180-230ºC, 15-35 bar steam, 1-20 min	Lignin recovery after enzymatic hydrolysis of insoluble fraction
AFEX lignin	NH_3, high pressure, 90-100ºC	Lignin recovery after enzymatic hydrolysis of insoluble fraction
Klason lignin	72% H_2SO_4, 30-50ºC, 1 hour	Lignin recovery after filtration and washing with water
Organosolve-lignin (Alcell process)	(Ethanol, ethanol: water solvent) 180-210ºC	Lignin recovery after evaporation of soluble fraction

Table 2. Comparison of different isolated polymeric lignins

Isolated polymeric lignin	Quality
Kraft lignin, lignosulfonates, Klason and dilute acid lignin	-High sulphur content -Barrier in catalytic downstream process -Psuedolignin formation in dilute acid process
Steam explosion lignin	-Psuedolignin formation
Soda alkali (NaOH) lignin	-Lignin contains a substantial amount of carbohydrate fraction -High-cost reagent and difficult recovery makes process uneconomical
AFEX lignin	-Lignin obtained is substantial pure -Energy and CAPEX intensive
Dilute ammonia lignin (ICT- lignin)	-Lower CAPEX and OPEX -No pseudolignin formation -Lignin obtained substantial pure -No sulphur content

Table 3. Antimicrobial effects of lignin fragments (minimal inhibitory concentration, µg/ml) (Zemek et al 1979)

Compound	*Escherichia coli*	*Saccharomyces cerevisiae*	*Candida albicans*	*Bacillus licheniformis*	*Micrococcus luteus*	*Aspergilus niger*
C_1	3000	3000	3000	250	250	3000
C_2	100	100	100	50	50	250
C_3	375	375	375	177	177	700
C_4	375	1400	2800	375	375	3000
C_5	375	187	375	375	375	700
C_6	375	375	375	187	187	750
C_7	375	375	375	375	375	750
C_8	180	90	180	90	90	640
C_9	150	150	150	150	150	600
C_{10}	150	150	150	150	150	600
C_{11}	375	375	375	375	375	750

C_1: Eugenol, C_2: Isoeugenol, C_3: Syringaldehyde, C_4: Coniferylalcohol, C_5: Ferulic acid, C_6: 4-hydroxy-3 methoxy-â-hydroxy-propiophenone, C_7: 1-(4-hydroxy-3-methoxyphenyl)-2-propanone, C_8: 2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methyl-5-propyl-coumaran ("dehydro-diisoeugenol"), C_9: Pinoresinoldione ("dehydrodiferulic acid"), C_{10}: Di-*O*-acetylpinoresinol, C_{11}: 2,3-Bis(á-hydroxyvanillyl)-1,4-butane-diol

fermentation in sheep and fecal concentrations of anaerobic, aerobic and coliform microorganisms in calves. Phenolic monomers are extensively metabolized by the rumen microbial flora to more chemically reduced forms which are subsequently absorbed and metabolized. So the antimicrobial action of lignin may be species-dependent and occurs mostly in non-ruminants.

Purified lignin acts as prebiotic

Prebiotics are indigestible feed ingredients which selectively stimulate growth or metabolic activity of a limited number of intestinal microorganisms in birds and mammals. *Lactobacilli* and *Bifidobacteria* are beneficial bacteria that limit intestinal colonization of pathogens by competing for nutrients and binding sites and by secreting anti-bacterial substances.

Prebiotics may also play important role in improving the intestinal morphology of animal species. Longer villi are generally correlated with better intestinal health and improved efficiency of digestion and absorption. Goblet cells are responsible for the production of mucins which destroy intestinal pathogens. In broiler, Alcell lignin (12.5 g/kg of DM) is beneficial to increase intestinal concentrations of

Lactobacilli and *Bifidobacteria* villi height and goblet cell number however not beneficial at a higher level (ie 25 g/kg of DM) suggesting that the prebiotic effect of Alcell lignin only occurs within a limited inclusion range.

CONCLUSION

Purified lignin has biological effects other than those of native lignin. Purified lignin such as Alcell lignin exhibits prebiotic effects in monogastric animals by favouring the growth of beneficial bacteria and improving morphological structures in the intestines. Additionally the bactericidal properties of lignin fragments may help in the control of intestinal pathogens thereby ensuring the safety of livestock products to humans.

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