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TANNINS IN FEEDSTUFFS FOR SIMPLE-STOMACHED ANIMALS

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INTRODUCTION

The term 'tannin' was originally used to describe substances in vegetable extracts used for converting animal skins into stable leather (Seguin, 1796). The substances essential in the tanning process (tannins) were later identified as polyphenolic compounds with various molecular weights and of varying complexity. It was also found that these polyphenolic compounds bind strongly not only to hide protein, but also to other proteins and to macromolecules such as polysaccharides. Tannins are present in a large number of products of vegetable origin used as human foods or animal feeds. During the past century a number of adverse nutritional effects has been attributed to tannins. This review will first summarize current knowledge of the chemistry, occurrence and natural function of plant tannins. Subsequently, special attention will be given to the harmful effects of tannins in animal feeds, particularly in simple-stomached farm animals such as poultry and pigs. The nutritional effects of tannins in ruminants have been reviewed recently by Kumar & Singh (1984), Mangan (1988), Kumar & Vaithiyanathan (1990), Leinmüller *et al.* (1991) and Menke & Leinmüller (1991).

CHEMISTRY OF TANNINS

Bate-Smith & Swain (1962) defined tannins as naturally occurring water-soluble polyphenolic compounds with a molecular weight between 500 and 3000 capable of precipitating alkaloids as well as gelatin and other proteins from aqueous solutions. From this definition it is clear that tannins are chemically not well defined substances but rather a group of substances with some common properties. Polyphenols referred to as tannins have a considerable number of phenolic groups. They are capable of forming effective cross-links with other molecules. Phenolic compounds with a low molecular weight (< 500) do not form stable cross-links with other molecules. On the other hand, compounds with a much higher molecular weight (> 3000) do not show tanning properties because they appear to be too large to penetrate into the collagen fibrils in hides (White, 1957).

Although tannins are chemically not well defined, they are usually divided into hydrolysable and condensed tannins (Freudenberg, 1920; Haslam, 1966). Hydrolysable tannins have a central carbohydrate core whose hydroxyl groups are esterified to phenolic carboxylic acids such as gallic acid, ellagic acid and hexahydroxydiphenic acid. Esters of the first two acids are referred to as gallotannins while combinations with the latter are referred to as ellagitannins. Fig. 1 shows a typical example of a hydrolysable tannin. Tannic acid is a well-known gallotannin and contains 8–10 moles of gallic acid per mole of glucose (Freudenberg & Weinges, 1962). These types of tannins are readily hydrolysed by acids, alkali or some enzymes. Upon hydrolysis they yield glucose or some other polyhydroxy alcohol and gallic acid or some phenolic acids related to it (Salunkhe *et al.* 1990).

Condensed tannins are mainly polymerized products of flavan-3-ol (catechin) and flavan-3,4-diol or a mixture of these. The full chemical nature of condensed tannins, however, has not been elucidated. Condensed tannins are also referred to as flavolans or procyanidins.

Flavan-3,4-diols belong to the class of leucoanthocyanidins because they polymerize upon heating in acid solutions not only to phlobaphene-like products (tannin reds), as flavan-3-ols do, but also to anthocyanidin. They are also designated as proanthocyanidins (Freudenberg & Weinges, 1962). Flavan-3,4-diol contains three asymmetric carbon atoms and hence eight stereoisomers.

Two simple precursors, acetate and phenylalanine, are needed for the synthesis of flavonoids, the group of substances to which most of the basic units of tannins belong. All flavonoids possess a typical $C_6-C_3-C_6$ structure. The precursors originate from

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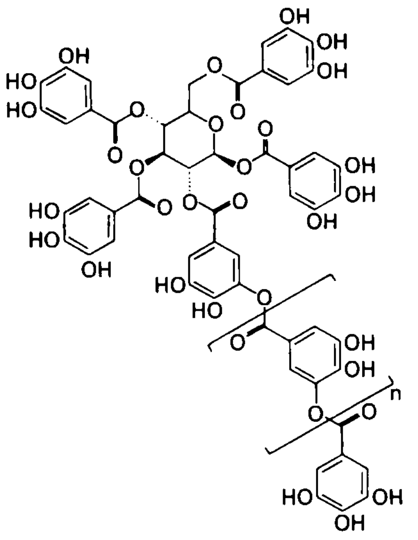

Fig. 1. Structure of a hydrolysable tannin.

carbohydrate and protein metabolism respectively (Mueller-Harvey & McAllan, 1992). Phenylalanine can also be synthesized in the shikimic acid pathway. Flavan-3,4-diol, with the typical $C_6-C_3-C_6$ carbon skeleton, is produced via chalcone, flavonone and dihydroflavonol intermediates. This is the immediate precursor of polymeric flavonols (Haslam, 1977).

The exact metabolic routes and intermediates for the formation of condensed tannins from flavonoid compounds are still unknown, but a large number of enzymes mediating the different steps in the condensation process have been identified (Mueller-Harvey & McAllan, 1992). The predominant bond between monomeric catechin molecules is a covalent 4,8 bond. However, 4,6 bonds have also been found in polyphenolic compounds in some plant species. In the condensation process during tannin formation, first dimeric compounds are formed, followed by trimeric, tetrameric and higher oligomers (Haslam, 1977).

Flavan-3-ols with a molecular weight below 3000 are soluble compounds. Higher polymerized procyanidins become insoluble and are often more closely linked to the structural tissue of the plant (Salunkhe *et al.* 1990). The final steps in the formation of condensed tannins in sorghum grain are shown in Fig. 2 (Haslam, 1977).

The chemistry of tannins has been extensively reviewed by Gupta & Haslam (1980), Porter (1988) and Mueller-Harvey & McAllan (1992).

OCCURRENCE OF TANNINS

GENERAL

The nature, content and location of tannins in plants vary considerably among species. Polyphenolics are of little importance in lower orders of plants such as fungi, algae and most of the monocotyledons such as grasses (McLeod, 1974). Tannins are most commonly found in dicotyledons, particularly in Leguminosae (Salunkhe *et al.* 1990).

Some important plant species used for human food or animal feeds contain considerable amounts of tannins, such as the food grains sorghum (*Sorghum vulgare*, Pers.), millet

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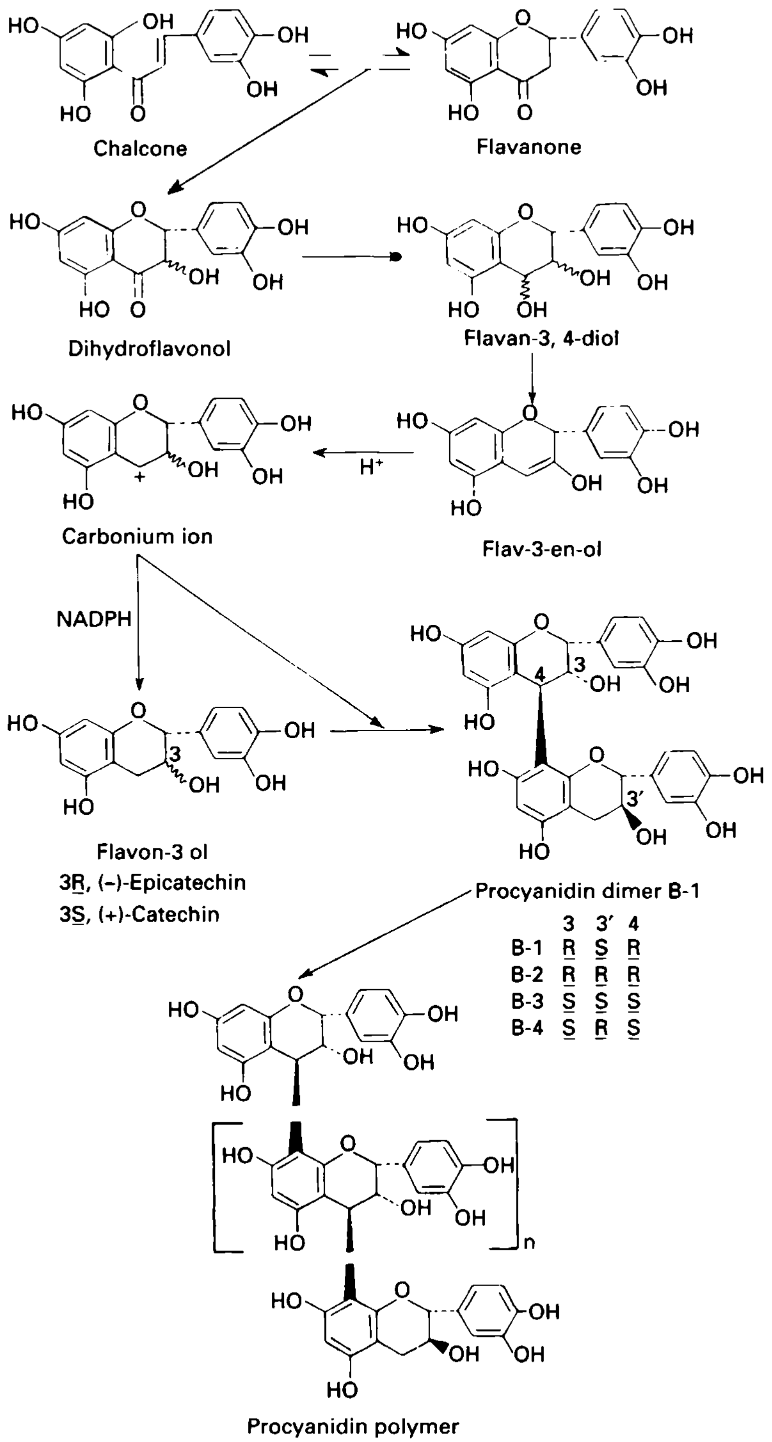

Fig. 2. The formation of condensed tannins in sorghum grain. (From: Haslam, E., 1977, with permission.)

(*Panicum miliaceum* L.), barley (*Hordeum vulgare* L.), rapeseed (*Brassica napus*) and a number of legume seeds. Bate-Smith & Lerner (1954) found leucoanthocyanidins (condensed tannins) in over 500 species of plants. Polyphenolic compounds are also found in beverages such as tea and wine (Hoff & Singleton, 1977). Furthermore, tannins and other polyphenolic compounds appear to be present in different fruits such as apple, banana, blackberry, date, grape, peach, pear, plum, raspberry and strawberry (Goldstein & Swain, 1963; Thompson *et al.* 1972).

Hydrolysable as well as condensed tannins are found in tree leaves, browse species and herbaceous legumes. These are known to be important feed sources for ruminants, particularly in arid and semi-arid regions (Kumar & Vaithiyanathan, 1990). The tannin content of leaves of trees and browse species varies considerably among species. The content of tannins (on a dry matter basis) can range from 1·5 to 30% (Leinmüller & Menke, 1990). The tannin content in forage leaves and the leaves of trees and browse species varies considerably during the season, as was shown by Feeny & Bostock (1968) for the leaves of oak (*Quercus robur* L.). On a dry matter basis, the tannin content changed from 0·5% in April to 5% in September.

Some legume herbages such as lucerne (*Medicago sativa* L.), lespedeza (*Lespedeza cuneata* L.), sainfoin (*Onobrychis viciaefolia* Scop.), sweet clover (*Melilotus officinalis* L.), red clover (*Trifolium pratense* L.), and white clover (*Trifolium repens* L.) are known to contain considerable amounts of hydrolysable and/or condensed tannins (Salunkhe *et al.* 1990).

In contrast to plants, with very few exceptions higher animals cannot synthesize compounds with benzenoid rings such as oestrone and related phenolic steroids from aliphatic precursors (Singleton, 1981). Plants are the main source of phenolic compounds found in animals.

TANNINS IN CEREALS AND LEGUME SEEDS

High concentrations of tannins have been found in sorghum grains. Tannin content (expressed as % catechin equivalents) has been reported to range from 3·6 to 10·2% (Harris & Burns, 1970), 4·8 to 8·2% (Harris *et al.* 1970) and 2·7 to 6·9% (Jambunathan *et al.* 1986). Tannin content appears to be related to the colour of the pericarp of the grain (Subramanian *et al.* 1983). The testa layer of the grain contains the polyphenolic compounds.

Strumeyer & Malin (1975) isolated the polyphenols from sorghum and found that they are of the condensed type. Hydrolysable tannins were absent. Williams *et al.* (1983) determined that the procyanidins in sorghum consist of 2–40 monomeric units.

Barley and millet are other cereals containing polyphenolic compounds. Millet contains some C-glycosyl flavones (carbohydrate C–C linked to a flavonoid nucleus) which appeared to be resistant to hydrolysis (Reichert *et al.* 1980).

Different polyphenolic compounds have been analysed in barley, but detailed information on the exact nature of these compounds is not yet available. Total phenolic content ranged from 0·55 to 1·23% in different varieties of barley (Eggum & Christensen, 1975).

With regard to the legume seeds, tannins have been found in dry bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), chickpea (*Cicer arietinum* L.), faba bean (syn. broad bean, field bean; *Vicia faba* L.), cowpea (*Vigna unguiculata* L.) and lentils (*Lens culinaris*). In most grain legumes tannins are present as condensed tannins (Salunkhe *et al.* 1990).

The tannin content of faba bean is shown in Table 1. Faba beans are classified in varieties

Table 1. *Tannin content (% equivalents*[1,2,3,4]*) of coloured- and white-flowering varieties of faba beans* (Vicia faba *L.*) *as measured with different colorimetric assays*

Whole beans		Cotyledons		Testa				
Mean	Range	Mean	Range	Mean	Range	*n*	Method	Reference
Coloured-flowering varieties								
1·77	1·34–2·00	0·86	0·84–0·91	7·10	5·34–7·70	6	Total phenols[1]	Griffiths, 1981
1·22	0·49–1·64	0·58	0·57–0·68	5·57	3·60–7·00	9	Total phenols[1]	Bos & Jetten, 1989
0·65	0·59–0·70	0·08	0·08–0·08	4·20		2	Condensed tannins[2]	Griffiths, 1981
1·54	0·95–2·40			10·82	6·59–14·97	17	Condensed tannins[2]	Wang & Ueberschär, 1990
0·84	0·48–1·31	0·05	0·04–0·07	5·46	4·29–6·39	9	Condensed tannins[2]	Bos & Jetten, 1989
2·15	0·76–3·54					46	Condensed tannins[3]	Cabrera & Martin, 1986
0·40	0·34–0·50	0·21	0·17–0·26	3·59	3·30–3·89	4	Total phenols[1]	Kadirvel & Clandinin, 1974
				3·7	2·0–6·0	5	Condensed tannins[4]	Marquardt *et al.* 1978
White-flowering varieties								
0·75	0·70–0·81	0·82	0·74–0·88	0·37	0·28–0·51	6	Total phenols[1]	Griffiths, 1981
0·06		0·07		0·04		1	Condensed tannins[2]	Griffiths, 1981
0·19	0·08–0·32			1·52	0·71 2·40	5	Condensed tannins[2]	Wang & Ueberschär, 1990
0·52	0·50–0·58					6	Total phenols[1]	Bos & Jetten, 1989
0·06	0·05–0·08	0·06	0·05–0·07	0·13	0·05–0·40	6	Condensed tannins[2]	Bos & Jetten, 1989
0·07	0·00–0·19					9	Condensed tannins[3]	Cabrera & Martin, 1986
				0·6	0·00–2·4	4	Condensed tannins[4]	Marquardt *et al.* 1978

[1] Tannic acid as reference.
[2] Catechin as reference.
[3] Calculated as the difference between total phenols and residual phenols after precipitation with polyvinylpolypyrrolidone; % tannic acid equivalents.
[4] Purified faba bean tannins as reference.

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producing coloured seeds and those yielding white-seeded beans. Condensed tannins are mainly present in the testa of the coloured seeds. When tannins are measured as total phenols considerable amounts are also found in the cotyledon fraction. This result, however, can be attributed to the presence of some non-tannin phenolics, such as phenolic amino acids, in this part of the seed.

Cabrera & Martin (1986) found a clear correlation between colour of the flower, seed colour and tannin content of faba beans. White-flowering varieties, with no pigments in the flowers, yielded white and grey seeds with low tannin contents. Coloured-flowering varieties yielded seeds of different colour with the amounts of tannins increasing progressively in seeds having green, red, beige or brown colours.

Martin-Tanguy *et al.* (1977) determined the chemical nature of tannins in coloured-flowering varieties of faba beans. They found polymers of flavan-3-ols (catechin and gallocatechin) and flavan-3,4-diols joined together by carbon-carbon linkages between the C4 of one unit and C6 or C8 of other units. Chains were linearly linked with a flavan-3-ol at the terminal end.

NATURAL FUNCTION OF TANNINS

Tannins in cereals and legume seeds appear to play a role in the crop's resistance to being eaten by birds, particulary in sorghum. They also play a role in their susceptibility to attack by fungi and pests and in the incidence of preharvest germination (Salunkhe *et al.* 1990).

In the early stages of maturity, low-tannin sorghum varieties are attractive as feeds for different avian species in many parts of the world. High-tannin varieties, on the other hand, tend to be less palatable and are sometimes referred to as being bird-resistant. This has been attributed to the astringent taste of tannins, caused by the complexation of tannins with saliva proteins and the mucous epithelium in the oral cavity, which reduces palatability (Bullard & Elias, 1980).

Low-tannin varieties of sorghum and faba bean are more susceptible to attack by fungi and pests in the field (Dreyer *et al.* 1981; Bond & Smith, 1989). However, some evidence indicates that monomeric phenols such as flavan-3-ols, and not tannins, are responsible for this higher resistance (Bullard & Elias, 1980).

It has been suggested that tannins in sorghum grains also play a role in prevention of preharvest germination. This phenomenon occurs in wet environmental conditions, especially in low-tannin varieties. Tannins may form a physical barrier, which prevents water imbibition necessary for germination (Salunkhe *et al.* 1990).

TANNIN ANALYSIS

A considerable number of different assays have been developed for the measurement of tannins in plants. However, these assays, due to the complex chemical nature of tannins, do not provide completely satisfactory results. They can, nevertheless, be categorized into three groups: colorimetric methods, protein binding methods and other methods.

COLORIMETRIC METHODS

Vanillin assay

The vanillin assay is widely employed as a method for the quantitative determination of condensed tannins in fruits, sorghum and forage legumes (Swain & Hillis, 1959; Burns, 1971; Broadhurst & Jones, 1978; Price *et al.* 1978*a*). The assay is specific for flavan-3-ols, dihydrochalcones and proanthocyanidins (Sarkar & Howarth, 1976). The principle of the assay is based on the substitution of vanillin for a phenolic hydroxyl group, yielding a red-coloured condensation product which is measured spectrophotometrically at 480–550 nm.

It is known that vanillin does not react in a stoichiometric way with the monomeric units in condensed tannins, since the reactive sites are not readily available after condensation. Vanillin therefore gives stronger reactions with monomeric flavans than with condensed tannins (Salunkhe *et al.* 1990). Catechin (monomeric flavan-3-ol) is often used as a standard in this assay.

Folin Denis assay

This assay is the most widely used type for measuring total phenol content in plant products and beverages. The principle is based on the reduction of phosphomolybdic–phosphotungstic acid (Folin Denis reagent) to a blue colour complex in alkaline solution by phenols (Folin & Denis, 1912). This assay is relatively non-specific as it also reacts with several other compounds including xanthine, proteins and some amino acids (Lowry *et al.* 1951). Tannic acid is commonly used as a standard. A second weakness of the Folin Denis method is that it does not yield stoichiometric results, even when the number of hydroxyl groups is taken into account (Goldstein & Swain, 1963).

Prussian blue assay

This assay is based on the reduction of the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) by tannins and other phenolic compounds to form ferric ferrocyanide ($Fe(III)\,[Fe(II)\,(CN)_6]^-$), which is known as Prussian blue. The absorption of this complex can be measured at 720 nm (Price & Butler, 1977). Polyphenolics with a varying hydroxylation pattern and degree of polymerization react differently in this assay.

Acid butanol assay

The acid butanol assay is a functional group assay which is specific for proanthocyanidins (condensed tannins), when used under the conditions described by Porter *et al.* (1986). The original procedure was described by Bate-Smith (1973). In this assay flavonoid subunits of the condensed tannins are oxidatively cleaved to yield anthocyanidin, which is measured spectrophotometrically. The method measures the total amount of subunits in the condensed tannin fraction.

Butler *et al.* (1982) described a method to estimate the degree of polymerization of condensed sorghum tannins by using a modified vanillin assay to measure the flavan-3-ol end groups in combination with the acid butanol assay which determines the total number of subunits in the tannin molecules.

None of the colorimetric assays for tannin determination is very specific. Most of them, however, are appropriate for screening purposes, with the vanillin and acid butanol assays being most widely used.

PROTEIN BINDING METHODS

Protein binding assays can be used to determine either the tannin content of a sample or the biological activity of tannins (Hagerman & Butler, 1989). For the measurement of tannins via protein binding, the amount of tannins precipitated by a standard protein is established. Different proteins such as gelatin, casein, bovine serum albumin, haemoglobin and different enzymes have been used for this purpose. Each protein binding assay gives a different response with tannins of different sources. This is due to the fact that the tendency of tannins to form insoluble complexes with proteins is influenced by many factors such as the characteristics of the tannins (molecular weight, structural heterogeneity), the protein source (degree of glycosylation, amino acid composition and molecular weight) and reaction conditions (pH, temperature, reaction time, relative concentrations of the reactants) (Hagerman & Butler, 1989). Tannins tend to complex with proteins such as gelatin or specific proline-rich proteins that have a high content of proline resulting in a protein with a loose structure (Asquith & Butler, 1986).

In some methods the amount of protein in the tannin–protein complexes is determined (Martin & Martin, 1982; Makkar *et al.* 1987; Marks *et al.* 1987). When the biological activity of tannins is measured, not only insoluble tannin–protein complexes should be measured but also the soluble ones. Competitive binding studies (Hagerman & Butler, 1981; Asquith & Butler, 1985) enable measurement of both soluble and insoluble tannin–protein complexes.

OTHER METHODS

More detailed information on the structure and nature of (poly)phenolic compounds and tannins can be obtained by using high-performance liquid chromatography (HPLC), mass spectral analysis, droplet countercurrent chromatography and centrifugal partition chromatography (Okuda *et al.* 1989). Mueller-Harvey *et al.* (1987) were able to identify condensed tannins, gallotannins and some low molecular weight phenolic compounds in aqueous extracts of different tropical browse species by using HPLC followed by absorption measurement at 280 and 350 nm. However, no individual compounds could be identified within the fraction of condensed tannins.

Okuda *et al.* (1989) showed that it was possible to estimate the approximate molecular weight of hydrolysable tannins by normal phase HPLC in plant extracts eluted from a gel permeation chromatography column. For condensed tannins, however, these possibilities were not shown.

Putman & Butler (1989) showed that it was possible to separate on a reversed phase HPLC system high molecular weight sorghum procyanidins having a relative degree of polymerization of up to 13 monomeric units.

Some attempts have been made to separate various tannin extracts chromatographically on gel permeation columns. The most successful results were obtained with columns of hydroxypropylated dextran gel such as Sephadex LH20. According to Okuda *et al.* (1989), tannins can be separated on the basis of differences in adsorptivity of polyphenolic compounds on the gel rather than on the basis of gel filtration. Strumeyer & Malin (1975) were among the first to use a Sephadex LH20 column to isolate and fractionate condensed tannins from sorghum. In a first step using 95% ethanol as eluent, non-tannin phenolic compounds were separated from the tannin-containing compounds. Subsequent elution of the tannin fractions from two sorghum varieties in aqueous acetone (50/50 v/v) yielded different chromatographic patterns. Marquardt *et al.* (1977) used the same procedure to purify and fractionate low molecular weight compounds (fraction A) and condensed tannins (fraction B) from extracts of hulls of faba bean. In fraction A at least 15 phenolic compounds were found. When applying the same chromatography to extracts of a white-flowering variety of faba bean, low molecular weight compounds were still found, but no peaks were found in the chromatograms which could be identified as condensed tannins (Marquardt *et al.* 1978).

Cansfield *et al.* (1980) further analysed the condensed tannin fraction of faba bean and found two major peaks in the LH20 chromatogram. Different fractions were collected from the chromatogram. The estimated relative degree of polymerization of the fractions differed markedly.

Kumar & Horigome (1986) used an LH20 column to fractionate tannins from black locust bean (*Robinia pseudoacacia*) using 70% aqueous acetone as eluent, which gave better separation than 50% aqueous acetone. The degree of polymerization of the tannins ranged from 4·1 for the first fraction to 1·5 in the final fraction. They concluded that the separation of tannins in Sephadex LH20 with 70% aqueous acetone is based on differences in molecular size between the tannins.

The structure of some individual (poly)phenols has been elucidated using advanced techniques of mass spectral analysis and nuclear magnetic resonance spectroscopy (for review see Okuda *et al.* 1989).

Excellent reviews on tannin analysis have been recently published by Deshpande *et al.* (1986), Makkar (1989) and Okuda *et al.* (1989).

NUTRITIONAL EFFECTS OF TANNINS

EFFECTS ON ANIMAL PERFORMANCE

Numerous studies have been conducted on the effects of tannins in feedstuffs on animal performance. Some of them have been carried out with isolated tannins from feedstuffs or with 'standards' of commercial tannins, such as tannic acid, which were thought to be representative of tannins in a number of feedstuffs. Most studies, however, were carried out with raw or fractionated feedstuffs (e.g. hulls of legume seeds) of the same plant species containing different levels of tannins as analysed by one of the available methods. In these studies the effects or differences found were fully or partly related to the differences in tannin level in the experimental diets.

Tables 2, 3 and 4 summarize the nutritional effects of tannins in several feedstuffs on the performance of rats, poultry and pigs respectively, and on nitrogen, amino acid and energy digestibility in these species. Some general observations presented in Tables 2, 3 and 4 are as follows:

(1) It has not been conclusively demonstrated that tannins, as found in conventional diets, can reduce feed intake in simple-stomached animal species.

(2) Tannins in diets generally reduce weight gain and impair feed conversion efficiency in growing animals.

(3) Tannins reduce the apparent digestibility of nitrogen (protein), amino acids and, to a lesser extent, energy.

The extent to which tannins reduce animal performance varies widely (Tables 2, 3 and 4). The following factors may determine the quantitative effects of tannins:

- response parameter chosen (weight gain, feed intake, feed conversion efficiency)
- source of tannins or feedstuffs used
- tannin concentration, which may also depend on the type of assay used
- length of the test period
- differences among animal species
- age of the animal
- diet composition (e.g. protein level and protein source)
- production level
- influence of factors other than tannins when using tannin-containing feedstuffs or tannin-rich fractions instead of isolated tannins.

The large number of variables that tend to modify the harmful effects of tannins limits the usefulness of direct comparison between the different studies.

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Table 2. *Some effects of dietary tannins in rats*

Source	Inclusion source (%)	Tannin level	Effect[1]		Reference
Sorghum	85–95	Low(1)/high(1)	DC_N	−17·5	Ford & Hewitt, 1979
Sorghum	95	0·5/0·7/1·3% S	FI	No effect	Muindi & Thomke, 1981
			DE	−4	
			DC_N	−15·4	
			DC_{Nfe}	−3·0	
Sorghum	?	0·33/2·50% S	$trDC_N$	−39·3%	Savage, 1989
			NPU	19·5%	
Sorghum	85	0·01–5·60% S	ADG	−59·0%	Elkin *et al.* 1990
			F/G	−55·0%	
Tannic acid	5		FI	−60%	Glick & Joslyn, 1970*a*
			ADG	−79%	
Carob tannins	6		ADG	−22·9%	Tamir & Alumot, 1969
			FI	−4·7%	
			FC	+23·3%	
			DC_N	−11·8	
Faba bean	28–32	Low(1)/high(1)	$trDC_N$	−3·1	Ford & Hewitt, 1979
			NPU	−11·8%	

[1] Difference with value for control or low tannin-group.

Abbreviations for Tables 2–4. FI, feed intake; ADG, average daily gain; FC, feed conversion efficiency; F/G, feed:gain ratio; end. N, endogenous N secretion; il.DC_{dm}, ileal dry matter digestibility (units); il.DC_N, ileal N digestibility (units); tr.DC_N, true N digestibility (units); DC_N, apparent N digestibility (units); DC_{dm}, apparent dry matter digestibility (units); DC_{Nfe}, apparent N-free extract digestibility (units); DC_{aa}, mean apparent amino acid digestibility (units); DC_{st}, apparent starch digestibility (units); AME_N, N corrected apparent metabolizable energy; DE, digestible energy (digestibility units); NPU, net protein utilization; ret. DM, retention dry matter; ret. N, retention N; D, level in diet (%); S, level in source (%).

EFFECTS ON FEED INTAKE

Conflicting reports have been published on the effect of dietary tannins on feed intake. On the one hand, tannins are known to have a bitter or astringent taste which reduces palatability and hence will negatively affect voluntary feed intake. In contrast, it has been suggested that a slightly astringent taste increases the palatability of feed and stimulates feed intake (Gupta & Haslam, 1980). Morton (1972) suggests that man has a 'taste for tannins' to explain man's preference for tannin-containing beverages such as tea and red wine.

The physical basis for astringency may be that tannins bind and perhaps precipitate salivary mucoproteins. This would reduce the lubricating property of saliva, give the mouth a feeling of dryness and affect the ability to swallow the food (Mole, 1989). A second more direct way by which tannins affect feed palatability may be that tannins directly bind to taste receptors (Mole, 1989).

Glick & Joslyn (1970*a*) and Vohra *et al.* (1966) showed a reduction in feed intake in rats and chickens due to supplementation with tannic acid. In contrast, an increased feed intake was found in chicks fed sal seed (*Shorea robusta*), a seed that contains high levels of hydrolysable tannins (Zombade *et al.* 1979). The opposite, however, was found by Ahmed *et al.* (1991).

The level and type of tannins as well as differences among animal species may explain the contrasting results with respect to the effect of tannins on feed intake. In natural ecosystems there is clear evidence that different herbivorous animal species select feeds of vegetable origin on the basis of their tannin level and that the normal or accepted tannin level in the diets of animals in their natural environment differs between species (Mole, 1989).

Table 3. *Some effects of dietary tannins in poultry*

Species	Source	Inclusion source (%)	Tannin level	Effect[1]		Reference
Chick	Sorghum	66	0·0/1·92% D	ADG	−9%	Dale *et al.* 1980
				FC	+20%	
	Sorghum	72	Low(2)/high(2) 0·0/0·27/0·48/ 1·13% D	ADG	−46·3%	Rostagno *et al.* 1973*a*
	Sorghum	90	0·33/1·41% D	DC_{aa}	−50·8	Rostagno *et al.* 1973*b*
	Sorghum	77–86	0·08/1·91/2·83% D	DC_N	−44·4	Mitaru *et al.* 1985
	Sorghum	80	Low(1)/high(1) 0·01/5·60% S	ADG	−30%	Elkin *et al.* 1990
				F/G	+21·9%	
	Sorghum	75	Low(1)/high(1) 0·05/4·82% S	ADG	−41·3%	Elkin *et al.* 1990
				FC	+23·2%	
	Sorghum	71–82	Low/high 0·15/1·20/1·90%	$trDC_N$	−53·6	Ford & Hewitt, 1979
	Sorghum	30 71	Low(1)/high(2)	ADG	No effect	Herstad, 1979
				FC	No effect	
	Tannic acid	1·41		end. N	+300%	Rostagno *et al.* 1973*b*
	Tannic acid	0·0–1·92		ADG	−12%	Dale *et al.* 1980
				FC	+10%	
	Faba bean	48	Low(1)/high(2)	DC_N meal	−14·5	Lacassagne *et al.* 1988
				DC_N pellet	−15·8	
				DC_{st} meal	+9·4	
				DC_{st} pellet	+3·8	
				AME_N meal	+3·0	
				AME_N pellet	+2·0	
	Faba bean	50	Low–high ($n = 10$)	DC_N	−18 (max)	Martin-Tanguy *et al.* 1977
	Faba bean	72–79	Low(1)/high(1)	$trDC_N$	−4·5	Ford & Hewitt, 1979
Laying hen	Sorghum	83–91	Low(1)/high(2) 0·2/0·7/0·7%	DC_N	−8·6	Herstad, 1979
				DE	−2·2	
Chick	Faba bean	85	Low(3)/high(2)	FI	+2·2%	Marquardt & Ward, 1979
				ADG	−7·5%	
				F/G	+9·3%	
	Faba bean hull extract		0–2·5% D	FI	−11·7%	Marquardt & Ward, 1979
				ADG	−33·3%	
				F/G	+31·7%	
				ret_{DM}	−10·3	
				ret_N	−19·0	
Laying hen	Faba bean	11–35	Low(1)/high(2)	Egg weight	No effect	Larbier, 1980
				Laying rate	No effect	
				ADG	No effect	
	Faba bean	30	Low(1)/high(2)	Egg weight	−6·3%	Martin-Tanguy *et al.* 1977
				Laying rate	−7·4%	
Muscovy duckling	Faba bean	50	Low high ($n = 6$)	ADG	−26·7%	Martin-Tanguy *et al.* 1977
Duck	Sorghum	80	Low(1)/high(1) 0·0/5·6% S	ADG	+5·7%	Elkin *et al.* 1990
				F/G	+8·7%	

[1] For abbreviations see Table 2.

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Table 4. *Some effects of dietary tannins in pigs*

Source	Inclusion source (%)	Tannin level	Effect[1]		Reference
Sorghum	72	Low(2)/high(2)	ADG	−5·4%	Myer & Gorbet, 1985
		0·1/0·1/3·6/3·8% S	FI	+5·9%	
			F/G	−14·6%	
Sorghum	90	Low(2)/high(1)	FI	+9·4%	Cousins *et al.* 1981
		0·83/0·88/3·40% S	ADG	No effect	
			FC	+10·2%	
Sorghum	90	Low(2)/high(2)	il. DC_{dm}	−4·0	Cousins *et al.* 1981
		0·83/0·88/3·17/	il. DC_N	−5·8	
		3·40% S	DC_{dm}	−3·6	
			DC_N	−4·8	
Sorghum	77–86	Low(1)/high(2)	il. DC_{dm}	+0·5	Mitaru *et al.* 1984
		0·08/1·91/2·83%	il. DC_N	−6·9	
			DC_{dm}	+7·4	
			DC_N	−10·6	
Sorghum	76	Low(1)/high(2)	ADG (23–60 kg)	−7·5%	Grosjean & Castaing, 1984
		0·2/1·0/1·4%	FC	+9·7%	
			ADG (60–103 kg)	−6·4%	
			FC	+6·7%	
Faba bean	30	Low(1)/high(2)	DC_{OM} bean	−1·3	Liebert & Gebhardt, 1983
		1·0/1·5/1·7% S	DC_N bean	−2·7	
Faba bean	30	Low(1)/high(2)	ADG	−5·9%	Bourdon & Perez, 1984
			FC	+11·8%	
			DC_N diet	−1·6	
			DC_N bean	−6·1	
Faba bean	51	Low(1)/high(1)	DC_N	−8·0	Duée *et al.* 1979
			DC_{aa}	−9·4	
			DE	−3·6	
Faba bean	15	Low(1)/high(2)	ADG	No effect	Fekete *et al.* 1985
			FC	No effect	

[1] For abbreviations see Table 2.

EFFECTS ON THE DIGESTIVE PROCESS

In vitro *interactions of tannins with proteins and carbohydrates*

Tannins are known for their ability to interact with different molecules such as proteins and carbohydrates. Tannins, by definition, form complexes with proteins which may lead to coagulation or precipitation. The strength and degree of interaction between tannins and proteins is determined by the nature of both the tannins and the proteins. The relative ratio of tannins and protein in solution, and physical and chemical conditions such as type of medium, temperature, pH, ionic strength and incubation time, also determine the degree of interaction between the two groups of compounds (Hagerman & Butler, 1989).

White (1957) suggests that the size of the tannin molecule is an important factor affecting its ability to cross-link with proteins. They should be small enough to penetrate into the conformational structure of the molecule but should also possess sufficient reactive groups to form effective cross-links with protein molecules.

Tannins bind to proteins by the interaction of their reactive hydroxyl groups with the carbonyl groups of proteins. Hydrogen bonds and hydrophobic interactions appear to be the principal linkages involved (Artz *et al.* 1987). Hydrogen bonding depends much more on pH than do hydrophobic interactions. Precipitation of proteins by tannins is found to be maximal for a number of proteins at pH values close to their isoelectric point (Hagerman & Butler, 1978).

Hydrophobic interactions between tannins and proteins tend to be enhanced at high ionic strengths and at high temperatures (Mueller-Harvey & McAllan, 1992). Some detergents are able to dissociate tannin–protein complexes (Hagerman & Butler, 1978) indicating that hydrophobic interactions are very important in tannin–protein associations.

In competitive binding studies, Hagerman & Butler (1981) clearly showed differences in binding affinities between proteins and tannins. Affinities of tannins from sorghum for bovine serum albumin and ovalbumin were much lower than for fetuin, gelatin and a mouse salivary proline-rich protein (GP-66sm). It was concluded that condensed tannins from sorghum had a particularly high affinity for proteins having a high content of proline, which gives an open and loose structure to the protein molecule.

Hagerman & Butler (1980) found that under optimal conditions sorghum tannins are able to bind and precipitate at least 12 times their own weight of protein. Hagerman & Robbins (1987) found different optima for the protein: tannin ratios for maximum protein precipitation by tannins from different sources.

Griffiths (1981) found that removal of tannin-containing hulls had a significant positive effect on the solubility of faba bean proteins. This effect on protein solubility was not found in low-tannin varieties of faba beans.

Tannins are also known to interact with carbohydrates, particularly starch. However, their affinity seems to be less than for proteins. Deshpande & Salunkhe (1982) studied the interaction of tannic acid and catechin with starches of different legumes. Processed amorphous amylose and amylopectin associated more with phenolic compounds than did native starch. The *in vitro* digestibility of starches associated with tannic acid or catechin was reduced by 9–17%.

More fundamental research should be carried out into the nature of the interactions of condensed tannins with starch and other carbohydrates. The interaction of tannins with certain nutrients may be one of the means by which tannins interfere with the digestive process.

Effects of tannins on the activity of digestive enzymes

Because tannins are able to form complexes with proteins, it is not surprising that they also bind to enzymes. This has implications for their biological activity. Griffiths (1979) reported that activities of trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1) and α-amylase (EC 3.2.1.1) in *in vitro* assays were reduced after addition of tannin-containing extracts from hulls of coloured-flowering varieties of faba bean. The inhibition was found to be reversible after addition of polyvinylpyrrolidone, a strong tannin binder. Extracts of white-flowering faba bean did not show enzyme inhibiting characteristics.

Also, tannin-containing extracts from rapeseed (Yapar & Clandinin, 1972), green gram and ripe carobs (Tamir & Alumot, 1969), chickpeas and pigeon peas (Singh, 1984) have been found to impair the *in vitro* activity of digestive enzymes.

Griffiths & Moseley (1980) have determined the activity of digestive enzymes in intestinal contents of rats fed diets containing hulls of high- and low-tannin varieties of faba bean. Activities of trypsin, chymotrypsin and α-amylase were reduced in animals fed the high-tannin diet.

Horigome *et al.* (1988) studied the effects of different leaves of fodder plants containing condensed tannins on the activity of trypsin, α-amylase and lipase (EC 3.1.1.3) in rats. The activities of the first two were significantly inhibited *in vivo*. All three enzymes were inhibited *in vitro*. A high positive correlation was found between the estimated degree of polymerization of the condensed tannins in the plants and the extent of enzyme inhibition.

Griffiths (1980) suggests that dietary tannins may also increase pancreatic secretion of digestive enzymes. This may complicate *in vivo* studies on the effects of tannins on enzyme activity. He suggests that in some animal species tannins may stimulate pancreatic secretion in a manner analogous to that of proteinase inhibitors from legume seeds (Liener, 1989). This could explain why dietary tannins in some cases increase activities of lipase in intestinal digesta. This observation is based on the assumption that total pancreatic enzyme secretion is increased by tannins and that the relative affinity of tannins is higher for trypsin and α-amylase than for lipase (Griffiths & Moseley, 1980; Horigome *et al.* 1988).

Ahmed *et al.* (1991) studied the effects of diets with sal seed meal, which contains hydrolysable gallotannins, on enzymes in the pancreas, in the intestinal lumen and in the intestinal mucosa of broiler cockerels. The size of the pancreas was significantly increased on a high-tannin sal seed meal diet. Also the activities of trypsin and α-amylase, expressed per kg of body weight, increased significantly in the animals on the high-tannin diet. The activities of trypsin and α-amylase in the intestinal lumen were reduced when the tannin content of the diet was increased from 0 to 25 g/kg. Mucosal dipeptidase (EC 3.4.13.11) and sucrose α-glucosidase (EC 3.2.1.20) (a disaccharidase) were both inhibited by tannins in the diet.

Blytt *et al.* (1988) found a much more pronounced effect of tannins on the activity of alkaline phosphatase (EC 3.1.3.1) and 5′-nucleotide phosphodiesterase isolated from the bovine intestinal mucosa than on the same activities tested as a crude particulate membrane fraction. Some authors (Blytt *et al.* 1988; Salunkhe *et al.* 1990) therefore stress that the *in vitro* effects of tannins on the activity of digestive enzymes cannot simply be extrapolated to *in vivo* conditions. Possible reasons for the difference are the large number of alternative binding sites that are available to tannins in the digestive tract and the different chemical and physical conditions in the two systems.

Oh & Hoff (1986) indicated that the effect of polyphenols on the digestive process might also be due to their inhibitory effect on the formation of active enzymes from inactive zymogen precursors.

Fahey & Jung (1989) stated that the extent of inhibition of digestive enzymes may depend on several factors such as the amount of dietary protein available, the formation of tannin–protein complexes prior to ingestion, the relative amounts of different enzymes present, the order in which they are encountered and differences in affinities of enzymes for tannins. Also species and age of the animal concerned may influence the magnitude of the effect of tannins on the activity of digestive enzymes. Information on the effects of tannins on the *in vivo* activity of digestive enzymes in species other than rats and chickens is limited.

Effects of tannins on nutrient digestibility

Effects of tannins on the digestibility of protein and energy. Results shown in Tables 2, 3 and 4 and discussed in the section 'Effects on animal performance' suggest that tannins in different feedstuffs reduced apparent protein and amino acid digestibilities. Also reduced energy digestion has been observed in some studies (digestible energy in pigs, apparent metabolizable energy in poultry). However, these effects seem to be less important than the effects on protein digestibility.

Effects of tannins on vitamin and mineral nutrition. Some studies reveal that tannins also affect vitamin and mineral metabolism. Suschenet (see review by Salunkhe *et al.* 1990) found a negative effect of feeding 3·2% tannic acid on the vitamin A (retinol) status of rats. It was suggested that vitamin A absorption from the small intestine was reduced by dietary tannic acid. Tannic acid has been shown to interact with thiamin (Rungruangsak *et al.* 1977) and to reduce vitamin B_{12} absorption in rats (Carrera *et al.* 1973).

Tannins are known to form insoluble complexes with divalent metal ions such as iron, rendering them less available for absorption. Rao & Prabhavathi (1982) suggest that tannins are responsible for the low bioavailability of iron in legume seeds. Garcia-Lopez *et*

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al. (1990) found a tendency for a lower iron absorption in rats after addition of tannin-containing hulls from kidney beans to their diets. Griffiths (1982) found a high iron-binding capacity of extracts from seed coats of coloured-flowering varieties of faba bean. White-flowering varieties did not show this property. The effect was attributed to the presence of condensed tannins in the extracts of the dark beans. In man, differences in iron availability have also been found between high- and low-tannin sorghum varieties (Radhakrishnan & Sivaprasad, 1980). However, in cereal grains the bioavailability of iron may also be affected by differences in level of phytic acid, a potent mineral binder. Information on interactions of tannins with other minerals is not available.

Effects of tannins on the gastrointestinal mucosa

Some studies have determined the effects of tannins of different origin on the morphology of the wall of the gastrointestinal tract and the absorptive capacity of the digestive tract. Vohra *et al.* (1966) fed various commercially available hydrolysable and condensed tannins to chicks. When feeding 4% or more tannic acid to chicks, mortality rate greatly increased and the dead animals showed, on autopsy, sloughing of the mucosa of the oesophagus, subcutaneous oedema and thickening of the crop. Mitjavila *et al.* (1973) observed a significant stimulatory effect of tannic acid infused into the stomach of rats on the secretion of pepsin (EC 3.4.23.1) and free acidity but found lower concentrations of mucin in the gastric juice. They suggested that the observed conditions were favourable for the development of gastric ulceration.

Mitjavila *et al.* (1977) fed 1% tannic acid and oxidized tannic acid to rats and found changes in the gastric and duodenal mucosa. Hypersecretion of gastric mucus and necrotic effects on the gastric mucosa were found as well as glandular atrophy. Alterations in the histological studies were accompanied by a reduction in cellular metabolism as measured by a decrease in oxygen consumption of the epithelial cells of the small intestine. This was paralleled by a reduction in succinic dehydrogenase (EC 1.3.99.1) activity, which was assumed to be a measure of mitochondrial activity. The activity of some other metabolic enzymes, and enzymes involved in the absorption of metabolites, was hardly affected by tannic acid. In the faeces increased levels of glucosamine and sialic acid were found, indicating that hypersecretion of mucus had occurred.

Motilva *et al.* (1983) studied glucose absorption in the small intestine of rats in the presence of saline extracts of different legume seeds (*Phaseolus vulgaris* and *Vicia faba* L.). They reported that there was an inverse relationship between the polyphenolic content of the extracts and the rate at which D-glucose was absorbed. Addition of polyamide, a strong tannin binder, only partly overcame the observed reduction in glucose absorption, indicating that other factors may have been involved. The authors suggest that polyphenols in the extracts might react with the brush border, thereby modifying membrane proteins, resulting in impaired glucose transport, without gross morphological changes.

Santidrian & Marzo (1989) found reduced intestinal absorption of D-galactose and L-leucine in growing chicks fed diets with 2·5 and 3% tannic acid. Mitjavila *et al.* (1970) observed reduced absorption of glucose and methionine in the small intestine of mice in the presence of tannic acid solutions. Tannic acid, chlorogenic acid and catechol, each in both unoxidized and oxidized form, reduced the Na^+-dependent D-glucose uptake in brush border membrane vesicles isolated from the rat small intestine (Welsch *et al.* 1989).

Sell *et al.* (1985) studied the effects of feeding high- and low-tannin sorghum on the morphology of the duodenum, ileum, caecum and colon of rats, chicks and laying hens. All intestinal sections were morphologically normal as examined by light microscopy. The only consistent effect appeared to be a slight reduction of the crypt depth and wall thickness of the duodenal tissue in animals fed the high-tannin sorghum. Both glucosamine and sialic acid excretion in faeces were elevated in rats on the high-tannin sorghum diet. The latter indicates an increased secretion of mucus from the intestinal tract.

From these studies it can be concluded that hydrolysable tannic acid exerts significant effects on the gut wall morphology and metabolism and, as a result, on the absorption of several nutrients. The effects of condensed tannins in this respect are less clear and need to be studied further.

Systemic effects of tannins

The description of effects of dietary tannins in this review has been confined so far to effects observed on processes in the lumen of the digestive tract or on the mucosa of the intestinal wall. Whether dietary tannins also cause systemic effects in the animal is related to the question whether dietary tannins are absorbed from the digestive tract.

Tannic acid, when fed to different animal species, has been shown not only to affect the digestibility and absorption of nutrients but also to affect different internal organs. Chang & Fuller (1964) observed fatty livers in chicks fed diets containing tannic acid. Karim *et al.* (1978) reported necrosis of the liver and kidneys of chicks fed diets containing 1–3% tannic acid. Also varying degrees of desquamation in the surface epithelium and necrosis of the epithelial layer of the small intestine were found in some birds.

The effects on the liver and kidneys indicate that either tannic acid itself or degradation products of tannic acid (e.g. gallic acid) are absorbed from the small intestine and cause toxic effects. At least some intact tannic acid absorption must have occurred since gallic acid given parenterally or orally did not cause liver damage as did tannic acid (Korpássy *et al.* 1951). The growth depressing effect of gallic acid in chicks was only 30% of that of tannic acid (Kratzer *et al.* 1975). This, however, could be due to the fact that tannic acid also affects digestibility. Gallic acid does not possess the same binding properties as tannic acid.

Tannic acid when injected into rats caused disaggregation of liver polyribosomes, altered microsomal enzyme activity and inhibited nucleic acid and protein synthesis at the cellular level (for a review see Singleton, 1981). Mitjavila *et al.* (1971), however, feeding 3·2% tannic acid to rats for six months, did not find effects on liver function, triglyceride concentration or oxidative enzyme content, although growth was retarded.

Metabolism of tannic acid in animals produces gallic acid derivatives, mainly 4-methoxy gallate (4-O-methyl gallic acid). Oral administration of tannic acid to chickens resulted in some gallic acid excretion in the urine but not in the faeces. Pyrogallol, a metabolite of gallic acid, was found in both the faeces and the urine (Kadirvel *et al.* 1969; Potter & Fuller, 1968). Methionine and choline have been found to alleviate tannic acid toxicity (Chang & Fuller, 1964). It is assumed that this is related to the ability of these nutrients to act as methyl group donors. Methyl groups are required in the process of methoxylation of gallic acid during its detoxification in the liver.

Not much is known about the toxicity of condensed tannins; it is generally assumed that they are relatively resistant to hydrolysis in the gut and are too large to pass the intestinal membranes (Fahey & Jung, 1989; Mole, 1989). Milić & Stojanović (1972) found that free gallotannins from lucerne and gallic acid are degraded in the lumen of the gastrointestinal tract of mice while the condensed tannins of lucerne remained intact. Laparra *et al.* (1977), however, showed that absorption from the gut lumen of dimeric radioactively labelled condensed tannins from grapes occurred in mice. Significant amounts of radioactivity were found in the blood within 10 minutes of oral administration of labelled tannins. It was assumed that the administered tannin fraction was free from labelled monomers and that the condensed tannins had remained intact during passage through the digestive tract.

Because the latter was an assumption, this experiment cannot be considered to be an absolute proof of the absorption of condensed tannins from the gut lumen.

Butler *et al.* (1986) fed ^{125}I-labelled condensed tannins from sorghum to rats. After six days of feeding, 61 % of the label was recovered from the faeces, 20 % was found in the urine and significant levels were found in the serum, liver and kidneys. This should indicate a significant absorption of intact condensed tannins or of their degradation products. In this experiment, however, some doubt was expressed as to the success of labelling of the tannins. Moreover, there is a possibility that modification of the tannins could have occurred during their extraction from the sorghum grain (L. G. Butler, pers. commun.).

Elkin *et al.* (1978) found that laying hens fed high-tannin sorghum diets developed leg abnormalities, characterized by bowing of the legs and swelling of the hock joints. They found that this was not the result of decreased bone mineralization caused by tannins. It was suggested that absorbed tannins from the gut lumen may have caused alterations in the organic matrix of the bones. If this is true, it could be an indication that condensed tannins from sorghum can pass the intestinal barrier. In chicks elevated levels of the liver enzyme UDP-glucuronosyltransferase (EC 2.4.1.17) were found when the animals received a diet containing high-tannin instead of low-tannin sorghum (Sell & Rogler, 1983). This observation was related to the absorption of tannins and their metabolic detoxification in the liver.

It is clear from this review that at least hydrolysable tannins may cause systemic toxic effects. These tannins may reach metabolically active tissues, either by direct absorption of intact tannins or by absorption of their degradation products. Particularly important are the effects on the liver. It is less clear if condensed tannins can cause systemic effects. The literature does not contain firm indications of the absorption of condensed tannins and related systemic effects.

Defensive response towards dietary tannins

A number of herbivorous species consume tannin-rich feedstuffs as a part of their natural diet, without showing severe toxic or otherwise detrimental effects. They have probably developed some type of adaptation towards these dietary constituents.

When rodents such as rats were fed tannin-containing diets, they showed an initial loss of body weight, but after four days the animals started to gain weight again. Such an adaptation has been shown by Glick & Joslyn (1970*b*) when feeding different types of tannins, including tannic acid, and by Mehansho *et al.* (1983) who fed high-tannin sorghum. The latter authors found that in the adapted animals the parotid glands had undergone dramatic hypertrophy, accompanied by an increase in production of a series of proline-rich proteins (PRP). The proteins had a high content of the non-essential amino acids proline, glycine and glutamic acid. It was shown that these proteins had a very high binding affinity for tannins, being ten times higher than the affinity of bovine serum albumin (Butler *et al.* 1986). It was assumed that the secreted PRP in animals receiving a tannin-rich diet act as binding agents for tannins, thereby preventing other harmful and antinutritional effects (Butler *et al.* 1986).

The response of the parotid glands in rats was found when feeding high-tannin sorghum, tannic acid and a number of other tannins. It could not be induced, however, by feeding gallic acid or catechin (Butler *et al.* 1986). Feeding tannins directly into the rat's stomach by tube did not produce a response of the parotid glands, possibly by bypassing the upper digestive tract or due to binding of tannins to dietary proteins before exposure to the digestive tract (Butler *et al.* 1986).

The PRP response due to dietary tannins was also found in mice (Mehansho *et al.* 1985), but not in hamsters (Mehansho *et al.* 1987). The lack of response in the latter species is probably the reason for the high sensitivity of this species to dietary tannins. Hamsters fed a diet with 2% tannins failed to grow over a period of six months. A diet with 4% Quebracho tannins had no effect in rats and mice but was fatal for hamsters (Mehansho *et al.* 1987).

The response of the parotid glands in rats and mice can also be induced by intraperitoneal injection of the β-agonist isoproterenol. Propranolol, a β-antagonist, was found to suppress the hypertrophy of the parotid glands and their PRP synthesis. The mechanism of PRP induction by dietary tannins is therefore most likely to be mediated via β-receptors, but the exact mechanism is unknown (Butler *et al.* 1986).

Proline-rich proteins have been found in the saliva of a number of other species, including man, hare, rabbit, koala, cow and pig. Levels in saliva of cat and dog were very low. The affinity of the PRP for sorghum tannins in the saliva of different species appeared to be rather varied (Mole *et al.* 1990). It is not clear to what extent these PRP play a role in the defence against dietary tannins. Other functions of these proteins have been described (Bennick, 1982) or suggested (Mole *et al.* 1990).

Besides the adaptive mechanism of the parotid glands of rodents towards dietary tannins, no information is available on adaptive mechanisms in other simple-stomached species, including those important in animal husbandry, such as pigs and poultry.

TECHNOLOGICAL TREATMENTS FOR REDUCING TANNIN CONTENT OF FEEDSTUFFS

Various treatments have been proposed to reduce the tannin content of feedstuffs or their biological effects. Where tannins are confined to a specific part of a feedstuff, such as in the hull portion of legume seeds, or in the testa layer just under the seed coat of sorghum, physical removal of the hull (dehulling) reduced the tannin content as shown for faba beans (Eggum, 1980; van der Poel *et al.* 1991) and sorghum (Eggum *et al.* 1983).

Soaking of tannin-containing feedstuffs in water or alkaline solutions may be a way to solubilize and/or modify tannins so that they can be separated from the most valuable part of the feedstuff or become nutritionally less active. Assayable tannin content of sorghum was shown to be reduced after soaking in water or alkaline solution (Price *et al.* 1979). Soaking grains in aqueous sodium hydroxide and washing out alkali and extracted material improved the nutritional value, increased *in vitro* protein digestibility (Chavan *et al.* 1979) and improved starch digestibility (Kock *et al.* 1985). Soaking winged beans (*Psophocarpus tetragonolobus* L.) with distilled water, sodium hydroxide or potassium hydroxide for 24 h reduced tannin content by 50–90% (Sathe & Salunkhe, 1981). Soaking cowpeas (*Vigna sinensis* L.) in aqueous acidic and akaline solutions for 24 h lowered assayable tannins by over 50% and also increased *in vitro* protein digestibility (Laurena *et al.* 1986). The positive effects of acid or alkali treatments as found in the former studies may be related to a change in content or structure of tannins; they may however also be attributed to direct effects of these treatments on the structure and digestibility of proteins. The latter has been reviewed for soyabean proteins by Pedersen (1986).

Reconstitution and anaerobic storage of moistened feedstuffs for 1–3 weeks reduce the assayable tannin content and improve the nutritional value of high-tannin sorghum grain for rats (Reichert *et al.* 1980), chickens (Mitaru *et al.* 1985; Teeter *et al.* 1986) and pigs (Mitaru *et al.* 1984). Anaerobic fermentation may change the structure and reactivity of tannins, thereby improving the nutritional value of sorghum.

Addition of chemicals with a high affinity for tannins, such as polyvinylpyrrolidone and polyethylene glycol or gelatin, may also reduce the nutritional effects of tannins (Butler *et* *al.* 1986; Salunkhe *et al.* 1990). The latter can be explained by the binding of chemicals to dietary tannins, which prevents the tannins binding to nutrients or endogenous proteins. Supplementation of high-tannin sorghum diets for broilers with 0·25 and 0·50% $NaHCO_3$ improved growth performance and nitrogen retention in broilers (Banda-Nyirenda & Vohra, 1990). An explanation for the observations, however, was not given. Spraying of solutions of calcium hydroxide (0–2%), sodium hydroxide (2–10%) and ferrous sulphate (2–10%) on sal seed meal reduced tannin content to various extents (Wah *et al.* 1977).

Tannins in general are rather heat-resistant. Dry heating of high-tannin sorghum did not reduce tannin content (Price *et al.* 1978*b*). Moist heating reduced assayable tannin content in sorghum (Price *et al.* 1980; Bressani *et al.* 1982). Price *et al.* (1980), however, showed in rats that the nutritive value of heat-treated high-tannin sorghum was not improved. The decrease in tannin content may be due to binding of tannins to proteins or other organic compounds, which reduces their extractability. A change in the chemical structure of tannins as a result of heating has never been shown.

Germination of high-tannin sorghum for 72 h reduced tannin content by over 70% (Chavan *et al.* 1981). A similar observation was made with faba beans (Savelkoul *et al.* 1992). This loss in tannins may be attributed to the activity of polyphenol oxidase (EC 1.10.3.2) or other enzymes (Rao & Deosthale, 1982). Others, however, attributed the reduction in tannin content after germination to a decrease in extractability (Bressani & Elias, 1980; Savelkoul *et al.* 1992). Nutritional studies with germinated high-tannin cultivars of cereal grains or legume seeds are scarce.

Although efforts have been made to eliminate or inactivate tannins in feedstuffs by technological treatments, most of them appear to be rather laborious, expensive or ineffective. A detailed review on the effects of various technological treatments on tannins has been recently published by Salunkhe *et al.* (1990).

CONCLUDING REMARKS

In the foregoing, information on plant tannins with respect to their chemistry, occurrence, natural function, analysis and nutritional effects has been reviewed. Although research on tannins has a long history, considerable additional research must be carried out before details of tannin chemistry are elucidated and the nutritional effects of tannins fully explained.

Limited information is available on the chemical nature of polyphenols referred to as tannins in foods and feedstuffs commonly used throughout the world. Advanced techniques such as HPLC and nuclear magnetic resonance should provide better and new information on the biosynthesis and structure of tannins in plant material.

Current information indicates that plant tannins play a protective role in the defence of plants against environmental influences. Increased concentrations of tannins have been found in plants under environmental stress (Mole, 1989).

New, low-polyphenol varieties of some important food and feed plants, such as sorghum and faba bean, have been developed by plant breeders. Although they show a good yield potential in most circumstances, they appear to be more susceptible to microbiological infestation and diseases, and the new sorghum varieties are more attractive to some seed predators such as birds. A certain level of (poly)phenols seems to be essential for adequate disease resistance.

On the other hand, tannins have antinutritional effects, particularly in simple-stomached animals. The main effects of tannins appear to be attributable to their protein-binding capacity. Reduced digestibility of protein and some other nutrients in different animal species has been observed in the presence of tannins in the diets.

Reduced activity of protein degrading enzymes has also been found in the presence of tannins both *in vitro* and *in vivo*. A large number of reports show detrimental effects of dietary tannins on growth performance and efficiency of food utilization in simple-stomached animals, such as rat, chicken and pig.

Generally, much more emphasis should be laid on research dealing with the relation between the chemical nature of the tannins within and between different plant species and their nutritionally harmful effects.

Information is also needed on whether and under what circumstances tannins interact with either feed proteins or endogenous proteins. This topic has been little studied. Answers to these questions could assist in understanding the mode of action of dietary tannins *in vivo*. Other points which remain to be studied are the effects of hydrolysable and condensed tannins on the histology and function of the mucosa of the wall of the digestive tract. It is also not known if intact condensed tannins or their degradation products cause systemic effects after absorption from the lumen of the digestive tract.

Information on the fate of dietary tannins themselves in the gastrointestinal tract is limited. Polyphenolic compounds, particularly condensed tannins, are assumed to be rather resistant towards endogenous enzymes and towards microbial fermentation (Swain, 1979). *In vitro* some bacterial strains were capable of degrading tannins of various origins (Leinmüller *et al.* 1991). *In vivo*, information on the capacity of intestinal or ruminal microflora to degrade condensed tannins is not available. Since microbial activity in the digestive tract of simple-stomached animals is relatively small compared with that in ruminants, degradation of condensed tannins by microflora may not be of quantitative importance. Moreover, most significant microbial activity in these species is found in the hindgut. It is not likely that any microbial degradation of tannins at this site of the digestive tract reduces the antinutritional effects of dietary tannins.

Most of the effects of dietary tannins in simple-stomached animals can be considered antinutritional. A few beneficial effects of tannins, however, have also been suggested. Singleton (1981) states that dietary tannins at appropriate levels may have a general antibiotic effect by suppressing the growth of detrimental flora in the alimentary tract. Although tannins generally reduce the growth of microorganisms (Takechi *et al.* 1985; Laks, 1989; Leinmüller *et al.* 1991), such a specific effect has never been shown. Steiner (1989) suggests that natural tannins active as antiviral and antibacterial agents have potential as future pharmaceuticals. It remains questionable, however, whether preventive or therapeutic effects can be expected from tannins occurring in common foods and feedstuffs.

Beneficial effects of dietary tannins appear to be more important in ruminants. Various authors have reviewed the positive effects of tannins in preventing excessive ruminal degradation of dietary proteins (Mangan, 1988; Leinmüller *et al.* 1991). Tannins also reduce the risk of bloat by binding proteins which are responsible for ruminal foam formation and decrease the activity of gas-producing microflora in the rumen (Mangan, 1988).

With respect to the antinutritional effects of tannins, more attention should be given to differences between animal species. Huisman *et al.* (1990*a*, *b*) reported significant differences between simple-stomached animal species in their sensitivity to other antinutritional factors, such as proteinase inhibitors and lectins. It may be assumed that such differences between species also exist with regard to polyphenolic compounds in plant feedstuffs. Particular attention should be paid to the adaptive response of animals to dietary tannins. Both rats and mice show a specific adaptive response by increasing secretion of PRP by the parotid glands when tannins are present in the diet. This adaptation probably facilitates the consumption of tannin-containing plants and may be associated with the relation between the plant species and the animal species preying on them. The existence of such an adaptation in other species or other adaptive mechanisms have not been reported but they may be important and should be studied (Marquardt, 1989). From the research carried out on tannins and their nutritional effects, most attention has been paid to effects in rats and chickens. Relatively little attention has been given to the effects in pigs, although they are well known as consumers of feedstuffs that may contain high concentrations of tannins. In this respect attention should also be given to the nutritional effects in pigs of condensed tannins present in the hulls of faba bean. The relationship between the chemical nature of the tannins and their nutritional effects has to be considered. Knowledge of the harmful effects of tannins will provide information on the importance of developing new faba bean varieties or other crops with low tannin levels or of looking for technological treatments for tannin inactivation. On the other hand, significant levels of nutritionally less harmful polyphenols may be maintained or increased to enhance the plant's resistance to disease and predators.

Knowledge of the nature of harmful tannins in plant foods and feeds would foster the development of new analytical techniques especially directed towards these compounds. In turn, such techniques will be important for animal nutritionists in determining maximum tolerance levels (threshold levels) for tannins in feedstuffs for simple-stomached animals. Such values are lacking at present.

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