TANNINS IN FABA BEANS (VICIA FABA L.)

- antinutritional properties in monogastric animals -

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr H.C. van der Plas,
in het openbaar te verdedigen
op dinsdag 22 juni 1993
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

Jansman, A.J.M., 1993. Condensed tannins are found in coloured-flowering varieties of faba beans (Vicia faba L.). They are considered as antinutritional factors for non-ruminant species. High-tannin hulls of faba beans and isolated tannins were shown to induce a rapid hypertrophy of the parotid glands in rats and increase the secretion of salivary proline-rich proteins with a high affinity for tannins. In this way rats are able to defend themselves against more harmful effects of tannins. The growth performance of chickens on diets containing 300 g/kg of either low-tannin or high-tannin faba beans did not differ. In contrast, the apparent ileal and faecal digestibility in pigs of protein and amino acids differed significantly between both types of beans, in favour of the low-tannin beans. Inclusion of hulls of faba beans with either a low or a high tannin content in diets for piglets revealed that tannins in faba beans reduce in particular the apparent faecal digestibility of protein and amino acids and the N balance. No systemic/toxic effects of feeding high-tannin hulls to pigs were found. The activity of trypsin and, to a lesser extent, chymotrypsin was reduced in digesta collected from the small intestine of pigs. It was shown with the ^{15}N isotope dilution technique that about half of the extra protein appearing in ileal digesta and faeces of pigs when feeding faba bean hulls with a high tannin content is of endogenous origin. The other half consists of dietary protein. Tannins from faba beans show some preference to interact with proteins with a high content of proline and histidine. A concept for the effects and mode of action of faba bean tannins in different monogastric animal species is discussed.

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The research described in this thesis was financially supported by TNO Nutrition and Food Research, Zeist, The Netherlands.

STELLINGEN

I

De variatie in nutritionele waarde van veldbonen voor éénmagige landbouwhuisdieren wordt mede bepaald door verschillen in het gehalte aan gecondenseerde tanninen (dit proefschrift).

II

De secretie van proline-rijke speekseleiwitten door ratten bij consumptie van tanninen geeft een zeer probate bescherming tegen schadelijke effekten van tanninen in het maagdarmkanaal (dit proefschrift).

III

Door het vermogen zich fysiologisch aan te passen aan tanninen in het rantsoen zijn ratten niet geschikt als modeldier voor onderzoek naar de antinutritionele effekten van tanninen in éénmagige landbouwhuisdieren (dit proefschrift).

IV

De antinutritionele effekten van gecondenseerde tanninen in veldbonen beperken zich bij het varken voornamelijk tot het verteringsproces (dit proefschrift).

V

Onderzoek naar de antinutritionele effekten van tanninen in veevoedergrondstoffen wordt in belangrijke mate belemmerd door gebrek aan adequate analysetechnieken voor tanninen.

VI

Het gebruik van looizuur als referentiestof in onderzoek naar de antinutritionele effekten van gecondenseerde tanninen in vlinderbloemigen, zoals toegepast door Santidrian en Marzo (J. Sci. Food Agric. 47, 435-442, 1989), gaat voorbij aan de wezenlijke verschillen die bestaan ten aanzien van het werkingsmechanisme van gecondenseerde en hydrolyseerbare tanninen.

VII

Het jarenlange onderzoek verricht door een promovendus valt in het niet bij het onderzoek dat een pasgeborene in enkele maanden uitvoert.

VIII

Gezien de geringe kans op honorering van projektvoorstellen ingediend voor bepaalde EG subsidieprogramma's en de inspanning die met de voorbereiding gemoeid is, moet men zich afvragen of een poging tot deelname verantwoord is.

IX

De hernieuwde belangstelling van de farmaceutische industrie voor het regenwoud als apotheek (Volkskrant, 11 juli 1992) is niet zonder gevaar.

X

Zowel het gebruik van doping in de sport als het beleid ten aanzien van de controle hierop is een vorm van oneerlijke concurrentie.

XI

Het veelvuldig misplaatst toepassen van de term "deskundige" roept scepsis op. Het afweren van deze "titel" is derhalve geen bescheidenheid.

XII

In tegenstelling tot hetgeen men zou mogen verwachten, neemt bij toenemende mate van welvarendheid het vermogen om te relativeren af.

XIII

Elke promovendus heeft tijdens de promotieplechtigheid een oogje op de pedel.

Stellingen behorende bij het proefschrift van A.J.M. Jansman,
Tannins in faba beans (Vicia faba L.) - antinutritional properties in monogastric animals.

Wageningen, 22 juni 1993.

Aan Octa
Aan Ineke

VOORWOORD

Bij het gereed komen van dit proefschrift, maak ik gaarne van de gelegenheid gebruik om verschillende personen te bedanken die hebben bijgedragen aan het tot stand komen van dit werk.
Allereerst wil ik mijn promotor Prof. Dr. Ir. M.W.A. Verstegen en co-promotor Dr. J. Huisman hartelijk danken voor hun steun gedurende de afgelopen vier en een half jaar. Martin en Joop, onze discussies in deze tijd zijn voor mij van grote waarde geweest. Drempels voor jullie kamers bleken niet te bestaan.
Vervolgens wil ik de begeleidingscommissie van het onderzoek onder voorzitterschap van Dr. Ir. A.F.B. van der Poel en verder bestaande uit Dr. Ir. H. Boer, Dr. K.D. Bos, Dr. Ir. G.J.M. van Kempen, Prof. Dr. Ir. S. Tamminga en Dr. Ir. E.J. van Weerden bedanken voor hun bereidheid regelmatig als klankbord te dienen bij discussies over de lijnen en de voortgang van het onderzoek.
I also like to thank Prof. Dr. R.R. Marquardt and Dr. A.A. Frohlich (University of Manitoba, Winnipeg, Canada) for offering the possibility to work in their lab for a period of six months and for assisting in the experiments. We will always remember the wonderful time we had in Canada.
De volgende personen zijn mij ook zeer behulpzaam geweest bij het verzetten van zoveel werk. Mijn dank gaat daarom ook uit naar:
- Piet van Leeuwen en Martje Fentener-van Vlissingen voor hun assistentie bij de operaties van de proefdieren;
- Gerard Beelen en Johan de Jong voor hun bijdrage in de coördinatie van het onderzoek;
- Kasper Deuring, Anne Hoek, Dick van Kleef, Johan de Zeeuw, Peter van der Togt en Tamme Zandstra. Jullie leverden als medewerkers van het ILOB en de Vakgroep Veevoeding technische assistentie bij de dierproeven;
- Rolf Coolen, Annemarie van den Driessche, Marian van 't End, Jane-Martine Muylaert, Truus Post, en Kees Oskam voor het analyseren van grote aantallen monsters uit de dierproeven;
- Leo Jacobs, Piet Roeleveld en Karel Siebers voor respektievelijk het ontschillen van de veldbonen en nauwgezet mengen van de proefvoeders;
- Jules Bos, Henk Enting en Jos Houdijk die als student een bijdrage hebben geleverd aan het in dit proefschrift beschreven onderzoek;
- Witek Grala. Thank you for your assistance!
- Jan Wiebenga en Giljam Derksen voor hun hulp bij de statistische analyse van de proefgegevens;
- Dirk van der Heij voor de controle van de Engelse taal;
- het secretariaat van het ILOB en de vakgroep Veevoeding voor de bijdrage aan de afronding van het geheel.
- alle niet met name genoemde personen die bij het onderzoek waren betrokken.
Allen hartelijk dank!

Verder wil ik mijn ouders bedanken voor alle mogelijkheden die zij mij geboden hebben. In het bijzonder wil ik echter Octa noemen. Je had begrip voor de mate waarin het werk mij bezighield en je begreep dat "vrije" tijd soms betrekkelijk was. Dank je wel voor je geduld, hulp en steun.

Het in dit proefschrift beschreven onderzoek is mede mogelijk gemaakt door de financiële ondersteuning van TNO-Voeding, de Landbouwuniversiteit, het Fonds Mestonderzoek en de Universiteit van Manitoba (Canada).

Alfons Jansman

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- antinutritional properties in monogastric animals -

INTRODUCTION

EFFECTS IN DIFFERENT ANIMAL SPECIES

MODE OF ACTION IN PIGS

DISCUSSION

INTRODUCTION

Chapter 1

GENERAL INTRODUCTION

General introduction

To cover the need for protein in animal feeds the European Community (EC) is heavily dependent on the import of protein-rich feedstuffs from outside the EC. Imports of full-fat soya beans and soya bean meal from North and South America are most important in this respect. In 1990, the EC and the Netherlands used 19.6 and 1.6 million tonnes of soya bean meal in animal feeds, respectively (Eurostat, 1991; Hoofd Produktschap Akkerbouw, personal communication). The high dependence on imports, which is accompanied with a large undesirable mineral input and deposition, prompted the EC to stimulate the production and use of home-grown plant protein sources by a price-supporting policy. This resulted in the past decade in an increase in production of legume seeds in western Europe, among which pea (Pisum sativum L.) and faba bean (synonym field or broad bean, Vicia faba L.) are the most important. The increase in use of these legume seeds in animal feeds in the EC in the past decade is shown in Figure 1. In the period 1987-1991 the annual use of peas and faba beans in animal feeds in the EC and the Netherlands amounted to 5.4 and 0.75 million tonnes, respectively (Eurostat, 1991; PGZP, 1991).

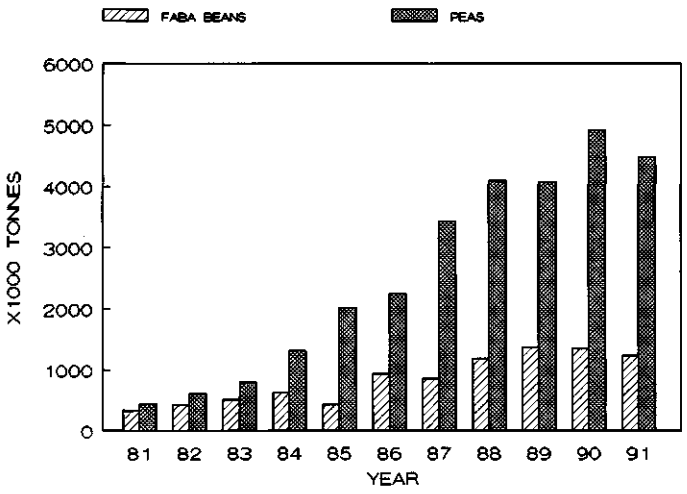

Figure 1. Use of faba beans and peas in animal feeds in the European Community during the past decade.

When alternative protein sources, such as peas and faba beans, were included in diets for pigs, performance of the animals sometimes remained below that obtained with traditional diets containing a large proportion of soya bean meal (Eggum, 1980; Fowler, 1980; Petersen & Schulz, 1980; Huisman, 1989). It was suggested that the presence of antinutritional factors (ANFs) in legume seeds is, at least partly, responsible for these observations. ANFs are defined as non-fibrous naturally occurring substances in feedstuffs which negatively affect performance or health in man and

animals (Huisman & Jansman, 1991). Examples of ANFs in legume seeds are protease inhibitors, lectins, amylase inhibitors, vicine/convicine, phytate, oligosaccharides and tannins (Liener, 1989; Pusztai, 1989; Birk, 1989; Marquardt, 1989; Huisman & Jansman, 1991). Most *in vivo* studies dealing with ANFs have been conducted with rats and poultry, while only a number of studies has been carried out with pigs. The latter, however, is an economically important species in animal production in western Europe and is a consumer of various alternative protein sources. The effects of ANFs in peas in monogastric animals have been extensively studied by Huisman (1990). It became apparent that the effects differ among the ANFs, and also among animal species. This was demonstrated for trypsin inhibitors as well as for lectins.

The effects of ANFs in faba beans, and of tannins in particular, interfering with the nutritional value of faba beans for monogastric animal species need to receive more attention. Tannins are widely occurring polyphenolic compounds in plants. They are known for their strong protein-binding properties. In faba beans they are found in the hull portion of the seed. A literature review (Chapter 2) reveals that tannins in feedstuffs may negatively affect animal performance and can reduce the efficiency of utilization of ingested nutrients. These negative effects of condensed tannins on the nutritive value of faba beans have forced plant breeders to select varieties with a reduced tannin content. As a result, some low-tannin varieties of faba beans have been developed (Bond & Smith, 1989). The low tannin content in these beans, however, revealed an important natural function of plant tannins. Some low-tannin varieties of faba beans show a poor early development. In addition, some have a lower yield performance than traditional high-tannin varieties, particularly under environmentally stressful conditions and in the absence of fungicides (Bond et al., 1986). Tannins play a role in the natural defence of plants and their seeds against environmental stress, such as infestation by micro-organisms and predation, and they may thus be involved in the plant's disease resistance (Salunkhe et al., 1990).

Bond et al. (1991) concluded that a certain level of condensed tannins in faba beans is needed for the plant's ability to cope with environmental stress. It needs to be established yet what level is required for sufficient protection of the seed. That level could be higher than the very low contents found in the present low-tannin varieties.

Although several studies on a range of effects of tannins in feedstuffs have been carried out (Chapter 2), a more detailed study on the effects of faba bean tannins in different animal species, including pigs, has not been performed. Rats and mice are known to have the ability to react to dietary tannins from sorghum by increasing the production and salivary secretion of proteins with a high affinity for tannins. In Chapter 3, it is studied whether rats respond in a similar way when they are fed tannins from faba beans. Subsequently, in Chapters 4 and 5, the nutritive value of faba beans with different levels of condensed tannins is evaluated in chickens and pigs on the basis of different criteria. In broiler chickens, growth performance was studied when feeding diets with faba beans with a different tannin content. In pigs, the apparent ileal and faecal digestibility of nutrients in faba beans varying in tannin content was determined. The results of these studies suggest that the effects of tannins on the nutritive value of faba beans are most pronounced in pigs.

Therefore, in additional studies some aspects of the mode of action of faba bean tannins in pigs were more thoroughly investigated (Chapters 6, 7 and 8). The effects of tannins in faba beans were determined on various physiological parameters (faecal digestibility of nutrients, N utilization, activity of digestive enzymes and organ weights) in pigs by comparing the effects of inclusion of hulls of faba beans with a low or high tannin content (Chapter 6). The results of this study indicate that tannins reduce the apparent digestibility of nutrients and suggest that tannins also decrease the activity of digestive enzymes in pigs. For that reason effects on the activity of two proteolytic enzymes (trypsin and chymotrypsin) in intestinal digesta were studied more extensively in pigs cannulated at the proximal and distal end of the small intestine (Chapter 7). The studies described in Chapters 6 and 7 indicate that tannins have the ability to bind to proteins of both dietary and endogenous origin. Therefore, it was investigated to what extent faba bean tannins influence the endogenous excretion of protein or affect the true digestibility of dietary protein at both the ileal and the faecal level (Chapter 8).

Finally, in Chapter 9 effects of condensed tannins, which were determined in the studies with the different animal species, are evaluated. Special attention is given to a concept for the effects and mode of action of faba bean tannins in different monogastric animal species.

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Chapter 2

TANNINS IN FEEDSTUFFS FOR SIMPLE-STOMACHED ANIMALS

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Nutrition Research Reviews, in press

Contents

Tannins in feedstuffs for simple-stomached animals

1 ABSTRACT

This paper reviews the current knowledge on the natural function, analysis and the nutritional effects of dietary tannins in simple-stomached animals. Tannins are naturally occurring water-soluble polyphenolic compounds capable of interacting with proteins. They appear to play a role in the defence of plants and their seeds against microbial infestation and predation. Their presence is related to the plant's disease resistance. Tannins are a rather heterogeneous group of substances which are generally divided into hydrolysable and condensed tannins. Their chemistry and biosynthetic pathways are not fully known. Analysis of tannins in foods and feedstuffs is often carried out with colorimetric assays, which have limitations in terms of sensitivity and specificity. Other assays for tannins determine biological activity by measuring the protein precipitation capacity of tannin extracts.

Tannins are of interest for nutritionists because of their occurrence in various commonly used foods and feedstuffs. Tannins in diets for simple-stomached animals may result in a reduced performance. In the cereal grain sorghum (Sorghum bicolor) and in legume seeds such as faba beans (Vicia faba L.) variable levels of, in particular, condensed tannins are found. In contrast to hydrolysable tannins, they appear to be relatively resistant to degradation in the gastrointestinal tract of simple-stomached animals. Their antinutritional effects can mainly be related to their ability to form complexes with proteins. As a result, a reduced apparent protein digestibility is often found. It is not clear whether this is due to a reduced digestibility of the feed protein itself or to an increased secretion of endogenous proteins. Clear effects of tannins on the activity of digestive enzymes have also been found, especially *in vitro*. Condensed tannins may also interact with carbohydrates (starch) as well as with certain minerals. Systemic effects have been found after feeding hydrolysable tannins but not, with a few exceptions, after the consumption of condensed tannins. In some rodent species an increased secretion of salivary proteins with a high affinity to tannins is observed after the consumption of tannin-rich diets. This appears to be an important adaptive detoxification mechanism towards tannins in the diets. This or other adaptive responses are unknown in other animal species. More research should be carried out to determine the precise mode of action of tannins of different origin in simple-stomached animals. More knowledge is also needed on the relation between tannin level in the diet and its harmful effects in different animal species. In research carried out in this field so far most attention has been given to effects of tannins in small rodent species, such as rat and mice, and in poultry. Reports on studies with pigs are scarce.

2 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 a variable complexity. It was also

found that these polyphenolic compounds do not only bind strongly to hide protein, but also to other proteins and 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 on 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).

3 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 ($M < 500$) do not form stable cross-links with other molecules. On the other hand, compounds with a much higher molecular weight ($M > 3000$) 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 (1) hydrolysable and (2) 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. Figure 1 gives the chemical structure of a few phenolic acids and 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 so far. 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), like

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. The chemical structure of flavan-3-ol and flavan-3,4-diol is given in Figure 2.

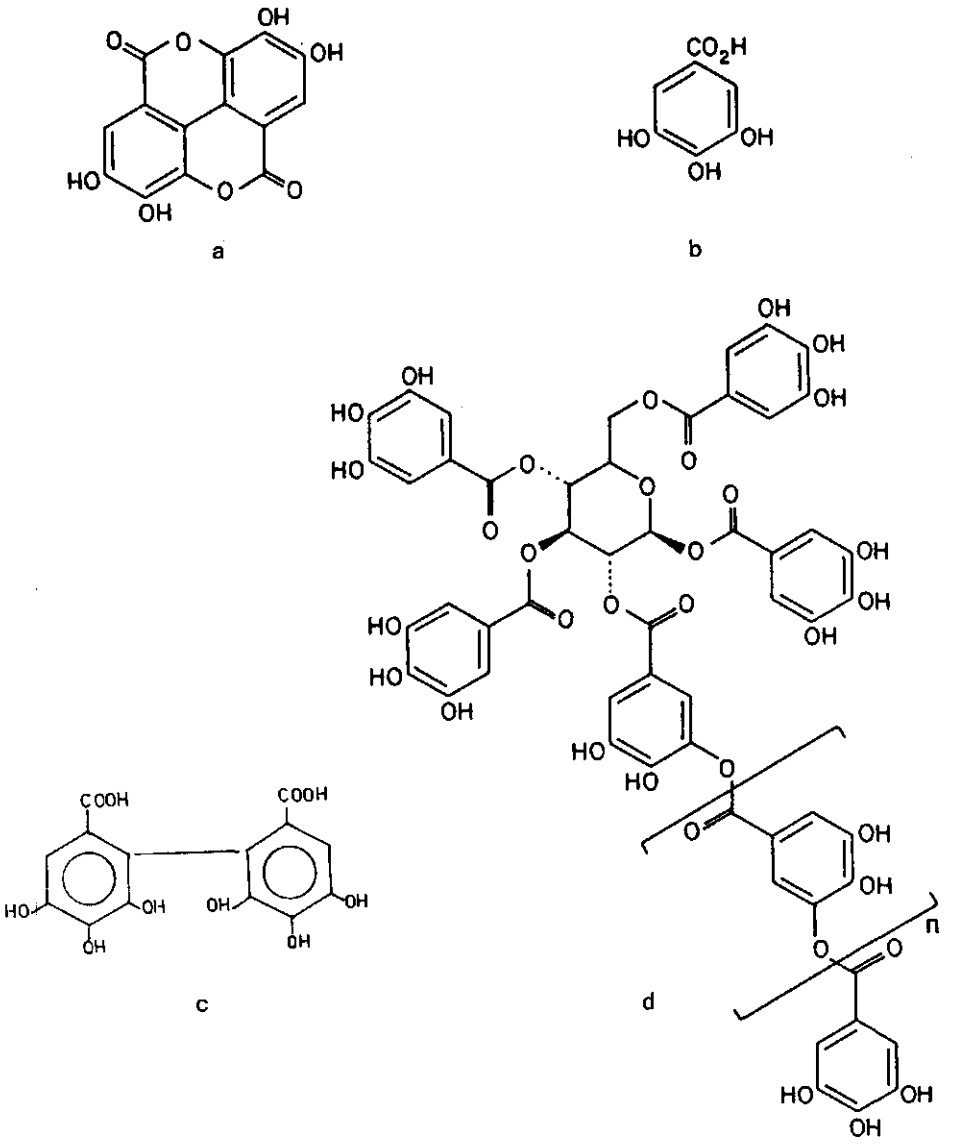
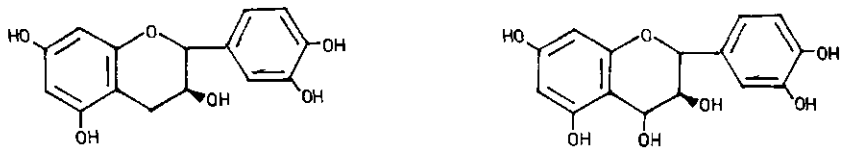


Figure 1. Structure of gallic acid (a) ellagic acid (b), hexahydroxydiphenic acid (c) and a structure of a hydrolysable tannin (d).

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 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).



a b

Figure 2. Structure of flavan-3-ol (a) and flavan-3,4-diol (b).

The exact metabolic routes and intermediates for the formation of condensed tannins from flavonoid compounds are still unknown. A large number of enzymes mediating the different steps in the condensation process, however, 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 Figure 3 (Haslam, 1977).

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

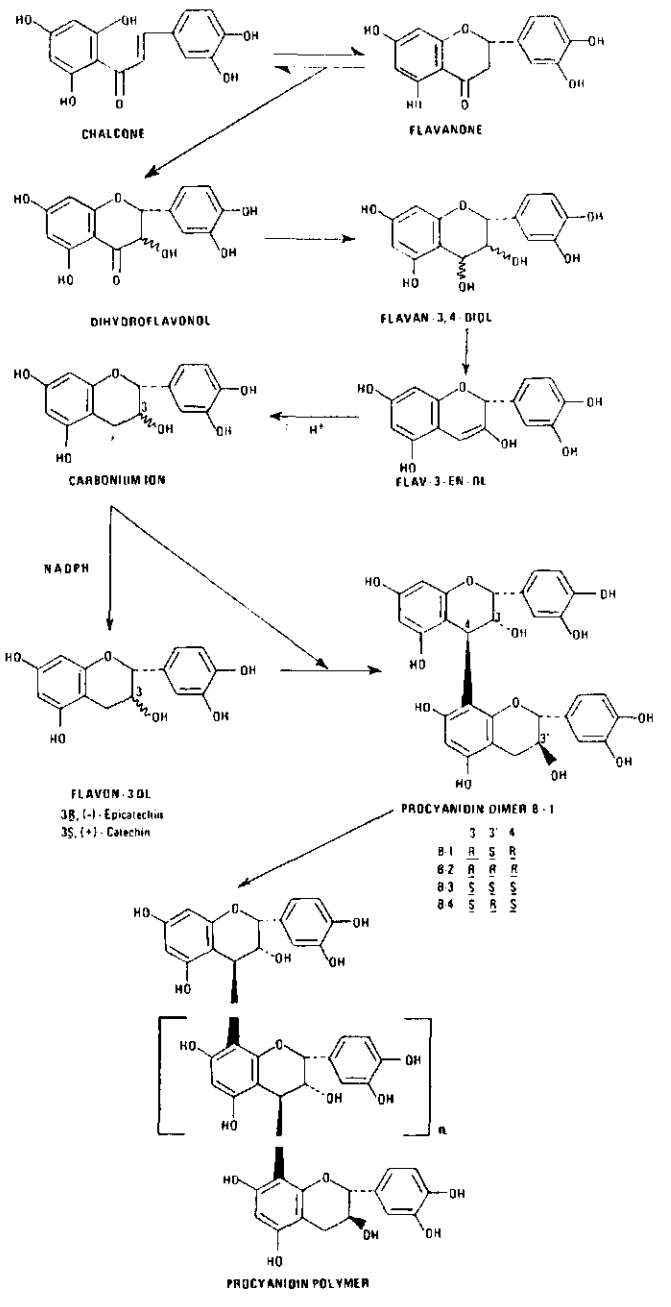


Figure 3. The formation of condensed tannins in sorghum grain.
(From: Haslam, E., 1977. Symmetry and promiscuity in procyanidin biochemistry, Phytochemistry, 16, 1625. With permission).

4 OCCURRENCE OF TANNINS

4.1 General

The nature, content and localisation of tannins in plants varies considerably among species. Polyphenolics are of little importance in low order 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 feed contain considerable amounts of tannins, such as the food grains sorghum (Sorghum bicolor), millet (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 vary considerably among species. The content of tannins (on a dry matter basis) can range from 1.5 to 30% (Leinmuller & Menke, 1990). The tannin content in forage leaves and the leaves of trees and browse species varies considerably during the growing 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 alfalfa (Medicago sativa L.), sericea (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, higher animals cannot synthesize compounds with benzenoid rings from aliphatic precursors with very few exceptions, such as estrone and related phenolic steroids (Singleton, 1981). Plants are the main source of phenolic compounds found in animals.

4.2 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 3.6-10.2% (Harris & Burns, 1970), 4.8-8.2% (Harris et al., 1970) and 2.7-6.9% (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 up to 40 monomeric units.

Barley and millet also contain 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 contents 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 L.), pea (Pisum sativum L.), chick pea (Cicer arietinum L.), faba bean (syn. broad bean, field bean; Vicia faba L.), cow pea (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 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 for seeds having a green, red, beige and brown colour.

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.

5 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, particularly in sorghum. They also play a role in their susceptibility to attack by fungi and pests and in the incidence of pre-harvest 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

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.

Coloured-flowering varieties								
Whole beans		Cotyledons		Testa				
mean	range	mean	range	mean	range	n	Method	Reference
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 & Ueberschar, 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								
Whole beans		Cotyledons		Testa				
mean	range	mean	range	mean	range	n	Method	Reference
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, 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.

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., 1982; 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 pre-harvest 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).

6 TANNIN ANALYSIS

A considerable number of different assays has 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.

6.1 Colorimetric methods

6.1.1 *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., 1978a). The assay is specific for flavan-3-ols, dihydrochalcones and proanthocyanidins (Sarkar & Hawordth, 1976). The principle of the assay is based on the substitution of vanillin for a phenolic hydroxyl group which yields 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 in condensed tannins are not readily available for reaction 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.

6.1.2 *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, not even when the number of hydroxyl groups is taken into account (Goldstein & Swain, 1963).

6.1.3 *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 ferro ferri cyanide ($Fe(III)[Fe(II)(CN)_6]^-$), which is known as Prussian blue. The absorption of this complex can be measured at 720 nm (Price & Butler, 1972). Polyphenolics with a varying hydroxylation pattern and degree of polymerization react differently in this assay.

6.1.4 *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.

6.2 Protein-binding methods

Protein binding assays can be used either to determine the tannin content of a sample or to determine 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 have been used for this purpose such as gelatin, casein, bovine serum albumin, haemoglobin and different enzymes. 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, structure 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; Marks et al., 1987; Makkar 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.

6.3 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 (MS), 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-MW 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 a LH20 column to fractionate tannins from black locust (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 (MS) and NMR spectroscopy (for review see Okuda et al., 1989).

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

7 NUTRITIONAL EFFECTS OF TANNINS

7.1 Effects on animal performance

Numerous studies have been conducted on the effects of tannins in feedstuffs on animal performance. Some of these 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 analytical 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:

1. It has not been conclusively demonstrated that tannins, as found in conventional diets, will reduce feed intake in simple-stomached animal species.
2. Tannins in diets generally will 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 be dependent on the type of assay used,
- length of the test period,
- the animal species selected,
- age of the animal,
- diet composition (e.g. protein level and protein source)
- production level and
- 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 tends to modify the harmful effects of tannins limits the usefulness of direct comparison among the different studies.

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, 1970a
			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 between the value for the high-tannin group(s) and the control or low-tannin group(s)

FI : feed intake
ADG : average daily gain
FC : feed conversion efficiency
F/G : feed : gain ratio
end. N : endogenous N secretion
egg w. : egg weight
$il.DC_{dm}$: apparent ileal dry matter digestibility (units)
$il.DC_N$: apparent ileal N digestibility (units)
$tr.DC_N$: true N digestibility (units)
DC_N : apparent faecal N digestibility (units)
DC_{dm} : apparent faecal digestibility of dry matter (units)
DC_{Nfe} : apparent faecal digestibility of N-free extract (units)
DC_{aa} : mean apparent faecal 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 (%)

Table 3. Some effects of dietary tannins in poultry[1].

Source	Inclusion source (%)	Tannin level	Effect[2]		Reference
sorghum	66	0.0/1.92% D	ADG FC	- 9% +20%	Dale et al., 1980
sorghum	72	low(2)/high(2) 0.0/0.27/0.48/ 1.13% D	ADG	-46.3%	Rostagno et al., 1973a
sorghum	90	0.33/1.41% D	DC_{aa}	-50.8	Rostagno et al, 1973b
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 F/G	-30% +21.9%	Elkin et al., 1990
sorghum	75	low(1)/high(1) 0.05/4.82% S	ADG FC	-41.3% +23.2%	Elkin et al., 1990
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 FC	no effect no effect	Herstad, 1979
tannic acid	1.41%		end. N	+300%	Rostagno et al., 1973b
tannic acid	0.0-1.92%		ADG FC	-12% +10%	Dale et al., 1980
faba bean	48	low(1)/high(2)	DC_N meal DC_N pellet DC_{st} meal DC_{st} pellet AME_N meal AME_N pellet	-14.5 -15.8 + 9.4 + 3.8 + 3.0 + 2.0	Lacassagne et al., 1988
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
sorghum	83-91	low(1)/high(2) 0.2/0.7/0.7%	DC_N DE	- 8.6[3] - 2.2[3]	Herstad, 1979
faba bean	85	low(3)/high(2)	FI ADG F/G	+ 2.2% - 7.5% + 9.3%	Marquardt & Ward, 1979
faba bean hull extract		0-2.5% D	FI ADG F/G ret_{DM} ret_N	-11.7% -33.3% +31.7% -10.3 -19.0	Marquardt & Ward, 1979
faba bean	11-35	low(1)/high(2)	egg weight laying rate ADG	no effect[3] no effect[3] no effect[3]	Larbier, 1980
faba bean	30	low(1)/high(2)	egg weight laying rate	- 6.3% - 7.4%	Martin-Tanguy et al., 1977
faba bean	50	low-high (n=6)	ADG	-26.7%[4]	Martin-Tanguy et al., 1977
sorghum	80	low(1)/high(1) 0.0/5.6% S	ADG F/G	+ 5.7%[5] + 8.7%[5]	Elkin et al., 1990

For abbreviations see Table 2.
[1]Effects in chickens unless stated otherwise [3]in laying hens; [4]in muscovy ducklings; [5]in ducks.
[2]Difference between the value for the high-tannin group(s) and the control or low-tannin group(s).

Table 4. Some effects of dietary tannins in pigs.

Source	Inclusion source (%)	Tannin level	Effect[1]		Reference
sorghum	72	low(2)/high(2) 0.1/0.1/3.6/3.8% S	ADG FI F/G	- 5.4% + 5.9% -14.6%	Myer & Gorbet, 1985
sorghum	90	low(2)/high(1) 0.83/0.88/3.40% S	FI ADG FC	+ 9.4% no effect +10.2%	Cousins et al., 1981
sorghum	90	low(2)/high(2) 0.83/0.88/3.17/ 3.40% S	il. DC_{dm} il. DC_N DC_{dm} DC_N	- 4.0 - 5.8 - 3.6 - 4.8	Cousins et al., 1981
sorghum	77-86	low(1)/high(2) 0.08/1.91/2.83%	il. DC_{dm} il. DC_N DC_{dm} DC_N	+ 0.5 - 6.9 + 7.4 -10.6	Mitaru et al., 1984
sorghum	76	low(1)/high(2) 0.2/1.0/1.4%	ADG (23-60kg) FC ADG (60-103 kg) FC	- 7.5% + 9.7% - 6.4% + 6.7%	Grosjean & Castaing, 1984
faba bean	30	low(1)/high(2) 1.0/1.5/1.7% S	DC_{OM} bean DC_N bean	- 1.3 - 2.7	Liebert & Gebhardt, 1983
faba bean	30	low(1)/high(2)	ADG FC DC_N diet DC_N bean	- 5.9% +11.8% - 1.6 - 6.1	Bourdon & Perez, 1984
faba bean	51	low(1)/high(1)	DC_N DC_{aa} DE	- 8.0 - 9.4 - 3.6	Duée et al., 1979
faba bean	15	low(1)/high(2)	ADG FC	no effect no effect	Fekete et al., 1985

For abbreviations see Table 2.
[1]Difference between the value for the high-tannin group(s) and the control or low-tannin group(s)

7.2 Effects on feed intake

Conflicting reports have been published on the role 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 capability of swallowing 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).

Maxson et al. (1973) report that the feed intake of ruminants was increased when the tannin content of the diet was raised. In contrast, Donnelly & Anthony (1969) report that fodder plants were rejected by ruminants when the tannin concentration of the plants was about 20 g/kg dry matter.

Glick & Joslyn (1970) and Vohra et al. (1966) showed a reduction in feed intake in rats and chickens due to the supplementation of 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 (Zambade 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 among species (Mole, 1989).

7.3 Effects on the digestive process

7.3.1 *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 both the nature of the tannins and that of 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 the ability of the molecule 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 due to 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-to-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.

7.3.2 *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 proteinaceous enzymes. This has implications for their biological activity. Griffiths (1979) reported that activities of trypsin, chymotrypsin and α-amylase 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 (PVP), a strong tannin binder. Extracts of white-flowering faba bean did not show enzyme-inhibiting characteristics.

Also, tannin-containing extracts from rapeseed (Yaper & 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 (1981) has 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.

In the bovine rumen, the activity of various microbial enzymes was reduced by supplementation of tannins from oak leaves (Quercus incano) (Makkar et al., 1988).

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 in rats. The activity of the first two was 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 protease 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 and sucrose α-glucosidase (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 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) indicate 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) state 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 of concern 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.

7.3.3 *Effects of tannins on nutrient digestibility*

7.3.3.1 Effects of tannins on the digestibility of protein and energy

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

7.3.3.2 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 due to dietary tannic acid. Tannic acid has been shown to interact with thiamin (Rungruangak 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. Roa & Prabhavathi (1982) suggest that tannins are responsible for the low bioavailability of iron in legume seeds. Garcia-Lopez et 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. This 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 (Wolters et al., 1992).

Information on interactions of tannins with other minerals is not available.

7.3.4 *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 gastro-intestinal 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 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 ulcerations.

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 with 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 succinate dehydrogenase 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 the 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 in the observed effects. The authors suggest that polyphenols in the extracts might react at the brush border, thereby modifying membrane proteins which results in an impaired glucose transport, without gross morphological changes.

Santidrian & Marzo (1989) found a 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 a 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.

7.3.5 *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 not only been shown 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 parenterally or orally given gallic acid did not cause liver damage as did tannic acid (Korpassy 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 (see for a review 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 activity, 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 gave 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 condensed tannins are relatively resistant to hydrolysis in the gut and are too large to pass the intestinal membranes (Fahey & Jung, 1989; Mole, 1989). Milic & Stojanovic (1972) found that free gallotannins from alfalfa and gallic acid are degraded in the lumen of the gastro-intestinal tract of mice while the condensed tannins of alfalfa 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 of labelled monomers and that the condensed tannins had remained intact during passage trough the digestive tract. Because the latter was an assumption, this experiment cannot be considered to provide an absolute proof for 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, modification of the tannins could have occurred during the extraction of tannins from the sorghum grain (Butler, personal communication).

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 a 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 to cause this effect. In chicks elevated levels of the liver enzyme UDP-glucuronyltransferase were found when the animals received a diet containing high-tannin sorghum 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 degradation products of these tannins. Particularly important are the effects on the liver. It is less clear if condensed tannins can cause systemic effects. The literature does not produce firm indications of the absorption of condensed tannins and related systemic effects.

7.3.6 *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 probably have developed some type of adaptation towards these dietary constituents.

When rodents such as rats are fed tannin-containing diets, they show an initial loss of body weight, but after four days the animals start to gain weight again. Such an adaptation has been shown by Glick & Joslyn (1970b) 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 with an increase in production of a series of proline-rich proteins (PRPs). The proteins had a high content of the non-essential amino acids proline, glycine and glutamic acid. It was subsequently 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., 1983). It is assumed that the secreted PRPs 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 after 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). Tube feeding tannins directly into the rats' stomach 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 ß-agonist isoproterenol. Propranolol, a ß-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 mediated via ß-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 PRPs for sorghum tannins in the saliva of different species appeared to be rather variable (Mole et al., 1990).

It is not clear to what extent these PRPs play a role in the defence against dietary tannins. Also 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.

8 TECHNOLOGICAL TREATMENTS FOR REDUCING TANNIN CONTENT OF FEEDSTUFFS

Various treatments have been proposed to reduce tannin content of feedstuffs or their biological effects. If tannins are confined to a specific part of a feedstuff, such as in legume seeds where tannins are found in the hull portion of the seed, or in the testa layer just under the seed coat, like in sorghum, physical removal of the hull (dehulling), will reduce 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 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 solutions (Price et al., 1979). Soaking grains in aqueous sodium hydroxide and washing out alkali and extracted material improved 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 destilled water, sodium hydroxide or potassium hydroxide for 24 h reduced tannin content by 50-90% (Sathe & Salunkhe, 1982). Soaking cowpeas (Vigna sinensis L.) in aqueous acidic and akaline solutions for 24 h lowered assayable tannins over 50% and also increased *in vitro* protein digestibility (Laurena et al., 1986). The positive effects of acid or alkali treatment as found in the former studies may be related to a change in content or structure of tannins, however, they may also be attributed to direct effects of these treatments on the structure and the digestibility of proteins. The latter has been reviewed for soybean proteins by Pedersen (1986).

Reconstitution, anaerobic storage of moistened feedstuffs for one till three weeks, reduces the assayable tannin content and improves 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 structure

and reactivity of tannins, thereby improving the nutritional value of sorghum.

Addition of chemicals with a high affinity for tannins, such as polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) or gelatin may also be useful to 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 to bind to nutrients or endogenous proteins. Supplementation of high-tannin sorghum diets for broilers with 0.25 and 0.50% of $NaHCO_3$ improved growth performance and nitrogen retention in broilers (Banda-Nyirenda & Vohra, 1990). An explanation for the observations, however, was not given. Spraying with solutions of calcium hydroxide (0-2%), sodium hydroxide (2-10%) and ferrous sulphate (2-10%) on Salseed meal (Shorea robusta) 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. 1978b). 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 of 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 with over 70% (Chavan et al., 1981). A similar observation was made in faba beans (Savelkoul et al., 1992). This loss in tannins may be attributed to the activity of polyphenol oxidase or other enzymes (Rao & Deosthale, 1982). Others, however, attributed the reduction in tannin content after germination to a decrease in their 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 using technological treatments most of these 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).

9 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, it can be stated that considerable additional research must be carried out before details of tannin chemistry are elucidated and the antinutritional effects of tannins are fully explained.

First of all, limited information is available on the chemical identity of polyphenols referred to as tannins in food and feedstuffs commonly used throughout the world. Advanced techniques such as HPLC, MS and NMR 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 levels 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 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. A reduced digestibility of protein and some other nutrients in different animal species has been observed due to the presence of tannins in the diets.

A 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 detrimental effects.

Also information is needed on whether and under which circumstances tannins interact with either feed proteins or endogenous proteins. This topic has been studied little. 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 are capable of degrading tannins of various origin (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 to that in ruminants, degradation of condensed tannins by microflora does not seem to 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 will reduce the antinutritional effects of dietary tannins.

Most of the effects of dietary tannins in simple-stomached animals can be considered as antinutritional effects. 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 as

active 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 among animal species. Huisman et al. (1990a,b) reported significant differences among simple-stomached animal species in their sensitivity to other antinutritional factors, such as protease inhibitors and lectins. It may be assumed that such differences among 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 proline-rich proteins by the parotid glands when tannins are present in the diets. This adaptation probably allows the consumption of tannin-containing plants and may be associated with the relation between the plant species and the animal species predating on those. The existence of such an adaptation in other species or other adaptive mechanisms has not been reported but 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 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 (Vicia faba L.). 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 diseases and predators.

Knowledge on 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, these 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 presently lacking.

Acknowledgements

The author wishes to thank Professor M.W.A. Verstegen and Professor R.R. Marquardt for their valuable comments on the manuscript.

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EFFECTS IN DIFFERENT ANIMAL SPECIES

Chapter 3

PRODUCTION OF PROLINE-RICH PROTEINS BY THE PAROTID GLANDS OF RATS FED DIETS CONTAINING TANNINS FROM FABA BEANS (VICIA FABA L.)

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Submitted for publication

Production of proline-rich proteins by the parotid glands of rats fed diets containing tannins from faba beans (Vicia faba L.)

Abstract

Feeding of a casein-based diet with either 400 g/kg of tannin-containing faba bean hulls (Vicia faba L.) (1.41 % condensed tannins) or 60 g/kg of a tannin-rich hull extract of faba beans (1.99 % condensed tannins) to rats over a period of 7 days resulted in a 4.6 and 2.3 fold increase in weight of the parotid glands, respectively, ($P<0.05$) and a corresponding 6.5 and 4.7 fold increase in the level of proline-rich proteins (PRPs) in the glands ($P<0.05$). In a dose-response experiment, increasing the level of tannin-rich hull extract in the diet (0.0, 3.8, 7.5, 15.0, 30.0 and 60.0 g/kg) resulted in a linear increase in both the size of parotid glands ($R^2 = 0.90$; $P<0.05$) and the quantity of PRPs in the glands ($R^2 = 0.89$; $P<0.05$). The apparent caecal digestibility of total ($R^2 = 0.97$) and individual amino acids (R^2 varied from 0.27 to 0.99) decreased linearly ($P<0.05$). The quantity of PRPs in the caecum of rats was estimated from the decrease in caecal digestibility of proline, glycine and glutamic acid. The estimated secretion of PRPs, when calculated on the basis of the three respective amino acids, were 3.40, 3.48 and 3.93 mg of PRPs per 10 mg of additional hull extract in the diet (21.8% of condensed tannins). The results indicate that tannins from faba beans stimulate the parotid glands to increase the secretion of PRPs in rats. The PRPs then interact with dietary condensed tannins to reduce their antinutritional effects.

Introduction

Tannins are defined as naturally occurring polyphenolic compounds with a molecular weight between 500 and 3000 daltons with the ability to precipitate proteins in aqueous solutions (Bate-Smith & Swain, 1962). They are found in various plant species, among them plants which are used as human food or animal feed, such as cereal grains, legume seeds, forages and browses (Salunkhe et al., 1990a; Leinmüller & Menke, 1990). Tannins are considered to play a role in the plant's ability to cope with environmental stressors, such as predation by rodents and birds, and infestation by micro-organisms, including moulds (Salunkhe et al., 1990a). When present in diets for livestock, tannins generally reduce growth performance and increase the excretion of nutrients. In diets for man and non-ruminant animal species, tannins can reduce the digestibility of protein, carbohydrates and minerals, may lower the activity of digestive enzymes, and some of them may cause damage to the mucosa of the digestive tract or exert systemic toxic effects (Mangan, 1988; Salunkhe et al., 1990b; Jansman, 1993). In addition, negative effects of dietary tannins on voluntary feed intake have been reported (Robbins et al., 1987).

The consumption of diets containing high-tannin sorghum was shown to specifically increase the size of the parotid glands and the synthesis and secretion of proline-rich proteins (PRPs) in rats (Mehansho et al., 1983). This response was not observed after

feeding low-tannin sorghum. Similar results were obtained with mice (Mehansho et al., 1985). Recently, Mehansho et al. (1992) showed that the response of the rat's parotid glands was also induced by isolated and purified condensed tannins from sorghum or tannic acid. They hypothesized that PRPs are secreted with the saliva and are bound with dietary tannins in the oral cavity to protect dietary protein. PRPs found in parotid glands of rats are soluble in trichloroacetic acid (TCA) and have molecular weights (MW) between 27,000 and 38,000, except for one glycoprotein with a MW of 200,000 (Mehansho et al., 1983). In a competitive binding assay PRPs were found to have a 1000-fold higher affinity for tannins than other proteins, such as lysozyme (Mehansho et al., 1983).

In the present paper the effects of condensed tannins from faba beans (Vicia faba L.), in native and isolated form, on the size of the parotid glands of rats and the production and secretion of PRPs and individual amino acids were studied.

Materials and methods

Feeding trials

Two feeding trials with rats were carried out. In *Exp. 1* the effects of feeding a tannin-rich extract of faba bean hulls (60 g/kg) were compared with those of feeding tannin-rich faba bean hulls (400 g/kg). In *Exp. 2* the dose-response relationships between the amount of hull extract in the diet (0.0, 3.8, 7.5, 15.0, 30.0, and 60.0 g/kg) and feed intake, body weight gain, weight of the parotid glands and the synthesis and secretion of PRPs were established.

In both experiments, male Sprague-Dawley rats were housed individually in wire cages at 22 °C. The relative humidity was about 60% and the lighting period from 07.00 to 19.00 h. Prior to introduction of the experimental diets, rats were fed a regular Chow diet (Wayne, Rodent Blox F6C). In *Exp. 1*, 15 weanling rats were purchased and after 3 weeks were fed a purified diet; diet I, *Exp. 1* (Table 1). After 18 days the rats were weighed and allocated to one of the 5 experimental diets (Table 1). Each group consisted of 3 rats and had a mean body weight of 218 ± 33 g. In the following 7 day period, feed intake and body weight were monitored daily. After the experimental period the rats were weighed and euthenasized using CO_2 and cervical dislocation. Both parotid glands of each rat were dissected. After stripping of connective tissue, the glands were weighed and stored at -20 °C. The caecum was removed and caecum digesta were transferred into small vials and stored at -20 °C.

In *Exp. 2*, 24 rats (110 g) were fed the regular Chow diet for 19 days and then diet I, *Exp. 2* (Table 1). After 8 days the animals were weighed and assigned to one of the 6 experimental groups. The mean body weight of rats in each group was 303 ± 27 g. Group I consisted of 6 rats and the other groups of 4 rats. Experimental diets were fed for a period of 7 days followed by dissection and collection of caecal digesta according the procedure described for *Exp. 1*. In both experiments feed and water were offered ad libitum.

Table 1. Composition (g/kg) of the experimental diets in *Exps. 1* and *2*.

	Experiment 1					Experiment 2					
	I	II	III	IV	V	I	II	III	IV	V	VI
Casein	200	200	200	200	200	150	150	150	150	150	150
Corn starch	150	150	150			150	150	150	150	150	150
Cellulose	50	110	50	400		110.0	106.2	102.5	95.0	80.0	50.0
Sucrose	497	437	437	297	297	489	489	489	489	489	489
Corn oil	50	50	50	50	50	50	50	50	50	50	50
Tannin extract			60				3.8	7.5	15.0	30.0	60.0
Faba bean hulls					400						
Mineral mix[1]	35	35	35	35	35	35	35	35	35	35	35
Vitamin mix[2]	12	12	12	12	12	10	10	10	10	10	10
DL-methionine	3	3	3	3	3	3	3	3	3	3	3
Cr_2O_3	3	3	3	3	3	3	3	3	3	3	3

[1]Bernhart Tomarelli Salt Mixture, modified; ICN Biochemicals, Cleveland, OH 44128. The mineral mix contains per kg: 21.0 g calcium carbonate, 735 g dicalcium phosphate, 2.27 g citric acid, 0.46 g cupric citrate, 5.58 g ferric citrate, 25.0 g magnesium oxide, 8.35 g manganese citrate, 0.01 g potassium iodide, 81.0 g potassium phosphate, 68.0 g potassium sulfate, 30.6 g sodium chloride, 21.4 g sodium phosphate, 1.33 g zinc citrate.

[2]Vitamin Diet Fortification Mixture, ICN Biochemicals, Cleveland, OH 44128. Cat. no. 904654. The vitamin mixture contains per kg: 1.8 g vitamin A acetate (500.000 IU/g), 0.125 g vitamin D concentrate (850.000 IU/g), 22.0 g α-tocopherol (250 IU/g), 45.0 g ascorbic acid, 5.0 g inositol, 75.0 g choline chloride, 2.25 g menadione, 5.0 g p-aminobenzoic acid, 4.25 g niacine, 1.0 g riboflavin, 1.0 g pyridoxine hydrochloride, 1.0 g thiamine hydrochloride, 3.0 g calcium pantothenate, 0.020 g biotin, 0.090 g folic acid, 0.00135 g vitamin B12.

Preparation of faba bean hulls

Faba beans (cv. Diana) were coarsely crushed in a plate grinder. A crude hull fraction was obtained by the use of a gravitity shaker. The hull fraction (testa) was further purified by air-classification. The hull fraction contained approximately 96% of testa and 4% of cotyledons. Prior to inclusion in the diets, hulls were ground in a hammer mill over a 1/16 inch screen and a laboratory mill with a 1 mm screen (Cyclotec, 1093 Sample Mill, Tecator). The tannin content of the hulls was 5.0% catechin equivalents (Kuhla & Ebmeier, 1981).

Preparation of a tannin-rich extract from faba bean hulls

The procedure used to obtain the tannin-rich extract from faba bean hulls has been described in detail by Marquardt et al. (1977). Faba bean hulls (cv. Diana) were extracted with 7 volumes of water. The aqueous solution, after removal of the solid parts, was subjected to a freeze-thaw cycle with the aim to denature the extracted protein. After reconstitution and removal of the precipitate, the solution was concentrated by reverse osmosis and further concentrated under reduced pressure. The extract was finally dried by lyophilization. The dried powder was stored in a dark, air tight container at -20°C. The tannin content of the extract was 21.8% catechin equivalents (Kuhla & Ebmeier, 1981).

Experimental diets

The composition of the diets of both experiments is shown in Table 1. Each of the diets in *Exp. 1* contained 200 g/kg of casein as the only source of protein. In diets II and III, 60 g/kg of corn starch were replaced by the same level of cellulose and the extract from the hulls of faba beans, respectively, compared to diet I. In diets IV and V, 400 g/kg of cellulose or tannin-containing faba bean hulls were included at the expense of corn starch and sucrose. As a result, diets IV and V had a lower content of digestible energy compared to diets I-III. Feed samples were analysed for their content of condensed tannins according the method of Kuhla & Ebmeier (1981), using catechin as a standard. Levels of condensed tannins in the experimental diets were below the detection limit (<0.10%) for diets I, II and IV (Table 2). Diet III with 60 g/kg of hull extract and diet V with 400 g/kg of faba bean hulls contained 1.41 and 1.99% of condensed tannins, respectively.

Diets in *Exp. 2* contained increasing concentrations of tannin-rich extract as replacement for cellulose. Levels of condensed tannins in the diets were below 0.10% for diets I-III, and 0.33, 0.66 and 1.32% catechin equivalents for diets IV, V and VI, respectively. Calculated values for the tannin content of the diets generally agreed well the analysed values. For diet III the calculated value (0.16%) was slightly higher than the analysed value (<0.10%) (Table 5). Casein was the only source of protein (150 g/kg) for all diets in *Exp. 2*.

In both experiments, diets contained vitamins and minerals according to the rat's requirements (NRC, 1978). Chromic oxide (Cr_2O_3) was added to the diets as a digestibility marker. The diets were prepared in a dough mixer (mixing time 10 min) and offered to the rats in meal form.

Determination of proline-rich proteins (PRPs) in parotid glands

PRPs were extracted from the parotid glands using the procedure of Mehansho & Carlson (Mehansho & Carlson, 1983) with slight modifications. Glands were homogenized for 1 min in 5 volumes of 10% trichloroacetic acid (TCA). Samples were centrifuged for 20 min at 17.000 g at 4°C and the supernatant was saved. The pellet was reextracted with two volumes of 10% TCA and centrifuged again. The combined

supernatants were extracted three times with three volumes of ether saturated with water to remove the TCA. The concentration of PRPs in the resulting samples were determined spectrophotometrically with the bicinchonimic acid (BCA) assay (Smith et al., 1985) using purified PRPs from parotid glands of rats fed faba bean tannins as a standard. The absorbancy of non-PRPs, BCA reactive, low molecular weight compounds present in the tissue extracts, was determined following membrane filtration (Centricon Microconcentrators, MW cut-off 10.000; Amicon Division, W.R. Grace & Co, Danvers MA 01923, USA). These values were substracted from those of the corresponding original samples. The quantity of PRPs in the parotid glands was calculated by multiplying the concentration of PRPs in the extracts by the volume of the extract.

Determination of apparent caecal digestibility values

Digesta from the caecum obtained during dissection were freeze-dried and finely crushed. Individual samples were analysed for dry matter, Cr_2O_3 (Williams et al., 1962) and amino acids, excluding methionine, cystine and tryptophan, using a 24 h hydrolysis with 6N HCl at 110°C (Andrews & Baldar, 1985). Feed samples were analysed according to the same procedures. Apparent caecal digestibility figures were calculated using the marker ratio technique.

Calculation of the quantitative secretion of PRPs by the parotid glands

In a preliminary study 6 rats were fed a diet with 60 g/kg of hull extract from faba beans (similar to diet III of *Exp. 1*) for 7 days. The parotid glands were collected and the PRPs in the glands were extracted from the tissue as described above. Low molecular weight compounds were removed from the extract by membrane filtration (Centriprep Concentrators, MW cut-off 10.000; Amicon Division, W.R. Grace & Co, Danvers MA 01923, USA). The purified extract was freeze-dried and amino acid analysis was performed. Proline, glutamic acid and glycine appeared to be the predominant amino acids found in the purified PRPs, contributing 33.8, 27.9 and 11.2 weight percentages, respectively (unpublished results). The quantitative increase in secretion of PRPs by the parotid glands of the rats fed tannin-containing diets in the present studies (*Exp. 1* and *2*) was estimated independently from the reduction in the apparent caecal digestibility values for proline, glutamic acid or glycine compared to the values for the same amino acids in their respective control groups (experimental groups II and IV in *Exp. 1*, group I in *Exp. 2*). It was assumed that the extra proline, glycine and glutamic acid in caecal digesta in the tannin-fed groups originated only from an increase in secretion of PRPs by the parotid glands. This assumption is based on the observation that PRPs, which contained over 70% of the above three amino acids, are selectively secreted and bound to tannins when tannins are present in the diet (Jansman, 1993).

Statistical analysis

Analysis of variance was carried out on all data from *Exps. 1* and *2* using treatment as a factor. If the treatment effect was significant, the differences between treatment means were tested with the Least Significance Difference (LSD) test (Snedecor & Cochran, 1980).

Results of *Exp. 2* were also analysed with a linear regression model using "inclusion level of hull extract" as the independent variable (SPSS, 1988).

Results

One rat each in group II, *Exp. 1* and in groups I and III of *Exp. 2* were excluded from the data base as they did not consume the diets.

Experiment 1

Feed intake of rats did not differ significantly between groups I-IV, but was significantly lower for group V ($P<0.05$) (Table 2). Weight gain over the same period was highest for rats in groups I and III, greatly reduced for rats in groups II and IV, and was negative for those in group V ($P<0.05$). The total amounts of tannins consumed by rats fed diets III and V were approximately the same.

Both the absolute and the relative weight (as a % of body weight) of the parotid glands were much higher in the groups which received tannin-containing diets (groups III and V) ($P<0.05$) compared to those fed the non tannin-containing diets (groups I, II and IV) (Table 2). The increase in size of the glands in these groups was accompanied by a significant increase in the amount of PRPs found in the glands of rats in the same groups ($P<0.05$). Amounts were 6.5 and 4.7 times higher than in their respective control groups II and IV (Table 2). A high cellulose content of the diet (diets I vs. II vs. IV) had no effect on the absolute or relative size of the parotid gland or their content of PRPs ($P>0.05$).

Increasing the level of cellulose from 50 (diet I) to 110 g/kg (diet II) did not reduce the apparent caecal digestibility of amino acids, whereas increasing it to 400 g/kg (diet IV) reduced digestibilities of all but three of the amino acids ($P<0.05$) with the overall decrease for all amino acids being 7% units (94-87%, $P<0.05$) (Table 3). Inclusion of the tannin-rich hull extract (diet III) or faba bean hulls (diet V) had a much greater effect on amino acid digestibility than cellulose, with the effects being different for rats fed diets containing the tannin extract (diet III vs. II) compared to those fed the diet containing faba bean hulls (diet V vs. IV). For most essential amino acids the difference in caecal digestibility between diets II and III was less than 10 percentage units. The largest differences were found for the non-essential amino acids proline (Pro), glutamic acid (Glu) and glycine (Gly) (-117, -33 and -114 percentage units, respectively). Inclusion of hulls of faba beans (group V) instead of cellulose (group IV) had a more pronounced effect on the apparent caecal digestibility of each of the amino acids (Table 3). However, again the decrease was highest for the digestibility of

Table 2. Tannin content of the diets, number of animals per group (n), feed intake and weight gain of rats over 7 days, final body weight, weight of parotid glands and their level of PRPs (means ± SE) in *Exp. 1.*

Diet	I	II	III	IV	V
Tannin content[1] (%)	<0.10	<0.10	1.41	<0.10	1.99
n	3	2	3	3	3
Feed intake (g)	141.6 ± 9.7[a]	118.6 ± 15.4[a]	117.9 ± 2.9[a]	131.8 ± 3.4[a]	76.1 ± 14.8[b]
Weight gain (g)	40.6 ± 4.7[a]	22.1 ± 3.6[b]	36.8 ± 1.7[a]	17.6 ± 3.5[b]	-18.7 ± 3.8[c]
Final body weight (g)	260 ± 20[a]	227 ± 14[ab]	254 ± 19[a]	235 ± 12[ab]	198 ± 19[b]
Parotid gland (mg)	720 ± 53[a]	476 ± 23[a]	2219 ± 202[c]	677 ± 60[a]	1550 ± 198[b]
Parotid gland (% of body weight)	0.28 ± 0.04[a]	0.22 ± 0.01[a]	0.87 ± 0.02[b]	0.29 ± 0.04[a]	0.78 ± 0.04[b]
PRPs (mg)	4.67 ± 0.35[a]	3.23 ± 0.27[a]	37.43 ± 3.93[b]	9.31 ± 1.06[a]	26.88 ± 5.88[b]

[b,c]Values with a different superscript in the same row differ significantly at $P<0.05$

[1]% catechin equivalents

Table 3. Apparent caecal digestibility values (%) (means ± SE) for amino acids and dry matter of the diets in *Exp. 1*.

Diet	I (<0.10%)[1]	II (<0.10%)	III (1.41%)	IV (<0.10%)	V (1.99%)
Arg	94.3 ± 0.5^{a}	93.0 ± 0.1^{a}	70.1 ± 1.1^{c}	81.8 ± 2.7^{b}	42.6 ± 1.3^{d}
His	96.4 ± 0.2^{a}	95.9 ± 0.4^{a}	87.2 ± 0.5^{c}	90.1 ± 1.3^{b}	60.2 ± 0.6^{d}
Ile	90.4 ± 1.1^{a}	89.4 ± 1.2^{ab}	88.2 ± 1.4^{ab}	83.1 ± 0.0^{b}	64.5 ± 3.5^{c}
Leu	95.8 ± 0.3^{a}	94.8 ± 0.4^{a}	92.1 ± 0.3^{b}	89.8 ± 1.0^{c}	71.5 ± 1.0^{d}
Lys	95.2 ± 0.5^{a}	93.8 ± 0.3^{a}	89.4 ± 0.2^{b}	87.0 ± 1.3^{b}	71.1 ± 1.6^{c}
Phe	95.7 ± 0.3^{a}	94.6 ± 0.4^{a}	90.6 ± 0.4^{b}	87.5 ± 1.3^{c}	66.0 ± 0.8^{d}
Thr	92.1 ± 0.8^{a}	90.5 ± 0.3^{a}	86.4 ± 0.8^{ab}	80.3 ± 2.3^{b}	54.0 ± 3.6^{c}
Val	93.2 ± 0.7^{a}	91.9 ± 0.8^{a}	89.3 ± 1.1^{ab}	84.8 ± 0.8^{b}	62.8 ± 3.2^{c}
Ala	87.1 ± 1.3^{a}	83.5 ± 0.6^{ab}	78.9 ± 0.6^{b}	67.5 ± 4.3^{c}	37.9 ± 2.4^{d}
Asp	91.6 ± 0.6^{a}	89.6 ± 0.1^{a}	77.2 ± 0.3^{b}	78.9 ± 3.3^{b}	46.0 ± 2.9^{c}
Glu	94.6 ± 0.4^{a}	93.6 ± 0.2^{a}	61.0 ± 1.6^{c}	91.6 ± 0.1^{a}	70.8 ± 1.4^{b}
Gly	83.1 ± 1.4^{a}	80.1 ± 0.7^{a}	-36.3 ± 19.3^{b}	56.7 ± 6.2^{a}	-20.0 ± 5.7^{b}
Pro	96.8 ± 0.4^{a}	95.7 ± 0.3^{a}	-21.1 ± 9.6^{c}	92.7 ± 1.7^{a}	52.5 ± 1.8^{b}
Ser	87.0 ± 0.8^{a}	86.1 ± 0.6^{ab}	79.4 ± 0.9^{c}	81.3 ± 0.3^{bc}	56.6 ± 2.9^{d}
Tyr	94.5 ± 0.2^{a}	93.1 ± 0.4^{a}	90.5 ± 0.4^{a}	85.7 ± 2.1^{b}	66.6 ± 1.7^{c}
ΣAA	93.7 ± 0.5^{a}	92.5 ± 0.3^{a}	66.3 ± 1.6^{c}	86.6 ± 1.1^{b}	60.4 ± 1.6^{d}
DM	89.0 ± 0.5^{a}	83.5 ± 0.3^{a}	84.1 ± 0.2^{a}	59.2 ± 2.3^{b}	50.7 ± 2.8^{c}

^{a,b,c}Values with a different superscript in the same row differ significantly at $P<0.05$.
[1] Values in brackets represent the tannin content of the diets (% catechin equivalents).

proline (-40 % units) and glycine (-77 % units).

Estimates for the increase in excretion of PRPs ranged from 34-69 mg PRPs per gram of feed intake for rats in group III, and from 26-35 mg PRPs per gram of feed intake for those fed the diet with faba bean hulls (diet V) (Table 4).

Experiment 2

Feed intake, body weight gain during the experimental period (7 days) and final body weight did not show large differences between the experimental groups (Table 5).

Feeding diets with increasing concentrations of the tannin-rich extract (X; mg/g) resulted in a linear increase in the relative weight of the parotid glands (as percentage of body weight; Y_1) (Figure 1) and in the quantity of PRPs in these glands (Y_2) (Figure 2). The corresponding prediction equations were $Y_1 = 0.0104\ X + 0.206$ ($R^2 = 0.90$) and $Y_2 = 1.12\ X + 6.07$ ($R^2 = 0.89$).

Table 4. Estimated increase of the excretion of PRPs (mg/g feed intake) in rats fed tannin containing diets, based on the reduction in caecal amino acid digestibility values for proline (Pro), glutamic acid (Glu) or glycine (Gly) (*Exp. 1*) (means ± SE).

	60 g/kg extract (group III)	400 g/kg tannin-rich faba bean hulls (group V)
Pro	69.4 ± 5.7	25.7 ± 1.2
Glu	49.8 ± 2.4	35.5 ± 2.4
Gly	33.8 ± 5.8	35.4 ± 2.6
Mean	51.0	32.2

The apparent caecal digestibility of amino acids of diets I-VI are presented in Table 6. For all amino acids, digestibility decreased linearly with an increasing level of hull extract in the diet. However, those amino acids which were affected to the greatest degree as assessed by the regression coefficient (a) were proline ($a = -7.9$, $R^2 = 0.98$) and glycine ($a = -18.0$, $R^2 = 0.99$). The increase in secretion of PRPs by the parotid glands was estimated from the decrease in apparent caecal digestibility of proline, glycine or glutamic acid in groups II-VI compared to the values for group I. The results show a linear positive relationship between the level of hull extract in the diet and the estimated increase in secretion of PRPs (Figure 3). Strikingly, the estimates based on the digestibility values for proline, glutamic acid and glycine were almost the same being 3.40, 3.48 and 3.93 mg per 10 mg hull extract (21.8% condensed tannins) in the diet, respectively.

Discussion

Condensed tannins in foods and feedstuffs have been shown to reduce the apparent digestibility of nutrients, particularly of crude protein. Included have been studies on the effects of tannins in tea, cacao and carob on rats (Shahkhalili et al., 1990); in faba beans on poultry (Martin-Tanguy et al., 1977; Ford & Hewitt, 1979; Mehansho & Carlson, 1983), on rats (Ford & Hewitt, 1979) and on pigs (Jansman et al., 1993); in sorghum on rats and poultry (Ford & Hewitt, 1979; Rogler et al., 1985); and in leaves from different fodder plants in rats (Horigome et al., 1988). The effect of tannins on N digestibility appeared to be dependent on both the level and the origin of the tannins, as well as on the animal species involved. Some authors found evidence for differences in the sensitivity among animal species for tannins in sorghum (Ford & Hewitt, 1979; Mehansho et al., 1987a; Elkin et al., 1990) and faba beans (Ford & Hewitt, 1979).

Table 5. Tannin content of the diets, number of animals per group (n), feed intake and body weight gain over a period of 7 days and body weight of rats at the time of dissection in *Exp. 2* (means ± SE).

Diet	I	II	III	IV	V	VI
Tannin content[1]	<0.10 (0.00)	<0.10 (0.08)	<0.10 (0.16)	0.28 (0.33)	0.66 (0.65)	1.32 (1.31)
n	5	4	3	4	4	4
Feed intake (g)	160.1 ± 5.3^{abc}	159.4 ± 6.5^{abc}	168.4 ± 5.0^{ab}	167.9 ± 6.4^{ab}	157.2 ± 9.3^{bc}	142.6 ± 4.7^{c}
Body weight gain (g)	28.2 ± 0.8^{ab}	30.6 ± 4.7^{ab}	31.3 ± 2.4^{ab}	32.2 ± 2.2^{a}	34.7 ± 4.2^{a}	23.3 ± 1.5^{b}
Final body weight (g)	328.7 ± 19.1^{a}	335.1 ± 11.4^{a}	326.6 ± 9.0^{a}	338.5 ± 12.1^{a}	338.5 ± 13.9^{a}	329.1 ± 9.8^{a}

[1] % catechin equivalents; in brackets calculated contents based on the analysed content of the tannin extract (21.8%).
^{a,b,c}Values with a different superscript in the same row differ significantly at $P<0.05$.

Table 6. Apparent caecal digestibility values (%) (means ± SE) for amino acids and dry matter of diets in *Exp. 2* and the total explained variance (R^2), linear regression coefficient (a) and constant (b) of the best fitting linear models for the apparent caecal digestibility data (Y; %) with the level of hull extract in the diet (X; 10 mg/g).

Diet	I (0.0)[1]	II (0.08)	III (0.16)	IV (0.33)	V (0.66)	VI (1.32)	R^2	a	b
Arg	90.4 ± 0.8^{a}	86.1 ± 1.1^{b}	85.3 ± 0.8^{bc}	82.8 ± 0.5^{c}	73.0 ± 1.9^{d}	62.0 ± 0.3^{c}	0.95	- 4.7	89.0
His	93.9 ± 0.5^{a}	92.0 ± 0.3^{b}	91.3 ± 0.5^{b}	89.7 ± 0.2^{c}	86.2 ± 0.4^{d}	81.7 ± 0.6^{e}	0.94	- 2.0	93.0
Ile	89.4 ± 0.9^{a}	87.6 ± 0.9^{ab}	87.3 ± 1.0^{abc}	87.1 ± 0.8^{abc}	85.1 ± 0.8^{bc}	84.7 ± 1.1^{c}	0.42	- 0.7	88.3
Leu	94.0 ± 0.4^{a}	92.8 ± 0.4^{ab}	92.3 ± 0.2^{b}	92.3 ± 0.5^{b}	90.5 ± 0.7^{c}	88.2 ± 0.7^{d}	0.79	- 0.9	93.5
Lys	93.1 ± 0.4^{a}	91.8 ± 0.5^{a}	92.2 ± 0.2^{a}	91.9 ± 0.5^{a}	89.8 ± 0.7^{b}	87.3 ± 0.2^{c}	0.82	- 0.9	92.8
Phe	89.4 ± 0.9^{a}	87.0 ± 0.5^{ab}	85.7 ± 0.2^{ab}	86.8 ± 0.5^{ab}	85.1 ± 0.7^{ab}	79.5 ± 0.2^{b}	0.27	- 1.4	88.5
Thr	88.7 ± 0.9^{a}	85.3 ± 1.0^{b}	85.4 ± 0.9^{b}	84.9 ± 0.9^{b}	80.6 ± 1.6^{c}	77.5 ± 0.9^{c}	0.74	- 1.7	87.2
Val	91.8 ± 0.4^{a}	89.6 ± 0.5^{ab}	89.9 ± 0.8^{ab}	89.3 ± 1.0^{b}	86.8 ± 0.9^{c}	85.2 ± 0.9^{c}	0.66	- 1.0	90.8
Ala	83.5 ± 1.1^{a}	80.7 ± 1.4^{ab}	81.0 ± 0.9^{ab}	80.7 ± 1.7^{ab}	77.8 ± 1.4^{b}	71.5 ± 1.4^{c}	0.71	- 1.8	82.8
Asp	87.3 ± 0.7^{a}	84.5 ± 1.1^{b}	83.9 ± 0.1^{b}	82.1 ± 0.9^{b}	76.6 ± 1.3^{c}	69.3 ± 0.9^{d}	0.92	- 2.9	86.4
Glu	93.4 ± 0.5^{a}	91.5 ± 0.6^{b}	90.9 ± 0.4^{bc}	88.9 ± 0.3^{c}	83.1 ± 0.7^{d}	73.8 ± 1.0^{e}	0.97	- 3.2	93.2
Gly	78.6 ± 1.0^{a}	69.8 ± 2.0^{b}	64.4 ± 0.8^{b}	54.8 ± 0.9^{c}	20.2 ± 3.4^{d}	-28.8 ± 3.7^{e}	0.99	-18.0	78.1
Pro	95.7 ± 0.4^{a}	92.2 ± 0.6^{a}	92.0 ± 0.6^{a}	86.4 ± 0.8^{b}	72.1 ± 1.8^{c}	49.6 ± 2.4^{d}	0.98	- 7.8	96.3
Ser	87.2 ± 1.2^{a}	84.0 ± 1.3^{b}	83.1 ± 1.2^{b}	81.9 ± 0.8^{b}	78.7 ± 0.9^{c}	72.9 ± 0.3^{d}	0.84	- 2.2	85.7
Tyr	92.9 ± 0.4^{a}	92.0 ± 0.8^{a}	90.3 ± 0.5^{ab}	90.9 ± 0.3^{a}	87.8 ± 1.6^{bc}	85.7 ± 1.0^{c}	0.68	- 1.2	92.3
ΣAA	91.5 ± 0.5^{a}	89.2 ± 0.7^{b}	88.8 ± 0.2^{bc}	87.1 ± 0.3^{c}	81.6 ± 0.8^{d}	73.6 ± 0.1^{e}	0.97	- 2.9	91.0
DM	84.3 ± 0.3^{a}	82.4 ± 0.3^{b}	83.1 ± 0.4^{b}	82.7 ± 0.4^{b}	82.2 ± 0.3^{b}	82.9 ± 0.3^{b}	ns		

[1]% catechin equivalents; in brackets calculated contents based on the analysed content of the tannin extract.
ns: not significant

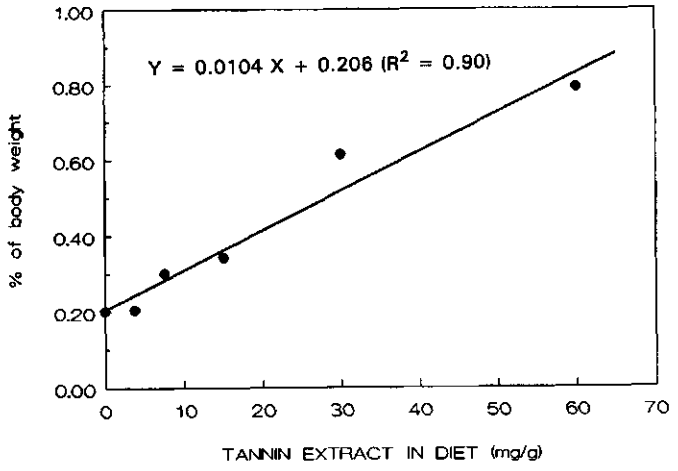

Figure 1. Effects of feeding diets containing various levels of a tannin-rich extract from faba bean hulls on the relative weight (% of body weight) of the parotid glands of rats (*Exp. 2*).

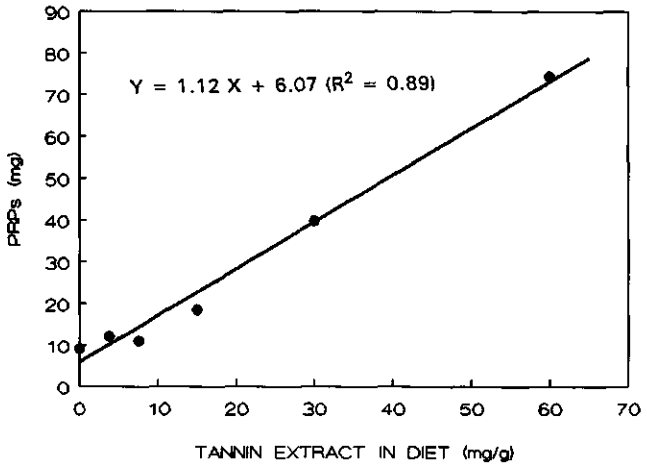

Figure 2. Effects of feeding diets containing various levels of a tannin-rich extract from faba bean hulls on the quantity of PRPs in the parotid glands of rats (*Exp. 2*).

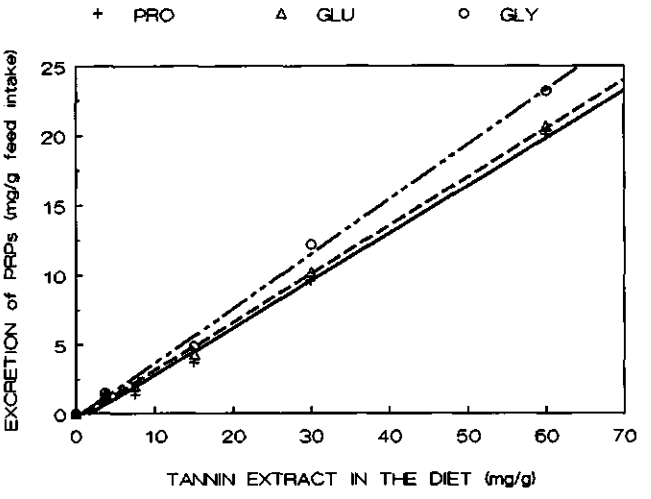

Figure 3. Estimates for the increase in excretion of PRPs in rats fed diets containing various levels of a tannin-rich hull extract from faba beans, based on the reduction of the caecal digestibility of proline (Pro), glutamic acid (Glu) or glycine (Gly) (*Exp. 2*).

Binding of tannins to both dietary and endogenous proteins, such as digestive enzymes and proteins located at the luminal side of the intestinal tract, have been used to explain the reduced apparent digestibility of protein in tannin-containing diets. Clear evidence for systemic effects in animals after feeding condensed tannins does not exist. Condensed tannins are hypothesized to be resistant to intestinal degradation and to be too large for intact absorption (Mole, 1989).

In the present study a significant reduction in the digestibility of protein (total amino acids) in rats receiving diets with either tannin-containing faba bean hulls or a tannin-rich extract from the same hulls was accompanied by an increase in both the size of the parotid glands and the level of PRPs in these glands. A similar response of parotid glands was found in rats and mice after feeding high-tannin sorghum (Mehansho et al., 1983; 1985) and later in rats after feeding tannic acid and isolated tannins from sorghum (Mehansho et al., 1992). Tannin induced PRPs were shown to have a very high binding affinity for tannins (Mehansho et al., 1983). Mehansho et al. (1987b) suggested that some species show this response as a first line of defense against these naturally occurring polyphenolic compounds. It was shown in our study that the same mechanism is triggered in rats within 7 days after the introduction diets containing condensed tannins from faba beans (Vicia faba L.).

Although diet V in *Exp. 1* had a higher content of condensed tannins than diet III, the response of the parotid glands in size and content of PRPs was slightly lower for the hull fed group (group V). This may be related to a higher solubility of tannins in the

extract compared to tannins in faba bean hulls, which may be capable of more efficiently stimulating the parotid glands. The effects on both gland size and the increase in the amount of PRPs in parotid glands in our study were somewhat less than found by Mehansho et al. (1983) using a diet with 926 g/kg of sorghum containing 7.7% of condensed tannins. This is probably attributable to the amount of tannins in the diet but other factors such as source of tannins, diet composition (protein content), age of the rats and the method used to determine PRPs in parotid glands may also affect the results obtained.

Digesta from the caecum of rats were used to estimate the digestibility of individual amino acids in the diets. Estimates with a very low standard error were obtained for most amino acids. In *Exp. 1* the differences in apparent caecal digestibility of amino acids between groups can be explained by differences in the level of dietary fibre, content of condensed tannins and accessibility of tannins to PRPs. Shah et al. (1982) showed effects of both the level and source of fibre (including cellulose) on the apparent faecal digestibility of nitrogen in rats. The lower faecal N digestibility with increasing levels of dietary fibre was explained by an increased secretion of endogenous proteins, as a result of an increased secretion of digestive enzymes, sloughing of the intestinal mucosa, and a reduced rate of intestinal reabsorption of amino acids from endogenous proteins. The same explanation can in part be used for the differences in *Exp. 1* in apparent caecal digestibility of amino acids in rats fed different amounts of cellulose. Another reason for the effects of hulls compared to hull extracts is that the tannins in rats fed the faba bean hulls not only interacted with PRPs but to a greater degree with those in the diet and other endogenous proteins. This would have increased their concentration in the caecal digesta relative to values obtained with the hull extract and thereby the apparent overall amino acid digestibilities.

The apparent digestibility for most essential amino acids was affected in the groups fed the tannin-containing diets to a lesser degree than for some of the non-essential amino acids, particularly proline, glycine and glutamic acid. It was hypothesized that the observed reduction in digestibility for these amino acids was mainly due to the interactions of tannins with the PRPs that were secreted by the parotid glands as these three amino acids comprise 72.9% of the weight of isolated PRPs from parotid glands of tannin fed rats. This assumption formed the basis of the calculation of the quantitative increase in excretion of PRPs (Table 4, Figure 3). In *Exp. 1*, the average estimated increase in excretion of PRPs was higher for the group fed the extracted tannins (51 mg/g feed intake) than for group fed tannins as faba bean hulls (32 mg/g feed intake). Proportional changes in both the size and the amounts of PRPs in the parotid glands for these groups were found (Table 2). These effects occurred even though the total amount of tannins that were consumed by the rats in both groups was about the same. The total excretion of all of the other amino acids, however, was much greater in rats fed the hull diet. These results suggest that tannins in faba bean hulls at the concentrations used more effectively decreased total amino acid digestibilities than tannins from the extract of hulls except for glycine, glutamic acid and proline, amino acids which are the main constituents of the PRPs. These results may be explained on the basis that tannins in the extract presumably were able to bind

to PRPs more rapidly than those in faba bean hulls, as a result the total amount of PRPs and the corresponding three amino acids excreted would be greater in rats fed the tannin extract compared to the faba bean hulls. Tannins from the faba bean hulls presumably did not have ready access to the PRPs and therefore tended to also interact with other proteins which contained all of the amino acids. This would result in an increased excretion of the other proteins and therefore result in reduced digestibilities of the corresponding amino acids that comprise these proteins.

In *Exp. 2*, consistent estimates were obtained for the increase in excretion of PRPs (Figure 3). The average estimate for the increase in excretion of PRPs at the highest inclusion level of hull extract (60 g/kg) was 21 mg per gram of feed intake. This value was much lower than found in *Exp. 1* (51 mg/g feed intake). The reason for this difference was not established but it may in part be attributed, as the data from Tables 3 and 6 would suggest, to an enhanced excretion of non-PRPs in the second study compared to the first study. Presumably the tannins in *Exp. 1* were able to interact more effectively with the PRPs than in the second study.

From the foregoing it is likely that a major part of the increase in amino acids in caecal digesta in the tannin fed groups is the result of the presence of proline-rich proteins secreted by the parotid glands. Shahkhalili et al. (1990) observed, using the ^{15}N isotope dilution technique, that the reduced faecal N digestibility in rats fed a diet with a polyphenol-rich tea extract is mainly due to an increased excretion of endogenous nitrogen, which is in agreement with observations from the present studies.

It would appear that the adaptive mechanism of the parotid glands responds in a dose-response manner towards an increase in tannin content of the diet since the size of the parotid glands, their content of PRPs and the caecal excretion of these proteins increases linearly with an increased content of tannin-rich extract in the diet. It may be assumed that the physiological limits of the response were not reached in the present studies, in which dietary tannin levels up to 2% catechin equivalents were used. The amount of PRPs estimated to be present in the caecum is well correlated with the dietary level of condensed tannins. In fact, higher levels of PRPs may have been secreted by the parotid glands but they may have been reabsorbed in the small intestinal tract after enzymatic or fermentative degradation if they had not interacted with dietary components such as tannins.

The mechanism by which tannins induce the hypertrophy of parotid glands of rats and mice and increase secretion of PRPs is not clear. It is known that propranolol, a ß-antagonist, is capable of inhibiting the response in rats. This suggests that ß-receptors are involved in the development of this physiological mechanism (Mehansho et al., 1992). Mole et al. (1990a) observed a much larger depression in growth of rats, when fed a diet with high-tannin sorghum and propranolol compared to the growth of those receiving the same diet without propranolol. The data on weight gain of the animals in the current study (Table 5) should be considered with care, because of the limited number of animals per group and the short time period (7 days). They nevertheless suggest that rats are able to maintain an adequate growth rate when fed diets with increasing level of condensed tannins from faba beans.

Dietary tannins that are effectively bound by PRPs present in the saliva probably do not interact with feed proteins or other endogenous proteins such as digestive enzymes. The latter may be particular harmful to the animal. The effectiveness of the PRP response, however, may be incomplete particularly when dietary tannins are not rapidly extracted from the diet during chewing. This may be expected when using tannin-containing feedstuffs instead of tannin extracts. Since tannins in diets for rats are capable of reducing the activity of several digestive enzymes in the small intestine (Horigome et al., 1988; Griffiths & Moseley, 1980) tannins may not always be effectively bound by PRPs.

Although saliva of various species contain proline-rich proteins (Mole et al., 1990b), it is not clear whether species others than rat and mice are able to develop a similar response when consuming tannin-containing diets. In hamster, this response was absent and used as an explanation for the high sensitivity of this species for dietary tannins (Mehansho et al. 1987a). If other species lack this response, it can be stated that rat and mice are invalid models for establishing the nutritional effects of dietary tannins in these species.

Overall it may be concluded that faba bean tannins increase the size of the parotid glands and the production of PRPs which in turn are capable of binding tannins. The degree that PRPs bind dietary tanins appears to be influenced by several factors. This can be estimated from the secretion of its principle constituent amino acids, glutamic acid, glycine and proline. Tannins that do not interact with PRPs probably bind exogenous or endogenous proteins resulting in an enhanced excretion of all amino acids.

Acknowledgements

The authors wish to thank P.A. Mills for analysis of amino acids and J. Wiebenga for assistance in the statistical analysis of data.

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Chapter 4

PERFORMANCE OF BROILER CHICKS FED DIETS CONTAINING DIFFERENT VARIETIES OF FABA BEAN (VICIA FABA L.)

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Archiv für Geflügelkunde, in press

Performance of broiler chicks fed diets containing different varieties of faba bean (Vicia faba L.)

Abstract

In two experiments faba beans (Vicia faba L.) with varying levels of condensed tannins were evaluated as a protein supplement in diets for broiler chicks (1-4 weeks of age). The inclusion level of the beans was 300 g/kg. Rations were carefully balanced on content of metabolizable energy (ME) and levels of digestible indispensible amino acids. No significant differences in weight gain and feed conversion efficiency of broilers were observed between diets containing varieties of faba beans varying in tannin content (Experiment 1). Inclusion of an autoclaved batch (30 min, 105°C) of low-tannin beans (300 g/kg) did not affect performance of the chickens as compared to those fed a control maize/soya bean meal ration (Experiment 1).

When protein and essential amino acid contents in the diets were reduced to slightly marginal levels (90% of requirements), still no difference in nutritive value between two varieties of faba beans with either a high or a low tannin content were established as measured by the growth performance of broiler chicks (Experiment 2). It was concluded that faba beans are a suitable protein source for broiler chicks. Levels of condensed tannins in coloured-flowering varieties of faba beans (up to 1% catechin equivalents) seem to be too low to exert antinutritional effects in growing chicks when bean inclusion levels do not exceed 300 g/kg and diets are nutritionally well balanced.

Introduction

Indigenous protein sources for animal feeds have gained considerable interest in Western Europe during the past decade. Among them are legume seeds such as faba beans (Vicia faba L.). Based on protein content and amino acid composition, faba beans seem to be a nutritionally well balanced alternative protein source in poultry diets. Only their content of methionine may be limiting (Griffiths, 1980). Inclusion of raw faba beans in broiler diets supplemented with methionine, however, reduced animal performance in different studies (Blair et al., 1970; Rubio et al., 1989). Others did not find negative effects on performance of broilers when raw faba beans up to a level of 450 g/kg were included in diets (Kadirvel & Clandinin, 1974; Ilian et al., 1985).

Differences in levels of antinutritional factors (ANFs) in faba beans have been associated with the reduced performance of broilers on some occasions. Trypsin inhibitors (Wilson et al., 1972a,b), lectins (Rubio et al., 1989, 1990), both of which are associated with the cotyledon fraction of the seed, and condensed tannins (Martin-Tanguy et al., 1977; Marquardt & Ward, 1979; Wareham et al., 1991), which are present in the hull portion of the seed, have been suggested to be involved. Marquardt & Ward (1979) suggest that over 50% of the observed reduction in performance of

broilers when fed raw faba beans was due to factors present in the hulls of coloured-flowering, tannin-containing faba beans.

Heat treatment generally improved the nutritive value of faba beans for poultry (Wilson et al., 1972a; Edwards & Duthie, 1973; Marquardt & Campbell, 1973; Rubio et al., 1989). Inactivation of the heat-sensitive antinutritive factors, such as protease inhibitors and lectins, may be a plausible explanation for this improvement after heating. Condensed tannins in different feedstuffs, however, are not or only partly inactivated by heat (Marquardt & Ward, 1979; Salunkhe et al., 1990).

In the present studies the nutritive value for growing chicks of different varieties of faba bean with a varying tannin content was evaluated. The faba beans were included in diets at a level of 300 g/kg. The protein level of the diet and the balance of levels of indispensible amino acids were taken into account. In one of the two experiments also a heat-treated batch of a low-tannin variety of faba bean was examined for its nutritive value for broiler chicks. This treatment was chosen in order to evaluate the possible detrimental effects of heat-labile antinutritional factors such as protease inhibitors and lectins.

Materials and methods

Experiment 1

Diets

In the first experiment eleven experimental diets were evaluated. Diet I was a control diet free of legume seeds. Diet II was considered to be a practical maize/soya bean meal diet. Diets III-VIII each contained 300 g/kg of faba beans of a different variety. Two low-tannin (cvs. Blandine and Toret), one medium-tannin (cv. Herz Freya) and three high-tannin varieties (cvs. Alfred, Mythos and Troy) were used. In order to evaluate the nutritive value of heat-treated low-tannin faba beans, a batch of cv. Blandine beans was autoclaved for 30 min at 105°C (bean moisture level 20%). The beans were subsequently dried at ambient conditions for 24 h. The dried beans were included in diet IX at a level of 300 g/kg. To determine whether possible detrimental effects on the nutritive value of high-tannin faba beans were cotyledon- or hull-associated, in two other diets (diets X and XI, respectively), cotyledons or hulls of the high-tannin variety Alfred were included. Inclusion levels of both fractions were 248 and 52 g/kg, respectively. These levels are equivalent to the inclusion of 300 g/kg of raw faba beans. Cotyledons and hulls were obtained by breaking whole beans on a roller mill (roller distance 3 mm) followed by partitioning in an air classifier.

All diets were balanced to contain 12.94 MJ ME/kg and 204 g/kg of crude protein (CP) and were assumed to contain similar levels of digestible indispensible amino acids (CVB, 1988). Calculated levels of digestible amino acids in the diets were 12.1 g/kg for lysine, 8.3 g/kg for methionine plus cystine, 7.2 g/kg for threonine, 2.4 g/kg for tryptophan and 12.3 g/kg for arginine.

Table 1. Composition of the diets (g/kg) in Experiment 1.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Maize	449.0	411.6	294.7	295.1	283.1	275.8	281.2	274.5	294.7	315.2	399.4
Barley	299.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	300.0
Casein	103.0	6.0	42.5	42.5	52.0	58.0	54.0	58.0	42.5	57.5	102.0
Herring meal	30.0	30.0	18.0	17.5	22.0	25.0	22.5	25.0	18.0	25.0	30.0
Meat meal	20.0	20.0	11.0	11.0	13.5	15.0	14.0	15.0	11.0	15.0	20.0
Soya bean meal		210.0									
Faba beans[1]			300.0	300.0	300.0	300.0	300.0	300.0	300.0		
Cotyledons of faba beans[2]										248.4	
Hulls of faba beans[2]											51.6
Cellulose	17.5	6.5								19.0	
Sunflower oil	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Animal fat		37.5	48.5	48.0	46.0	45.0	46.0	45.5	48.5	37.0	16.5
Cane molasses	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Premix[3]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
$CaCO_3$	13.5	11.0	13.0	13.0	13.0	12.6	12.7	13.0	13.0	13.0	13.5
$CaHPO_4$	10.5	12.5	15.5	15.5	14.0	13.5	14.0	13.5	15.5	13.7	10.5
NaCl	3.0	2.5	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-lysine HCl		4.0	2.9	3.0	2.3	1.5	2.0	1.8	2.9	1.6	
DL-methionine	1.4	2.8	3.4	3.4	3.2	2.9	3.0	3.0	3.4	2.9	1.5
L-threonine		0.9	1.2	1.2	1.0	0.7	0.9	0.8	1.2	0.8	
L-tryptophan		0.5	0.7	0.7	0.6	0.5	0.5	0.5	0.7	0.5	
L-arginine	4.1	1.7	0.6	1.1	1.8	2.0	1.7	1.9	0.6	1.9	4.0
$NaHCO_3$	2.0	2.5	2.5	2.5	2.0	2.5	2.0	2.0	2.5	2.0	2.0
$KHCO_3$	7.0		2.5	2.5	2.5	2.0	2.5	2.5	2.5	3.5	6.0

[1]Included faba beans: low-tannin varieties Blandine (diet III) and Toret (diet IV), medium-tannin variety Herz Freya (diet V), high-tannin varieties Alfred (diet VI), Mythos (diet VII) and Troy (diet VIII); diet IX contained autoclaved faba beans of the low-tannin variety Blandine.

[2]Cotyledons and hulls of the high-tannin variety Alfred.

[3]The premix supplied per kg feed: riboflavin 4 mg; nicotinamide 40 mg; d-panthotenic acid 12 mg; choline chloride 800 mg; cyanocobalamin 15 μg; DL-tocopherol acetate 15 mg; menadione 5mg; retinyl acetate 3.44 mg; cholecalciferol 50 μg; biotin 0.1 mg; folic acid 1.0 mg; $FeSO_4.7H_2O$ 300 mg; MnO_2, 100 mg; $CuSO_4.5H_2O$ 100 mg; $ZnSO_4.7H_2O$ 150 mg; Na_2SeO_3 0.15 mg; ethoxyquin 100 mg; avoparcin 10 mg.

During diet formulation the analysed content of CP and amino acids of the faba beans were used (Table 2). The beans of all varieties were assumed to contain 9.70 MJ ME/kg and to have similar digestibility values for essential amino acids (CVB, 1988). ME content of faba bean cotyledons and faba bean hulls were estimated to be 10.66 and 2.63 MJ ME/kg, respectively. The diets were calculated to contain 9.6 g/kg Ca and 5.2 g/kg available P (CVB, 1988). Diets were prepared in a cold pelleting process ($T < 60°C$).

The diet composition is given in Table 1. The composition of the faba beans and faba bean fractions is shown in Table 2.

The tannin content of the faba beans was measured according to the Folin Denis method (Swain & Hillis, 1959), the vanillin-sulphuric acid method (Kuhla & Ebmeier, 1981) and the ISO method (ISO, 1987). The activity of trypsin inhibitors was determined according to the method of van Oort et al. (1989).

Activity of lectins was measured by their haemagglutination activity (Valdebouze et al., 1980).

Animals and experimental procedures

One-day-old Hybro male broiler chicks were randomly divided over 66 electrically heated battery cages with wire floors. The cages were situated in an insulated room with facilities to control temperature and relative humidity. Chicks were subjected to continuous artificial fluorescent illumination. The first five days after arrival all animals received control diet I. At day 6 after hatching (experimental day 0) the animals were weighed and allocated to one of the eleven experimental diets. Each treatment was assigned to six cages with 15 chickens each in a randomized block design. At day 0 average body weight of the animals was 123 g. Average body weight was similar for each cage. In the three following days the animals became used to the experimental diets.

Both feed and water were offered ad libitum throughout the experiment.

At days 7, 14 and 21 the animals were weighed individually and feed consumption per cage was recorded.

At the end of the experiment (day 21) 18 animals of the dietary treatments I, III, VI and VII were randomly selected for the determination of pancreatic weight.

Performance of the birds was determined by measuring feed intake, body weight gain and feed conversion efficiency (FCE).

Experiment 2

Diets

Experiment 2 was set up to evaluate the effect of tannins in faba beans under conditions of marginal supply of protein and essential amino acids (90% of requirements; NRC, 1984). Eight dietary treatments were tested. The composition of the diets is given in Table 3. Both diets I and II were control diets free of legume seeds and containing 200 and 180 g/kg of CP, respectively. Levels of all indispensible

Table 2. Chemical composition (g/kg) of the faba bean varieties[1] and faba bean fractions used in the experimental diets.

	BL[1]	TO[1]	HF[1]	AL[1]	MY[1]	TR[1]	aBL[1]	cAL[1]	hAL[1]
Dry matter	869.6	885.4	872.4	854.0	866.2	877.1	873.3		865.5
Crude protein	300.3	300.9	262.0	238.5	256.6	238.0	310.0		
Ether extract	14.0	15.0	14.0	13.8	15.9	11.0	15.6		5.0
Ash	35.2	36.1	32.9	44.5	31.7	36.2	34.4		27.9
Crude fibre	81.5	83.0	87.0	86.0	86.0	96.0	75.0		315.5
Nitrogen-free extract	438.6	450.4	476.5	471.2	476.0	495.9	438.3		
Starch	217.6		191.9	260.7	220.9		238.6		
Alanine	11.0	11.4	9.7	9.3	9.8	9.0		10.8	4.4
Arginine	28.1	26.6	21.6	19.4	21.7	20.0		23.4	7.9
Aspartic acid	30.0	31.3	25.5	24.7	25.8	23.1		29.1	11.1
Glutamic acid	48.3	48.5	39.3	38.4	41.1	35.8		40.0	13.7
Glycine	11.4	11.7	10.0	9.8	10.0	9.5		11.0	6.4
Histidine	7.1	7.0	6.0	5.6	6.1	5.5		6.9	3.0
Isoleucine	12.5	12.9	10.7	10.4	10.9	9.6		11.9	4.7
Leucine	21.4	22.0	18.5	17.7	18.4	16.4		20.3	7.7
Lysine	17.5	17.5	14.9	14.7	15.1	14.0		17.5	6.9
Methionine	2.3	2.4	1.7	1.7	1.8	1.7		2.1	1.0
Cystine	3.8	3.9	3.3	3.3	3.8	3.1		3.6	1.6
Phenylalanine	12.0	12.2	9.9	9.6	10.2	9.3		11.1	4.3
Proline	12.5	12.9	10.6	10.1	11.9	10.1		11.2	4.2
Serine	14.6	14.9	12.5	11.7	12.6	11.0		13.9	5.6
Threonine	10.4	10.7	8.9	8.5	9.0	8.3		9.9	4.1
Tryptophan	2.4	2.4	2.1	2.0	2.1	1.9		2.3	1.0
Tyrosine	9.7	9.4	8.2	7.7	8.2	7.5		8.8	4.2
Valine	13.5	14.2	11.6	11.4	11.8	9.0		13.2	5.3
Tannins (FD)[2]	0.57	0.59	1.19	1.55	1.52	1.60		0.66	5.42
Tannins (VAN)[3]	<0.05	<0.05	0.40	0.96	0.98	0.96		0.06	4.20
Tannins (ISO)[4]	<0.05	<0.05	0.34	0.78	0.86	0.88	<0.05	<0.05	4.64
TIA[5]	1.33	1.05	1.44	0.70	1.55	0.73	0.30		
Lectins[6]	5	5	5	2	5	5			

[1]BL, cv. Blandine; TO, cv. Toret; HF, cv. Herz Freya; AL, cv. Alfred; MY, cv. Mythos; TR, cv. Troy; aBL, autoclaved cv. Blandine; cAL, cotyledons cv. Alfred; hAL, hulls cv. Alfred.
[2]expressed in % tannic acid equivalents
[3]expressed in % catechin equivalents
[4]expressed in % tannic acid equivalents
[5]expressed in mg trypsin inhibited per gram of sample
[6]expressed in haemagglutination units per mg of sample

Table 3. Composition of the diets (g/kg) in Experiment 2.

	I	II	III	IV	V	VI	VII	VIII
Maize	414.6	456.3	275.7	278.4	297.3	299.5	316.2	318.5
Barley	250.0	250.0	200.0	200.0	200.0	200.0	200.0	200.0
Casein	47.5	36.0	59.0	59.0	40.0	40.0	23.0	23.0
Herring meal	30.0	25.0	25.0	25.0	18.0	18.0	12.0	12.0
Meat meal	20.0	15.0	15.0	15.0	12.0	12.0	7.0	7.0
Soya bean meal	125.0	100.0						
Faba beans cv. Toret[2]							300.0	300.0
cv. Alfred[2]			300.0	300.0	300.0	300.0		
Cellulose	11.0	12.0						
Sunflower oil	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Animal fat	22.0	20.0	44.0	44.0	47.0	47.0	50.0	50.0
Cane molasses	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Premix[1]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
$CaCO_3$	12.5	13.0	13.0	13.0	13.0	13.0	13.5	13.5
$CaHPO_4$	11.5	13.5	13.5	13.5	15.5	15.5	17.5	17.5
NaCl	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-lysine HCl	0.8	1.7			1.0	1.0	2.6	2.6
DL-methionine	1.9	1.8	2.6	2.6	2.6	2.6	3.1	3.1
L-threonine	0.4	0.7	0.7		1.0		1.5	0.4
L-tryptophan		0.2	0.2		0.3		0.5	0.2
L-isoleucine		0.3	0.1		0.4		0.9	0.5
L-arginine	2.3	2.5	1.7		1.4	0.9	0.5	
L-valine							0.7	0.7
$NaHCO_3$	2.0	2.0	2.0	2.0	3.0	3.0	2.5	2.5
$KHCO_3$	2.5	4.0	1.5	1.5	1.5	1.5	2.5	2.5
Calculated composition								
Crude protein	204	180	203	201	182	180	182	180
ME (MJ/kg)	12.98	12.97	12.99	12.97	12.97	12.97	12.97	12.97
Calcium	9.8	9.8	9.7	9.7	9.7	9.7	9.8	9.8
Av. phosphorus	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
Dig. isoleucine	7.9	7.1	7.9	7.8	7.1	6.7	7.1	6.7
Dig. lysine	11.0	9.8	11.0	11.0	9.8	9.8	9.8	9.8
Dig. met & cys	8.0	7.2	8.0	8.0	7.2	7.2	7.2	7.2
Dig. threonine	7.2	6.5	7.2	6.5	6.5	5.5	6.5	5.5
Dig. tryptophan	2.1	1.9	2.1	1.9	1.9	1.6	1.9	1.6
Dig. arginine	12.0	10.8	12.0	10.4	10.8	10.3	10.8	10.3
Dig. valine	9.5	8.1	9.4	9.4	8.0	8.0	7.3	7.3

[1]See Table 1. [2]cv. Toret, low-tannin cultivar; cv. Alfred, high-tannin cultivar.

digestible amino acids in diet I were matched with the animal's requirements (NRC, 1984) using synthetic amino acids. In diet II contents of digestible arginine, isoleucine, lysine, methionine & cystine, threonine, tryptophan and valine were equivalent to 90% of the animal's requirements. Diets III-VI each contained 300 g/kg of faba beans of the high-tannin cv. Alfred. Diet III had a protein level of 200 g/kg. In this diet all indispensible amino acids were added up to the levels of digestible amino acids in diet I. Diet IV was similar to diet III, except that in diet IV only synthetic lysine and methionine were supplemented up to the animals's requirement (diet I). Both diets V and VI had a crude protein content of 180 g/kg. In diet V all essential amino acids were supplemented up to levels in diet II, equivalent to 180 g/kg of CP. In diet VI only lysine and methionine were added up to the levels in diet II. Diets VII and VIII were identical to diets V and VI, except that the high-tannin faba beans cv. Alfred were replaced with cv. Toret beans with a low tannin content. During ration formulation similar values for the composition of faba beans were used as in Experiment 1. Diets were fed as pellets as in Experiment 1.

Animals and experimental procedures

The type of birds used and the housing conditions were similar to those in Experiment 1. The dietary treatments were evaluated in four replicates consisting of 15 male chicks per cage each. Allocation of the diets took place after an adaptation period of six days in which the animals received control diet I. Average body weight of the animals at the start of the experiment (experimental day 0; day 7 after hatching) was 169 g. Both water and feed were supplied ad libitum. Individual body weight of the animals and feed consumption per cage were determined at days 7, 14 and 19 of the experiment.

Statistical analysis

The experimental data were analysed using analysis of variance (ANOVA) with treatment as the only factor. If the treatment effect was significant, the differences between means were tested with the least significance difference (LSD) test (Snedecor & Cochran, 1980).

Results

Protein content as well as levels of condensed tannins varied considerably among varieties (Table 2). Both low-tannin varieties had a markedly higher protein content (Nx6.25) than the medium- and high-tannin varieties.

Measurement of levels of tannins in faba beans according to the three methods mentioned gave different results, but the relative order of tannin contents within methods was the same. These differences in results between methods can be attributed to differences in the extraction technique applied and to differences in reagents and standards used in the tannin assay procedures.

Activity of trypsin inhibitors (TIA) ranged from 0.70 to 1.55 mg trypsin inhibited per

gram of bean. TIA seems to be unrelated to tannin content. In cv. Blandine autoclaving reduced the TIA value from 1.33 to 0.30.

The mean lectin activity in the beans was low and did not vary widely among varieties.

Results on the performance of the birds in Experiment 1 are presented in Table 4. Feed intake over a period of 21 days differed slightly among experimental groups. In some cases differences were statistically significant ($P<0.05$). Feed intake ranged from 72.4 (group IX) to 76.9 g/day (group II). Comparison of the feed consumption of animals fed diets containing 300 g/kg raw faba beans (diets III-VIII) revealed a tendency of a higher intake of groups receiving a diet with high-tannin faba beans (V-VIII vs. II-IV; +2.9%; $P<0.05$). Feed intake of chicks receiving a diet with either 248 g/kg cotyledons (diet X) or 52 g/kg hulls (diet XI) of the cv. Alfred did not differ from that of chicks receiving 300 g/kg raw whole faba beans of the same variety (diet VI).

Weight gain was highest for the group receiving the maize/soya bean meal diet (diet II). The lowest weight gain was obtained in the group fed the diet with 300 g/kg heat-treated faba beans cv. Blandine (4.4% below value of control group II; $P<0.05$). Average weight gain of the birds receiving 300 g/kg beans of a low-tannin variety

Table 4. Average feed intake (g/day), body weight gain (g) and feed conversion efficiency (FCE; g/g) of broiler chicks in Experiment 1[1].

Group	Days 0-7			Days 0-14			Days 0-21		
	Feed intake	Weight gain	FCE	Feed intake	Weight gain	FCE	Feed intake	Weight gain	FCE
I	43.1^{a}	230^{a}	1.313^{ab}	59.3^{ab}	584^{a}	1.423^{ab}	75.6^{ab}	1039^{abc}	1.528^{ab}
II	45.2^{b}	248^{c}	1.274^{c}	62.4^{cd}	625^{c}	1.398^{bcd}	76.9^{a}	1070^{c}	1.509^{abcd}
III	43.9^{ab}	239^{b}	1.288^{bc}	59.3^{ab}	597^{ab}	1.391^{cd}	73.3^{cd}	1038^{abc}	1.483^{d}
IV	44.1^{ab}	237^{b}	1.302^{abc}	60.0^{abc}	592^{ab}	1.419^{abcd}	74.1^{bcd}	1031^{ab}	1.509^{abcd}
V	44.5^{ab}	241^{b}	1.295^{abc}	61.0^{cd}	599^{b}	1.425^{ab}	75.7^{ab}	1047^{abc}	1.520^{abc}
VI	44.5^{ab}	237^{b}	1.314^{ab}	61.0^{cd}	600^{b}	1.424^{ab}	75.2^{abc}	1046^{abc}	1.511^{abcd}
VII	45.5^{b}	241^{b}	1.324^{ab}	61.4^{cd}	606^{b}	1.419^{abc}	76.1^{a}	1058^{bc}	1.510^{abcd}
VIII	44.8^{b}	236^{b}	1.330^{a}	61.3^{cd}	600^{b}	1.429^{a}	76.3^{a}	1042^{abc}	1.538^{a}
IX	43.9^{ab}	237^{b}	1.297^{abc}	58.7^{a}	592^{ab}	1.390^{d}	72.4^{d}	1023^{a}	1.486^{cd}
X	44.7^{b}	238^{b}	1.315^{ab}	60.4^{bc}	601^{b}	1.406^{abcd}	75.1^{abc}	1056^{bc}	1.495^{bcd}
XI	44.5^{ab}	235^{b}	1.325^{ab}	61.0^{cd}	600^{b}	1.425^{ab}	76.2^{a}	1049^{abc}	1.525^{ab}
LSD (P=0.05)	1.62	5.4	0.038	1.45	14.1	0.029	1.94	32.3	0.034

[1]Values with a different superscript within a column differ significantly at $P<0.05$.

(diets III and IV) was slightly, but not significantly, lower than for those fed a diet with 300 g/kg beans of medium- and high-tannin varieties (diets V-VIII) (average difference -1.3%).

Feed conversion efficiency was lowest for the animals receiving either raw (diet III) or autoclaved faba beans cv. Blandine (diet IX), and highest for those receiving diet VIII (300 g/kg high-tannin beans cv. Troy) (relative difference +3.5%). Except for the group fed diet III (raw beans cv. Blandine), FCE of the groups receiving high- or low-tannin beans did not differ significantly. The FCE was slightly higher for the animals fed a diet containing 52 g/kg hulls (diet XI) than the value for the group receiving a diet containing 248 g/kg cotyledons of the same variety (diet X).

The results over the periods 0-7 and 0-14 days showed similar trends as described above.

Table 5. Relative weights of the pancreas of chickens in the experimental groups I, III, VI and VII in Experiment 1.

Group	Faba bean cultivar	Tannin level	Rel. pancreas weight (%)[1]
I	-	-	0.183[a]
III	Blandine	low	0.210[b]
VI	Alfred	high	0.213[b]
VII	Mythos	high	0.209[b]

[1]Values with a different superscript within a column differ significantly at $P<0.05$.

Table 5 shows the relative weights of the pancreas of birds of four experimental groups in Experiment 1. The relative pancreatic weight was higher when 300 g/kg faba beans of either a low-tannin (diet III) or a high-tannin variety (diets VI and VII) were included compared to the value for the group fed the control diet I ($P<0.05$).

Results of the second experiment are shown in Table 6. Feed intake of the birds over 19 days was significantly higher in the group fed diet II (180 g/kg CP) than in those receiving diet I (204 g/kg CP) (+3.2%). Feed intake of the broilers receiving diets III-VIII did not differ from that of the control group I.

Weight gain over a period of 19 days was highest for group I. Inclusion of 300 g/kg high-tannin faba beans in nutritionally balanced diets did not affect weight gain of the birds. Feeding diets containing either high-tannin (diets V and VI) or low-tannin beans (diets VII and VIII) under conditions of marginal supply of protein and essential amino acids did not result in a difference in weight gain.

The FCE was lowest for groups receiving control diet I and highest for those fed control diet II (difference +5.6%) ($P<0.05$). The efficiency values of birds fed diets containing 300 g/kg of faba beans with either a high (diets V and VI) or a low tannin content (diets VII and VIII) under conditions of marginal supply (180 g/kg) of protein and essential amino acids did not differ significantly.

Discussion

Condensed tannins in feedstuffs are known for their nutrient binding properties. The presence of condensed tannins in faba beans seem to be an important determinant for their nutritional quality for poultry (Marquardt et al., 1977; Martin-Tanguy et al., 1977; Marquardt & Ward, 1979). However, studies were generally conducted with extremely high inclusion levels of either raw tannin-containing beans or tannin fractions isolated from the beans. The studies reported here were carried out using a more moderate inclusion level of 300 g/kg of faba beans.

Table 6. Average feed intake (g/day), body weight gain (g) and feed conversion efficiency (FCE; g/g) of broiler chicks in Experiment 2[1].

Group	Days 0-7			Days 0-14			Days 0-19		
	Feed intake	Weight gain	FCE	Feed intake	Weight gain	FCE	Feed intake	Weight gain	FCE
I	51.6^{ab}	266^{a}	1.360^{a}	66.8^{a}	658^{a}	1.421^{a}	78.5^{a}	995^{a}	1.499^{a}
II	53.7^{c}	259^{ab}	1.449^{d}	69.9^{b}	645^{abc}	1.517^{f}	81.0^{b}	972^{abc}	1.583^{e}
III	51.0^{a}	261^{ab}	1.368^{ab}	66.9^{a}	653^{a}	1.434^{ab}	78.2^{a}	985^{ab}	1.508^{a}
IV	51.6^{ab}	261^{ab}	1.382^{abc}	67.3^{a}	650^{ab}	1.449^{abc}	78.4^{a}	974^{abc}	1.530^{bc}
V	52.4^{abc}	260^{ab}	1.414^{bcd}	68.2^{a}	645^{abc}	1.480^{de}	78.9^{a}	969^{bc}	1.546^{cd}
VI	52.7^{bc}	259^{b}	1.427^{cd}	67.9^{a}	630^{c}	1.508^{ef}	79.0^{a}	954^{c}	1.572^{de}
VII	51.5^{ab}	259^{ab}	1.392^{abc}	66.6^{a}	636^{bc}	1.465^{bcd}	78.4^{a}	958^{c}	1.556^{cde}
VIII	51.6^{ab}	256^{b}	1.410^{bcd}	66.5^{a}	633^{c}	1.472^{cd}	77.3^{a}	951^{c}	1.544^{cd}
LSD (P=0.05)	1.57	6.9	0.047	1.66	15.39	0.032	1.86	23.99	0.029

[1]Values with a different superscript within a column differ significantly at $P<0.05$.

In Experiment 1, six varieties of faba beans with different levels of condensed tannins were evaluated in diets for growing chicks. Feed intake of birds receiving the diets with high-tannin beans was slightly higher than for those fed the diets with low-tannin beans. Marquardt et al. (1977) reported a reduced feed consumption when adding a tannin-containing extract from faba beans to broiler diets. Tannin content in their diets, however, exceeded by far those found in our rations.

Weight gain of the chickens in our experiment was not affected by feeding different varieties of faba beans and was similar to those receiving a maize/soya bean meal based diet. The FCE values were also similar to the control group value over a period of three weeks. In contrast, Marquardt & Ward (1979) found differences in performance of chicks when fed diets with 900 - 950 g/kg of faba beans varying in

tannin level. They compared diets containing either high-tannin faba bean varieties or low-tannin varieties and found that weight gain was reduced (-7.5%) and the FCE was increased (+9.3%) by the high-tannin beans. These results were associated with a reduced retention of dry matter, protein, fat, ash and calcium. In an additional experiment of Marquardt & Ward (1979) tannin-containing aqueous extracts from faba bean hulls were included in experimental diets, the highest level of condensed tannins in the diets being 2.5%. A markedly reduced feed intake and weight gain and an increased feed conversion efficiency were found. In contrast, in our experiments levels of condensed tannins in diets were calculated to be below 0.3%, which may explain differences in results.

The results of our experiment do not agree either with those of Blair et al. (1970) who found a reduced performance of growing chicks on diets with increasing levels of faba beans up to 450 g/kg as compared to birds receiving a standard wheat/soya bean ration. Unfortunately, the tannin content of the beans was not mentioned. The presence of different antinutritive factors such as trypsin inhibitors in combination with the relatively poor quality of the faba bean protein were held responsible for their observation. Blair et al. (1970), however, only supplemented methionine while possibly also other essential amino acids could have been limiting. In Experiment 1 care was taken that all essential amino acids were added up to the animal's requirements.

Lacassagne et al. (1988) found a significantly lower apparent digestibility of the protein in diets containing high-tannin faba beans as compared to those with low-tannin beans at a level of 960 g/kg in 20-day-old chicks in both mash and pelleted diets. Martin-Tanguy et al. (1977) established a negative correlation between levels of tannins in faba beans, which were included in diets at a level of 500 g/kg, and the apparent digestibility coefficients of the dietary nitrogen in growing chicks.

Our results seem in agreement with those of Wiseman et al. (1991) who did not find differences in carcass nitrogen retention in broiler chicks when comparing the inclusion of two isogenic lines of faba beans with a low and high tannin content at levels of 200, 400 and 600 g/kg.

Inclusion of hulls of a high-tannin variety at a level of 52 g/kg (equivalent to 300 g/kg whole beans) did not reduce performance of the broilers in this experiment (Experiment 1). Wareham et al. (1991) investigated the effects of condensed tannins in faba bean hulls in chick diets by exchanging hulls of a high- and a low-tannin variety at a level of 200 g/kg. A small but significant depressive effect on the nitrogen retention of chickens and nitrogen corrected metabolizable energy (ME_N) of the diets was noticed with increasing levels of high-tannin hulls. Reduced performance due to high levels of tannin-containing hulls from faba beans in the diets of broiler chicks was also reported by Ward et al. (1977). Our experiment suggests that the presence of high-tannin hulls at relatively low levels does not result in a reduced performance of growing chicks.

To establish whether a limited supply of protein and amino acids could enhance possible harmful effects of feeding high-tannin faba beans, Experiment 2 was carried out. The protein level of some of the experimental diets was restricted to 180 g/kg and the level of supplementation of some essential amino acids was reduced. Animals

receiving the nutritionally suboptimal control diet (diet II) increased feed intake to overcome limited protein supply and were able to attain a similar weight gain as birds fed control diet I. The FCE, however, increased in the birds fed diet II. When feeding a nutritionally balanced diet with 300 g/kg of faba beans (diet III) performance was not affected as compared to control group I. Similar results were obtained in Experiment 1.

When only lysine and methionine were supplemented (diet IV) weight gain was not affected but the feed conversion efficiency increased as compared to supplementation of all essential amino acids (diet III). When the diet contained 300 g/kg of faba beans with either a high or a low tannin content and a protein level of 180 g/kg, feed consumption was not affected. Even under conditions of marginal supply of protein and indispensible amino acids, no difference in performance could be observed between groups receiving diets containing either low- or high-tannin faba beans (groups V vs. VII, VI vs. VIII).

Heat treatment of a low-tannin variety resulted in a reduced TIA of the beans. Heat sensitivity of protease inhibitors in legume seeds has been demonstrated by various authors (Abbey et al., 1979; Liener & Kakade, 1980; Liener & Tomlinson, 1981). However, heat treatment of low-tannin faba beans in our experiment did not increase the performance of broiler chicks. Shanon & Clandinin (1977) found a 15% increase of metabolizable energy content in faba beans after autoclaving the beans for 45 and 60 min at an unknown temperature. Marquardt & Campbell (1973) also reported a positive effect on the nutritive value of faba beans in broiler diets due to heat processing (15 min, 121°C). It is not likely that heat treatment of faba beans in our experiment had a positive effect on ME content since this should have been reflected in an improved feed conversion efficiency for group IX relative to group III. A slight overtreatment of the faba beans in our study may have occurred. This could have reduced protein availability and may explain the somewhat decreased weight gain of the group fed the diet with heat-treated faba beans (group IX).

Inclusion of faba beans in the diets led to hypertrophy of the pancreas of the chicks (Table 5). Most likely, protease inhibitors are responsible for this effect. Rubio et al. (1989) also observed an increased size of the pancreas in broiler chicks after the inclusion of raw faba beans in diets. They further noticed interlobular oedema with hypertrophy of the primary granules and also found indications for the onset of degeneration of pancreatic cells. Similar effects of protease inhibitors from legume seeds on the pancreas of small animal species have been found by others (Abbey et al., 1979; Liener et al., 1985). Heat treatment of faba beans was shown to overcome the hypertrophic effects on the pancreas in chicks (Marquardt et al., 1976; Rubio et al., 1990) and rats (Abbey et al., 1979).

From the present experiments it can be concluded that the inclusion of either low- or high-tannin faba beans at a level of 300 g/kg does not affect growth and feed conversion efficiency of broiler chicks when the diets are carefully balanced for essential amino acids. Antinutritional factors (ANFs) present in faba beans, particularly condensed tannins, only seem to exert detrimental effects on the chick's performance when extremely high inclusion levels of either raw faba beans or concentrated or

isolated ANFs are used. At practical inclusion levels faba beans, independent of their tannin content, seem to be a suitable protein supplement in chick diets.

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Chapter 5

ILEAL AND FAECAL DIGESTIBILITY IN PIGLETS OF FABA BEANS (VICIA FABA L.) VARYING IN TANNIN CONTENT

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Animal Feed Science and Technology, in press

Ileal and faecal digestibility in piglets of faba beans (Vicia faba L.) varying in tannin content

Abstract

The ileal and faecal digestibility of nutrients of four varieties of faba beans was determined in piglets (15-30 kg) fitted with a post-valvular T caecum cannula. The varieties differed in their content of condensed tannins. Beans of a white-flowering variety (cv. Blandine) had a low content of condensed tannins (<0.1%). The other beans originated from coloured-flowering varieties and had a medium (0.4%) (cv. Herz Freya) or high tannin content (1.0%) (cv. Mythos and cv. Alfred). The low-tannin beans were evaluated in raw form and after autoclave treatment (30 minutes, 105°C). Beans were included in the diets at a level of 300 g/kg. Diets were tested in eighteen piglets in two experimental periods (n=6). The ileal N digestibility (%) was higher ($P<0.05$) for the low-tannin beans (85.7) than for the medium- and high-tannin varieties (73.7, 72.4, 71.5 for Herz Freya, Mythos and Alfred, respectively). Faecal N digestibility values were 89.3, 85.7, 82.4 and 79.4 for Blandine, Herz Freya, Mythos and Alfred beans, respectively. The values for Blandine and Alfred beans differed significantly ($P<0.05$). For individual amino acids differences in digestibility between Blandine beans and the average value for beans of the three tannin-containing varieties ranged from 8 units for aspartic acid to 18 units for cystine. Autoclaving the low-tannin faba beans Blandine did not improve their N digestibility at the ileal (85.7 (raw) versus 82.3 (autoclaved)) and the faecal level (89.3 versus 88.9). Apparent ileal digestibility of starch was similar for all raw and the autoclaved beans.

The absence of condensed tannins in faba beans seems to have a positive effect on ileal and faecal digestibility of particularly crude protein and amino acids of faba beans in pigs. Autoclaving low-tannin faba beans did not improve the nutritional value for pigs.

Introduction

The animal feed industry is dependent on an adequate supply of protein-rich ingredients for animal feeds. Traditionally, both full-fat soya beans and extracted soya bean meal have served as important protein sources in feeds for non-ruminant species. Other legume seeds such as faba beans (Vicia faba L.) could be an alternative source of proteins in these diets (Gatel, 1992).

The nutritive value of faba beans, based on the chemical composition, is fairly high with protein levels ranging between 200 and 320 g/kg DM. Apart from a low methionine level, the protein has a well balanced pattern of indispensible amino acids (Thacker, 1990). The major factor that limits the use of raw legume seeds in feeds for monogastrics is the presence of antinutritional factors (ANFs). In faba beans, protease inhibitors, lectins, tannins, vicine/convicine and flatus-producing factors are found (Hussein, 1982). Most of these substances interfere with the digestion of nutrients in several animal species. Therefore, these substances may impair growth of pigs fed

diets containing high levels of faba beans (Aherne et al., 1977; Fowler, 1980).

Among the ANFs present in faba beans major attention has been given to condensed tannins. Tannins are polyphenolic compounds with a molecular weight between 500 and 3000 that are capable of precipitating proteins from aqueous solutions (Bate-Smith & Swain, 1962). Various antinutritional effects of dietary tannins have been reported. The negative effects on protein digestibility are most emphasized. Tannins may bind to dietary proteins, thereby preventing enzymatic digestion. In addition, tannins may inhibit the activity of digestive enzymes or increase the secretion of endogenous proteins (Marquardt, 1989).

Coloured-flowering varieties of faba beans contain between 0.4 and 1.3 % catechin equivalents of condensed tannins (Bos & Jetten, 1989). Recently, low- tannin varieties of faba beans have been developed. These white-flowering varieties are assumed to have a higher nutritive value than the traditional cultivars (Bond & Smith, 1989). Faba beans also contain low levels of trypsin inhibitors and lectins, which may at least partly be inactivated by heat (Hussein, 1982). Therefore heat treating faba beans may increase their nutritional value.

This paper presents the results of the evaluation of the ileal and faecal digestibility in pigs of four varieties of faba beans. Of these, three are traditional varieties, two with a high and one with a medium tannin content, and one is a low-tannin variety. In addition, autoclaved beans of the low-tannin variety were evaluated as a low-ANF batch of beans.

Materials and methods

Faba bean varieties

The faba bean varieties Blandine, Herz Freya, Mythos and Alfred were used in the experiment. The batches were kindly donated by, respectively, Maribo Seeds, Holeby, Denmark; Barenbrug Holland B.V., Oosterhout, The Netherlands; Norddeutsche Pflanzenzucht, Holtsee, Germany; and Cebeco Handelsraad, Rotterdam, The Netherlands. The batches of Blandine, Mythos and Alfred were harvested in the year preceding the experiment. The batch of cv. Herz Freya was at least three years of age at the start of the experiment. Prior to use the beans were stored in dark conditions at ambient temperature.

The beans of cv. Herz Freya had a very dark, almost black seed coat. The beans of Alfred and Mythos were brown-coloured, whereas seeds of the variety Blandine were white.

Part of the batch of Blandine beans was heated isothermically in an autoclave for 30 min at 105°C and at a final moisture level of 20%. After this treatment beans were dried in an oven for 6 h at 40°C. Each batch of beans was ground in a hammer mill with a 3 mm screen.

Both the chemical composition and the levels of various antinutritional factors in the beans are given in Table 1.

Table 1. Analysed chemical composition (g/kg[1]) of faba beans and their contents of some antinutritional factors.

	Blandine	Herz Freya	Mythos	Alfred	autoclaved Blandine
Dry matter	896.6	872.4	866.2	854.0	883.6
Crude protein[2]	300.3	262.0	256.6	238.5	313.6
Ether extract	14.0	14.0	16.3	13.8	15.0
Ash	35.2	32.9	31.7	44.5	34.8
Crude fiber	81.5	87.0	86.0	86.0	76.0
NFE	438.6	477.5	475.6	471.2	444.2
Starch	217.6	191.9	220.9	260.7	-
Alanine	11.0	8.7	9.8	9.3	-
Arginine	28.1	21.6	21.7	19.4	-
Aspartic acid	30.0	25.5	25.8	24.7	-
Glutamic acid	48.3	39.3	41.1	38.4	-
Glycine	11.4	10.0	10.0	9.8	-
Isoleucine	12.5	10.7	10.9	10.4	-
Leucine	21.4	18.5	18.4	17.7	-
Lysine	17.5	14.9	15.1	14.7	-
Methionine	2.3	1.7	1.8	1.7	-
Cystine	3.8	3.3	3.8	3.3	-
Serine	14.6	12.5	12.6	11.7	-
Threonine	10.4	8.9	9.0	8.5	-
Valine	13.5	11.6	11.8	11.4	-
Tannins (FD)[3]	0.57	1.19	1.52	1.55	0.59
Tannins (VAN)[4]	0.02	0.40	0.98	0.96	-
Tannins (ISO)[3]	0.00	0.34	0.86	0.78	-
TIA[5]	1.33	1.44	1.55	0.70	0.30
Lectins[6]	5	5	5	2	-

[1] all analytical figures on a "as fed" basis
[2] N x 6.25
[3] % tannic acid equivalents
[4] % catechin equivalents
[5] mg trypsin inhibited per gram of sample
[6] haemagglutination units per mg of sample
- not analysed

Experimental diets

Six experimental diets were used. The composition of the basal diet (BA) is given in Table 2. The diets BL, HF, MY, AL and BLau were prepared by substituting 30% of the basal diet for 30% of one of the batches of faba beans.

Diet BA : basal diet
Diet BL : 70% basal diet + 30% faba beans cv. Blandine
Diet HF : 70% basal diet + 30% faba beans cv. Herz Freya
Diet MY : 70% basal diet + 30% faba beans cv. Mythos
Diet AL : 70% basal diet + 30% faba beans cv. Alfred
Diet BLau : 70% basal diet + 30% autoclaved faba beans cv. Blandine

Chromic oxide was added to each of the diets as a digestibility marker at a level of 5 g/kg.

Animals and experimental procedure

The piglets used were male castrates of the crossbred Dutch Landrace x Dutch Yorkshire. At arrival the animals weighed between 8 and 10 kg. During the experiment the animals were housed individually in metabolism cages. After four days of adaptation to the cages, 18 animals were surgically fitted with a post-valvular T caecum (PVTC) cannula (van Leeuwen et al., 1991). After a recovery period of 4-10 days after cannulation the animals were assigned to six experimental groups of three animals each. Each group received one of the six experimental diets. After nine days of adaptation to the diets, faeces were collected quantitatively 24 h per day on five successive days using faeces collection bags attached to the animals. The collection bags were changed twice daily and stored at -20°C until further handling. The faeces collection period was followed by a period of five days during which ileal digesta were collected for 12 h a day (08.00-20.00 h). For collection small plastic bags were used which were fixed on the cannula with plastic strips. Bags were changed at least every two hours. The contents were immediately frozen in plastic containers at -20°C.

Four days after finishing the first collection period, the animals fed the BL, HF, MY, AL or BLau diet were randomly reassigned over five experimental groups for the second experimental period. The animals in the control group were kept on the diet BA throughout the whole experiment. After another ten-day adaptation period subsequently faeces and chyme were collected as in the first collection period.

The average body weight of the animals in the first and second experimental period was 17.5 and 28.0 kg, respectively.

Throughout the experiment the animals were fed at a level of 2.7 times their assumed maintenance requirements for energy (2.7 x 420 kJ ME/kg$^{0.75}$; ARC, 1981). The diets were offered in pelleted form twice daily. Pellets had been prepared in a cold pelleting process (T < 60°C). Water was freely available from drinking nipples.

Room temperature was kept between 21 and 25°C.

Table 2. Formulation (g/kg) and calculated chemical composition (g/kg) of the basal diet.

Formulation		Chemical composition	
Barley	304.4	Dry matter	884.1
Maize	350.0	Crude protein	166.3
Herring meal (74% CP)	50.0	Net energy (MJ/kg)	9.9
Skim milk powder	100.0	Ether extract	43.7
Whey powder	50.0	Crude fibre	18.2
Meat meal tankage	20.0	Ash	63.8
Maize starch	50.0	Ca	10.2
Sunflower oil	15.0	Available P	5.7
Cane molasses	20.0	Lys	10.8
Vitamin/mineral mix[1]	14.3	Met	4.5
$CaCO_3$	8.0	Cys	2.4
$Ca(H_2PO_4)_2$	7.0	Thr	7.5
NaCl	3.0	Trp	2.0
L-lysine	2.0		
DL-methionine	1.0		
L-threonine	1.0		
L-tryptophan	0.3		
$KHCO_3$	4.0		

To each of the experimental diets 5 g/kg of Cr_2O_3 was added as a marker.

[1]The premix supplied per kg feed: 9000 IU vitamin A; 1800 IU vitamin D_3; 40 mg vitamin E; 5 mg riboflavin; 30 mg niacinamide; 12 mg d-pantothenic acid; 150 mg choline chloride; 40μg vitamin B_{12}; 3 mg menadione; 50 mg ascorbic acid; 0.3 mg folic acid; 100 mg $CuSO_4.5H_2O$; 200 mg $ZnSO_4.H_2O$; 70 mg MnO_2; 400 mg $FeSO_4.7H_2O$; 2.5 mg $CoSO_4.7H_2O$; 0.2 mg $Na_2SeO_3.5H_2O$; 0.5 mg KI; 40 mg tylosin.

Sample preparation and analysis

Samples of faba beans and feeds were ground on a laboratory mill with a 1 mm screen prior to analysis. Pooled samples of faeces and chyme per animal were freeze-dried. Dry matter and nitrogen were determined in the fresh samples. Other analyses in faeces and chyme were carried out on the freeze-dried material.

The contents of dry matter (DM), ash, N, ether extract (EE) and crude fibre (CF) were determined following standard procedures. Crude protein content was calculated as N x 6.25. Organic matter (OM) was calculated as dry matter - ash, whereas nitrogen-free extract (NFE) was calculated as dry matter - (ash + N x 6.25 + ether extract + crude fibre). Starch in the beans and the feed was determined

polarimetrically according to NEN (1974). Starch content in chyme samples was determined enzymatically using a kit method (Boehringer cat. No. 207748).

The tannin content of bean samples was measured according to the Folin Denis method (Swain & Hillis, 1959), the vanillin-sulphuric acid method (Kuhla & Ebmeier, 1981) and the ISO method (ISO, 1987). The activity of trypsin inhibitors in the beans was determined according to the method of van Oort et al. (1989). Lectins were determined by measuring haemagglutination of red blood cells according to the procedure described by Valdebouze et al. (1980).

Amino acids were determined using ion exchange chromatography after acid hydrolysis (6 M HCl for 22 h at 100°C).

Chromium was analysed by flame atomic absorption spectrometry after sample hydrolysis in a mixture of perchloric acid and nitric acid.

Digestibility of the beans was calculated from the difference in digestibility of the basal diet and the experimental diets.

Statistical analysis

Analysis of variance (ANOVA) on all data showed that the period effect was not significant. Therefore digestibility coefficients of the diets and the faba beans were assessed using analysis of variance with "Diet" or "Variety" as the only experimental factor. If the treatment effect was significant, the differences between means were tested with the least significance difference (LSD) test (Snedecor & Cochran, 1980).

Results

The composition of the faba beans is given in Table 1. The crude protein content (N x 6.25) of the low-tannin variety Blandine is markedly higher than that of the other varieties (average difference 48 g/kg). This difference is reflected in the contents of amino acids in the beans (Table 1). The starch content of the beans varied between 192 g/kg for cv. Herz Freya and 261 g/kg for cv. Alfred.

Trypsin inhibitor activity (TIA) in the raw beans varied from 0.70 (cv. Alfred) to 1.55 mg/g (cv. Mythos). Lectin activity in raw beans was low and did not vary widely. Tannin contents varied markedly among the four untreated varieties. Levels of condensed tannins as measured by the vanillin-sulphuric acid method (VAN) and the ISO procedure (ISO) were highest for Mythos and Alfred. The values for Herz Freya were about half of those determined for Alfred and Mythos. Condensed tannins were apparently absent in the beans of Blandine and were consequently not measured after autoclaving. The Folin Denis (FD) method does not only determine (condensed) tannins but includes other phenolic compounds as well. Therefore, values obtained with this assay were higher than for the other methods. Among varieties similar trends in tannin contents were observed.

The autoclave treatment of beans of cv. Blandine did not affect their chemical composition. The TIA in the heat-processed beans, however, was reduced from 1.33 to 0.30 mg trypsin inhibited per gram of bean.

Tables 3 and 4 show the results for the apparent ileal and faecal digestibility of the experimental diets. At the ileal level, for DM, ash, OM and NFE the highest digestibility values were found for the basal diet, although differences with values for other experimental diets were not significant ($P<0.05$) in all cases. Apparent ileal

Table 3. Apparent ileal digestibility (%) of the experimental diets.

Group	DM	Ash	OM	N	EE	CF	NFE	Starch
BA	76.8^{a}	43.9^{a}	79.3^{a}	75.5^{bc}	85.8^{a}	24.9^{a}	83.0^{a}	98.7^{a}
BL	74.8^{ab}	37.3^{b}	77.3^{ab}	80.1^{a}	88.5^{a}	19.0^{ab}	80.7^{b}	98.6^{a}
HF	73.3^{b}	33.1^{b}	75.9^{bc}	74.8^{c}	88.2^{a}	22.5^{ab}	80.5^{b}	98.6^{a}
MY	72.8^{b}	35.4^{b}	75.2^{c}	74.2^{c}	86.5^{a}	18.8^{b}	79.9^{b}	98.4^{a}
AL	72.7^{b}	34.9^{b}	75.2^{bc}	73.9^{c}	87.8^{a}	20.9^{ab}	80.1^{b}	98.5^{a}
BLau	74.0^{b}	36.5^{b}	76.4^{bc}	78.6^{ab}	87.8^{a}	19.4^{ab}	80.0^{b}	98.1^{a}
SEM	0.75	1.55	0.72	1.12	0.96	2.07	0.57	0.38

^{a,b,c,d} Values with a different superscript within a column differ significant at $P<0.05$.
SEM : Standard error of the mean.

Table 4. Apparent faecal digestibility (%) of the experimental diets.

Group	DM	Ash	OM	N	EE	CF	NFE
BA	86.6^{a}	57.6^{a}	88.7^{a}	84.9^{ab}	86.4^{a}	39.9^{ab}	92.6^{ab}
BL	86.6^{a}	58.3^{a}	88.5^{ab}	86.9^{a}	85.2^{a}	46.1^{a}	93.0^{a}
HF	85.7^{ab}	59.0^{a}	87.3^{bc}	85.2^{ab}	86.5^{a}	40.2^{ab}	92.6^{ab}
MY	85.0^{bc}	57.8^{a}	86.7^{cd}	83.9^{b}	86.5^{a}	38.3^{ab}	92.1^{bc}
AL	83.9^{c}	57.5^{a}	85.8^{d}	83.0^{b}	85.7^{a}	35.0^{b}	91.8^{c}
BLau	86.2^{ab}	59.5^{a}	88.0^{abc}	86.8^{a}	86.4^{a}	40.8^{ab}	92.9^{a}
SEM	0.48	1.21	0.45	0.94	1.04	2.92	0.19

^{a,b,c,d} Values with a different superscript within a column differ significant at $P<0.05$.
SEM : Standard error of the mean.

N digestibility was clearly highest for the diets containing the low-tannin beans (diets BL and BLau) ($P<0.05$). Between diets containing beans with substantial levels of condensed tannins, no differences in ileal digestibility were observed. Starch in all diets was nearly completely digested in the small intestine.

At the faecal level, significant differences among diets in apparent digestibility of

DM, OM, N and NFE were found ($P<0.05$). For DM, OM, N and NFE lowest values were found for the diets containing the high-tannin beans cv. Mythos and Alfred (diets MY and AL). The faecal digestibility for DM, ash, N, CF and NFE was on average 12, 21, 9, 19 and 12 percentage units higher, respectively, than the ileal digestibility figures of the same diets. The apparent digestibility of EE was about equal at the ileal and the faecal level.

Beans of the low-tannin variety Blandine had a higher N digestibility at the ileal level than the medium- and high-tannin varieties ($P<0.05$) (Table 5). The apparent ileal

Table 5. Apparent ileal digestibility (%) of faba beans.

Beans	DM	Ash	OM	N	EE	CF	NFE	Starch
Blandine	70.0^{a}	12.2^{a}	72.6^{a}	85.7^{a}	108.6^{a}	13.4^{a}	73.7^{a}	98.1^{a}
Herz Freya	65.1^{a}	-10.9^{b}	68.1^{a}	73.7^{b}	107.6^{a}	20.6^{a}	73.3^{a}	98.1^{a}
Mythos	63.1^{a}	-0.5^{ab}	65.7^{a}	72.4^{b}	91.5^{a}	13.4^{a}	71.0^{a}	97.4^{a}
Alfred	62.8^{a}	5.8^{ab}	65.8^{a}	71.5^{b}	104.3^{a}	17.7^{a}	71.7^{a}	97.9^{a}
Autoclaved Blandine	67.4^{a}	9.5^{ab}	70.0^{a}	82.3^{a}	103.0^{a}	14.4^{a}	70.9^{a}	96.3^{a}
SEM	2.67	7.85	2.51	2.89	8.16	4.36	2.78	0.73

^{a,b,c,d} Values with a different superscript within a column differ significant at $P<0.05$.
SEM: Standard error of the mean.

Table 6. Apparent faecal digestibility (%) of faba beans.

Beans	DM	Ash	OM	N	EE	CF	NFE
Blandine	86.8^{a}	61.3^{a}	88.1^{a}	89.3^{a}	77.4^{a}	52.2^{a}	94.3^{a}
Herz Freya	83.7^{ab}	65.5^{a}	84.3^{ab}	85.7^{ab}	86.6^{a}	40.4^{ab}	92.6^{ab}
Mythos	81.3^{bc}	58.6^{a}	82.1^{bc}	82.4^{ab}	86.6^{a}	36.9^{ab}	90.8^{bc}
Alfred	77.6^{c}	57.5^{a}	79.0^{c}	79.4^{b}	80.6^{a}	30.5^{b}	89.4^{c}
Autoclave d Blandine	85.3^{ab}	67.8^{a}	86.4^{ab}	88.9^{a}	86.2^{a}	41.7^{ab}	94.1^{a}
SEM	1.66	4.82	1.59	2.62	7.20	5.55	0.81

^{a,b,c,d} Values with a different superscript within a column differ significant at $P<0.05$.
SEM: Standard error of the mean.

digestibilities tended also to be higher for DM, ash and OM. Heat treatment had a slight, not significant, negative effect on the digestibility of DM and N of the low-tannin beans (3.4 and 0.4 percentage units, respectively). Between the medium- and

Table 7. Apparent ileal digestibility (%) of amino acids of different varieties of faba beans.

	Ala	Arg	Asp	Cys	Glu	Gly	Ile	Leu	Lys
Blandine	83.3[a]	94.8[a]	88.2[a]	66.0[a]	93.6[a]	80.1[a]	87.0[a]	88.7[a]	91.6[a]
Herz Freya	70.5[b]	89.8[b]	80.1[b]	46.5[b]	85.2[b]	67.3[b]	78.4[b]	80.8[b]	83.9[b]
Mythos	67.7[b]	88.0[b]	79.9[b]	45.2[b]	81.7[b]	62.9[b]	74.8[b]	76.6[b]	82.1[b]
Alfred	70.5[b]	88.7[b]	81.0[b]	53.1[ab]	83.8[b]	64.4[b]	78.6[b]	79.8[b]	84.3[b]
SEM	3.69	0.96	2.16	4.76	1.86	3.20	2.44	2.32	2.18

	Met	Ser	Thr	Val
Blandine	79.6[a]	88.6[a]	82.3[a]	85.8[a]
Herz Freya	66.4[b]	79.4[b]	72.9[b]	76.7[b]
Mythos	68.1[ab]	79.1[b]	71.1[b]	73.1[b]
Alfred	72.7[ab]	80.9[b]	73.0[b]	76.5[b]
SEM	4.14	2.43	2.72	2.55

[a,b,c,d] Values with a different superscript within a column differ significant at $P<0.05$.
SEM: Standard error of the mean.

high-tannin varieties of faba beans no significant differences in nutrient digestibilities were observed (Tables 5). The apparent faecal digestibilities of the faba beans showed similar differences as the ileal digestibilities (Table 6). In some cases the differences were slightly smaller at the faecal level. The small EE fraction in faba beans was completely digested at the ileal level. CF digestibility of faba beans did not differ among varieties at the ileal level, values ranging between 13 and 21 percentage units (Table 5). At the faecal level, digestibility figures for CF varied between 30 and 52. The apparent faecal digestibility of CF of cv. Blandine was significantly higher than that of the high-tannin variety Alfred (Table 6).

The differences in ileal amino acid digestibilities between the low-tannin variety on the one hand and the medium- and high-tannin cultivars on the other are obvious (Table 7). The average differences in ileal digestibility ranged from 7.9 and 8.2 percentage units for aspartic acid and lysine to 15.2 and 17.7 units for glycine andcystine, in favour of the low-tannin variety Blandine ($P < 0.05$).

The apparent ileal amino acid digestibility did not differ significantly among the three tannin-containing varieties Herz Freya, Mythos and Alfred.

Discussion

In the present study the nutritional value of four untreated varieties of faba beans with various contents of condensed tannins and one autoclaved batch of beans was determined in piglets by measuring both their ileal and faecal digestibility.

It is assumed that amino acids disappearing from the small intestine are absorbed as amino acids and can be used for protein synthesis by the animal. In the large intestine, the microbial fermentation disturbs a clear pattern of amino acid absorption owing to degradation of amino acids of feed origin and net synthesis of microbial protein (Fuller, 1991). Evaluation of the nutritive value of feedstuffs for pigs, especially for amino acids, should therefore preferentially be carried out by determining their ileal digestibility.

Regarding the levels of antinutritional factors present in the untreated faba beans, condensed tannins appear to be important. Condensed tannins are known to exert particularly negative effects on protein digestibility in different feedstuffs as shown for sorghum in pigs (Cousins et al., 1981), faba beans in poultry (Martin-Tanguy et al., 1977; Lacassagne et al., 1988) and faba beans *in vitro* (Marquardt et al., 1978). In these studies low-tannin varieties appeared to have a higher nutrient digestibility than varieties with higher tannin levels. In a study with pigs (body weight 25-30 kg) Duée et al. (1979) found a significantly higher apparent faecal digestibility of N in a low-tannin variety of faba beans than in a high-tannin cultivar (+ 9 percentage units). Using older pigs (50 kg), Liebert & Gebhardt (1983) found in a similar trial a difference of only 3 percentage units for apparent faecal N digestibility of faba beans in favour of a low-tannin cultivar.

In our study the largest differences among the bean varieties were found for protein and amino acid digestibilities at the ileal level. Average differences in N digestibility

between the low-tannin variety on the one hand and the three tannin-containing varieties on the other at the ileal and the faecal level were 13.2 and 6.8 percentage units, respectively. The larger difference at the ileal level stresses the importance of evaluating feedstuffs on the basis of their ileal digestibility, assuming that N absorbed from the large intestine cannot be utilized.

The effects of condensed tannins on apparent protein digestibility may be explained either by direct binding of condensed tannins to dietary proteins, by a reduced activity of protein-degrading enzymes (Longstaff & McNab, 1991) or by an increased secretion of endogenous proteins (digestive enzymes, mucus or mucosal cells) (Mangan, 1988; Marquardt, 1989). It should be noted that when comparing feedstuffs with different tannin levels, as in our study, other factors than the level of tannins, such as level and composition of structural carbohydrates and intrinsic protein quality, may also affect the differences. In this respect it should be mentioned that Marquardt et al. (1978) found small differences in the composition of the hulls of faba beans between low- and high-tannin varieties.

Differences in apparent ileal digestibility among individual amino acids in faba beans are obvious. This means that condensed tannins may have a different affinity for proteins with a different amino acid profile. Asquith & Butler (1986) noticed *in vitro* different affinities of condensed tannins of different origins for various proteins. Hagerman & Butler (1981) stated that sorghum tannins have a high affinity for proteins that are relatively large, have an open, loose structure and are rich in hydrophobic amino acids, particularly proline. Cousins et al. (1981) showed in a study with sorghum with different levels of condensed tannins that the apparent ileal digestibility of tryptophan, histidine, glycine and proline were more depressed in high-tannin varieties. They suggested that the low proline and glycine digestibilities were the result of an increased endogenous protein secretion. In our experiment digestibility was not measured for all amino acids, so that such a conclusion cannot be drawn from the present data.

Although tannins can bind to starch and consequently inhibit starch digestion *in vitro* (Desphande & Salunkhe, 1982), no difference in starch digestibility among the low-, medium- and high-tannin varieties of faba beans were found. Under the physical and chemical conditions prevailing the gastrointestinal tract of pigs, condensed tannins seem to have a preference to complex with proteins rather than with carbohydrates.

It should be remarked, however, that the ileal digestibility of starch may have been slightly overestimated as a result of the digesta collection procedure used. Although collection bags were changed at least every two hours, some fermentation of starch after collection may have occurred.

Among the three varieties containing condensed tannins no difference was found in apparent ileal digestibility and only slight differences at the faecal level although the content of condensed tannins in beans of cv. Herz Freya was about half of that of the varieties Mythos and Alfred (Table 1). The fact that the batch of Herz Freya beans was at least three years old at the time of the experiment might be important in this respect. Storage of high-tannin beans may lead to a reduction in assayable tannin

content due to oxidative polymerization of tannins. This process can reduce the extractability of tannins during the assay as was found by Sievwright & Shipe (1986) for Phaseolus beans. The fact that no significant difference in nutritional quality was observed between beans of the cv. Herz Freya on the one hand and the freshly harvested beans of Mythos and Alfred on the other may suggest that the polymerized tannins still maintain their nutrient-binding properties.

Autoclaving low-tannin beans of the cv. Blandine did not improve their ileal and faecal digestibility in young pigs. The values obtained were even below those obtained for the untreated batch of the same variety. Ivan & Bowland (1976) studied the effect of autoclaving faba beans for 30 and 60 minutes on ileal and faecal digestibility in pigs weighing 25 kg. No significant improvement in the ileal digestibility of dry matter, N, amino acids (except for arginine) and energy could be observed. They concluded that heat-labile antinutritional factors do not play an important role in the nutritive value of faba beans in pigs. Van der Poel et al. (1992) came to the same conclusion after observing that steam heating of dehulled faba beans (103°C for 20 min) did not improve their ileal and faecal digestibility in piglets.

In conclusion, it can be stated that significant differences exist in the nutritive value of faba beans for young piglets. The absence of condensed tannins seems to be favourable for the nutritive value of faba beans, particularly with regard to apparent crude protein and amino acid digestibility. However, more research should be carried out to determine the precise mode of action of condensed tannins in faba beans and to establish the influence of other factors that may interfere with the digestibility of nutrients in faba beans in pigs.

Acknowledgements

The authors wish to thank P. van Leeuwen for surgical assistance and J. Wiebenga for assistance in statistical analysis of data.

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MODE OF ACTION IN PIGS

Chapter 6

EFFECTS OF DIETARY INCLUSION OF HULLS OF FABA BEANS (VICIA FABA L.) WITH A LOW AND HIGH CONTENT OF CONDENSED TANNINS ON SOME PHYSIOLOGICAL PARAMETERS IN PIGLETS

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Animal Feed Science and Technology, in press

Effects dietary inclusion of hulls of faba beans (Vicia faba L.) with a low and high content of condensed tannins on some physiological parameters in piglets

Abstract

In an experiment with young piglets (8-17 kg) the effects of condensed tannins in hulls of faba beans (Vicia faba L.) on faecal nutrient digestibility, N utilization, organ weights and some other physiological parameters were studied. Hulls of a white-flowering variety (cv. Blandine) with a low content of condensed tannins (<0.10% catechin equivalents) and of a coloured-flowering variety (cv. Alfred) with a high content of condensed tannins (3.3 % catechin equivalents) were included at a level of 200 g/kg in experimental diets II and III, respectively. In a control ration (diet I) tannin-free, autoclaved (120°C, 20 min) hulls of peas were incorporated at a level of 152 g/kg. Diets were balanced as to calculated contents of net energy, crude protein (N x 6.25 (CP)), lysine, methionine and cystine, threonine, tryptophan, vitamins and minerals. Diet I showed the highest faecal digestibility coefficients for dry matter (DM), organic matter (OM), crude fibre (CF) and nitrogen-free extract (NFE) ($P<0.05$). Diet III had a lower apparent faecal digestibility for DM, CP, OM, CF, NFE and individual amino acids than both diets I and II ($P<0.05$). Apparent faecal digestibility was most severely reduced for histidine, glycine and proline. The higher faecal N excretion in the high-tannin group ($P<0.05$), was accompanied by a lower N excretion with the urine ($P<0.05$). N retention tended to be lower for the animals receiving diet III (10.0 g N/day (III) versus 10.9 and 11.0 g N/day for diets I and II, respectively). The wet empty weight of the caecum was lower for the group fed the high-tannin diet ($P<0.05$). Activity of trypsin and chymotrypsin in digesta from the small intestine was lower in animals fed the high-tannin diet ($P<0.05$). Faecal mucin excretion, as measured via faecal glucosamine and galactosamine content, did not differ between treatments.

It was concluded that condensed tannins from faba beans in diets for piglets exert antinutritional effects by reducing the apparent digestibility of nutrients, particularly of protein and amino acids. This can lower N retention in pigs. Faba bean condensed tannins do not seem to cause systemic effects, when the dietary content does not exceed 0.6% catechin equivalents.

Introduction

The nutritive value of feedstuffs for non-ruminant species is largely dependent on the presence of nutrients which can be digested and, after absorption, can be utilized. The nutritive value is limited by substances which interfere with the digestive process itself. Non-starch polysaccharides (NSP) and antinutritional factors (ANFs) are examples of such factors.

In many parts of the world faba beans (Vicia faba L.) have been recognized as a

potential high-quality protein feedstuff for both pigs and poultry (Eggum, 1980). However, their nutritive value seems to be limited by the presence of condensed tannins (Martin-Tanguy et al., 1977; Marquardt et al., 1977; Jansman et al., 1993). Condensed tannins are polyphenolic compounds with the ability to precipitate proteins from aqueous solutions. Tannins are found particularly in the hull fraction of faba beans of coloured-flowering varieties (Bos & Jetten, 1989). Condensed tannins of various origins have been shown to reduce apparent digestibility, particularly of protein in diets for non-ruminant animal species (Salunkhe et al., 1990). Moreover, in *in vitro* studies and in some *in vivo* studies condensed tannins inhibited the activity of digestive enzymes (Longstaff & McNab, 1991). This is due to the formation of tannin-enzyme complexes which are biologically inactive (Griffiths, 1979; Griffiths & Moseley, 1980). Suggestions have been made that hydrolysable tannins in particular, or their degradation products, may be absorbed and cause systemic effects on some metabolic organs such as liver and kidneys (Karim et al., 1978). In rats, sorghum tannins caused hypertrophy of the parotid glands accompanied by an increased secretion of proline-rich proteins (PRPs) (Mehansho et al. 1983). Some authors suggest that tannins may cause detrimental effects on the morphology and histology of the mucosa of the gastro-intestinal tract (Mitjavila et al., 1977). Tannins may also induce increased losses of mucins in the faeces (Sell et al., 1985).

The effects of condensed tannins in faba beans have been studied particularly in chickens (Marquardt et al., 1977; Longstaff & McNab, 1991), but hardly in pigs. In the present study the effects of hulls of faba beans with a low and high tannin content on nutrient digestibility and nitrogen utilization were determined in pigs. In addition, their effects on protein-degrading enzymes, organ weights, faecal hexosamines excretion and some characteristics of the gastro-intestinal tract were studied.

Materials and methods

Experimental diets

The composition and some analytical figures of the three experimental diets are given in Table 1. The three diets differed with respect to their content of hulls of pea and faba bean. Each diet contained barley and maize as major ingredients. In diet II 200 g/kg of hulls of a white-flowering, low-tannin variety of faba bean, cv. Blandine, were included while diet III contained 200 g/kg of hulls of a coloured-flowering, high-tannin variety, cv. Alfred. In the tannin-free control diet I 152.5 g/kg of autoclaved hulls of a commercial batch of peas (Pisum sativum L.) were included. The crude fibre contents of diets I, II and III were 82, 115 and 103 g/kg, respectively.

Diets I to III were further balanced as to contents of crude protein (CP; N x 6.25), net energy, Ca, P, lysine, methionine & cystine, threonine, tryptophan, vitamins and minerals (Table 1). Each of these indispensable amino acids were included at a level of 90% of their ileal digestible requirement value according to Dutch feeding standards (CVB, 1990). All diets contained vitamins and minerals according to requirements (ARC, 1981).

Table 1. Composition (g/kg) and analysed/calculated contents (g/kg) of the experimental diets.

	I	II	III
Barley	300	300	300
Maize	265.2	206.8	206.8
Maize gluten meal (59% CP)	40	38	38
Skim milk powder	100	100	100
Meat meal	40	40	40
Pea hulls, autoclaved	152.5		
Faba bean hulls cv. Blandine		200	
Faba bean hulls cv. Alfred			200
Soya oil	37.5	52.0	52.0
Vitamin/mineral mix[5]	10.0	10.0	10.0
Cane molasses	20.0	20.0	20.0
$CaCO_3$	12.0	12.0	12.0
$CaHPO_4$	6.0	5.5	5.5
NaCl	3.0	3.0	3.0
$KHCO_3$	4.0	3.0	3.0
$NaHCO_3$	4.0	4.0	4.0
DL-methionine	0.5	0.7	0.7
L-lysine HCl	3.7	3.5	3.5
L-threonine	0.8	0.8	0.8
L-tryptophan	0.5	0.5	0.5
L-isoleucine	0.3	0.2	0.2
Dry matter[1]	900.1	907.7	901.8
Net energy[2] (MJ/kg)	9.01	9.00	9.00
Crude protein[1] (Nx6.25)	180	174	173
Ether extract[1]	75.0	85.5	85.5
Crude fibre[1]	82.3	114.6	102.9
Neutral detergent fibre[1]	142.2	175.6	162.5
Ash[1]	63.7	62.1	62.7
Ca[2]	9.9	9.9	9.9
P[2]	6.2	6.2	6.2
Available P[2]	3.5	3.5	3.5
Lys[1]	10.1	9.8	9.8
Met[1]	3.5	3.5	3.4
Met & Cys[2]	6.1	6.1	6.1
Thr[1]	6.8	6.5	6.5
Trp[2]	2.1	2.1	2.1
Condensed tannins[3]	<0.1	<0.1	0.54
TIA[4]	0.31	0.31	0.34

[1]Analysed values
[2]Calculated values
[3]% catechin equivalents
[4]Trypsin inhibitor activity, mg trypsin inhibited per gram sample
[5]The vitamin/mineral mix delivered per kg feed: 9000 IU vitamin A; 1800 IU vitamin D_3; 40 mg vitamin E; 5 mg riboflavin; 30 mg niacinamide; 12 mg d-pantothenic acid; 150 mg choline chloride; 40μg vitamin B_{12}; 3 mg menadione; 50 mg ascorbic acid; 0.3 mg folic acid; 100 mg $CuSO_4.5H_2O$; 200 mg $ZnSO_4.H_2O$; 70 mg MnO_2; 400 mg $FeSO_4.7H_2O$; 2.5 mg $CoSO_4.7H_2O$; 0.2 mg $Na_2SeO_3.5H_2O$; 0.5 mg KI; 40 mg tylosin.

Hull sources and preparation

A commercial batch of pea hulls was autoclaved at 120°C for 20 min to decrease trypsin inhibitor activity, which was reduced from 0.76 mg to 0.17 mg trypsin inhibited per gram of hulls. Hulls were subsequently dried in an oven for 24 h at 60°C.

Hulls of both batches of faba beans were obtained by coarsely breaking the beans on a Variostuhl (Modell CEX 2, 1982) equipped with fluted rolls (roll gap 4 mm, 4 flutes per cm, flute angles 45°/65°, differential 600:400 rpm). The breaking procedure was repeated using a roll gap of 2.5 mm. The hulls were separated from the cotyledon fraction by using aspiration on a mini-Petkus cleaning machine (Röher, No. 20113). The hull fraction was ground on a hammermill (Kamas) with a 2.5 mm screen prior to inclusion in the diets.

The hull fractions made up 10.6 and 16.4 % of the weight of the original beans of the cvs. Blandine and Alfred, respectively.

An analytical characterization of the hulls of pea and faba bean is given in Table 2. Pea hulls, as used in diet I, contained somewhat more crude protein than faba bean hulls. This may be related to the presence of some residues of cotyledons in the hull fraction. The hulls of both peas and faba beans appeared to be high in non-starch structural polysaccharides (NSP) as can be seen from their high content of crude fibre, neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Table 2). The lignin content in both the pea and faba bean hulls was low. The level of condensed tannins in the hulls of faba beans appeared to be the main difference between both batches. Hulls of cv. Blandine and cv. Alfred contained <0.10 and 3.30% catechin equivalents, respectively. Activity of trypsin inhibitors in the hulls of peas and faba beans was low and nearly equivalent (Table 2).

Table 2. Analysed chemical composition (g/kg) of hulls of peas (Pisum sativum L.) and faba beans (Vicia faba L.).

Hull type	Peas autoclaved	Faba beans cv. Blandine	Faba beans cv. Alfred
Dry matter	927.2	899.4	887.0
N x 6.25	105.0	53.7	62.3
Crude fibre	389.6	494.8	380.8
NDF	573.2	659.1	547.4
ADF	470.6	614.3	535.6
Lignin	0.0	5.0	22.3
Tannins[1]	<0.10	<0.10	3.30
TIA[2]	0.17	0.37	0.16

[1]according to the vanillin/sulphuric acid method (% catechin equivalents)
[2]TIA: trypsin inhibitor activity, mg trypsin inhibited per gram sample.

Animals and experimental procedures

Twenty four newly weaned castrated male piglets (4 weeks of age; BW 8.4 ± 0.8 kg) of the cross bred Dutch Landrace x Dutch Yorkshire were placed in metabolism cages. The piglets were fed a commercial weaning diet. After 9 days of adaptation to the cages the animals were allocated to one of the three experimental groups. Mean body weight of animals in each group was 10.1 ± 0.8 kg. The animals were then changed over to the experimental diets. During the pre-experimental period (11 days) the feeding level was gradually increased from 2.2 to 2.7 times their maintenance requirements for energy (ARC, 1981). During the balance period (6 days) daily feed intake was registered, and both faeces and urine of the individual animals were collected quantitatively. Faeces were collected using stoma bags attached around the anus. Bags were changed twice daily and stored at -20°C. Urine was collected in containers via funnels underneath the cages. In each of the containers 5 ml of 25% sulphuric acid was added to prevent volatilization of nitrogenous compounds. Urine was stored at 4°C. Throughout the experiment the animals were fed twice daily at 08.00 and 17.00 h. Water was freely available via drinking nipples.

Following the balance period the animals were weighed and sacrificed for dissection. The animals to be dissected received their regular morning meal in two equal portions at 09.00 and 12.00 h. Average interval between final meal and dissection was 3 ± 1.4 h. The animals first received inhalation anaesthesia with O_2/N_2O and halothane. The abdomen was opened and plastic strips were used to separate different segments of the gastro-intestinal tract. Pairs of strips 5 cm apart were positioned anterior and posterior to the stomach, 0.5 m and 5.5 m distal of the Treitz ligament and 0.5 m proximal of the ileocaecal ligament. Subsequently, the digesta in the stomach, the duodenum (including 0.5 m of the proximal jejunum), the jejunum (between 0.5 and 5.5 m distal of the Treitz ligament) (Jejunum 1), the distal jejunum and ileum (Jejunum 2) and the caecum were collected quantitatively by manual stripping, and were weighed. Furthermore, a representative sample of the colon content was taken. Samples were immediately stored in a freezer at -20°C. The empty wet weight of the different parts of the gastro-intestinal tract was measured. Subsequently, the pancreas, liver, spleen, right kidney, parotid gland and submandibular gland were dissected and weighed. The pancreas and both the parotid and submandibular glands were frozen immediately at -20°C.

Chemical analysis

Feeds and faecal samples were analysed for dry matter (DM) (ISO 6496, 1983), N (ISO 5983, 1979), ash (ISO 5984, 1978), ether extract (EE, ISO 6492, 1985) and crude fibre (CF, NEN 5417, 1988). Crude protein (CP) was calculated as N x 6.25. Organic matter (OM) was defined as dry matter - ash, and nitrogen-free extract as dry matter - (ash + N x 6.25 + ether extract + crude fibre). Dry matter and N were analysed in the fresh faeces. Other determinations in faeces were carried out in freeze-dried material after grinding on a laboratory mill with a 1 mm screen.

NDF, ADF and acid detergent lignin (ADL) in the hull samples were analysed according to Goering & Van Soest (1970).

Amino acids, excluding cystine and tryptophan, in feeds and faecal samples were analysed following the method of Andrews & Baldar (1985).

Hulls and diets were analysed on level of condensed tannins according to the vanillin-sulphuric acid method of Kuhla & Ebmeier (1981) and on trypsin inhibitor activity (TIA) as described by van Oort et al. (1989).

Digesta samples obtained from different parts of the gastro-intestinal tract at the time of dissection were freeze-dried. From losses of weight during freeze-drying, the dry matter content of digesta samples was derived. Digesta samples from the segments referred to as Duodenum, Jejunum 1 and Jejunum 2 were analysed on the activity of trypsin and chymotrypsin according to Bergmeyer (1974). Samples of pancreatic tissue were activated with enterokinase (EC 3.4.21.9) prior to analysis of activity of both enzymes.

Galactosamine and glucosamine contents of faeces samples were analysed according to procedures described by Rotter et al. (1989) and Mills et al. (1989).

Statistical analysis

One-way analysis of variance was caried out on the experimental data using treatment as a factor. If the treatment effect was significant, the differences between means were tested with the Least Significance Difference (LSD) test (Snedecor & Cochran, 1980).

Results

Results for apparent faecal digestibility values of the experimental diets for DM, CP, Ash, OM, CF, EE and NFE are presented in Table 3. Control diet I had a higher faecal

Table 3. Apparent faecal digestibility of the experimental diets.

	I	II	III	SEM[1]
DM	0.863[a]	0.816[b]	0.759[c]	0.010
CP	0.829[a]	0.828[a]	0.747[b]	0.012
Ash	0.648[a]	0.665[a]	0.628[a]	0.018
OM	0.877[a]	0.826[b]	0.767[c]	0.010
CF	0.680[a]	0.448[b]	0.209[c]	0.039
EE	0.845[a]	0.874[a]	0.871[a]	0.013
NFE	0.919[a]	0.888[b]	0.847[c]	0.005

[a,b,c]Values with different superscripts in the same row differ significantly at $P<0.05$.
[1]SEM: standard error of the mean.

digestibility for DM, OM, CF, and NFE than the two diets with faba bean hulls (II and III) ($P<0.05$). Apparent faecal CP and Ash digestibility did not differ between diets I and II. The faecal digestibility of DM, CP, OM, CF and NFE for diet III was lower than for diet II ($P<0.05$), with 6, 8, 6, 24 and 4 units, respectively.

Table 4 shows the apparent faecal digestibility of amino acids for the three experimental diets. Between diet I with pea hulls and diet II with low-tannin faba bean hulls no differences were found for any of the amino acids determined. The apparent digestibility of all amino acids was lower for diet III ($P<0.05$) than for diets I and II. The difference in digestibility between diets II and III was lowest for lysine (5.1 units) and highest for proline (18.8 units). Total amino acid digestibility differed by 8 units which is in agreement with the difference in CP digestibility (Table 3).

Table 4. Apparent faecal digestibility of amino acids of the experimental diets.

	I	II	III	SEM[1]
Arg	0.888[a]	0.879[a]	0.808[b]	0.008
His	0.878[a]	0.874[a]	0.766[b]	0.010
Iso	0.842[a]	0.851[a]	0.777[b]	0.014
Leu	0.882[a]	0.890[a]	0.827[b]	0.010
Lys	0.880[a]	0.878[a]	0.827[b]	0.009
Met	0.841[a]	0.850[a]	0.797[b]	0.014
Phe	0.862[a]	0.872[a]	0.801[b]	0.010
Thr	0.843[a]	0.843[a]	0.777[b]	0.011
Val	0.850[a]	0.854[a]	0.780[b]	0.012
Ala	0.836[a]	0.839[a]	0.764[b]	0.012
Asp	0.832[a]	0.825[a]	0.761[b]	0.012
Glu	0.909[a]	0.912[a]	0.845[b]	0.008
Gly	0.842[a]	0.815[a]	0.706[b]	0.010
Pro	0.911[a]	0.910[a]	0.722[b]	0.008
Ser	0.868[a]	0.869[a]	0.810[b]	0.009
Tyr	0.842[a]	0.835[a]	0.765[b]	0.011
ΣAA	0.873[a]	0.873[a]	0.793[b]	0.009

[a,b]Values with different superscripts in the same row differ significantly at $P<0.05$.
[1]SEM: standard error of the mean.

Data on the performance of the piglets during the period in which the animals received the experimental diets (17 days) are given in Table 5. Feed intake tended to be higher for control group I because of small feed rests for a few animals in groups II and III in the pre-experimental period and because of small differences in body weight at the start of the experimental period. Weight gain was lowest in group III

($P<0.05$) and also tended to be lower for group II than for control group I. Feed conversion efficiency was highest in piglets in group III ($P<0.05$).

Table 5. Feed intake (g/day), weight gain (g/day) and feed conversion efficiency of the piglets over 17 days.

	I	II	III	SEM[1]
Feed intake	591[a]	575[a]	571[a]	16
Weight gain	421[a]	386[ab]	356[b]	15
Feed conversion efficiency	1.41[a]	1.50[a]	1.61[b]	0.03

[a,b]Values with different superscripts in the same row differ significantly at $P<0.05$.
[1]SEM: standard error of the mean.

Data on nitrogen utilization during the N balance period are given in Table 6. N intake was highest for animals in group I and lowest for those in group III, because of slight differences in mean body weight at the start of the experimental period. Piglets fed the diet with high-tannin faba bean hulls showed the highest faecal and the lowest urinary N excretion ($P<0.05$). N retention in the piglets did not differ significantly between the three groups, but the value tended to be lower for the animals in group III. In Table 6 also presents some relative figures for the N metabolism of the animals. Utilization of the apparently digested N (N retained/N digested x 100%) was highest for group III relative to group I ($P<0.05$). It also tended to be higher than the value for group II.

Table 6. Nitrogen utilization of the experimental animals during the balance period (6 days).

	I	II	III	SEM[1]
N intake (g/day)	17.10[a]	16.51[ab]	15.79[b]	0.4
N faeces (g/day)	2.90[a]	2.83[a]	3.99[b]	0.2
N urine (g/day)	3.32[a]	2.68[b]	1.85[c]	0.2
N retention (g/day)	10.88[a]	10.99[a]	9.96[a]	0.4
N ret./N int. * 100	63.5[a]	66.6[a]	63.1[a]	1.5
N ret./N dig. * 100	76.5[a]	80.5[ab]	84.5[b]	1.5
N uri./N int. * 100	19.5[a]	16.2[a]	11.6[b]	1.2

[a,b,c]Values with different superscripts in the same row differ significantly at $P<0.05$.
[1]SEM: standard error of the mean.

N ret. : N retention
N dig. : N apparently digested
N int. : N intake
N uri. : N in urine

The results for group III show that the lower apparent protein digestibility is compensated by an increased utilization of digested protein. Urinary N excretion as a percentage of N intake was lower for group III (11.6 %) than for the other groups (19.5 and 16.2 % for group I and II, respectively; $P<0.05$).

Table 7 presents the weights of some organs at dissection. The body weight of the animals receiving the diet with high-tannin faba bean hulls tended to be lower. Results on organ weights are expressed on both an absolute and a relative basis (% of BW). In both ways no differences were found for the weight of the pancreas, liver, spleen or parotid gland. The absolute weight of the kidneys and the submandibular glands of the piglets in group III was somewhat lower than in groups I and II ($P<0.05$). In relative terms, the submandibular glands of piglets in group III were smaller than in group II. No gross morphological changes were observed for any of these organs in either of the experimental groups.

Table 7. Body weight and absolute and relative organ weights.

	I	II	III	SEM[1]
Absolute weight				
Body weight (kg)	17.5[a]	16.8[a]	16.1[a]	0.5
Pancreas (g)	32.0[a]	34.7[a]	29.9[a]	2.2
Liver (g)	444[a]	435[a]	418[a]	15.6
Spleen (g)	46.1[a]	49.1[a]	51.9[a]	5.1
Kidney (g)	80.8[ab]	85.2[a]	73.0[b]	3.5
Parotid gland (g)	66.9[a]	73.9[a]	69.4[a]	8.8
Submandibular gland (g)	6.2[ab]	6.4[a]	5.5[b]	0.3
Relative weight (% of BW)				
Pancreas	0.183[a]	0.206[a]	0.185[a]	0.009
Liver	2.549[a]	2.598[a]	2.602[a]	0.067
Spleen	0.264[a]	0.299[a]	0.326[a]	0.035
Kidney	0.462[a]	0.509[a]	0.455[a]	0.014
Parotid gland	0.378[a]	0.447[a]	0.432[a]	0.051
Submandibular gland	0.035[ab]	0.038[a]	0.034[b]	0.001

[a,b]Values with different superscripts in the same row differ significantly at $P<0.05$.
[1]SEM: standard error of the mean.

In Table 8 the absolute and relative wet empty weights of different segments of the gastro-intestinal tract are given. The wet empty weight of the stomach, both in absolute and in relative terms, was highest for animals receiving faba bean hulls ($P<0.05$). The weight of the duodenum and colon was higher for group I than for group III ($P<0.05$). No differences in relative weight of these segments were found. The weight of the caecum was lower in group III than in group II ($P<0.05$).

Table 8. Wet empty weights of some segments of the gastro-intestinal tract of the piglets (absolute and as a % of body weight).

	I	II	III	SEM[1]
Absolute (g)				
Stomach	126[a]	148[b]	144[b]	6
Duodenum	51[a]	49[ab]	46[b]	2
Jejunum 1	196[a]	211[a]	210[a]	15
Jejunum 2	345[a]	339[a]	316[a]	29
Colon	323[a]	284[ab]	272[b]	18
Caecum	34[ab]	36[a]	27[b]	3
Relative (% of BW)				
Stomach	0.72[a]	0.88[b]	0.90[b]	0.02
Duodenum	0.29[a]	0.30[a]	0.29[a]	0.02
Jejunum 1	1.13[a]	1.27[a]	1.32[a]	0.10
Jejunum 2	1.98[a]	2.02[a]	1.97[a]	0.17
Colon	1.85[a]	1.70[a]	1.70[a]	0.11
Caecum	0.19[ab]	0.22[a]	0.17[b]	0.02

[a,b]Values with different superscripts in the same row differ significantly at $P<0.05$.
[1]SEM: standard error of the mean.

Fresh weight, dry matter weight and dry matter content of digesta collected from various segments of the gastro-intestinal tract are given in Table 9. Data on fresh or dry matter weight did not differ statistically among experimental groups. Dry matter content of digesta in the stomach and the caecum was higher in both groups receiving diets with faba bean hulls ($P<0.05$). Dry matter content of colon digesta in group III was significantly higher than in the control group I.

The activity of trypsin and chymotrypsin measured in duodenal digesta at the time of dissection did not differ significantly among treatments (Table 10). Activity values, however, tended to be highest for group II. For the proximal part of the jejunum (Jejunum 1), trypsin activity was higher in group II ($P<0.05$) than in both other groups. For chymotrypsin, activity at this site only differed between groups I and II. In the distal part of the jejunum and the ileum (Jejunum 2) trypsin activity was lowest in the high-tannin group III. Activity of chymotrypsin in digesta was higher in groups II and III than in group I ($P<0.05$). In pancreatic tissue the activity of both trypsin and chymotrypsin was higher for control group I ($P<0.05$).

No clear differences in the content of glucosamine and galactosamine in faeces were found among experimental groups (Table 11). Only the content of galactosamine in faeces of animals in group III (2.16 mg/g) was significantly lower than in group I (3.31 mg/g) ($P<0.05$).

Table 9. Fresh weight, dry matter weight and dry matter content of digesta in different segments of the gastro-intestinal tract.

	I	II	III	SEM[1]
Fresh weight (g)				
Stomach	347[a]	309[a]	330[a]	20
Duodenum	41[a]	28[a]	35[a]	5
Jejunum 1	94[a]	96[a]	93[a]	17
Jejunum 2	267[a]	283[a]	280[a]	30
Caecum	109[a]	128[a]	100[a]	13
Dry matter weight of digesta (g)				
Stomach	89[a]	102[a]	110[a]	10.0
Duodenum	0.7[a]	0.5[a]	0.7[a]	0.2
Jejunum 1	7.5[a]	6.0[a]	6.6[a]	1.6
Jejunum 2	31[a]	35[a]	30[a]	3.8
Caecum	18[a]	24[a]	20[a]	2.8
Dry matter content of digesta (%)				
Stomach	25.3[a]	33.0[b]	33.1[b]	1.9
Duodenum	2.1[a]	2.1[a]	2.0[a]	0.6
Jejunum 1	7.6[a]	5.9[a]	7.2[a]	1.1
Jejunum 2	11.9[a]	12.4[a]	10.9[a]	1.1
Caecum	16.5[a]	18.7[b]	19.4[b]	0.7
Colon	23.5[a]	26.1[ab]	27.6[b]	1.0

[a,b]Values with different superscripts in the same row differ significantly at $P<0.05$.
[1]SEM: standard error of the mean.

Discussion

Composition of the diets

The present experiment was carried out to evaluate the effects of condensed tannins in hulls of faba beans on nutrient digestibility in pigs. Also N metabolism, activity of digestive enzymes in digesta, and systemic effects on metabolic organs were determined. To evaluate the nutritional effects of condensed tannins from faba beans, the use of isolated and purified tannins from these seeds is preferred. However, large-scale isolation of tannins for this purpose is not possible so far. In various studies, tannic acid, a hydrolysable tannin, has been used to study nutritional effects of tannins (Liebert & Gebhardt, 1980; Kubena et al, 1983; Santidrian & Marzo, 1989). Results of these studies are used to quantify the effects of tannins in various feedstuffs. This is probably not correct since the effects of hydrolysable tannins are

Table 10. Activities of trypsin and chymotrypsin in pancreatic tissue and digesta from various parts of the intestinal tract (units[1]/g air-dry matter).

	I	II	III	SEM[2]
Trypsin				
Pancreas	5910[a]	4224[b]	4115[b]	519
Duodenum	214[a]	556[a]	258[a]	129
Jejunum 1	278[a]	443[b]	274[a]	51
Jejunum 2	391[a]	372[a]	252[b]	29
Chymotrypsin				
Pancreas	708[a]	475[b]	452[b]	79
Duodenum	28[a]	83[a]	35[a]	17
Jejunum 1	32[a]	55[b]	42[ab]	7
Jejunum 2	33[a]	41[ab]	44[b]	3

[a,b]Values with different superscripts in the same row differ significantly at $P<0.05$.
[1]see Bergmeyer (1974).
[2]SEM: standard error of the mean.

markedly different from those of condensed tannins (Jansman, 1993). Tannins in faba beans have been shown to be of the condensed type, being polymers of merely flavan-3-ols and flavan-3,4-ols (Martin-Tanguy et al., 1977). By using faba bean hulls in the present experiment, the effects of native tannins in this legume seed were studied.

In control diet I, autoclaved pea hulls were included to obtain a diet with a similar crude fibre content as in the diets with faba bean hulls. Analytical results, however, showed that the crude fibre content was lower in control diet I than in diets II and III,

Table 11. Faecal content of glucosamine and galactosamine of the experimental groups.

	I	II	III	SEM[1]
Glucosamine (mg/g DM)	5.23[a]	4.63[a]	4.69[a]	0.60
Glucosamine (mg/mg N)	0.147[a]	0.175[a]	0.160[a]	0.018
Galactosamine (mg/g DM)	3.31[a]	2.61[ab]	2.16[b]	0.34
Galactosamine (mg/mg N)	0.093[a]	0.098[a]	0.074[a]	0.010

[a,b]Values with different superscripts in the same row differ significantly at $P<0.05$.
[1]SEM: standard error of the mean.

by 32 and 21 g/kg, respectively (Table 1).

Both pea and faba bean hulls are rich in non-starch polysaccharides (NSP) (Table 2). Faba bean hulls appeared to consist mainly of cellulose (ADF minus lignin) (513-609 g/kg) and to contain some hemicellulose (NDF minus ADF) (12-45 g/kg). These observations agree with results of Marquardt et al. (1978), Longstaff & McNab (1991) and Longstaff et al. (1991). The level of condensed tannins was assumed to be the most important difference between both batches of faba bean hulls.

Faecal digestibility of diets containing pea- and faba bean hulls

The higher apparent faecal digestibility of dry matter, organic matter and crude fibre of the control diet with pea hulls compared to the diets containing faba bean hulls, may partly be explained by the difference in level of crude fibre between the diets. In addition, the composition of structural carbohydrates in hulls of peas and faba beans differ to some extent. Cellulose content (ADF minus lignin content) seems to be slightly lower, while the hemicellulose content (NDF minus ADF content) is somewhat higher in pea hulls (Table 2). Stanogias & Pearce (1985a) have shown that hemicellulose is somewhat higher digestible than cellulose in legume seed hulls in pigs. Moreover, hulls of both peas and faba beans are known to contain some soluble pectic polysaccharides (Longstaff & McNab, 1989; 1991). Their digestibility may be higher than that of polysaccharides with a low solublity, such as cellulose and hemicellulose (Longstaff & McNab, 1989). Differences in content of cellulose, hemicellulose and pectins between pea and faba bean hulls could thus have been contributed to differences in dry- and organic matter digestibility of the diets.

The reduced crude protein digestibility of the diet containing the tannin-rich faba bean hulls may be explained by the action of tannins. A relatively low apparent protein digestibility of diets containing high-tannin, instead of low-tannin sorghum has been found in various animal species, such as rats (Ford & Hewitt, 1979; Muindi & Thomke, 1981), chickens (Rostagno et al., 1973; Mitaru et al., 1984) and pigs (Cousins et al., 1981; Mitaru et al., 1985). Longstaff et al. (1991) determined the effects of feeding diets with low- and high-tannin faba bean hulls on nutrient digestibility in adult cockerels. In their experiment, apparent protein digestibility of diets with 200 and 500 g/kg of high-tannin hulls was dropped by 12 and 20 units, respectively, compared to the control diets containing similar levels of low-tannin faba bean hulls. In the present experiment with piglets, a hull inclusion level of 200 g/kg was associated with a 8-unit difference in apparent faecal N digestibility between low- and high-tannin hulls.

The extent of decrease in apparent digestibility of individual amino acids in the high-tannin diet varied markedly and was highest for proline, glycine and histidine. A similar observation was made by Cousins et al. (1981), who determined the ileal and faecal digestibility of low- and high-tannin sorghum in pigs. From a study with pigs, Mitaru et al. (1985) concluded that tannins in sorghum have a higher affinity for hydrophobic amino acids. This may also explain the difference in effect of tannins among individual amino acids in our study.

The reduced apparent faecal digestibility of the nitrogen-free extract of the diet containing high-tannin faba bean hulls compared to the diet with low-tannin hulls may

be the result of carbohydrates (starch) complexing with tannins. *In vitro*, binding of tannins to carbohydrates have been shown (Desphande & Salunkhe, 1982).

Effects of fibre and tannins from faba beans on protein digestibility and enzyme activity in digesta and the pancreas

The most common explanation for the effects of dietary tannins on protein digestibility is the binding of dietary tannins to feed proteins, rendering them unavailable for digestion (Salunkhe et al., 1990). Interaction of tannins with digestive enzymes may explain their effect as well. We found in most cases a reduced activity of both trypsin and chymotrypsin in digesta collected from various parts of the small intestine in pigs fed the tannin-rich diet, as compared to pigs receiving low-tannin faba bean hulls. Griffiths (1979) has shown *in vitro* the inhibitory properties of tannins from coloured-flowering varieties of faba beans on the activity of trypsin, chymotrypsin and α-amylase. Extracts of hulls of low-tannin white-flowering varieties did not show such properties. Griffiths & Moseley (1980) found similar effects *in vivo* in digesta from rats. Recently, Longstaff & McNab (1991) and Yuste et al. (1992) found significantly reduced activities of trypsin, α-amylase and lipase in digesta of cockerels and young chicks fed diets with tannin-containing faba bean hulls and tannin-rich extracts from faba beans.

It is not clear from literature whether effects of tannins on the activity of digestive enzymes is only due to inhibition of secreted enzymes. They could reduce the pancreatic secretion of enzymes as well. Pigs fed either diets with low- or high-tannin faba bean hulls, however, had similar weights of the pancreas. The activities of trypsin and chymotrypsin in pancreatic tissue were similar as well. This suggests that the pancreatic function remains unaffected. In chickens, Longstaff & McNab (1991) did not find an effect of diets containing 400 g/kg of high-tannin faba bean hulls on the size of the pancreas. Unfortunately, in their experiment protease activity was not measured in the pancreatic tissue. Also Griffiths & Moseley (1980) and Marquardt et al. (1977) did not observe pancreatic enlargement in chickens after feeding diets with tannin-rich faba bean hulls. This suggests that dietary condensed tannins do not affect pancreatic secretion of digestive enzymes to a large extent.

The lower activity of trypsin and chymotrypsin in digesta in some parts of the intestinal tract from pigs fed on the diet with pea hulls compared with the activity found in digesta of pigs receiving the low-tannin faba bean hulls may be explained by inhibition of enzymes in the gut lumen. Schneemann (1978) found *in vitro* differences between fibre sources in inhibitory properties towards the activity of digestive enzymes in digesta.

As a third option, effects of dietary tannins on the apparent protein digestibility in pigs may be explained by increased losses of endogenous proteins, others than digestive enzymes, as suggested by Cousins et al. (1981). They assumed that the strongly reduced digestibility of proline and glycine was the result of an increased secretion of endogenous protein. Some of these proteins are relatively rich in these amino acids (Souffrant, 1991). The marked effect of tannins on proline digestibility may be due to an increased secretion of specific proline-rich proteins (PRPs) by the

salivary glands. When feeding rat and mice high-tannin sorghum, Mehansho et al. (1983, 1985) found a marked hypertrophy of the parotid glands accompanied by increased synthesis and secretion of PRPs. The parotid and submandibular glands of the piglets fed the high-tannin diet in our study, however, did not show hypertrophy. This may indicate an absence of such a response to dietary tannins in pigs. Alternatively, the secretion of salivary proteins may be increased in this group without showing hypertrophy of the salivary glands.

Both the glucosamine and galactosamine content of faeces of the pigs in this experiment were determined as a measure of mucin excretion. Mucin is considered to be an endogenous glycoprotein with a high resistance to proteolysis. It contains glucosamine and galactosamine as important hexosamines (Mantle & Allen, 1981). Faecal content of both did not differ between the groups receiving the different faba bean hulls. Sell et al. (1985) found an increased faecal glucosamine content in rats fed a high-tannin sorghum diet. Fuller & Cadenhead (1991) suggested that the galactosamine content in ileal digesta of pigs may provide an estimate of mucin glycoprotein secretion. They showed that the galactosamine content in ileal digesta of pigs was affected by both feeding level and diet composition. It can be concluded from our study that both faecal glucosamine and faecal galactosamine content are not largely affected by diets containing either low- or high faba bean hulls compared to the control diet with pea hulls. It is thus not likely that differences in mucin excretion can explain the reduced apparent digestibility of crude protein in the high-tannin diet.

Effects of fibre and tannins from faba beans on N retention and organ weights

The reduced apparent digestibility of protein and amino acids in the high-tannin diet, tended to decrease N retention and weight gain and increased feed conversion efficiency of the piglets fed this diet. The N retention, however, was not as low, as could be expected from the decrease in N digestibility since the utilization of absorbed N, tended to be increased in the group fed the high-tannin diet. A similar observation was made by Cousins et al. (1981) after feeding high-tannin sorghum to pigs. This suggest that condensed tannins from faba beans do not disturb the metabolism of absorbed amino acids.

In our experiment, condensed tannins in faba bean hulls did not cause severe systemic effects in pigs. This was concluded from the unchanged relative weights of the pancreas, liver, spleen and kidney in pigs fed the high-tannin diet. Systemic effects could be the result of absorption of intact tannins or their degradation products. Karim et al. (1978) found metabolic effects on the liver and kidneys of chicks after feeding hydrolysable tannic acid at a dietary level of 1-3%. Kubena et al. (1983) found a reduced body weight gain and a lower relative weight of the liver and the spleen after feeding chicks 1.5% commercial tannic acid.

The relatively low empty weight of the stomach of the group fed on the pea hull diet, compared to those fed with faba bean hulls, is in agreement with results of Stanogias & Pearce (1985b). They found a significant effect of both type and level of dietary fibre on the wet empty weight of the stomach, small intestine, caecum and proximal colon of pigs. They stated that the empty weight of various segments of the

gastro-intestinal tract is related to the labour performed by the digestive tract. The lower wet empty weight of the caecum of piglets fed the high-tannin faba bean hulls compared to those receiving low-tannin hulls may be related to the extent of fermentative processes in this part of the intestinal tract. Tannins are known to inhibit the activity of enzymes synthesized by the ruminal microflora in ruminants or to decrease the availability of substrates for rumen fermentation (Makkar et al., 1988). Similar effects may occur in the hind gut of pigs where fermentative digestion is predominant. This would agree with the reduced apparent faecal digestibility of crude fibre in the high-tannin diet.

In conclusion, it can be stated that condensed tannins in faba beans are, at least partly, responsible for the reduced nutritive value of some varieties of faba beans in pigs as observed by van der Poel et al. (1991) and by Jansman et al. (1993). The effects of condensed tannins on the apparent digestibility of nutrients, amino acids in particular, are most evident. Severe systemic effects of faba bean tannins at dietary levels below 0.6% catechin equivalents were not found. More research is needed to determine whether the effects on apparent digestibility of nutrients are due to a reduced availability of nutritional substrates, inhibition of the digestive capacity of pigs, or an increased secretion of endogenous compounds into the digestive tract.

Acknowledgements

The authors wish to thank J. Bos, D.J. van Kleef, P. van Leeuwen and T. Zandstra for their technical assistance during the experiment and Dr R.R. Marquardt (University of Manitoba, Winnipeg, Canada) for the analysis of amino acids and hexosamines.

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Chapter 7

EFFECT OF CONDENSED TANNINS IN HULLS OF FABA BEANS (VICIA FABA L.) ON THE ACTIVITY OF TRYPSIN AND CHYMOTRYPSIN IN DIGESTA COLLECTED FROM THE SMALL INTESTINE OF PIGS

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Submitted for publication

Effect of condensed tannins in hulls of faba beans (Vicia faba L.) on the activity of trypsin and chymotrypsin in digesta collected from the small intestine of pigs

Summary

The effects of condensed tannins in hulls of faba beans (Vicia faba L.) on the activity of trypsin and chymotrypsin in digesta obtained from the small intestine of pigs were studied.

In four castrated male pigs (mean body weight 83 kg) fitted with both a simple T-cannula in the duodenum and a post-valvular T-cannula at the terminal ileum, two experimental diets were tested in a Latin square design. The low-tannin diet (LT) contained 200 g/kg of hulls of faba beans cv. Blandine with a low content of condensed tannins (<0.1% catechin equivalents). The high-tannin diet (HT) contained 200 g/kg of faba bean hulls cv. Alfred with a content of condensed tannins of 3.5% catechin equivalents. Spot samples of fresh duodenal digesta were taken daily at 15 time points between 08.00 and 20.00 h on four subsequent days. Ileal digesta were collected nearly quantitatively on the same days between 08.00 and 20.00 h over periods of 2 h.

Trypsin and chymotrypsin activity in duodenal digesta did not differ between treatments at any time point ($P>0.05$). In ileal digesta of pigs fed diet HT the mean activity of trypsin was reduced ($P<0.05$). The activity of chymotrypsin in ileal digesta did not differ between treatments. The ratio between trypsin and chymotrypsin activity was somewhat lower in ileal digesta of pigs receiving the HT diet ($P<0.10$). The apparent ileal digestibility of crude protein (N x 6.25) was lower for the HT than for the LT diet (0.614 vs. 0.728) ($P<0.05$).

Condensed tannins are probably responsible for the lower activity of trypsin in ileal digesta of pigs fed high-tannin faba bean hulls. Various explanations for the absence of effects of condensed tannins on enzyme activity in duodenal digesta are discussed.

Introduction

Faba beans (Vicia faba L.) are of interest as a protein supplement for pig diets (Thacker & Bowland, 1985). However, the nutritive value of faba beans is lower than predicted on the basis of their chemical composition (Fowler, 1980). This is thought to be due to the presence of several antinutritional factors. Protease inhibitors (Abbey et al., 1979; Griffiths, 1981; 1984), haemagglutinins (lectins) (Marquardt et al., 1974), vicine, convicine (Marquardt, 1989) and condensed tannins (Marquardt et al., 1977) have been found in faba beans. Among these, condensed tannins appear to be particularly important in relation to the nutritive value of faba beans (Griffiths, 1981; Marquardt & Bell, 1988). In faba beans, these polyphenolic compounds are found in the hulls of coloured-flowering varieties (Griffiths & Jones, 1977; Ward et al., 1977;

Newton & Hill, 1983).

Dietary inclusion of significant levels of high-tannin faba beans or their hulls reduced body weight gain, impaired feed conversion efficiency and reduced the apparent digestibility of crude protein in rats (Moseley & Griffiths, 1979; Griffiths & Moseley, 1980), chickens (Marquardt et al., 1977; Longstaff & Nab, 1991) and pigs (Jansman et al., 1993). The negative effects of condensed tannins are thought to arise from their interactions with dietary and endogenous proteins such as digestive enzymes (Leinmüller & Menke, 1990; Salunkhe et al., 1990). Condensed tannins can inhibit the activity of digestive enzymes both *in vitro* (Griffiths, 1981; Oh & Hoff, 1986; Horigome et al., 1988) and *in vivo* (Griffiths & Moseley, 1980; Horigome et al., 1988; Longstaff & McNab, 1991). However, Mole & Waterman (1987), Blytt et al. (1988) and Butler (1989) indicated that antinutritional effects of dietary condensed tannins are not due to binding and inhibiting digestive enzymes.

In most studies dealing with the antinutritional effects of tannins, rats and chickens are used. In the present experiment the effect of condensed tannins in faba beans on the activity of trypsin and chymotrypsin was studied in digesta of pigs obtained from two sites in the small intestine.

Materials and methods

Animals and housing

Four castrated male pigs (Dutch Landrace x Dutch Yorkshire) with a mean body weight of 83.0 kg were housed individually in metabolism cages at an ambient temperature of 24°C and a relative humidity of 60%. At a body weight of about 40 kg, the pigs had been surgically fitted with a post-valvular T-caecum cannula (van Leeuwen et al., 1991). In addition, a simple T-cannula was positioned approximately 10 cm caudal of the stomach pylorus, opposite the bile and pancreatic ducts.

Experimental diets

Two diets with hulls of two different varieties of faba beans were formulated. The basis of the diets consisted of barley, maize and dried skim milk. The LT diet contained 200 g/kg of hulls of faba beans of the white-flowering cv. Blandine with a low content of condensed tannins. The HT diet contained the same level of hulls of cv. Alfred with a high tannin content. The hulls were prepared as described by Jansman et al. (1993).

The composition and some analytical data of the diets are given in Table 1. The diets were balanced with regard to net energy, total content of lysine, methionine & cystine, threonine, tryptophan, isoleucine, vitamins and minerals. Crude protein content was slightly higher in the HT diet (166 g/kg) than in the LT diet (158 g/kg). The level of crude fibre was lower in the HT diet than in the LT diet (104 vs. 126 g/kg). The content of condensed tannins, analysed according to the method of Kuhla & Ebmeier (1981), was below the lowest detection limit (<0.10%) for the LT diet and was 0.68% catechin equivalents for the HT diet. The activity of trypsin inhibitors in

Table 1. Composition (g/kg) of the experimental diets.

	LT	HT
Barley	300.0	300.0
Maize	206.8	206.8
Maize gluten meal (590 g CP/kg)	38.0	38.0
Dried skim milk	100.0	100.0
Meat meal	40.0	40.0
Vicia faba hulls (cv. Blandine)	200.0	
Vicia faba hulls (cv. Alfred)		200.0
Soya oil	52.0	52.0
Vitamin/mineral mix[1]	10.0	10.0
Cane molasses	20.0	20.0
Limestone	12.0	12.0
$CaHPO_4.2H_20$	5.5	5.5
NaCl	3.0	3.0
$KHCO_3$	3.0	3.0
$NaHCO_3$	4.0	4.0
DL-methionine	0.7	0.7
L-lysine HCl	3.5	3.5
L-threonine	0.8	0.8
L-tryptophan	0.5	0.5
L-isoleucine	0.2	0.2
Calculated and analysed contents		
Net energy[2] (kJ/kg)	9012	9012
Dry matter[3]	947.8	938.0
Crude protein[3]	158.2	165.9
Ether extract[2]	74.5	74.5
Crude fibre[3]	125.8	104.3
Ash[2]	60.4	60.4
Ca[2]	9.9	9.9
P[2]	6.2	6.2
Condensed tannins[4]	<0.10	0.68
Trypsin inhibitor activity[5]	0.32	0.27

[1]The vitamin/mineral mix supplied per kg of diet: 9000 IU vitamin A; 1800 IU cholecalciferol; 40 mg vitamin E; 5 mg riboflavin; 30 mg niacinamide; 12 mg d-pantothenic acid; 150 mg choline chloride; 40 μg vitamin B-12; 3 mg menadione; 50 mg ascorbic acid; 0.3 mg folic acid; 100 mg $CuSO_4.5H_2O$; 200 mg $ZnSO_4.H_2O$; 70 mg MnO_2; 400 mg $FeSO_4.7H_2O$; 2.5 mg $CoSO_4.7H_2O$; 0.2 mg $Na_2SeO_3.5H_2O$; 0.5 mg KI; 20 mg tylosin.
[2]Calculated content
[3]Analysed content
[4]% catechin equivalents (analysed)
[5]mg trypsin inhibited per g of diet (analysed)

the diets, as determined by the method of van Oort et al. (1989), appeared to be similarly low for both diets. Cr_2O_3 (2.5 g/kg) was added to the diets as a digestibility marker.

The pigs were daily fed two equal portions, 1050 g per feeding time, at 08.00 and 20.00 h. The unpelleted feed was mixed with water (1:2 w/v) just before feeding.

Digesta collection procedures

The pigs were adapted to the experimental diets during a period of ten days. Two pigs were randomly assigned to the LT diet, whereas the other two pigs received the HT diet. In the first collection period of four days (P1), about 50 g of fresh duodenal digesta was obtained at each time (08.00, 08.30, 09.00, 10.00 and subsequently every hour up to 20.00 h) by collecting outflow of the duodenal cannula. Each time the cannula was opened for a maximum of 5 min. Fresh digesta samples were weighed and their pH was measured. They were pooled per time point per animal over four days and stored at -20°C.

Ileal digesta were collected on the same days between 08.00 and 20.00 h over periods of 2 h. A PVC tube connected the cannula with a container in which digesta were collected. The container was cooled with ice. Ileal digesta were pooled immediately per animal per 2 h period over four days. Samples were then stored at -20°C. pH of the pooled samples was measured afterwards.

After the first collection period, animals received the other diet (change-over). During the next period of ten days the pigs were adapted to the diets. Then a second collection period followed. This period (P2) also lasted four days. The procedures for digesta collection and sampling were as in P1.

Afterwards a representative part of the ileal digesta and all duodenal digesta samples were freeze-dried.

For the determination of the apparent ileal digestibility of dry matter and crude protein of the diets, freeze-dried ileal digesta per 2 h collection period were pooled per animal for each of the collection periods.

Samples of feed and freeze-dried digesta were ground in a laboratory mill with a 1 mm screen. Digesta samples were stored under gaseous nitrogen in small air-tight plastic flasks at -20°C until analysis.

Chemical analyses

The diets were analysed on contents of dry matter (ISO 6496, 1983), nitrogen (ISO 5983, 1979) and Cr_2O_3 using atomic absorption spectroscopy. The dry matter content of duodenal digesta was determined from the weight loss during freeze-drying. The nitrogen content of duodenal digesta was analysed in the freeze-dried samples. Fresh ileal digesta were analysed on dry matter and nitrogen content. In the pooled samples of freeze-dried ileal digesta per animal for each of the collection periods, dry matter, N and Cr_2O_3 content were determined. The crude protein (CP) content of samples was calculated as N x 6.25.

Trypsin and chymotrypsin activities in freeze-dried duodenal and ileal digesta were

determined spectrophotometrically according to Bergmeyer (1974). Trypsin activity was determined using α-N-toluene-p-sulphonyl-L-arginine methyl ester (10 mM) as substrate in Tris-HCl buffer (46 mM, pH 8.1, 11.5 mM $CaCl_2$) at 25°C. Chymotrypsin activity was measured using N-benzoyl-L-tyrosine ethyl ester (0.96 mM) as substrate in Tris-HCl buffer (80 mM, pH 7.8, 0.1 M $CaCl_2$), also at 25°C. Activity is defined as μmol of substrate converted by the enzyme per minute. The activity is expressed as units (U) per gram freeze-dried sample.

Statistical analyses

The results with regard to the data on digesta for each individual time point or collection period and the overall mean values per animal over four days were analysed statistically according to the following model (SAS-GLM procedure; SAS, 1990):

$$y_{ijkl} = \mu + period_i + diet_j + animal_k + e_{ijkl}$$

where:

y_{ijkl}	- dependent variable;
μ	- overall mean;
$period_i$	- collection period, i = 1,2;
$diet_j$	- diet, j = 1,2;
$animal_k$	- effect of the k^{th} animal, k = 1-4;
e_{ijkl}	- residual error

The factor "period" had no significant effect on either of the parameters analysed. The results in Tables 2 and 3 are presented as least-square means for diets with the standard errors of models, which did not include "period" as a factor. Results in Figures 1-8 are shown as means with their standard deviations.

Results

General remarks

Statistical analysis of the experimental data (dry matter content and pH of digesta, trypsin and chymotrypsin activity and their activity ratio in freeze-dried digesta and crude protein content of digesta) for each time point of collection of duodenal digesta revealed a significant animal effect in 9 of the 90 cases (6 variables x 15 time points). For data on ileal digesta per 2 h collection period in 5 of the 30 cases (5 variables x 6 collection periods of 2 h) a significant animal effect was found. For the mean values per animal over four days, only a significant animal effect was found for the dry matter content of fresh duodenal and ileal digesta.

Duodenal digesta

Mean size of the samples of duodenal digesta at each time point of sampling was 49.1 and 47.7 g for treatments LT and HT, respectively. Dry matter content of the duodenal digesta did not differ (Table 2). Between 08.00 and 20.00 h, 6.9 and 6.7% of total dry matter of one meal (1050 g) was sampled via the duodenal cannula for the LT and the HT treatment, respectively. Mean values for crude protein content, pH and activity of trypsin and chymotrypsin in duodenal digesta are given in Table 2. Mean crude protein content of freeze-dried duodenal digesta was higher for the HT treatment ($P<0.01$). Mean pH of duodenal digesta was similar for treatments LT and HT. The course of the pH over the 12 h period of sampling was similar for both diets (Figure 1). Values were highest just after and prior to feeding (pH 5.5 - 6.0). Lowest values were measured 2-3 h after feeding (pH 4 - 4.5).

Table 2. Mean content of dry matter and crude protein, pH and enzyme activity of duodenal digesta of pigs fed a diet with 200 g/kg of hulls of faba beans with either a low (LT) or high content of condensed tannins (HT).

	LT	HT	SE	P
Dry matter (g/kg)	93.7	92.4	2.9	
Crude protein (g/kg fdm[1])	167.3	178.1	2.3	**
pH	4.94	5.06	0.06	
Trypsin activity (U/g fdm[1])	156.7	142.6	20.2	
Chymotrypsin activity (U/g fdm[1])	38.3	38.0	6.6	
Trypsin/chymotrypsin ratio	4.20	4.18	0.31	

** $P<0.01$ [1]fdm: freeze-dried matter

For both treatments, trypsin and chymotrypsin activity in freeze-dried duodenal digesta varied considerably over the 12 h of sampling. Neither overall mean values (Table 2) nor individual values per time point (Figures 2 and 3) differed significantly between treatments. Standard deviations for the measurements on enzyme activities were relatively high, particularly for samples collected between 12.00 and 20.00 h.

Mean values for the ratio between activity of trypsin and chymotrypsin in freeze-dried duodenal digesta did not differ significantly between diets. Values tended to increase during the first hours after feeding and to decrease later until a subsequent feeding (Figure 4).

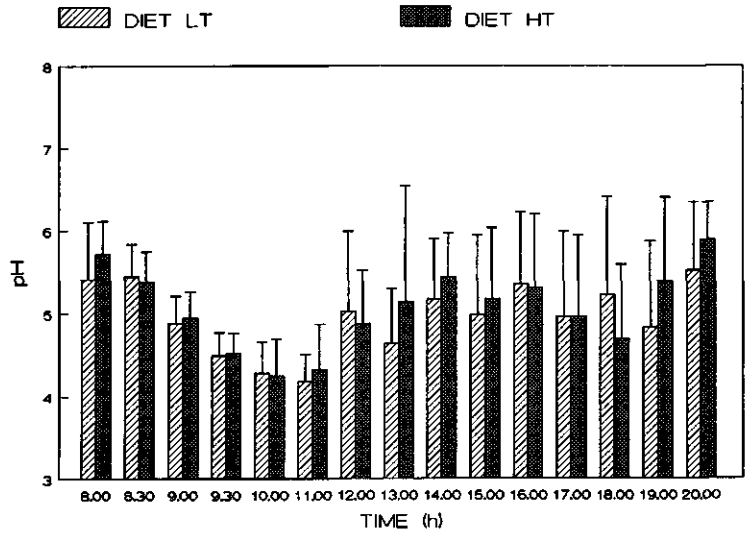

Figure 1. pH of duodenal digesta of pigs fed a diet with 200 g/kg of low-tannin (LT) or high-tannin (HT) faba bean hulls.

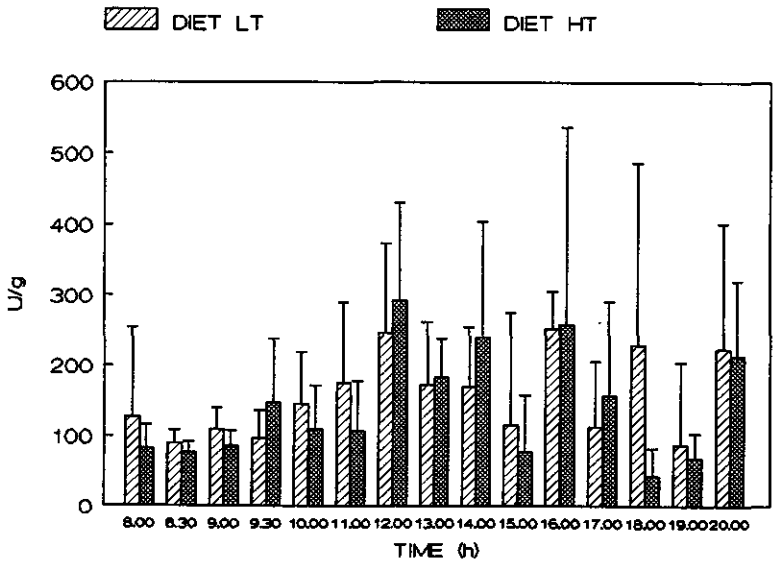

Figure 2. Trypsin activity (U/g) in freeze-dried duodenal digesta of pigs fed a diet with 200 g/kg of low-tannin (LT) or high-tannin (HT) faba bean hulls.

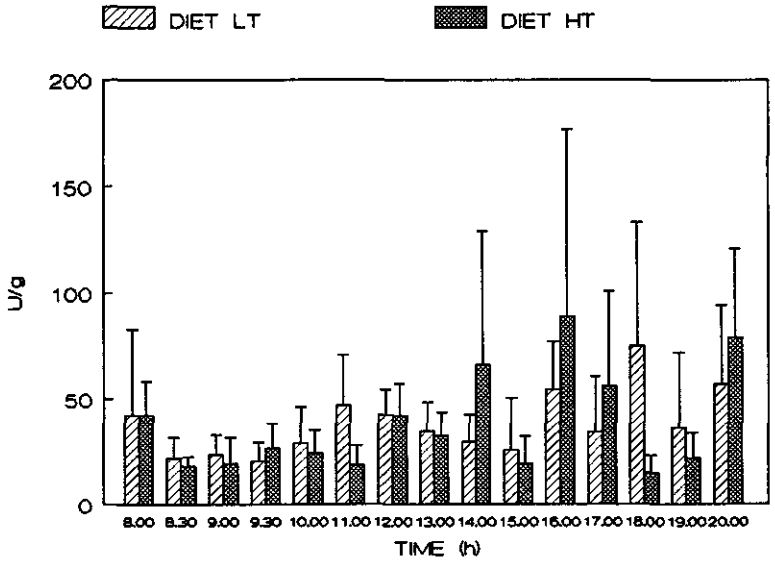

Figure 3. Chymotrypsin activity (U/g) in freeze-dried duodenal digesta of pigs fed a diet with 200 g/kg of low-tannin (LT) or high-tannin (HT) faba bean hulls.

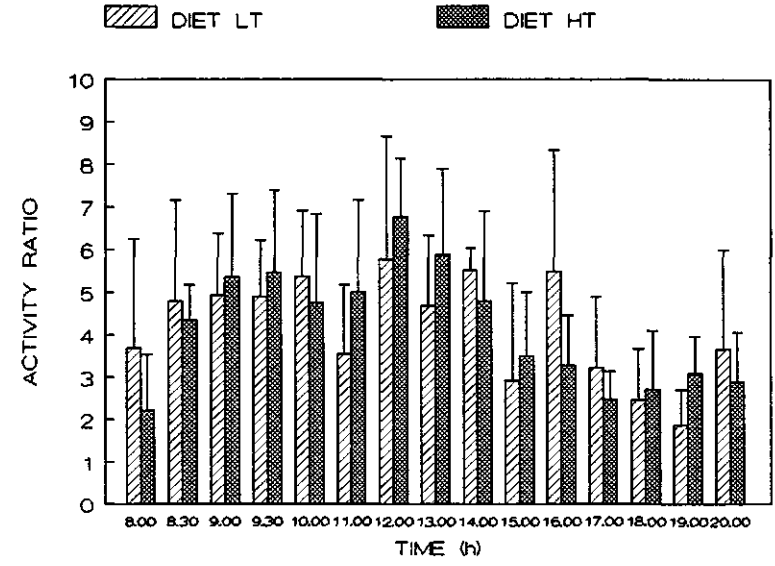

Figure 4. Ratio of the activity between trypsin and chymotrypsin in freeze-dried duodenal digesta of pigs fed a diet with 200 g/kg of low-tannin (LT) or high-tannin (HT) faba bean hulls.

Ileal digesta

The total amount of fresh digesta collected at the terminal ileum per 12 h did not differ between treatments with 2119 and 2037 g for diets LT and HT, respectively. The quantity of fresh digesta collected per interval of 2 h varied between 265 and 432 g for diet LT and between 268 and 391 g for diet HT. Dry matter content of ileal digesta was slightly lower for treatment HT ($P<0.05$) (Table 3). The CP content in ileal digesta (on a DM basis) was rather constant over the day (Figure 5). Overall and during each of the 2 h periods the CP content was higher for pigs receiving the HT diet ($P<0.05$) (Table 3; Figure 5). pH of ileal digesta for both treatments was similar (Table 3). Overall and between 12.00 and 20.00 h, the trypsin activity in ileal digesta was significantly lower for the HT treatment (Table 3; Figure 6). Chymotrypsin activity in freeze-dried ileal digesta did not differ between the diets, except for a lower activity found 6-8 h postprandially for the HT diet ($P<0.05$) (Table 3; Figure 7). Between 16.00 and 18.00 h the trypsin to chymotrypsin activity ratio was significantly lower in ileal digesta for the HT treatment (Figure 8) ($P<0.05$). Overall and in digesta collected over most of the other 2 h periods, the ratio tended to be lower for the HT diet.

The apparent ileal digestibility of dry matter, calculated using the marker (Cr_2O_3) ratio, was similar for both diets. For crude protein, the ileal digestibility value was 11 units lower for the HT treatment ($P<0.05$) (Table 3).

Table 3. Mean content of dry matter, crude protein, pH and enzyme activity in ileal digesta and apparent ileal digestibility values (DC) for dry matter (DM) and crude protein (CP) in pigs fed a diet with 200 g/kg of hulls of faba beans with either a low (LT) or high content of condensed tannins (HT).

	LT	HT	SE	P
Dry matter (g/kg)	122.8	109.9	3.6	*
Crude protein (g/kg dm)	118.3	176.0	3.6	***
pH	7.18	7.15	0.05	
Trypsin activity (U/g fdm[1])	110.2	76.7	5.7	**
Chymotrypsin activity (U/g fdm[1])	27.9	25.8	6.0	
Trypsin/chymotrypsin ratio	4.22	3.11	0.65	
DC_{DM}	0.571	0.570	0.014	
DC_{CP}	0.728	0.614	0.013	*

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

[1] *fdm: freeze-dried matter*

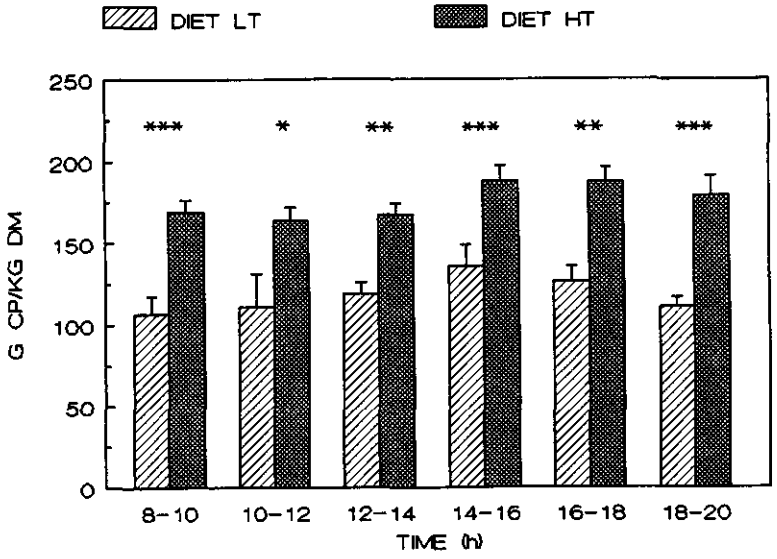

Figure 5. Crude protein content of ileal digesta of pigs fed a diet with 200 g/kg of low-tannin (LT) or high-tannin (HT) faba bean hulls (Differences between treatments * $P<0.05$; ** $P<0.01$; *** $P<0.001$).

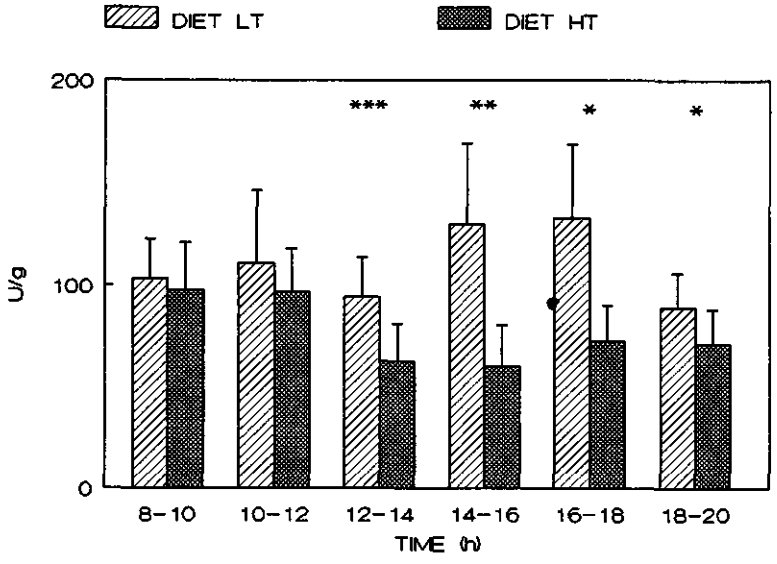

Figure 6. Trypsin activity (U/g) in freeze-dried ileal digesta of pigs fed a diet with 200 g/kg of low-tannin (LT) or high-tannin (HT) faba bean hulls (Differences between treatments * $P<0.05$; ** $P<0.01$; *** $P<0.001$).

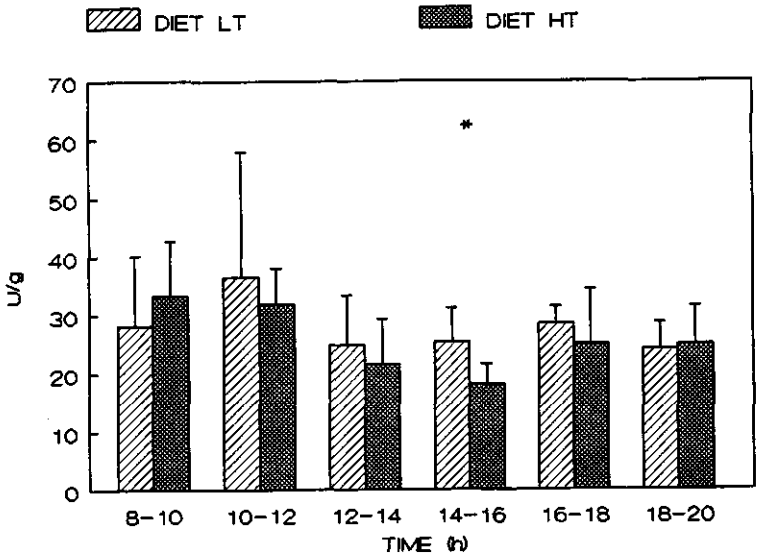

Figure 7. Chymotrypsin activity (U/g) in freeze-dried ileal digesta of pigs fed a diet with 200 g/kg of low-tannin (LT) or high-tannin (HT) faba bean hulls (Difference between treatments * $P<0.05$).

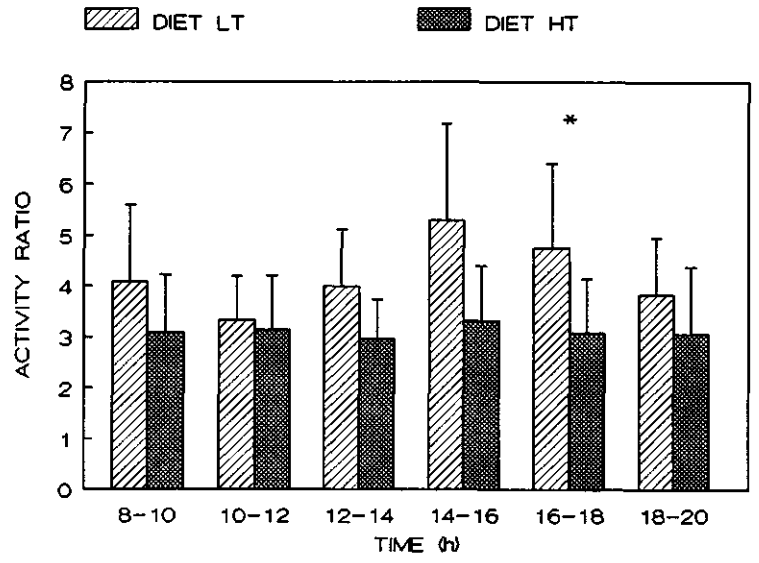

Figure 8. Ratio of the activity between trypsin and chymotrypsin in freeze- dried ileal digesta of pigs fed a diet with 200 g/kg of low-tannin (LT) or high-tannin (HT) faba bean hulls (Difference between treatments * $P<0.05$).

Discussion

General

Composition and enzyme activity of digesta in the small intestine of pigs vary widely over the day (Braude et al., 1976; Low et al., 1978; Low, 1979). Therefore, single-spot sampling of digesta or digesta collection over a short period of time will not provide representative samples. The use of re-entrant cannulae allows of quantitative collection of digesta at various sites of the digestive tract in pigs. However, re-entrant cannulation has some drawbacks in terms of practical application and maintainance of a normal physiological status of the intestines. This was shown by Köhler et al. (1992) in pigs fitted with a re-entrant cannula in the terminal ileum. Therefore, in the present study frequent spot sampling of duodenal digesta via a T-cannula has been used to study composition and enzyme activity of digesta in the proximal part of the duodenum of pigs. Both dry matter content (results not shown) and pH of duodenal digesta in our study followed the same pattern over the day as found by Braude et al. (1976) with quantitative collection. Therefore, we assumed that our samples of duodenal digesta were representative. The PVTC cannula at the terminal ileum of pigs allows almost complete collection of ileal digesta (van Leeuwen et al., 1991). Therefore, digesta collected via this cannula over 12 h per day is assumed to be representative of the digesta reaching the terminal ileum.

To study the effects of condensed tannins in faba beans, hulls of faba beans with a different tannin content were included in the diets. In faba beans, condensed tannins are found in the hulls of the seeds (Bos & Jetten, 1989). Furthermore, hulls of faba beans have a low CP content and consist merely of non-starch polysaccharides, cellulose in particular (Cerning et al., 1975). The slightly higher content of crude fibre and the lower level of CP in the LT diet (Table 1) are associated with small differences in the levels of fibre and protein between both hull fractions. The content of condensed tannins can be considered as the major difference between the diets.

Digesta composition and pancreatic enzyme secretion

The fall in pH of duodenal digesta in the first hours after feeding (Figure 1) is associated with the high rate of gastric emptying shortly after feeding as found by Braude et al. (1976). Mean pH values of duodenal digesta in our study were similar to those found by Braude et al. (1976) for duodenal digesta collected quantitatively over 24 h. The relatively low dry matter content of duodenal digesta for both treatments is related to endogenous secretions in the proximal part of the digestive tract in the form of saliva, gastric juice, bile and pancreatic juice (Braude et al., 1976). In our study the dry matter content of duodenal digesta was not influenced by the type of faba bean hulls included in the diets.

The mean CP content of duodenal digesta (on a DM basis) (Table 2) was almost equal to the level in the diets (Table 1). Some authors have found an increase in protein flow at the duodenal level in pigs compared to protein intake, as a result of endogenous protein secretion in the proximal digestive tract. This phenomenon has been reviewed by Low (1979). Nitrogen absorption prior to the proximal duodenum

was not observed in pigs (Zebrowska et al., 1983).

In our study activities of digestive enzymes in duodenal digesta fluctuate markedly over the day. Similar observations were made by Low (1982). This is probably related to within-day fluctuations in the secretion of pancreatic juice and/or the concentration of digestive enzymes in the juice and the dilution of enzymes by digesta flow. Corring & Saucier (1972) and Partridge et al. (1982) showed that both secretion of pancreatic juice and its composition vary widely over the day and that both are affected by the composition of the diet.

Information on the possible effects of dietary tannins on the secretion activity of the pancreas is scarce. Marquardt et al. (1977), Longstaff & McNab (1991) and Jansman et al. (1993) did not observe a change in pancreas weight in chickens and pigs, respectively, after feeding diets with tannin-rich hulls from faba beans.

We have conducted a small-scale experiment in a change-over design with three pancreas-cannulated pigs (BW ca. 40 kg; cannulation according to Hee et al., 1985) fed diets containing skimmed milk powder (180 g/kg), faba bean cotyledons (300 g/kg), maize starch (327 g/kg) and 100 g/kg of faba bean hulls with either a low or a high content of condensed tannins as the major ingredients (1100 g/day). The pancreatic juice secretion did not differ between treatments (1363 and 1317 g per 12 h, respectively). Mean output of enzymes (U/12 h), measured in only one of the two experimental periods, was 126.925 and 115.265 for trypsin and 60.686 and 53.210 for chymotrypsin in pigs fed the low- and high-tannin faba bean hulls, respectively. We concluded from this experiment that condensed tannins of faba beans do not affect the volume of pancreatic secretion and the pancreatic output of trypsin and chymotrypsin in pigs.

The small difference in crude fibre content between the diets (Table 1) does not likely affect digestive enzyme secretion by the pancreas. Mosenthin & Sauer (1991) found that inclusion of Alphafloc or straw at a level of 100 g/kg did not affect pancreatic enzyme output in pigs.

Enzyme activity in small intestinal digesta

Enzyme activity in digesta is influenced by physical-chemical conditions in digesta, dietary components and denaturation and autodigestion of enzymes (Low, 1982). The latter two, however, do not seem to be very important in the proximal duodenum. Low (1982) reported that total enzyme activity (U) in duodenal digesta of pigs was similar to the total activity found in the jejunum. Among the dietary components, fibre may reduce the activity of digestive enzymes due to adsorption of enzymes to the fibre matrix, as shown *in vitro* by Schneeman (1978), and to the effect of fibre-associated components (pectins, gums) on viscosity and pH (Isaksson et al., 1982). The pH of duodenal digesta in our study differed only slightly between treatments.

Corring et al. (1972), who measured total pancreatic output, and Low (1982), who measured enzyme activity in total duodenal digesta (U), found an increased activity of both trypsin and chymotrypsin in the first hours after feeding. The low activity of both enzymes, expressed as U/g freeze-dried duodenal digesta (U/g fdm), in the first hours after feeding in our study, may be the result of a dilution of pancreatic enzymes due to an increased flow of digesta during this period. However, duodenal digesta flow

itself could not be measured in our study. At the ileal level, the enzyme activities (U/g fdm) (Figures 6 and 7) were more steady over the day. The same was observed by Low (1982).

In several studies condensed tannins in diets reduced the activity of digestive enzymes in digesta obtained from various sites of the intestinal tract of rats and chickens. Horigome et al. (1988) found reduced activities of trypsin and α-amylase in the upper, middle and lower small intestine of rats after feeding tannin-rich extracts from various fodder plants. Similar results were obtained by Griffiths & Moseley (1980) after feeding rats tannin-rich faba bean hulls. Longstaff & McNab (1991) found a reduced activity of trypsin and lipase in small intestinal digesta of chickens fed high-tannin faba bean hulls compared to low-tannin faba bean hulls. Yuste et al. (1992) found a reduced activity of trypsin, α-amylase and lipase in jejunal digesta of young chickens after feeding tannin-containing faba bean hulls or tannin-rich extracts from the same hulls. Formation of tannin-enzyme complexes may explain the effects of dietary tannins on enzyme activity. Addition of polyvinylpyrrolidone (PVP), a potent tannin binder, to digesta extracts of tannin-fed rats restored trypsin activity to values of the control group. This indicates that the enzyme-tannin complex formation is reversible (Griffiths, 1980). In our study no differences between treatments were found with regard to the activity of trypsin and chymotrypsin in duodenal digesta.

Trypsin activity in ileal digesta (U/g fdm) was 70 and 54% of the level measured in duodenal digesta for the LT and HT diet, respectively. For chymotrypsin activity, the corresponding values were 73 and 68%. Quantitatively (U/24 h), using apparent ileal dry matter digestibility values from Table 3 for estimating ileal dry matter flow, the activity of trypsin was 30 and 23% of the estimated activity in the duodenum for the LT and HT treatment, respectively. Corresponding values for chymotrypsin activity were 31 and 29%, respectively. These values are in agreement with estimates made by Low (1982) using the re-entrant cannulation technique for quantitative digesta collection at different sites of the small intestine.

Trypsin activity was significantly reduced in ileal digesta of pigs fed the high-tannin hulls. There may thus be a difference in response to trypsin activity with regard to tannins at different sites of the digestive tract in pigs. These can be related to several factors, such as differences in solubility of tannins at various sites, the nature and quantity of dietary or other endogenous components in digesta which may bind to tannins, the length of the interval between enzyme activation and encounter of tannins available for interaction, and differences in physical-chemical circumstances. With respect to the latter, Hagerman & Butler (1978), found in *in vitro* studies that maximum precipitation of tannin-protein complexes occurred at a pH close to the isoelectric point (pI) of the proteins involved. Jones & Mangan (1977) observed that the major part of the Fraction 1 leaf protein of lucerne (Medicago sativa L.) that was complexed with condensed tannins from sainfoin (Onobrychis viciifolia Scop.) in the rumen of sheep was released in the proximal duodenum at a pH of 2.5. Therefore condensed tannins may be available for enzyme binding in the proximal duodenum. However, the pH as measured in duodenal digesta of pigs could still be relatively low for maximum protein binding since pI values for the porcine zymogens, trypsinogen

and chymotrypsinogen A, are 7.5 and 7.2, respectively (Walsh & Wilcox, 1970). The pH of ileal digesta in pigs was shown to be slightly higher than 7. This may be favourable for tannin-enzyme interactions.

Tannins have a different binding affinity for various proteins (Asquith & Butler, 1986). Thus they may affect the activity of various enzymes to a different extent. From our study it may be concluded that condensed tannins in faba bean hulls have a higher affinity for trypsin than for chymotrypsin in ileal digesta of pigs. This can be derived from the tendency of a reduction in trypsin/chymotrypsin activity ratio in ileal digesta of pigs fed the HT diet. A low affinity of tannins for lipase relative to trypsin and α-amylase was suggested by Horigome et al. (1988) to explain the difference in effects of condensed tannins in digesta of rats on these enzymes.

The mean digestibility of DM and CP of the diets was low. This may be due to the indicator method used. The relatively low recovery of the marker as found in this study may be related to the double cannulation of the pigs and the way of sample collection and sample treatment. The apparent ileal digestibility of CP was significantly reduced in the pigs receiving the high-tannin diet. A decrease in digestibility of CP was also found in other studies with chickens and pigs fed diets containing faba bean tannins (Martin-Tanguy et al., 1977; Lacassagne et al., 1988; Longstaff & McNab, 1991; Jansman et al., 1993).

Both trypsin and chymotrypsin are important for the breakdown of both dietary and endogenous proteins into peptides, and also for the activation of various digestive enzymes (Rinderknecht, 1986). Therefore the reduced apparent ileal digestibility of protein in high-tannin diets in non-ruminant animals may be related to a reduced activity of important digestive enzymes. However, various authors (Low, 1982; Zebrowska et al., 1983) suggested that the total amount of proteases secreted by the pancreas of pigs exceeds by far the amount required for complete hydrolysis of dietary proteins. This indicates that a reduction in activity of proteolytic enzymes in digesta obtained from a particular site of the digestive tract may have limited consequences for the animal's capacity to degrade dietary proteins through the whole digestive tract. Binding of tannins to dietary proteins or an increased excretion of endogenous proteins therefore seems a more logical explanation for the reduced apparent digestibility of protein in high-tannin diets. Moreover, Partridge et al. (1982) determined that the amount of N secreted by the pancreas of pigs (BW 50 kg) represents only 3-6% of total daily N troughput in the duodenum. This would suggest that dietary tannins have a large number of alternative binding sites from dietary proteins. The low ratio between enzyme protein and total protein in digesta *in vivo* led Blytt et al. (1988) to suggest that the main antinutritional effects of dietary tannins are not due to inhibition of digestive enzymes.

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Chapter 8

EFFECTS OF HULLS OF FABA BEANS (VICIA FABA L.) WITH A LOW OR HIGH CONTENT OF CONDENSED TANNINS ON THE APPARENT ILEAL AND FAECAL DIGESTIBILITY OF NUTRIENTS AND THE EXCRETION OF ENDOGENOUS PROTEIN IN ILEAL DIGESTA AND FAECES OF PIGS

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Effects of hulls of faba beans (Vicia faba L.) with a low or high content of condensed tannins on the apparent ileal and faecal digestibility of nutrients and the excretion of endogenous protein in ileal digesta and faeces of pigs

Abstract

In three experiments (*Exps. 1-3*) with young piglets (10-26 kg) the effects of inclusion of hulls of faba beans (Vicia faba L.) (200 g/kg) with a low (<0.1% catechin equivalents; LT) or high tannin content (3.3% catechin equivalents; HT) on the apparent ileal (*Exps. 1-2*) and faecal (*Exp. 3*) digestibility of nutrients were determined. In addition, the true digestibility of protein of the diets and the endogenous excretion of protein (Nx6.25) in ileal digesta and faeces of pigs were determined, using the ^{15}N isotope dilution technique (*Exp. 3*). Diets contained either casein or faba bean cotyledons as highly soluble (HS) protein sources (*Exps. 1-3*) or potato protein, soya concentrate, sunflower meal, meat meal or fish meal as protein sources with a low solubility (LS) (*Exp. 1*). Control diets contained cellulose as fibre source (64-73 g/kg). Inclusion of either type of hulls decreased the apparent ileal digestibility for dry matter (DM), organic matter (OM) and non-protein organic matter (NPOM) ($P<0.05$). Inclusion of LT hulls instead of cellulose only reduced the apparent ileal digestibility of crude protein (Nx6.25; CP) in *Exp. 3* ($P<0.05$). Inclusion of HT instead of LT hulls reduced the apparent ileal digestibility for CP (by 7-10 percentage units) and amino acids (by 4-29 units) ($P<0.05$). LT hulls (200 g/kg) decreased apparent and true ileal digestibility of CP from 88 to 83 and from 97 to 94, respectively ($P<0.05$). Inclusion of HT instead of LT hulls decreased apparent and true ileal CP digestibility from 83 to 74 and 94 to 91 ($P<0.05$). Endogenous CP excretion in pigs fed the diet with HT hulls increased from 22 to 32 and from 13 to 23 g/kg DM intake at the ileal and faecal level, respectively ($P<0.05$).

It is concluded that condensed tannins in faba beans interact with both dietary and endogenous proteins in the digestive tract of pigs. This reduces true digestibility of dietary protein and increases the excretion of endogenously secreted proteins. Tannins from faba beans show some preference to interact with proteins with a high content of proline and histidine.

Introduction

The use of faba beans (Vicia faba L.) in feeds for non-ruminant species has emphasized the need to evaluate antinutritional factors (ANFs) in these seeds. ANFs include condensed tannins that are found in particular in the hulls of coloured-flowering varieties (Bos & Jetten, 1989). Tannins are water-soluble polyphenolic compounds, which are able to precipitate proteins from aqueous solutions (Butler, 1989). *In vivo* tannins may cause a range of different antinutritional effects. Tannins decrease in particular the apparent digestibility of protein in non-ruminant animal species

(Jansman, 1993). In the gastrointestinal tract they can interact with both dietary and endogenous proteins. In rats, consumption of high-tannin sorghum or high-tannin faba bean hulls increased the relative weight of the parotid glands. These glands increased the synthesis and secretion of proline-rich proteins with a high affinity for tannins (Mehansho et al., 1983; Jansman et al., 1993a). As a result, a higher amount of endogenous nitrogen is found in the faeces. The existence of a similar mechanism in pigs after feeding tannin-rich faba bean hulls has not been demonstrated so far (Jansman et al., 1993b). Condensed tannins from faba beans reduced the activity of trypsin in digesta obtained from the small intestine of pigs (Jansman et al., 1993c). Similar effects of faba bean tannins on the activity of digestive enzymes were found in chickens (Longstaff & McNab, 1991). This suggests that tannins interact with digestive enzymes.

Information on the effects of condensed tannins from faba beans on the apparent ileal digestibility of protein and amino acids in pigs is scarce. The origin of the extra protein excreted in digesta and faeces is not known.

In the present studies the effects of hulls of faba beans containing a low or high level of condensed tannins on the apparent ileal digestibility of nutrients in piglets were evaluated. In addition, the effects of low- and high-tannin faba bean hulls on the endogenous excretion of crude protein (Nx6.25) in ileal digesta and faeces was investigated using the ^{15}N isotope dilution technique.

Materials and methods

Three digestibility experiments were carried out. The composition of the experimental diets is given in Table 1. *Exp. 2* is in part a replicate of *Exp. 1*. In *Exps. 1 & 2* the apparent ileal digestibility was measured of diets that contained either a low level of cellulose (64-69 g/kg) or hulls of faba beans with a low (cv. CEB 90904) or high content of condensed tannins (cv. Alfred) (200 g/kg) as the main fibre source. Cellulose was chosen as a fibre source in the control diets since cellulose, up to a level of 100 g/kg, does not affect the apparent ileal digestibility of protein (Sauer et al. 1991) or the ileal excretion of endogenous protein in pigs (Furuya & Kaji, 1991). Diets contained either protein sources with an assumed high solubility (casein and faba bean cotyledons) or with a low solubility (fish meal, soya concentrate, meat meal, potato protein and sunflower meal) *in vivo*. Diets were formulated at similar contents of net energy (NE), crude fibre, Ca and P. Vitamins, minerals and indispensable amino acids were included up to the animal's requirements (NRC, 1988). Chromic oxide (Cr_2O_3) was added to the diets as a digestibility marker at a level of 2.5 g/kg.

Hulls of faba beans were obtained and included in the diets as described by Jansman et al. (1993b). The composition of the faba bean hulls is given in Table 2. In *Exps. 1 & 2*, the apparent ileal digestibility of dry matter, ash, organic matter, crude protein, non-protein organic matter, amino acids (*Exp. 1*) and crude fibre (*Exp. 2*) were measured.

Schematically, diets in *Exps. 1* and *2* are described below.

Experiment 1

Diet HSC_1 : Control diet containing protein sources with a high solubility (HS; casein and faba bean cotyledons) and 69 g/kg of cellulose;

Diet LSC_1 : Control diet containing protein sources with a low solubility (LS) (fish meal, soya concentrate, meat meal, potato protein and sunflower meal) and 64 g/kg of cellulose;

Diet $HSHT_1$: Protein sources as in diet HSC_1 + 200 g/kg of high-tannin (HT) faba bean hulls, cv. Alfred.

Diet $LSHT_1$: Protein sources as in diet LSC_1 + 200 g/kg of high-tannin (HT) faba bean hulls, cv. Alfred

Diet $LSLT_1$: Protein sources as in diet LSC_1 + 200 g/kg of low-tannin (LT) faba bean hulls, cv. CEB 90904.

Experiment 2

Diet HSC_2 : Control diet containing protein sources with a high solubility (casein and faba bean cotyledons) and 69 g/kg of cellulose (= diet HSC_1);

Diet $HSLT_2$: Protein sources as in diet HSC_2 + 200 g/kg of low-tannin (LT) faba bean hulls, cv. CEB 90904;

Diet $HSHT_2$: Protein sources as in diet HSC_2 + 200 g/kg of high-tannin (HT) faba bean hulls, cv. Alfred (= diet $HSHT_1$).

Diets in *Exp. 3* contained either 73 g/kg of cellulose or 200 g/kg of low-tannin (cv. Toret) or high-tannin faba bean hulls (cv. Alfred) as the source of fibre. Protein originated from casein and faba bean cotyledons. Levels of indispensable amino acids were about 90% of the animal's requirements (NRC, 1988), in order to measure the effects on the N balance of the animals (results not shown). Diets differed slightly in composition from those in *Exp. 2*.

Experiment 3

Diet HSC_3 : Control diet containing protein sources with a high solubility (casein, faba bean cotyledons) and 73 g/kg of cellulose;

Diet $HSLT_3$: Protein sources as in diet HSC_3 + 200 g/kg of low-tannin (LT) faba bean hulls, cv. Toret;

Diet $HSHT_3$: Protein sources as in diet HSC_3 + 200 g/kg of high-tannin (HT) faba bean hulls, cv. Alfred.

The apparent ileal and faecal digestibilities of nutrients were measured. The true digestibility of dietary crude protein in diets and the endogenous excretion of protein by pigs were determined using the ^{15}N isotope dilution technique.

Animals and experimental procedures

In each of the three experiments castrated male piglets of the crossbred Dutch Landrace x Dutch Yorkshire were used.

Table 1. Composition of the experimental diets (g/kg).

	Experiment 1					Experiment 2			Experiment 3		
	HSC_1	LSC_1	$HSHT_1$	$LSHT_1$	$LSLT_1$	HSC_2	$HSLT_2$	$HSHT_2$	HSC_3	$HSLT_3$	$HSHT_3$
Maize starch	312.0	448.8	143.3	279.1	279.1	312.0	143.3	143.3	338.1	184.6	184.6
Dextrose	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Potato protein		60.0		60.0	60.0						
Soya concentrate		80.0		80.0	80.0						
Sunflower meal		35.0		35.0	35.0						
Meat meal		30.0		30.0	30.0						
Fish meal		50.0		50.0	50.0						
Casein	110.0		110.0			110.0	110.0	110.0	90.0	90.0	90.0
Dehulled faba beans, cv. Alfred	250.0		250.0			250.0	250.0	250.0	250.0	250.0	250.0
Hulls, faba beans, cv. Alfred			200.0	200.0				200.0			200.0
Hulls, faba beans, cv. CEB 90904					200.0		200.0				
Hulls, faba beans, cv. Toret										200.0	
Sunflower oil	17.0	6.5	56.0	46.5	46.5	17.0	56.0	56.0	16.0	50.5	50.5
Cane molasses	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Cellulose	69.0	64.0	5.5			69.0	5.5	5.5	73.0		
$CaCO_3$	16.8	10.0	17.0	10.2	10.2	16.8	17.0	17.0	11.0	12.0	12.0
$CaHPO_4$	20.5	14.0	19.0	12.8	12.8	20.5	19.0	19.0	19.0	15.0	15.0
NaCl	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
$KHCO_3$	9.0	10.0	3.5	5.0	5.0	9.0	3.5	3.5	9.0	4.0	4.0
$NaHCO_3$	5.0	2.5	5.0	2.5	2.5	5.0	5.0	5.0	5.0	5.0	5.0
DL-methionine	2.2	1.6	2.2	1.3	1.3	2.2	2.2	2.2	1.3	1.3	1.3

L-threonine	1.0		1.0			1.0	1.0	1.0	0.1	0.1	0.1
L-tryptophan		0.1		0.1	0.1						
Vitamin/mineral mix[1]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Cr_2O_3	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

Analysed[2]/calculated[3] composition

	Experiment 1					Experiment 2			Experiment 3		
	HSC_1	LSC_1	$HSHT_1$	$LSHT_1$	$LSLT_1$	HSC_2	$HSLT_2$	$HSHT_2$	HSC_3	$HSLT_3$	$HSHT_3$
Dry matter[2]	866.8	882.9	872.2	879.7	882.4	895.2	895.2	892.6	893.8	881.5	886.1
Crude protein[2]	180.3	165.0	196.3	185.1	174.1	181.3	184.4	182.5	163.1	168.8	171.9
Net energy[3] (kcal/kg)	2219	2218	2217	2220	2220	2219	2217	2217	2228	2227	2227
Crude fibre[2]	63	64	74	81	104	54	114	106	52	118	99
Calcium[3]	10.1	10.1	10.1	10.2	10.2	10.1	10.1	10.1	7.6	7.6	7.6
Available phosphorus[3]	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.4	4.8	4.8
Lysine[3]	11.4	10.9	12.2	11.7	11.7	11.4	12.2	12.2	11.3	11.3	11.3
Methionine & Cystine[3]	7.1	7.1	7.4	7.1	7.1	7.1	7.4	7.4	5.9	5.9	5.9
Threonine[3]	7.5	7.4	7.9	7.8	7.8	7.5	7.9	7.9	6.6	6.6	6.6
Tryptophan[3]	2.0	2.2	2.1	2.2	2.2	2.0	2.1	2.1	2.1	2.1	2.1

[1]The vitamin/mineral mix in experiments 1 & 2 delivered per kg feed: 9000 IU vitamin A; 1800 IU vitamin D-3; 40 mg vitamin E; 5 mg riboflavin; 30 mg niacinamide; 12 mg d-pantothenic acid; 150 mg choline chloride; 40μg vitamin B-12; 3 mg menadione; 50 mg ascorbic acid; 0.3 mg folic acid; 100 mg $CuSO_4.5H_2O$; 200 mg $ZnSO_4.H_2O$; 70 mg MnO_2; 400 mg $FeSO_4.7H_2O$; 2.5 mg $CoSO_4.7H_2O$; 0.2 mg $Na_2SeO_3.5H_2O$; 0.5 mg KI; 40 mg tylosin.
The vitamin/mineral in experiment 3 delivered per kg feed: 9000 IU vitamin A; 1800 IU vitamin D-3; 40 mg vitamin E; 5 mg riboflavin; 30 mg niacinamide; 12 mg d-pantothenic acid; 150 mg choline chloride; 40μg vitamin B-12; 3 mg menadione; 50 mg ascorbic acid; 0.3 mg folic acid; 40 mg $CuSO_4.5H_2O$; 200 mg $ZnSO_4.H_2O$; 15 mg MnO_2; 400 mg $FeSO_4.7H_2O$; 2.5 mg $CoSO_4.7H_2O$; 0.2 mg $Na_2SeO_3.5H_2O$; 0.5 mg KI; 40 mg tylosin.
[2]analysed content
[3]calculated content

Experiments 1 & 2

In *Exp. 1* piglets arrived at the laboratory at an age of about 6 weeks and a body weight (BW) of 10.5 ± 0.7 kg. They were housed individually in metabolism cages and were fed a commercial weaning diet. The piglets were fitted with a post-valvular T-caecum cannula at the terminal ileum 6-10 days after arrival (van Leeuwen et al., 1991). After a recovery period of 6-9 days, animals were assigned randomly to one of the five experimental groups, each comprising five piglets. Mean BW of animals by then was 13.9 ± 0.7 kg. In four days the animals were then gradually adapted to the experimental diets. Twelve days later ileal digesta were collected on four consecutive days between 08.00 and 20.00 h. The digesta were collected in plastic bags fixed to the cannula. Bags were changed at least every hour and digesta were frozen at -20°C. The estimated BW of the animals during the collection period was 18 kg. Diets were

Table 2. Composition of faba bean hulls cvs. CEB 90904 (*Exps. 1,2*), Toret (*Exp. 3*) and Alfred (*Exps. 1-3*) (g/kg).

	CEB 90904	Toret	Alfred
Type[1]	LT	LT	HT
Dry matter	890	895	887
Crude protein (Nx6.25)	51	41	46
NDF	647	702	591
ADF	623	674	571
Lignin	8	5	15
Condensed tannins[2]	<0.1	<0.1	3.5

[1]LT: low-tannin variety, HT: high-tannin variety
[2]% catechin equivalents

fed at a level of 2.7 times the animal's maintenance requirement for energy (ARC, 1981). They were fed at 08.00 and 20.00 h. Room temperature and relative humidity were 25-28°C and 35-50%, respectively.

Eighteen piglets were used in *Exp. 2*. They had been surgically fitted with a PVTC cannula at an age of about 6 weeks and a mean BW of 10 kg. Mean BW of the animals at the start of the experiment was 18.8 ± 0.9 kg. They were assigned to three experimental groups, each consisting of 6 piglets. Nine days after the introduction of the experimental diets, digesta were collected via the PVTC cannula over two 2-day periods (12 h per day) one day apart. Collection procedures, feeding level and feeding times were as for *Exp. 1*. Mean BW of the piglets during the period of digesta collection was 26 kg.

Diets for both experiments were manufactured in a cold pelleting process (T<60°C). Feed was offered to the piglets in a dry form. Water was freely available from drinking nipples.

One of the piglets in *Exp. 2* (group II) was withdrawn from the experiment due to health problems.

Experiment 3

Piglets were purchased at an age of about 5 weeks and a BW of 8.5 ± 0.6 kg (day 0). The animals were housed individually in metabolism cages and fed a commercial weaning diet. On days 6 and 7, twelve piglets were surgically fitted with a PVTC cannula (van Leeuwen et al., 1991). On day 10 the animals were assigned randomly to three experimental groups. Mean BW of animals by then was 8.8 ± 0.6 kg. The animals were changed to the experimental diets on days 11 and 12. Until then the animals were fed twice a day at 08.00 and 16.00 h. From day 12 onwards, they were fed four times per day at 06.00, 12.00, 18.00 and 24.00 h.

For details on the use of the ^{15}N isotope dilution technique in pigs, the reader is referred to de Lange et al. (1990) and Huisman et al. (1992). Briefly, on days 13 and 14 two silicone catheters were implanted in each piglet, one into the external jugular vein and one into the carotid arteria. On day 14 the animals were continuously infused with sterile physiological saline solution (NaCl, 9 g/l) via the catheter in the carotid arteria at a rate of 2 ml/h using perfusion pumps (Fr. B. Braun Melsungen AG, FRG, Art. 871732/0). From day 15 onwards, the piglets were continuously infused arterially with a L-leucine solution with a 50% ^{15}N enrichment. The mean infusion rate was 0.502 ± 0.020 mg L-leucine (50% ^{15}N enrichment) per kg BW per hour. To minimize the time for reaching a steady state of the ^{15}N enrichment of N in the precursor pool for endogenous protein (TCA-soluble blood plasma), the animals were primed with 0.774 mg ^{15}N-L-leucine (50% ^{15}N enrichment) per kg BW per hour during the first 7 hours of infusion. L-leucine (50% ^{15}N enrichment) was dissolved in a sterile non-pyrogenic solution of physiological saline at a concentration of 2.4 mg/ml. About 2 ml of solution was infused per animal per hour.

Blood samples of 5 ml were taken from each of the piglets via the catheter in the jugular vein on day 14 (before ^{15}N infusion) and on subsequent days at 09.00, 15.00 and 21.00 h. The blood samples were immediately centrifuged for 10 minutes at 800 *g*, 4°C. The plasma samples were pooled per animal per day and frozen at -20°C.

On days 16-20, faeces of the animals were collected quantitatively, using plastic bags attached to the animals. Faeces were stored in the freezer (-20°C). Faeces collected on day 20 were used for ^{15}N analysis. On days 21-23, ileal digesta were collected via the PVTC cannula for 12 h per day (08.00 - 20.00 h). Collection procedures were as in *Exps. 1* and *2*.

The feeding level was kept constant during the infusion period and was equivalent to 2.7 times the animal's maintenance requirement for energy (ARC, 1981). Pelleted diets were mixed with water (1:2; w/w) just before feeding. No additional water was supplied. Average room temperature was 23°C.

Sample preparation and chemical analysis

Samples of hulls of faba beans and diets were ground on a laboratory mill with a 1 mm screen prior to analysis. Hulls were analysed on content of neutral detergent fibre (NDF), acid-detergent fibre (ADF) and lignin (ADL) according to Goering & Van Soest (1970). Samples of ileal digesta (*Exps. 1-3*) were pooled per animal within experimental periods. Samples of fresh material were taken for analysis for dry matter and nitrogen (N) content. Part of the ileal digesta (*Exps. 1-3*) and faeces (*Exp. 3*) was freeze-dried and ground over a 1 mm screen. Digesta and feed samples were subsequently analysed for contents of dry matter, N, ash and Cr_2O_3 (*Exps. 1-3*), crude fibre (*Exps. 2* and *3*) and amino acids, tryptophan excluded (*Exp. 1*), following procedures described by Jansman et al. (1993b).

The nitrogen solubility index (NSI) and the protein dispersibility index (PDI) of feed samples (*Exps. 1* and *2*) were analysed following the methods of Dale et al. (1983) and AACC (1983), respectively.

Prior to analysis of ^{15}N enrichment of blood plasma samples (pooled per animal per day), 0.1 ml trichloroacetic acid (TCA) (40%) was added to 0.5 ml of plasma. The samples were mixed thoroughly and left overnight at 4°C. Samples were centrifuged for 20 min at 3000 *g* (4°C). The supernatants (TCA-soluble plasma) were neutralized with 1 M sodium hydroxide and freeze- dried. Freeze-dried samples of TCA-soluble plasma, feed samples and freeze-dried ileal digesta and faeces were analysed for ^{15}N enrichment using mass spectrometry.

Calculation of the true ileal and faecal digestibility of crude protein

The endogenous nitrogen in ileal digesta and faeces were calculated from the ^{15}N-enrichment in these samples and in the TCA-soluble plasma according to the following formula (de Lange et al., 1990):

$$N_e = N_d \times [(^{15}N_{dig} - ^{15}N_{diet})/(^{15}N_{pl} - ^{15}N_{pl(0)})]$$

N_e	: endogenous N in ileal digesta or faeces (g/day)
N_d	: total N in ileal digesta or faeces (g/day)
$^{15}N_{dig}$	: ^{15}N enrichment of ileal digesta or faeces (%)
$^{15}N_{diet}$	: natural ^{15}N enrichment of the diet (%)
$^{15}N_{pl}$	: ^{15}N enrichment of the TCA-soluble blood plasma (%)
$^{15}N_{pl(0)}$	: natural ^{15}N enrichment of the TCA-soluble blood plasma (%)

The ^{15}N enrichment excess in the endogenous protein secretion was assumed to be similar to the average ^{15}N enrichment excess in the TCA-soluble plasma taken during the corresponding days of faeces and ileal digesta collection (de Lange et al., 1990). The true ileal and faecal digestibility of crude protein (Nx6.25) was calculated

from the apparent ileal or faecal digestibility values and the recoveries of endogenous protein (Nx6.25) in ileal digesta and faeces (Souffrant et al., 1981; Schulze et al., 1993).

Calculation of the composition of tannin-associated protein (TAP)

Estimates for the molar composition of amino acids of tannin-associated protein (TAP) at the ileal level in *Exp. 1* were made following the method of Mitaru et al. (1984). It was assumed that the difference in amounts of amino acids in ileal digesta of animals fed the control diets (HSC_1 and LSC_1) or the diets containing high-tannin faba bean hulls ($HSHT_1$ and $LSHT_1$) were the result of binding of tannins to both dietary and endogenous proteins during the digestive process. The composition of these TAPs is expressed on a molar basis. The molar composition of TAPs for both types of diets (HS and LS) are indicated as TAPa and TAPb, respectively. In this calculation it was assumed that microbial protein synthesis and the transformation of amino acids by microbes in the small intestine was negligible.

The percentage of amino acids with non-polar side chains (NPS) in dietary protein, TAPa and TAPb and endogenous protein was calculated from the sum of the molar percentages of isoleucine, tyrosine, phenylalanine, proline, leucine and valine (Mitaru et al., 1984). These percentages were calculated as it was assumed that tannins have a high affinity for hydrophobic amino acids.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out on all data using treatment as a factor. If the treatment effect was significant, the differences between treatment means were tested with the least significance difference (LSD) test (Snedecor & Cochran, 1980).

Results

The nitrogen solubility index (NSI) and the protein dispersibility index (PDI) were markedly higher for diets HSC_1, HSC_2 than for diet LSC_1 (*Exps. 1 & 2).* Inclusion of 200 g/kg of faba bean hulls, instead of cellulose as a source of fibre, did not change markedly NSI and PDI for both types of diets (Table 3).

The hull-free diets HSC_1 and HSC_2 had a 10 units higher apparent ileal digestibility for crude protein (CP) than diet LSC_1 (Table 3; $P<0.05$). Diets HSC_1 and LSC_1 did not differ in apparent ileal digestibility for dry matter (DM), ash, organic matter (OM) and non-protein organic matter (NPOM). The diets with 200 g/kg of either low- or high-tannin faba bean hulls (HSLT, HSHT, LSLT and LSHT) had a lower apparent ileal digestibility for DM, OM and NPOM than the hull-free diets HSC and LSC (Tables 3 and 7; *Exp. 1-3*) ($P<0.05$). The type of hull in the diet, either LT or HT, had no effect on apparent ileal and faecal digestibility of DM, OM and NPOM (*Exp. 1-3*) and ash (*Exp. 1-2*).

Table 3. Nitrogen solubility index (NSI), protein dispersibility index (PDI) and apparent ileal digestibility coefficients of dry matter (DM), ash, organic matter (OM), crude protein (CP) and non-protein organic matter (NPOM) of the experimental diets (*Exps. 1 & 2*).

	Experiment 1						Experiment 2			
	HSC_1	LSC_1	$HSHT_1$	$LSHT_1$	$LSLT_1$	SEM[1]	HSC_2	$HSLT_2$	$HSHT_2$	SEM[1]
Hulls	-	-	HT[2]	HT[2]	LT[2]		-	LT[2]	HT[2]	
Cond. tannins	<0.1	<0.1	0.56	0.65	<0.1		<0.1	<0.1	0.62	
NSI	97	41	96	44	40		96	98	97	
PDI	66	16	42	13	15		72	69	69	
n	5	5	5	5	5		6	5	6	
DM	77.8^a	76.5^a	64.4^b	64.9^b	64.2^b	1.2	77.4^a	65.8^b	63.8^b	0.8
Ash	42.3^a	39.3^{ab}	35.1^{ab}	34.8^{ab}	32.4^b	3.3	47.1^a	36.6^b	34.0^b	2.3
OM	80.4^a	79.3^a	66.5^b	67.2^b	66.7^b	1.0	79.6^a	67.9^b	65.9^b	0.9
CP	81.4^a	71.8^b	71.3^b	64.5^c	70.8^b	1.6	79.2^a	77.5^a	70.6^b	0.9
NPOM	80.4^a	81.2^a	65.0^b	68.0^b	65.5^b	1.1	78.7^a	67.9^b	66.2^b	0.9
CF	-	-	-	-	-		-5.0^a	8.1^b	9.7^b	2.8

[a,b,c] Values with a different superscript within a row in the same experiment differ significantly at $P<0.05$.

[1] SEM: standard error of the mean

[2] LT: low-tannin hulls; HT: high-tannin hulls.

Table 4. Apparent ileal digestibility of amino acids in the experimental diets (*Exp. 1*) and differences in digestibility of amino acids between control (C) diets and hull-containing diets (LT or HT).

	HSC_1	LSC_1	$HSHT_1$	$LSHT_1$	$LSLT_1$	SEM[1]	HSC-HSHT	LSC-LSHT	LSC-LSLT
Arg	89.9^{a}	84.9^{bc}	82.6^{c}	78.0^{d}	87.8^{ab}	1.0	7.3	6.9	- 2.9
His	84.2^{a}	74.5^{b}	74.0^{b}	64.6^{c}	71.5^{b}	1.2	10.2	9.9	3.0
Ile	85.6^{a}	80.0^{b}	77.2^{b}	72.4^{c}	77.7^{b}	1.4	8.4	7.6	2.3
Leu	88.9^{a}	80.1^{b}	81.7^{b}	72.8^{c}	78.9^{b}	1.4	7.2	7.3	1.2
Lys	85.1^{a}	68.8^{cd}	76.9^{b}	63.4^{d}	72.9^{bc}	2.0	8.2	5.4	- 4.1
Met	84.0^{a}	76.2^{bc}	72.3^{cd}	68.2^{d}	78.1^{b}	0.8	7.8	8.0	- 1.9
Cys	31.6^{ab}	43.3^{a}	2.5^{c}	20.4^{b}	43.7^{a}	5.9	29.1	22.9	- 0.4
Phe	87.7^{a}	81.1^{b}	81.2^{b}	73.2^{c}	80.3^{b}	1.3	6.5	7.9	0.8
Thr	73.2^{a}	66.6^{b}	62.7^{bc}	59.7^{c}	67.6^{ab}	2.1	10.5	6.9	- 1.0
Val	85.3^{a}	77.0^{b}	78.4^{b}	69.0^{c}	75.6^{b}	1.6	6.9	8.0	1.4
Ala	77.7^{a}	73.8^{a}	63.3^{b}	63.1^{b}	72.5^{a}	2.1	14.4	10.7	1.3
Asp	83.6^{a}	72.0^{bc}	75.1^{b}	67.7^{c}	70.2^{c}	1.6	8.5	4.3	1.8
Glu	89.9^{a}	80.2^{b}	85.9^{a}	74.7^{c}	80.7^{b}	1.4	4.0	5.5	- 0.5
Gly	65.7^{a}	57.9^{ab}	43.9^{c}	49.6^{bc}	62.4^{a}	3.5	21.8	8.3	- 4.5
Pro	84.1^{a}	56.3^{bc}	71.4^{ab}	41.3^{c}	66.3^{b}	5.0	12.7	15.0	-10.0
Ser	80.7^{a}	71.6^{b}	71.4^{b}	64.0^{c}	72.6^{b}	2.0	9.3	7.6	- 1.0
Tyr	88.7^{a}	79.7^{b}	79.2^{b}	70.0^{c}	76.1^{b}	1.4	9.5	9.7	3.6
ΣAA	85.1^{a}	72.5^{b}	76.8^{b}	66.4^{c}	74.7^{b}	1.8	8.3	6.1	- 2.2

[1]SEM: standard error of the mean

The apparent ileal digestibility for CP (*Exps. 1 & 2*) (Table 3) and amino acids (*Exp. 1*) (Table 4) of diets HSLT and LSLT did not differ significantly from diets HSC and LSC, respectively. Only in *Exp. 3*, apparent ileal digestibility of CP was lower for diet $HSLT_3$ than for HSC_3 ($P<0.05$) (Table 6). HT diets had a lower apparent ileal digestibility for CP ($P<0.05$) than for the control (C) and LT diets. For HSHT diets digestibility values for CP were 10, 9 and 14 units lower than for the respective HSC diets (*Exps. 1-3;* Tables 3 and 7) ($P<0.05$). CP digestibility was 7 units lower for LSHT than for LSC ($P<0.05$) (*Exp. 1*). The HT diets had a lower apparent ileal digestibility for all amino acids than the C diets (Table 4). The differences were significant for all amino acids ($P<0.05$), except for glutamic acid and proline in HSHT diet and for lysine, aspartic acid and glycine in LSHT diet. The differences in digestibility values between diet HSC_1 and diet $HSHT_1$ ranged from 4 units for glutamic acid to 29 units for cystine. Differences between diets LSC_1 and $LSHT_1$ varied between 4 units for aspartic acid and 23 units for cystine.

Comparison of the amino acid composition of tannin-associated proteins (TAPs) and dietary protein (Table 5) reveals that TAPa (calculated from the results of the HS diets) is relatively low in arginine and glutamic acid and rich in alanine, glycine and proline. TAPb (calculated from the results of the HS diets) is low in lysine, aspartic and glutamic acid and rich in alanine and proline (Table 5). The molar percentage of amino acids with non-polar side chains (NPS) did not differ between protein in HS diets and in TAPa and was about 5 units higher for TAPb than for protein in the LS diets (Table 5).

Inclusion of 200 g/kg of LT hulls tended to increase the endogenous excretion of CP in ileal digesta and faeces compared to the control diet with cellulose ($P>0.05$) (6 and 4 g CP/kg DM intake) (Table 6). Inclusion of HT hulls markedly increased the excretion of endogenous protein at both levels compared to the LT diet (Table 6) ($P<0.05$) (10 g CP/kg DM intake). The proportion of dietary protein in ileal digesta and faeces tended to be higher in pigs fed HT diets (0.37 and 0.50, respectively) than in pigs fed LT hulls (0.32 and 0.47) (Table 6) ($P>0.05$).

The true ileal and faecal CP digestibility of diet HSC_3 was about 97 (Table 7) (*Exp. 3*). Diet $HSLT_3$ (*Exp. 3*) had a lower apparent and true digestibility for CP (about 5 and 3 units at the ileal level, 5 and 2 units at the faecal level, respectively) than diet HSC_3. For diet $HSHT_3$ the apparent and true ileal CP digestibility was 8 and 4 units lower, respectively, than for diet HSC_3. At the faecal level the digestibility values were 12 and 6 units lower for the diet HSC_3 (Table 7).

Discussion

Dietary composition

The inclusion of 64-73 g/kg of cellulose in the control diets, instead of 200 g/kg of LT or HT hulls of faba beans, was meant to formulate diets with a similar crude fibre content. Analysis of the diets (Table 1) showed, however, that the crude fibre content in hull-containing diets was higher (by 10-70 g/kg) than in the control diets with

Table 5. Molar percentages of amino acids in diets, in tannin-associated proteins[1] (TAPa and TAPb), difference in composition between dietary and TAPs (*Exp. 2*), calculated TAP values[2] (TAPa and TAPb calc.) and molar composition endogenous ileal protein in pigs (de Lange et al., 1990).

	HS	TAPa[1]	TAPa-HS	TAPa calc.[2]	LS	TAPb[1]	TAPb-LS	TAPb calc.[2]	End. pr.
Arg	13.7	11.5	-2.2	11.5	14.4	13.0	-1.4	11.8	9.2
His	5.6	6.5	0.9	4.3	6.1	7.9	1.8	4.6	3.0
Ile	3.9	3.7	-0.2	3.6	3.9	3.9	0.0	3.6	3.3
Leu	7.0	5.8	-1.2	6.1	6.9	6.6	-0.3	6.1	5.2
Lys	7.9	7.4	-0.5	7.5	7.4	5.2	-2.2	7.3	7.1
Met	0.8	0.7	-0.1	1.0	0.9	0.9	0.0	1.0	1.1
Cys	0.4	1.4	1.0	1.8	0.8	2.3	1.5	2.0	3.1
Phe	2.9	2.1	-0.8	2.9	3.2	3.3	0.1	3.0	2.8
Thr	3.4	4.1	0.7	5.3	4.0	3.6	-0.4	5.6	7.1
Val	5.6	4.4	-1.2	5.4	5.3	5.6	0.3	5.3	5.2
Ala	4.0	6.6	2.6	5.8	6.2	8.6	2.4	6.9	7.5
Asp	8.7	8.6	-0.1	8.6	8.7	4.9	-3.8	8.6	8.4
Glu	16.7	7.7	-9.0	12.7	12.3	8.9	-3.4	10.5	8.7
Gly	4.1	10.2	6.1	8.3	8.4	9.1	0.7	10.4	12.4
Pro	7.3	10.7	3.4	6.9	4.2	8.3	4.1	5.4	6.5
Ser	5.6	6.0	0.4	6.0	5.2	5.2	0.0	5.9	6.5
Tyr	2.4	2.6	0.2	2.6	2.1	2.7	0.6	2.5	2.8
NPS[3]	29.1	29.3		27.5	25.6	30.4		25.9	25.8

[1]Molar % of amino acids in tannin-associated proteins (TAP). Composition of TAPa and TAPb are based on the differences in digestibility of amino acids between the diets HSC, LSC and HSHT and LSHT, respectively.

[2]Calculated composition of tannin-associated proteins (TAPa calc. and TAPb calc.), assuming that 50% of the effect of tannins on apparent digestibility of amino acids is due to non-selective binding of dietary protein and 50% is due to non-selective binding of endogenous proteins. Data from the de Lange et al. (1990) are used for the amino acid composition of endogenous protein.

[3]NPS: sum of molar % of amino acids with non-polar side chains

Table 6. Endogenous excretion of crude protein (CP) and ratio of endogenous to dietary protein in ileal digesta and faeces (*Exp. 3*).

	Ileal digesta				Faeces			
	HSC_3	$HSLT_3$	$HSHT_3$	SEM[1]	HSC_3	$HSLT_3$	$HSHT_3$	SEM[1]
Endogenous CP excretion (g/kg DM intake)	16.4[a]	22.3[a]	31.9[b]	1.9	8.6[a]	12.9[a]	23.2[b]	2.4
Endogenous CP excretion (g/kg CP intake)	89.8[a]	116.7[a]	164.2[b]	9.7	47.2[a]	67.3[a]	119.7[b]	12.5
Endogenous CP (g/g)	0.77[a]	0.68[ab]	0.63[b]	0.03	0.60[a]	0.53[ab]	0.50[b]	0.03
Dietary CP (g/g)	0.23[a]	0.32[ab]	0.37[b]	0.03	0.40[a]	0.47[ab]	0.50[b]	0.03

[1]SEM: standard error of the mean

Table 7. Apparent ileal and faecal digestibility of dry matter (DM), Ash, organic matter (OM), crude protein (CP), non-protein organic matter (NPOM) and crude fibre (CF) and true ileal and faecal digestibility of CP of the diets (*Exp. 3*).

	Ileal digestibility				Faecal digestibility			
	HSC_3	$HSLT_3$	$HSHT_3$	SEM[1]	HSC_3	$HSLT_3$	$HSHT_3$	SEM[1]
Hulls	-	LT	HT		-	LT	HT	
Condensed tannins	<0.1	<0.1	0.69		<0.1	<0.1	0.69	
n	4	4	4		4	4	4	
DM	83.3[a]	64.2[b]	63.9[b]	1.2	87.9[a]	80.7[b]	75.8[b]	1.9
Ash	57.6[a]	30.8[b]	17.9[c]	4.0	69.9[a]	69.7[a]	65.2[a]	3.2
OM	84.9[a]	66.4[b]	66.8[b]	1.1	89.0[a]	81.4[b]	76.5[b]	1.8
CP, apparent	88.2[a]	82.7[b]	74.1[c]	1.3	92.1[a]	87.7[a]	76.1[b]	1.8
CP, true	97.2[a]	94.4[b]	90.5[c]	0.8	96.8[a]	94.5[a]	88.1[b]	0.7
NPOM	84.1[a]	62.2[b]	64.9[b]	1.1	88.3[a]	79.8[b]	76.6[b]	1.9
CF	-0.9[a]	-15.6[b]	1.5[a]	2.4	1.0[a]	27.3[b]	17.7[ab]	7.2

[1]SEM: standard error of the mean

cellulose. The values for crude fibre content of faba bean hulls used in diet formulation were apparently underestimated. Therefore, differences in results between hull-free and hull-containing diets in the present studies might be due to both qualitative and quantitative differences in dietary fibre. It was thought that cellulose in the hull-free control diets did not affect the apparent digestibility of protein and amino acids in pigs (Sauer et al., 1991).

Hulls of faba beans contain the major part of the seed's fibre (Hill-Cottingham, 1983) and all condensed tannins (Bos & Jetten, 1989). Faba bean hulls are rich in structural carbohydrates as indicated by their high NDF and ADF contents (Table 2). Longstaff & McNab (1991) showed that they merely consist of cellulose and some hemicellulose. Differences in composition of fibre between LT and HT hulls are small. The content of condensed tannins differed markedly between LT and HT hulls (Table 2). Therefore, inclusion of either LT or HT hulls mainly affects the level of condensed tannins in the diets.

Effects of fibre and tannins on apparent ileal and faecal digestibility

The reduced apparent ileal digestibility for DM, OM and NPOM in hull-containing compared to hull-free diets can be explained by the low ileal digestibility of DM in faba bean hulls. Due to their high fibre content, they are poorly degraded enzymatically in the small intestine of pigs. This is confirmed by the low ileal digestibility of crude fibre in hull-containing diets (< 10%) in *Exps. 2 & 3*. This also indicates that microbial activity is limited in the small intestine of pigs receiving these diets. Other workers have also found a low ileal digestibility of NDF in soya bean hulls (Laplace et al., 1989) and in wheat bran (Graham et al., 1986; Laplace et al., 1989) and of NDF isolated from wheat bran (Schulze et al., 1993). Only up to 30% of the fibre in faba bean hulls was degraded fermentatively in the large intestine. This can be concluded from the faecal digestibility of CF in the hull-containing diets in *Exp. 3* (Table 7).

Inclusion of LT faba bean hulls, instead of cellulose, as a source of fibre did not reduce the apparent ileal apparent digestibility of CP (*Exp. 1 & 2*) and amino acids (*Exp. 1*), and resulted in a decreased apparent ileal digestibility of CP in *Exp. 3*. Sauer et al. (1991) did not find effects of inclusion of either 100 g/kg of powdered cellulose or barley straw on the apparent ileal digestibility of CP and amino acids in pigs. Dietary fibre may decrease digestibility of protein and amino acids by stimulating the production of bacterial protein, through adsorption of amino acids and peptides onto the fibre matrix, and by increasing the excretion of endogenous protein (Schulze et al., 1993).

The low apparent digestibility of CP and amino acids in diets with HT hulls can be attributed to the effects of condensed tannins. Other studies with pigs (Jansman et al., 1993b) and chickens (Longstaff & McNab, 1991) also showed negative effects of HT faba bean hulls on apparent (faecal) digestibility of CP and amino acids.

Effects of fibre and tannins on the excretion of endogenous protein and the true digestibility of feed protein

The endogenous excretion of CP was slightly increased at the ileal level (from 16 to 22 g/kg DM intake) and at the faecal level (from 9 to 13 g/kg DM intake) when the diet included 200 g/kg of LT faba bean hulls instead of 73 g/kg of cellulose (*Exp. 3*). A similar increase in excretion of endogenous protein in ileal digesta was found by Schulze et al. (1993) when feeding piglets diets with up to 180 g/kg of NDF isolated from wheat bran. Furuya & Kaji (1991), however, did not observe an increase in content of N and amino acids in ileal digesta of pigs when including up to 150 g/kg of wood cellulose in protein-free diets. This shows that the endogenous excretion of these nutrients was not increased in their study. In rats, the inclusion of fibre increased sloughing of intestinal mucosal cells (Bergner et al., 1975) and enhanced mucus production (Schneeman et al., 1982). It can be concluded from the present studies that fibre from faba beans has a limited effect on apparent digestibility of protein and amino acids and endogenous protein excretion in pigs.

It is shown here that condensed tannins in faba beans increase the excretion of both exogenous (dietary) and endogenous protein in ileal digesta and faeces of pigs. About 55 and 45% of the lowered apparent digestibility of CP in high-tannin diet at the ileal and the faecal level, respectively, can be attributed to a decrease in the true digestibility of dietary protein (Table 7). Endogenous excretion of protein increased by about 10 g/kg DM intake both at ileal and the faecal level in pigs fed the high-tannin diet. The increase may be due to an enhanced secretion of endogenous proteins or to a reduced degradation and reabsorption of endogenously secreted proteins. The latter could be relevant since Souffrant et al. (1986) found that 70 and 82% of endogenous secreted proteins in the alimentary tract of pigs are reabsorbed up to the terminal ileum and the rectum, respectively.

Selectivity of faba bean tannins in binding proteins *in vivo*

The difference in solubility of feed proteins, as suggested by differences in NSI and PDI, between LSHT and HSHT diets (*Exp. 1*) might have affected the ratio of binding of faba bean tannins to dietary and endogenous proteins. Tannins probably bind preferably to solubilized proteins. The amount of soluble feed proteins was likely larger in the HSHT diet. Sufficient amounts of soluble dietary proteins, however, may still have been present in the LSHT diet to prevent excessive binding of tannins to endogenous proteins. Extensive binding of tannins to digestive enzymes or mucosal proteins could interfere with pancreatic enzyme secretion or disturb nutrient absorption. This could have more striking effects on the digestibility of nutrients than merely binding to dietary proteins. The effects of tannins on the digestibility of CP and amino acids in the LS diet compared to the HS diet do not suggest a larger interference of tannins with the digestive process, when feeding a diet with low amounts of soluble protein. The slightly lower effect of tannins on the digestibility of CP in the LS diet may be the result of differences in relative affinity and binding capacity of faba bean tannins to the various dietary and endogenous proteins.

Tannins from faba beans increase the secretion of proline-rich proteins (PRPs) by the rat's parotid glands (Jansman et al., 1993a). This results in a large decrease in the apparent caecal digestibility of the non-essential amino acids proline, glycine and glutamic acid. Mole et al. (1990) indicated that pigs also secrete basal levels of proline-rich proteins.

The secretion of these proteins in pigs may be increased only to a small extent, compared to rats, when feeding tannins, since only about half of the additional protein excreted in the HT group was found to be of endogenous origin. The extra endogenous protein may also consist of digestive enzymes, mucins and proteins from sloughed-off mucosal cells. Jansman et al. (1993c) and Longstaff & McNab (1991) have shown a decrease in activity of various digestive enzymes in the small intestine of pigs and chickens, respectively, when feeding faba bean tannins. This is likely the result of tannins binding to enzymes. Enzymes could therefore be part of the extra excreted endogenous protein in tannin-fed pigs.

The molar amino acid composition of dietary protein and TAPs (*Exp. 1*) differed for some amino acids. Both TAPa and TAPb had a markedly higher content of alanine and proline and a lower content of glutamic acid, compared to the respective dietary proteins. When tannins interact non-selectively to both dietary and endogenous proteins, the aforementioned differences in composition of TAPs and dietary proteins should be reflected in the composition of endogenous protein. The molar amino acid composition of TAPs was therefore also calculated from the composition of dietary and endogenous protein (de Lange et al. (1990), assuming that TAPs consisted for 50% of dietary and for 50% of endogenous protein and that no selectivity in binding occurred (Table 5). The values for the ratio of tannins binding to dietary and endogenous protein were used since both sources of protein contributed to roughly the same extent in the reduced digestibility of the HT diet in *Exp. 3* (Table 7). The composition of TAPa and TAPb calculated in this way are indicated as TAPa calc. and TAPb calc., respectively (Table 5). Compared to the calculated values (TAPa calc. and TAPb calc.), TAPa is high in histidine, glycine and proline and low in glutamic acid, whereas TAPb is high in histidine and proline, and low in lysine, threonine, aspartic acid and glutamic acid, respectively. Our data suggest that condensed tannins in faba bean hulls selectively bind proteins in the intestinal tract. *In vitro*, differences in binding affinity were found of up to four orders of magnitude between tannins from sorghum (Hagerman & Butler, 1981) and other tannins (Asquith & Butler, 1986) for various proteins relative to bovine serum albumin. Mitaru et al. (1984) found in a digestibility trial with pigs fed untreated high-tannin sorghum and reconstituted sorghum, in which tannins were inactivated, that TAPs had a higher percentage of NPS than dietary protein. They concluded that this was due the hydrophobic interactions involved in tannin-protein interactions. In our study only TAPb had a slightly higher NPS content than protein in diets LSC_1 and $LSHT_1$.

It can be concluded that condensed tannins in faba bean hulls affect the apparent digestibility of protein and amino acids in pigs. They can increase the amounts of both dietary and endogenous protein in ileal digesta and faeces of pigs. In this respect, the

effect of condensed tannins from faba bean in pigs seems to differ from that in rats, since in rats faba bean tannins increase the excretion of mainly salivary (endogenous) proteins. Our data also suggest a certain selectivity of condensed tannins from faba beans for binding dietary or endogenous proteins in the intestinal tract of pigs. This selectivity will make it difficult to derive effects of condensed tannins on the apparent digestibility of individual amino acids from the reduction in apparent digestibility of crude protein.

Acknowledgements

The authors wish to thank P. van Leeuwen and D.J. van Kleef for surgery on the experimental pigs and H. Schulze and T. Zandstra for their assistance during the experiment.

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DISCUSSION

Chapter 9

GENERAL DISCUSSION

General discussion

Tannins in feedstuffs

The nutritive value of feedstuffs of plant origin depends on the contents of various nutrients, their apparent digestibility and their utilization after absorption. Both the digestibility and the utilization of nutrients can be affected by the presence of antinutritional factors (ANFs). ANFs generally decrease performance and/or negatively affect health of animals. The use of (unprocessed) feedstuffs with high levels of ANFs in feeds for non-ruminants therefore limits the maximum inclusion levels in diet formulation. In the past decade research on ANFs, including tannins, in feedstuffs for non-ruminant animal species has been stimulated by the search for alternatives to soya as a protein source in animal feeds. Among the alternatives are peas (Pisum sativum L.) and faba beans (Vicia faba L.). Both are known to contain several antinutritional factors (Huisman & Tolman, 1992). ANFs do not only limit the nutritional and economic value of feedstuffs for animal production. Most of them also increase the excretion of nitrogen and minerals into the environment. The necessity to limit the mineral surplus has become an important issue in intensive animal production in the past decade. Therefore, the reduction of levels or activity of ANFs in plant feedstuffs by breeding or processing has recently received considerable attention.

ANFs, including tannins, can play an important role in the plant's defence against environmental stresses and in disease resistance. Hence, from an agronomic point of view, their presence may be desirable, as has been shown for condensed tannins in faba beans and sorghum (Chapter 2). Katoh et al. (1989) found that the tannin content of leaves of Japanese cedar (Cryptomeria japonica D. Don) was reduced in areas with severe environmental pollution. This was accompanied with an increased predation of larvae of a herbivorous moth upon these leaves. This stresses the possible function of tannins in plants and their interaction with environmental factors.

Faba beans contain several ANFs, including condensed tannins, protease inhibitors, lectins, vicine/convicine, phytate, saponins and anti-niacin (Wareham, 1991). The level of condensed tannins is considered to be of most significance in relation to the nutritive value of this feedstuff.

Tannins in general may potentially exert a large number of antinutritional effects (Table 1). The separate importance of each of them is not well known. Interpretation of results of studies on the antinutritional effects of tannins is generally confounded by a large number of variables, such as the animal species involved, evaluation criteria used, origin and chemical nature of the tannins (condensed/hydrolysable, degree of polymerization), level of tannins in the diets, diet composition and treatment of the control group(s).

Table 1. Summary of the potential antinutritional effects of dietary tannins in monogastric animals (Chapter 2).

Antinutritive effects of tannins

1. reduced feed intake
2. decreased digestibility of protein and amino acids
3. decreased digestibility of starch and other carbohydrates
4. reduced availability of vitamins and minerals
5. reduced activity of digestive enzymes
6. alterations in histology and damage of the mucosa of alimentary tract
7. toxic effects on internal organs
8. disturbed metabolism of nutrients after absorption

From the literature the following mode of action of dietary condensed tannins in non-ruminant species is hypothesized.

Mouth — Solubilization of tannins and binding of tannins to salivary proteins and taste receptors lead to an astringent taste of the diet. Specific binding to salivary proline-rich proteins (PRPs) secreted by the parotid glands occurs in rats; it is unknown whether other species show a similar response or secrete basal levels of similar proteins with a high affinity for tannins.

Stomach — Solubilization of tannins and binding to both endogenous and feed proteins and other nutrients can occur. The interactions between tannins and proteins, however, depend on pH, and on the structure of both tannins and proteins.

Duodenum — Solubilization of tannins and binding of tannins to either endogenous proteins, such as pancreatic digestive enzymes and mucosal proteins, or dietary nutrients, particularly proteins. Tannins may interfere with pancreatic secretory regulation.

Jejunum — As in the duodenum, tannins can bind to either endogenous proteins, such as digestive enzymes and mucosal proteins, or dietary nutrients, particularly proteins. Binding of tannins to the mucosa may disturb absorption of nutrients.

Ileum — Also in the ileum tannins can bind to either endogenous proteins, such as digestive enzymes and mucosal proteins or dietary nutrients, particularly proteins. Harmful effects on the mucosa of the digestive tract may occur. This can cause negative effects on the apparent ileal digestibility of nutrients due to interactions between tannins and nutrients of both dietary and endogenous origin up to the terminal ileum.

Large intestine — Interference of tannins with microbial activity can occur and thereby with fermentation of those nutrients which pass from the small intestine into the large intestine.

Consequences for performance

The reduced apparent digestibility of nutrients, in particular protein and amino acids results in a decreased availability of nutrients and an increased faecal excretion of nutrients. The literature does not reveal systemic and/or toxic effects of condensed tannins.

In this thesis attention is given to several aspects of this concept for the effects and mode of action of tannins in faba beans. They were studied in different animal species, including rats, chickens and pigs. Because rats have served as a model for many other species in nutritional studies, including those aiming at the elucidation of effects of dietary tannins, first some studies with rats were performed. The effects of condensed tannins of faba beans on the secretion of salivary proteins by the parotid glands of rats and on the digestibility of amino acids were determined. Since the literature indicates negative effects of tannins in poultry, the growth performance of chickens fed high- and low-tannin faba beans was evaluated. Information on the nutritional effects of tannins in pigs is scarce. Therefore, the main attention in the present investigations was directed towards the effects in this species. Especially the digestive process in pigs and the possible interference by tannins was studied. First the effects of faba bean tannins on the apparent ileal and faecal digestibility of nutrients was measured. In subsequent studies, the effects on N retention, activity of digestive enzymes and the endogenous excretion of protein were evaluated.

Tannins in faba beans

From the literature it was concluded (Chapter 2) that the level of condensed tannins in faba beans is highly variable. There are large differences in tannin content between white-coloured beans and those with a darker seed coat. Low-tannin beans originate mainly from white-flowering varieties, whereas high-tannin beans come from coloured-flowering cultivars. In fact, condensed tannins may be almost absent in seeds of white-flowering cultivars (Bos & Jetten, 1989). The analysed content of condensed tannins in faba beans is below 0.2% catechin equivalents for white-flowering cultivars and ranges between 0.5 and 1.5% catechin equivalents for coloured-flowering varieties, as determined by the vanillin-sulphuric acid method (Chapter 2). Part of the variation in tannin content of faba beans reported in the literature can be related to the inadequacy and inaccuracy of commonly used methods for assaying tannins.

When evaluating the nutritional effects of tannins different approaches can be used with respect to the way tannins are included in the diets. With the inclusion of complete (whole) feedstuffs with a different tannin content, negative effects on nutritive value can possibly be associated with differences in tannin content. Such an

approach has been followed for two studies described in this thesis (Chapters 4 and 5). Using such an approach tannins are applied in their native form. The effects measured, however, are often confounded by differences in other dietary components. Alternatively, tannin-rich extracts or purified tannins can be used. Care should be taken, however, that either the extraction of tannins from the feedstuff is complete or the tannins obtained should be at least representative of total tannins found in that particular feedstuff. In addition, one should be sure that isolated tannins are not changed in nature during extraction. Generally, isolated tannins are easily subjected to oxidation. This may change their reactivity and their biological activity (Steiner, 1989). Structural changes in tannins due to isolation are difficult to determine. Moreover, large-scale isolation and purification of tannins for studies with relatively large animal species is difficult and time-consuming. Commercial purified tannins are not available, although tannic acid is sometimes considered to be a suitable reference substance for tannins in feedstuffs. Tannic acid is a hydrolysable tannin and the nutritional effects of hydrolysable and condensed tannins are different (Chapter 2). Therefore, tannic acid cannot be used as a reference substance for studying the effects of condensed tannins. Condensed tannins are found in faba beans. Several studies have shown that the chemistry of tannins in plants is of a very complex nature. Tannins in feedstuffs consist of different polyphenolic compounds with regard to chemical structure (Laks, 1989). The extent of structural heterogeneity, however, is not known due to a lack of suitable analytical methods for individual polyphenolic compounds. Horigome et al. (1988) found that the degree of polymerization of tannins can affect their biological activity in terms of enzyme-inhibiting capacity. They showed that the biological activity of tannins increased with increasing molecular size of tannins in fodder plants. Porter & Woofruffe (1984) found that the capacity to precipitate haemoglobin in a standard astringency test increased when the degree of polymerization of some defined condensed tannins increased. Therefore, evaluation of *in vivo* effects of native tannins in feedstuffs by a single reference compound, such as tannic acid, is not a realistic approach.

In faba beans tannins are confined to the hull portion of the seed (Marquardt et al., 1978; Bos & Jetten, 1989). Hulls can be separated from the cotyledons using a windsifting process (Meijer & Muuse, 1989; Chapter 6). Hulls represent between 100-150 g/kg of total dry matter of faba beans (A.J.M. Jansman, unpublished results; Marquardt et al., 1978). The chemical composition of hulls of low- and high-tannin faba beans is given in Table 2. Hulls consist mainly of structural carbohydrates as their levels of crude fibre, neutral detergent fibre (NDF) and acid detergent fibre (ADF) are high. Since glucose is the predominant monomeric sugar after acid hydrolysis (Table 2), it appears that cellulose is the main non-starch polysaccharide. This can be inferred also from the difference between their ADF and acid detergent lignin (ADL) content, which is 720-740 g/kg for hulls of low-tannin cultivars and 620-650 g/kg for hulls of high-tannin varieties of faba beans. The low level of non-glucose monomeric sugars and the small difference between NDF and ADF content of hulls (< 100 g/kg) suggest that the hemicellulose content is low.

Table 2. Chemical composition of low- and high-tannin faba bean hulls (g/kg dry matter).

	Marquardt et al. (1977)		Schmandke (1988)		Jansman (1993)[1]		Longstaff & McNab (1991)	
	Low (n=1)	High (n=1)	Low (n=1)	High (n=1)	Low (n=3)	High (n=4)	Low (n=1)	High (n=2)
Condensed tannins	1[2]	44[2]	<1[3]	50[3]	<1[4]	44[4]	0[5]	18[5]
Protein (Nx6.25)	34	48	46	43	50	49	-	-
Ash	35	31	23	24	-	-	-	-
Ether extract	3	2	-	-	-	-	-	-
Crude fibre	602	530	628	564	-	-	-	-
NDF	-	-	860	847	765	704	-	-
ADF	710	677	756	784	725	682	-	-
ADL	44	78	25	137	7	62	-	-
Rhamnose	-	-	-	-	10	8	8	5
Fucose	-	-	-	-	2	1	2	2
Arabinose	-	-	-	-	13	12	16	12
Xylose	-	-	-	-	110	76	144	84
Mannose	-	-	-	-	1	1	2	2
Galactose	-	-	-	-	10	1	21	19
Glucose	-	-	-	-	468	407	597	552
Uronic acids	-	-	-	-	115	98	171	139
Total	-	-	-	-	729	613	961	815

[1]A.J.M. Jansman, unpublished results; results on monomeric composition of sugars of faba bean hulls (g/kg) are corrected for loss of H_2O during hydrolysis.
[2]vanillin-hydrochloric acid method (Burns, 1971), purified faba bean tannins as a standard
[3]vanillin-sulphuric acid method, phloroglucinol as a standard
[4]vanillin-sulphuric acid method, catechin as a standard
[5]vanillin-acetic acid method (Butler et al., 1982), catechin as a standard
- not determined

The lignin (ADL) content in faba bean hulls is also low, i.e. 7 g/kg for low-tannin cultivars and 62 g/kg for high-tannin cultivars (A.J.M. Jansman, unpublished results). Estimates of other workers (Table 2) confirm these observations. Contents of crude protein (N x 6.25), ash and crude fat (ether extract) in faba bean hulls are low and do not differ between low- and high-tannin varieties. From the data presented in Table 2

and those published by Jansman (Chapter 2) it is concluded that, apart from small differences in composition of structural carbohydrates, the level of condensed tannins is the most striking difference between hulls of low-tannin beans and high-tannin beans.

Therefore, in several studies described in this thesis (Chapters 3, 6, 7 and 8) hulls of faba bean cultivars have been used as a means to include faba bean tannins in experimental diets. In this way tannins are included in their native form. The level of dietary fibre, however, is also increased with the inclusion of hulls. This may affect some parameters related to the digestive process in simple-stomached animals. By comparing the effects of experimental groups receiving either low- (or zero-) or high-tannin faba bean hulls, it is likely that mainly effects caused by differences in tannin content are measured.

Nutritional effects of tannins in faba beans in rats, chickens and pigs

Tannins and secretion of PRPs

In rats, both high-tannin faba bean hulls and a tannin-rich extract of hulls rapidly induced an increase in salivary secretion of proline-rich proteins (PRPs) synthesized in the parotid glands. The latter was accompanied with a 2-5 fold increase in relative size (g/g of body weight) of these glands. At the same time the apparent digestibility of, in particular proline, glycine and glutamic acid decreased. When feeding low-tannin faba bean hulls, effects on either gland size or secretion of PRPs were absent (A.J.M. Jansman, unpublished results). This again indicates that tannins in faba bean hulls are associated with the effects on the parotid glands. Similar effects were found in rats after feeding high-tannin sorghum and purified tannins from sorghum (Mehansho et al., 1992). The magnitude of the response of the parotid glands was linearly dependent on the dietary level of tannins from faba beans. The complexes formed between faba bean tannins and salivary PRPs in rats appear to be stable under the conditions in the digestive tract. This can be concluded because the profile of amino acids, as found in purified PRPs, was reflected also in caecal digesta (Chapter 3). Mehansho et al. (1983) showed that PRPs *in vitro* have a high binding affinity to tannins. It is concluded therefore that in rats PRPs can act as tannin-binding agents. This adaptive mechanism prevents dietary tannins from being biologically active in the gastro-intestinal tract where digestion takes place. It results in rats in an increase in faecal contents of the non-essential amino acids proline, glycine and glutamic acid. In addition, tannin-fed rats maintained a growth rate comparable to control animals. The prevention of harmful effects of tannins in rats by the action of PRPs agrees with results obtained in studies in which dietary additives, such as polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG), were used to reduce the effects of tannins. Both PVP and PEG have a high affinity for tannins, and both reduce the harmful effects of tannins from faba beans in chickens (Marquardt et al., 1977) and from sorghum in rats (Ford & Hewitt, 1979).

In the literature no studies are known which indicate that chickens and pigs, in analogy with rats, are able to decrease the harmful effects of condensed tannins by increasing the secretion of salivary proteins. In pigs, the weights of the parotid glands did not increase after feeding tannin-rich faba bean hulls (Chapter 6). Mole et al. (1990), however, found in the parotid glands of pigs also low amounts of PRPs. Their affinity towards tannins, however, was low compared to PRPs from hare, rabbit and rat. This may indicate that PRPs have not evolved exclusively as a defence against tannins, but that they may well have other functions, such as a role in stabilizing minerals in the teeth (Mole et al., 1990). The decrease in apparent ileal and faecal digestibility of proline in pigs was larger than for most other amino acids when fed tannin-rich faba bean hulls (Chapters 6 and 8). However, this does not necessarily mean that the reduction in apparent protein digestibility is largely due to an increased excretion of PRPs. Only about half of the increased excretion of crude protein in tannin-fed pigs was shown to be of endogenous origin (Chapter 8). More research is therefore required to determine the possible role of PRPs in pigs in relation to dietary tannins.

When tannins are not bound to PRPs after ingestion of the meal, tannins may bind to other substrates of either endogenous or exogenous origin or remain unbound. Free tannins principally may be excreted, fermentatively degraded or absorbed intactly. A diet with 200 g/kg of tannin-rich faba bean hulls to pigs did not affect the relative weights of some important metabolic organs, such as liver, kidneys and spleen (Chapter 6). These observations confirm that condensed tannins from faba beans (up to 1% catechin equivalents in the diet) are not toxic to pigs when fed over a limited period of time. Toxic effects could occur after absorption of either intact tannins or their degradation products. Studies on long-term effects of faba bean tannins in pigs are not found in the literature. In contrast, a reduced growth and severe toxic effects on liver and kidneys were found when feeding tannic acid, a hydrolysable tannin, to chickens (Karim et al., 1978). Rats were able to consume diets with considerable levels of tannic acid without showing toxic effects in the organs (Mitjavila et al., 1971). This may be explained by the inactivation of tannic acid by PRPs secreted by the parotid glands. The results described in this thesis seem to confirm the suggestion (Salunkhe et al., 1990) that condensed tannins, in contrast to hydrolysable tannins, are fairly stable during their passage through the digestive tract of monogastric animals. Tannin molecules may be too large to be absorbed intactly, thus preventing systemic toxic effects. Intravenous injection of both hydrolysable and condensed tannins of various origin in rats were shown to exert various systemic and toxic effects (Singleton, 1981). No information is available on the effects of feeding tannic acid to pigs.

Effects of tannins in faba beans in the small intestine and on the digestive process

Effects on the activity of pancreatic enzymes

In the small intestine several digestive enzymes, either in free form or membrane-bound, may interact with free, solubilized tannins from the diet. The high pH at this

site of the intestinal tract, compared to that in the stomach, may be favourable for tannin-protein interactions. Proteins complex with tannins preferentially at pH values close to their iso-electric point (Hagerman & Butler, 1978). For example, Jones & Mangan, (1977) showed that complexes between Fraction 1 leaf protein and tannins from sainfoin (Onobrychis viciifolia Scop.) were most stable at pH values between 4 and 7. At a lower pH tannin-protein complexes dissociated. The same may happen with other dietary proteins, bound to tannins in the oral cavity. Free tannins may interact with other dietary components, digestive enzymes or other endogenous proteins in the small intestine.

The effects of condensed tannins in faba beans on the activity of various digestive enzymes in digesta of pigs from our studies and other species from studies in the literature, are summarized in Table 3. In pigs, chickens and rats, tannins in faba beans reduce the activity of trypsin to a variable extent. In pigs, the reduction in trypsin activity was only significant in digesta obtained from the distal part of the small intestine, but not in duodenal digesta. Activity of chymotrypsin in digesta obtained from either part of the small intestine of pigs was not affected significantly by tannins (Chapters 6 and 7). The different effects of tannins on enzyme activity in various parts of the digestive tract can probably be explained by differences in conditions such as pH. Moreover, the time interval between enzyme secretion and tannin encounter may be important. In adddition, there may be differences in the state of activity of enzymes, the presence of detergents, such as bile salts, and the nature and levels of both tannins and substrates available for interactions with tannins. Each of these factors may affect the formation of complexes between tannins and proteins (Hagerman, 1989). It can be assumed that tannin-bound enzymes lose their biological activity. If conditions for tannin-trypsin complexation are less favourable in the proximal small intestine of pigs, it is also likely that condensed tannins do not interfere with the cholecystokinin-pancreozymin (CCK-PZ) feedback mechanism for secretion of pancreatic enzymes. The latter was shown to be important in explaining the antinutritional effects of proteinaceous trypsin inhibitors found in many feedstuffs of plant origin (Liener & Kakade, 1980; Birk, 1989). It also seems likely that the activities of trypsin and chymotrypsin in pancreatic tissue are not affected to a large extent in pigs fed tannin-rich hulls (200 g/kg) (Chapter 6). Longstaff & McNab (1991) did not find effects of feeding tannin-rich faba bean hulls to chickens on the weight of pancreas and the activity of lipase and sucrase in pancreatic tissue. α-Amylase activity, however, was reduced in their study. Pancreatic weight of chickens was not changed either after feeding a tannin-rich extract from faba bean hulls (Marquardt et al., 1977). Dietary inclusion of whole faba beans, both with a low or a high tannin content, increased relative pancreatic weight in chickens (Chapter 4). This can be attributed to the presence of proteinaceous trypsin inhibitors in faba beans.

In chickens and rats the activity of α-amylase in small intestinal digesta was significantly reduced by faba bean tannins (Griffiths & Moseley, 1980; Longstaff & McNab, 1991; Yuste et al., 1992). Lipase activity decreased in digesta of chickens fed high-tannin faba bean diets (Longstaff & McNab, 1991; Yuste et al., 1992), but increased in rats (Griffiths & Moseley, 1980) (Table 3). The differences in effect of tannins on the activity of various digestive enzymes may be the result of variation in

Table 3. Effects of condensed tannins in faba beans (Vicia faba L.) on the activity of digestive enzymes[1] in digesta in different animal species.

Species	Source	Level (g/kg)	Site of sampling	Trypsin[1]	Chymotrypsin[1]	α-amylase[1]	Lipase[1]	Reference
pigs	HT hulls[2]	200	duodenum prox. jejunum dist. jejunum	46 (ns) 62 (*) 68 (*)	42 (ns) 76 (ns) 107 (ns)			This thesis (Chapter 6)
pigs	HT hulls[2]	200	duodenum ileum	91 (ns) 70 (*)	99 (ns) 92 (ns)			This thesis (Chapter 7)
chickens	HT hulls[2]	400	prox. jejunum	28 (*)		35 (*)	61 (*)	Longstaff & McNab, 1991
chickens	HT hulls[2]	300	jejunum	38 (*)		22 (*)	68 (*)	Yuste et al., 1992
chickens	tannin extract	30	jejunum	45 (*)		25 (*)	68 (*)	Yuste et al., 1992
rats	HT hulls[2]	100	small intestine	70 (*)		28 (*)	290 (*)	Griffiths & Moseley, 1980

[1]expressed as values relative to activities measured in digesta of control groups fed diets with similar levels of low-tannin hulls of faba beans or diets without faba bean hulls. The activity for control groups is set at 100.
[2]HT hulls: hulls of faba beans of coloured-flowering, high-tannin varieties.
ns: not significant
* : $P<0.05$

affinity of faba bean tannins for the enzymes and other substrates for interaction *in vivo*. In this respect it is interesting to note that enzyme activity in digesta from tannin-fed animals could be increased by adding PVP. PVP has a very high affinity for tannins (Griffiths & Moseley, 1980; Longstaff & McNab, 1991). They concluded that formation of tannin-enzyme complexes is reversible. Significant effects of dietary tannins on the activity of digestive enzymes *in vivo* are mostly found when the level of tannins in the diets is high (Table 3). It may thus be questioned whether enzyme inhibition by tannins is of nutritional importance with moderate levels of tannins in the diet. It is assumed that pigs secrete digestive enzymes far in excess to what is required for degradation of nutritional substrates (Low, 1982). This may also be true for other monogastric species.

In rats it has been assumed that a significant part of dietary tannins is bound to PRPs in the proximal digestive tract. It leaves them unavailable for enzyme binding in the small intestine. Significant inhibition of digestive enzymes in rats in some cases suggests that the binding of PRPs with tannins is not complete. This may be related to the low solubilization rate of native tannins in feedstuffs *in vivo* (Chapter 3).

Effects on the mucosa of the small intestine

The literature suggests that dietary condensed and hydrolysable tannins may affect the activity of brushborder-bound enzymes and also the histology of the mucosa of the digestive tract (Chapter 2). A detailed study was made on biopsies from different sites of the small intestinal wall of piglets from the experiment described in Chapter 6. Results did not reveal histological changes in the mucosa of the small intestine of piglets receiving either low- or high-tannin faba bean hulls (200 g/kg). The activity of mucosa-bound sucrase-isomaltase was not affected by the inclusion of faba bean hulls in the diets. Longstaff & McNab (1991) did also not observe an effect on sucrase activity in jejunal mucosa in chickens when feeding high-tannin faba bean hulls at a level of 300 g/kg compared to that measured in chickens fed the same level of low-tannin hulls. In contrast, the activity of mucosal aminopeptidase was significantly reduced in pigs when high-tannin hulls were fed with the diet (P. van Leeuwen, personal communication).

Results of *in vitro* incubation studies with purified faba bean tannins and differentiated human colon carcinoma (Caco-2) cells indicated that condensed tannins reduce the length of the microvilli. There was also a decrease in activity of brushborder sucrase-isomaltase and an interference with the metabolism of these cells by tannins. This was derived from measurements of the incorporation of radioactively labelled precursors (J.F.J.G. Koninkx, personal communication). It appears therefore that free tannins from faba beans can exert harmful effects on mucosal cells in the intestines *in vivo*. It is likely, however, that the *in vivo* effects on the histology and function of mucosal cells are limited because of the large number of alternative binding sites for dietary tannins.

Effects of tannins on ileal and faecal digestibility of nutrients

In a study with piglets (Chapter 5), the ileal and faecal digestibilities of a low-, a medium- and two high-tannin varieties of faba beans were measured. The apparent faecal digestibility differed, particularly for nitrogen and amino acids, in favour of the low-tannin variety. These effects were mainly attributed to differences in tannin content of the beans.

When including only hulls of faba beans with a different tannin content in diets for pigs, again particular effects were found on both the ileal and faecal digestibility of crude protein (N x 6.25) and amino acids (Chapters 6 and 8). It was shown that about half of the decrease in protein digestibility could be attributed to a reduced true digestibility of the dietary protein. In addition, condensed tannins in faba beans increased the endogenous excretion of protein in ileal digesta and faeces in pigs. Our studies do not indicate any particular source of endogenous protein in pigs whose secretion is largely increased due to tannins. Therefore, it seems that tannins particularly decrease the intestinal reabsorption of endogenously secreted proteins.

It was also shown that faba bean tannins selectively bind to proteins *in vivo*. This results in marked differences in the effect of tannins on digestibility of individual amino acids. They seem to bind preferentially to proteins with a relatively high content of proline and histidine (Chapter 8).

Compared to the effects of tannins on protein digestibility the significance of effects on the digestibility of other nutrients such as ash, crude fat and carbohydrates is relatively small (Chapters 6 and 8). The former results agree with findings of other workers who studied effects of faba bean tannins on nutrient digestibility in chickens (Marquardt et al., 1977; Longstaff & McNab, 1991; Yuste et al., 1992; Trevino et al., 1992). A slight decrease of the digestibility of carbohydrates such as starch due to tannins, however, may contribute to the lower energetic value of high-tannin varieties of feedstuffs for different animal species (Cousins et al., 1981; Wareham, 1991). The relatively large difference in faecal digestibility of crude fibre in pigs fed low- or high tannin hulls (Chapter 6) may have some importance. It is hypothesized that fermentation of fibre-rich high-tannin hulls in the hindgut may be hampered by tannins. This can also contribute to a lower energetic value of high-tannin faba beans for pigs.

A supplementary study with pigs showed that tannins in faba beans also reduce the faecal availability of iron and copper, but not of calcium, zinc and magnesium (A.J.M. Jansman, unpublished results). Similar conclusions were drawn by Wolters et al. (1992) from *in vitro* studies. They evaluated these to predict the bioavailability of minerals in foods and feeds. The effects of tannins on mineral availability seem only important when limited amounts of minerals are available and/or their ratios are not optimal. In practice, adequate mineral supply in concentrates for monogastric animals is normally guaranteed by a slight oversupplementation of the diets.

In vitro tannins were shown to bind to carbohydrates (Ya et al., 1989) and minerals (Faithfull, 1984). It can be concluded that tannins from faba beans *in vivo* have a relatively low affinity for non-protein substrates when large amounts of proteins are available for interactions.

Effects of tannins on animal performance

In studies with chickens (Chapter 4), growth performance was measured using diets with 300 g/kg of either low- or high-tannin faba beans. No differences in feed intake, average daily gain and feed conversion efficiency were observed, provided that protein and essential amino acids were supplied at either 100 or 90% of their assumed requirements. Diets with 300 g/kg of faba beans contain between 30 and 45 g/kg of faba bean hulls. This means that the content of condensed tannins in the diets does not exceed 0.30% catechin equivalents. This concentration is markedly below that used in other studies with rats and chickens, in which the effects of faba bean tannins were evaluated. When feeding diets with 500 or 850-900 g/kg of whole faba beans (Martin-Tanguy et al., 1977; Marquardt & Ward, 1979), 300 or 400 g/kg of faba bean hulls (Yuste et al., 1992; Longstaff & McNab, 1991), or 30 or 60 g/kg of tannin-rich hull extract (Yuste et al., 1992; Marquardt et al., 1977) condensed tannins in faba beans had significant negative effects on growth performance, amino acid digestibility and activity of digestive enzymes. It was concluded from our studies that tannins in faba beans do not seem to be extremely harmful to growing chicks when high-tannin faba beans are included at levels below 300 g/kg. In addition, this inclusion level far exceeds levels commonly used in practice. Wiseman et al. (1991) and Wareham (1991) arrived at a similar conclusion after studying the effects of condensed tannins in hulls of faba beans on the metabolizable energy and apparent metabolizable nitrogen of diets in chickens. Only inclusion of more than 150 g/kg of high-tannin faba bean hulls significantly decreased these parameters.

When appreciable levels of condensed tannins are present in the diet for non-ruminant animals, which are not effectively bound by salivary proteins, complex formation with dietary and other endogenous proteins of tannins occurs. The resulting reduced intestinal absorption of amino acids limits the availability of amino acids for body protein synthesis. As a consequence, N retention and growth is reduced. This occurs when dietary supply of essential amino acids does not exceed the animal's requirements, as was shown for pigs (Chapter 6). The extent of the reduction in N retention depends on effects of tannins on the digestibility of individual essential amino acids. The latter may vary considerably (Chapters 6 and 8). The mode of action of condensed tannins seems to be similar for pigs and poultry. When high-tannin beans instead of low-tannin varieties are included at moderate levels (below 300 g/kg) in diets for pigs, effects of tannins on growth performance may thus be small. This is confirmed in studies with pigs, in which growth performance only tended to be reduced when diets with moderate levels of high-tannin beans were fed (Bourdon & Perez, 1984; Fekete et al., 1985).

In vitro and *in vivo* binding of tannins to proteins

Condensed tannins in faba beans appear to affect particularly the digestibility of protein *in vivo*. This is the result of the formation of stable complexes between tannins and proteins in the alimentary tract. Both hydrogen bonds and hydrophobic interactions

are responsible for the formation of complexes between tannins and proteins (Hagerman, 1989). Presumably, tannin-complexed proteins are less susceptible to degradation by proteolytic enzymes. As a result there is an increased excretion of protein. *In vitro* interactions between tannins and proteins are dependent on a large number of factors, including pH, presence of detergents, concentrations and nature of both tannins and proteins (Hagerman, 1989). It is assumed that the optimum pH for the formation of insoluble tannin-protein complexes is around the isoelectric point of the protein involved. *In vivo* a large number of diverse proteins are available for tannin binding. The pH in the digestive tract of monogastrics varies from 2-3 in the stomach to 8 in the hindgut. Therefore, tannins may bind to different proteins during their passage through the digestive tract. It also means that tannins complexed with dietary proteins in the oral cavity during chewing may be released in the stomach or proximal duodenum at low pH, and subsequently bind to other proteins of either exogenous (feed) or endogenous origin. In pigs binding of faba bean tannins to both exogenous and endogenous proteins was shown (Chapter 8).

There are a few studies on the *in vitro* binding capacity of tannins of different origin, in which only bovine serum albumin (BSA) was used as substrate. Some data from the literature are summarized in Table 4. Binding capacity, expressed as mg BSA bound per mg of purified tannin or tannin equivalent, ranges from 0.55 to 4.83. Variation in results can be explained by differences in reaction conditions, nature and origin of the tannins, ratio between tannins and proteins in the assay and purity of the tannins used. Protein-binding capacity of tannins as measured with BSA may be different from

Table 4. Estimated *in vitro* binding of protein by purified tannins and tannin-rich extracts.

Tannins	Proteins	Protein binding[1]	Units	Reference
Tannic acid	BSA[2]	2.60	g/g	Makkar et al., 1987
Quercus leaves extracts	BSA	2.89 - 3.76	g/g eq.[3]	Makkar et al, 1987
Quercus leaves extracts	BSA	2.40 - 3.90	g/g eq.[4]	Martin & Martin, 1982
Quercus incana leaves	BSA	4.18 - 4.83[5]	g/g eq.[3]	Makkar et al., 1988
Sorghum	BSA	2.05	g/g purified tannins	Asquith & Butler, 1986
Pinto beans	BSA	0.98		
Quebracho	BSA	0.55		
Wattle	BSA	0.63		

[1]Protein binding expressed as g protein bound per g of tannins or tannin equivalents.
[2]BSA: bovine serum albumin
[3]partly purified sorghum tannins
[4]bisulphited Quebracho tannins
[5]one value excluded

results obtained with other proteins. Similar studies with different commonly used feed proteins are needed to calculate the potential protein-binding capacity *in vivo*. It should be stressed, however, that reaction conditions *in vitro* can be rather different from those *in vivo*.

From the digestibility of crude protein and amino acids in tannin-fed animals and animals fed control diets, estimates were made for the *in vivo* binding of isolated condensed tannins, and tannins in extracts or in faba bean hulls. In Table 5 results of estimates obtained in different animal species are given. It was assumed that the total decrease in apparent digestibility of crude protein or amino acids in the tannin-fed animals was the result of tannins binding to proteins of either dietary or endogenous origin, rendering them indigestible. For calculating the data on protein binding the following formula was used:

$$B = [DC_{control} - DC_{test}] * P/T$$

B : protein binding (g/g equivalent of tannins or per g of tannin extract or tannin-rich faba bean hulls)

$DC_{control}$: ileal or faecal digestibility coefficient for CP or total amino acids for the control, low-tannin diet

DC_{test} : ileal or faecal digestibility coefficient for CP or total amino acids for the high-tannin experimental diet

P : mean protein content of the control and the high-tannin diet (g/kg)

T : difference between the control and the high-tannin diet in tannin content (g equivalent/kg) or in content of tannin-rich extract or high-tannin faba bean hulls (g/kg)

Data are expressed as grams of protein per unit of tannin equivalent, per gram of tannin-rich extract or per gram of tannin-rich faba bean hulls. The estimates for *in vivo* protein-binding capacities of condensed tannins from faba beans and sorghum ranged from 1.84 to 4.25 and from 0.14 to 1.32 g per g of tannin equivalents, respectively (Table 5a). Similar estimates from studies using tannin-rich extracts from faba beans or tannin-rich faba bean hulls range from 0.41 to 1.60 g per g of extract and from 0.06 to 0.18 g/g of hulls (Table 5, b and c). A significant part of the variation in results in Table 5 can be attributed to differences in the assay procedure used for tannin analysis (a), diet composition (a,b,c), origin and composition of tannins (a,b,c), purity of tannin extracts (b), animal species involved (a,b,c) and variety of the feedstuff used (a,b,c). It can be concluded that condensed tannins in different feedstuffs may bind *in vivo* up to similar amounts of protein as determined *in vitro* with BSA (Table 4). This means that *in vitro* protein binding by tannins may be a useful parameter for the prediction of *in vivo* effects on protein digestibility. In addition, it appears that the *in vivo* binding capacity of condensed tannins does not differ systematically between rats, chickens and pigs. To evaluate this the apparent digestibility of protein of identical diets should be measured in each of the animal species.

Table 5. Estimated *in vivo* binding of proteins by purified tannins, tannin-rich extracts or feedstuffs containing condensed tannins[1].

Tannins	Species	Protein binding[2]	Units[2]	Reference
Per unit of tannin equivalents (a)				
Faba beans	chickens	4.25	g/g eq.[2]	Marquardt et al., 1979
Hulls faba beans	pigs	3.36[5] 1.97[5]	g/g eq.[4]	This thesis (Chapter 8)
Hulls faba beans	pigs	2.14[7]	g/g eq.[4]	This thesis (Chapter 6)
Hulls faba beans	rats	2.78[6]	g/g eq.[4]	This thesis (Chapter 3)
Tannin extract faba beans	rats	3.38[6] 1.84[6]	g/g eq.[4] g/g eq.[4]	This thesis (Chapter 3)
Sorghum	chickens	4.63	g/g tannic acid eq.	Rostagno et al., 1973
Sorghum	chickens	1.22	g/g eq.[4]	Mitaru et al., 1985
Sorghum	pigs	0.14[5]	g/g eq.[4]	Mitaru et al., 1984
Sorghum	pigs	0.32[5] 0.42[5] 0.24[5]	g/g eq.[4]	Cousins et al., 1981
Per unit of tannin extract (b)				
Hull extract faba beans	chickens	1.39	g/g extract	Yuste et al., 1992
Hull extract faba beans	chickens	1.60	g/g extract	Marquardt et al., 1979
purified tannin extract faba beans	chickens	1.42	g/g extract	Marquardt et al., 1977
Hull extract faba beans	rats	0.80[6] 0.41[6]	g/g extract	This thesis (Chapter 3)
Extract black locust leaves	rats	0.62[7]	g/g extract	Horigome et al., 1988
Per unit of tannin-rich hulls (c)				
Hulls faba beans	chickens	0.18	g/g hulls	Yuste et al., 1992
Hulls faba beans	chickens	0.17	g/g hulls	Longstaff & McNab, 1991
Hulls faba beans	pigs	0.08[5] 0.06[5]	g/g hulls	This thesis (Chapter 8)
Hulls faba beans	pigs	0.07[7]	g/g hulls	This thesis (Chapter 6)
Hulls faba beans	rats	0.13[6]	g/g hulls	This thesis (Chapter 3)

[1]Estimates are based on differences in digestibility of crude protein (Nx6.25) or total amino acids (AA)between tannin-fed groups and control groups, assuming that these can be fully attributed to differences in tannin content or inclusion of a tannin-rich ingredient.
[2]*in vivo* protein binding is expressed as grams of protein per gram of tannin equivalent, per gram of tannin-rich extract or per gram of tannin-rich faba bean hulls.
[3]purified faba bean tannins
[4]catechin equivalents
[5]based on ileal digestibility figures
[6]based on caecal digestibility figures
[7]based on faecal digestibility figures

In vivo proteins bind to polyphenolic compounds present in feedstuffs or extracts. They are analysed as tannins in commonly used procedures for tannin determination. The individual compounds may vary in binding affinity and capacity and in sensitivity in assays for tannin analysis. It thus depends on the structural heterogeneity of tannins whether the *in vivo* effects of condensed tannins on protein digestibility can be predicted from their analysed tannin content. The affinity and capacity of tannins to bind to dietary and endogenous proteins can vary largely. Hagerman & Butler (1981) showed that condensed tannins from sorghum differ markedly from BSA in affinity for various proteins. It is likely that similar differences exist for faba bean tannins.

The *in vivo* effects of tannins on protein digestibility may also be assessed in an *in vitro* system. Figure 1 shows the relationship between the apparent ileal digestibility of crude protein (N x 6.25) in pigs of diets with different levels of hulls of low- and high-tannin varieties of faba bean in pigs and the *in vitro* crude protein digestibility values of the same diets. The latter were measured by the method of Babinszky et al. (1990). The *in vitro* protein digestibility appears to be highly correlated with *in vivo* results (r = 0.94). Garrido et al. (1991) found a strong negative correlation between the *in vitro* digestibility of 24 varieties of faba beans and their tannin content (r = -0.88). Robbins et al. (1987) measured the digestibility of different tannin-containing forages in large herbivorous ruminants (deer, moose, caribou and elk). They found that the reduction in protein digestibility of forages was proportional to the *in vitro* BSA-precipitating capacity of extracts from these forages. These data suggest that *in vivo* effects of (condensed) tannins in faba beans on crude protein digestibility are similar to those in *in vitro* protein digestibility studies. Effects of faba bean tannins on mineral availability *in vivo* were also measured in *in vitro* studies (Wolters, 1992).

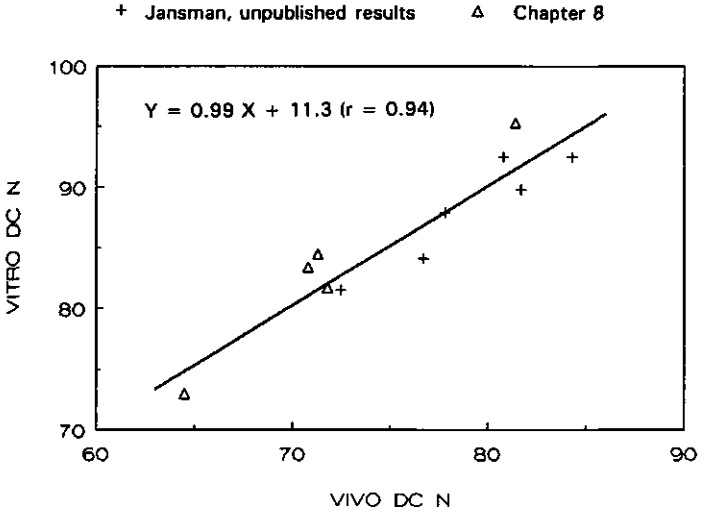

Figure 1. Relationship between digestibility of crude protein *in vitro* and the apparent ileal digestibility *in vivo* (in pigs) of diets containing different levels of low-tannin and high-tannin faba bean hulls.

Concluding remarks

The results described in this thesis show that, in rats and pigs, tannins in faba bean hulls reduce the apparent digestibility of nutrients, in particular of protein and amino acids.

In rats an increased excretion of protein after feeding faba bean tannins is mainly the result of an increased secretion of tannin-induced proline-rich proteins by the parotid glands. These proteins likely bind to dietary tannins thereby protecting other endogenous and feed proteins.

In contrast, in pigs only about half of the increased ileal and faecal excretion of protein when feeding faba bean tannins was shown to be of endogenous origin. Part of the additional excreted endogenous protein in pigs may consist of other proteins such as digestive enzymes and proteins from sloughed-off mucosal cells, which are complexed with tannins. No clear indications were found that pigs, like rats, protect themselves against tannins by increasing the secretion of specific proline-rich proteins.

A dose-response study with pigs fed increasing levels of high-tannin faba bean hulls, which were exchanged against low-tannin hulls, showed that negative effects of tannins on ileal and faecal digestibility of protein and amino acids and on activity of trypsin and chymotrypsin were linear (A.J.M. Jansman; unpublished results). This indicates that a threshold level for faba tannins in diets for pigs does not exist. A threshold level is defined as the maximum level of a factor in the diet that can be tolerated without causing antinutritional effects. The existence of such values has been suggested for other antinutritional factors, such as trypsin inhibitors and lectins (Huisman, 1990). The mode of action of these ANFs, however, differs markedly from that of condensed tannins.

The general concept for effects and mode of action of condensed tannins in feeds for non-ruminant animals, as defined in the beginning of this chapter, appears to be also largely valid for condensed tannins from faba beans in pigs. Literature data and results presented in this thesis show similar antinutritional effects and a similar mode of action of condensed tannins from faba beans in chickens and pigs.

Some of the *in vivo* effects of faba bean tannins in non-ruminant animal species may be well predicted from *in vitro* assays.

It can be concluded that condensed tannins in faba beans reduce the nutritive value of faba beans for pigs, and probably also for poultry. More information is required, however, on the structural heterogeneity of tannins in faba beans, their separate biological effects and the sensitivity of individual compounds in commonly used assays for tannins. A higher level of condensed tannins in faba beans than commonly found in white-flowering varieties may be desirable for agronomic reasons. This may especially become important when the use of pesticides in agricultural crop production has to decrease. Consequences for a reduced nutritive value of faba beans for non-ruminant species should then be further evaluated. It may be that antinutritional effects and the seed-protecting properties are fulfilled by various types or fractions of tannins. They need to be evaluated individually. The chemical structure of faba bean tannins and the antinutritional effects of each of the individual compounds have not

been studied extensively so far because of a lack of suitable analytical methods and large-scale isolation and purification methods for tannins.

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Summary

The search for alternative protein sources replacing soya in feeds for monogastric farm animals has increased in western Europe in the past decade. Among the alternatives, faba bean (Vicia faba L.) has attracted considerable attention. Faba beans contain fairly high levels of protein and can be grown well under climatic conditions prevailing in western Europe. The potential nutritive value of faba beans for monogastric animals is, however, not fully exploited because of the presence of antinutritional factors (ANFs). In faba beans, trypsin inhibitors, lectins, vicine/convicine and condensed tannins have been found as ANFs. Condensed tannins are considered to be particularly important with regard to the nutritive value of faba beans. The levels of condensed tannins in this legume seed vary widely between different varieties. Coloured-flowering varieties often contain fairly high levels of tannins (up to 1.5% catechin equivalents), whereas beans of white-flowering cultivars are known as low-tannin beans (<0.1% catechin equivalents). Condensed tannins can play a role in the defence of plants and seeds against predation, infestation by moulds and micro-organisms and in disease resistance. Hence, for agronomic reasons, a certain level of condensed tannins in faba beans may be desirable, especially in view of the aim to reduce the use of pesticides.

Tannins are defined as water-soluble polyphenolic compounds with the ability to precipitate proteins in aqueous solutions. A distinction can be made between hydrolysable and condensed tannins. The former have a central carbohydrate core to which phenolic carboxylic acids, such as gallic acid and ellagic acid are esterified. Condensed tannins are polymers of mainly flavan-3-ol (catechin), which are covalently bound. Tannins are found in many plant species and can be found in different morphological parts, such as leaves, stems, fruits and seeds. The chemistry of tannins is considered to be very complicated. Most commonly used analytical methods (colorimetric methods) give an estimate of the total tannin content. Procedures for analysing individual molecular structures, which together are referred to as tannins, are lacking. Tannins (hydrolysable and condensed) in diets for monogastric animals can exert a large number of antinutritional effects (Chapter 2). These include a reduction of feed intake as a result of their astringent taste, a decrease of the apparent digestibility of nutrients, a reduction of the activity of digestive enzymes, damage to the mucosa of the gastro-intestinal tract and systemic/toxic effects on the organism (some organs). These effects result in a reduced performance of animals after tannin consumption.

In this thesis studies on the antinutritional properties of condensed tannins in faba beans in monogastric animals (rats, chickens and pigs) are reported. Particular emphasis has been laid on the nutritional effects in pigs, since information on this species is scarce. Rats have been included as well because this species is often used as a model for evaluating the antinutritional effects of tannins.

In rats the effects of faba bean tannins on the salivary secretion of proteins synthesized in the parotid glands were studied (Chapter 3). Both tannin-rich faba bean hulls and isolated condensed tannins from faba beans increased the size of the parotid glands and the secretion of salivary proline-rich proteins (PRPs). Tannins reduced the apparent digestibility of crude protein and of proline, glycine and glutamic acid in

particular. These amino acids form the major constituents of PRPs. It is concluded that PRPs are secreted by the parotid glands to bind tannins rapidly after ingestion, thereby preventing harmful effects of these compounds during their passage through the gastro-intestinal tract. PRPs can therefore be seen as a mode of defence of rats against dietary tannins. As a consequence, tannin-fed rats were able to maintain an adequate growth rate.

In subsequent investigations, research was carried out with chickens and pigs. Both can be considered as target species because faba beans are considered to be a suitable protein source for these animals. In two growth trials with chickens the nutritive value of faba beans with different levels of condensed tannins was evaluated (Chapter 4). It appeared that inclusion in the diets of 300 g/kg of whole faba beans with either a low or high tannin content did not affect growth performance of broiler chickens. In a digestibility trial with piglets, however, the ileal and faecal digestibilities of faba beans, in particular of protein and amino acids, were significantly lower for high-tannin faba beans than for a low-tannin variety (Chapter 5). Therefore further research was carried out to establish the nature and extent of effects of tannins in faba beans in pigs. Since tannins in faba beans are confined to the hulls (testa) of the seeds, this fraction of the seed was used as a source of tannins. Hulls make up 100-150 g/kg of faba beans. In control diets, hulls of low-tannin faba beans were included. In a study with young piglets (Chapter 6), effects of tannins in faba beans on the apparent faecal digestibility of nutrients, on nitrogen (N) retention, on enzyme activity in digesta from the small intestine, and on organ weights were evaluated. Tannins reduced the apparent digestibility of nutrients, of protein and amino acids in particular, and tended to reduce N retention. Activity of trypsin and chymotrypsin in digesta from the small intestine was lower in tannin-fed pigs. Weight and gross histology of liver, kidneys, spleen and parotid glands were not affected when diets with 200 g/kg of high-tannin faba bean hulls were fed. It could be concluded that faba bean tannins interfere with the digestive process itself, but do not cause systemic toxic effects in pigs. In a further study (Chapter 7), the effects of faba bean tannins on the activity of the proteolytic enzymes trypsin and chymotrypsin were more thoroughly investigated. The pigs used had been cannulated in the proximal duodenum and in the terminal ileum. Activity of both enzymes remained unaffected in duodenal digesta. Condensed tannins from faba beans significantly reduced the activity of trypsin in ileal digesta of pigs, but did not significantly lower the activity of chymotrypsin. It was thought that tannins have a higher binding affinity for porcine trypsin than for chymotrypsin. It is questionable whether a decrease of the digestive capacity of tannin-fed pigs is responsible for the effects of tannins on nutrient (protein) digestibility since pigs secrete digestive enzymes in far excess of the amount required for nutrient degradation.

Further experiments were performed to establish the effects of tannins in faba bean hulls on the ileal digestibility of nutrients in pigs. In addition, the effects of tannins on the true ileal and faecal digestibility of dietary protein were measured by determining the endogenous excretion of protein in ileal digesta and faeces. This was done by using the ^{15}N isotope dilution technique (Chapter 8). This technique allows to distinguish endogenous N from undigested N of dietary origin in ileal digesta and

faeces. Results show a significant reduction in apparent ileal digestibility of protein and amino acids in diets containing 200 g/kg of high-tannin instead of low-tannin faba bean hulls. It appeared that about half of the effect of tannins on apparent protein digestibility was due to a decrease in true digestibility of dietary proteins. In addition, faba beans tannins increased the excretion of endogenously secreted proteins. These may consist in part of secretions from digestive organs (enzymes) and of proteins from sloughed-off mucosal cells. It has been hypothesized that tannins from faba beans reduce apparent protein digestibility in pigs by binding to both dietary and endogenous proteins. Binding of tannins to proteins prevents enzymatic digestion of the dietary protein and degradation and reabsorption of endogenously secreted proteins. Tannins from faba beans showed *in vivo* some selectivity in binding to certain proteins. This was concluded from the differences in effects of tannins on the apparent digestibility of individual amino acids. They seem to bind preferentially to proteins with a high content of proline and histidine. This can be related to the hydrophobic bonds involved in tannin-protein interactions.

From the results of the studies presented in this thesis a concept for the mode of action of condensed tannins in monogastric animals was developed. This is discussed in Chapter 9. It is assumed that in the alimentary tract a significant part of the tannins in feedstuffs are released by solubilization. This process starts in the oral cavity after ingestion of the diet. Here the dissolved tannins may bind to both nutrients from the diet, particularly protein, and to salivary proteins. Rats rapidly increase the production of salivary proteins with a high affinity of tannins after consumption of high-tannin diets. No evidence was found in our studies or in investigations reported in the literature that pigs and poultry are able to respond to a similar extent. The process of solubilization of tannins and their binding to dietary nutrients and endogenously secreted substances continues throughout the gastro-intestinal tract. The literature indicates that interactions between tannins and proteins depend on the pH of the environment, and also on the nature of the tannins and the proteins involved. Since the pH varies along the gastro-intestinal tract, nutrients complexed with tannins in the proximal part may be released at a low pH in the stomach. Therefore, tannins may subsequently interact with digestive enzymes and mucosal proteins in the small intestine. Tannins were assumed to complex enzymes *in vivo*. This was concluded from the reduction in activity of digestive enzymes when feeding tannins. The decrease in activity of digestive enzymes in the proximal digestive tract of pigs is relatively small. As a consequence, faba bean tannins do not interfere with the regulation of secretion of pancreatic enzymes in pigs. Histological changes, which could occur as a result of binding of tannins to the mucosa, were not observed in pigs on a diet containing faba bean tannins. This can be explained by the large number of alternative binding sites that tannins have *in vivo*. Effects of tannins on the performance of monogastric animals are largely due to a reduction of the apparent digestibility of nutrients, of in particular protein and amino acids. When supply of essential amino acids is marginal, tannins can decrease N retention and performance in growing animals. In addition, tannins are assumed to reduce the energetic value of the diet because they interfere with the digestibility of energy-containing nutrients. Based on an evaluation of the antinutritional effects of tannins in pigs and poultry

described in this thesis and in the literature, it is concluded that the mode of action and effects of tannins from faba beans in pigs and poultry do not differ largely. The absence of effects of faba bean tannins on performance in growth trials with chickens (Chapter 4) may be related to the low tannin content of the diets used (<0.3% catechin equivalents). Literature data show that faba bean tannins at higher levels in diets for poultry can reduce nutrient digestibility and performance in a similar way as in pigs.

It is concluded that condensed tannins significantly reduce the nutritive value of faba beans for monogastric animals, in particular by reducing the apparent digestibility of protein and amino acids. This can lead to a reduction in performance, in particular when diets are formulated in agreement with the animal's physiological need for nutrients. When low levels of high-tannin faba beans are present in the diets, however, antinutritional effects may be relatively small. Tannins also increase faecal excretion of nitrogen. This can be considered as an additional negative effect from the environmental point of view. In future research the chemical structure and nature of tannins in faba beans should be further elucidated. The antinutritional effects of each of the individual polyphenolic compounds found in faba beans, in particular the variation in affinity and capacity to bind nutrients, should be studied. Since some of the effects of tannins on nutrient digestibility can be evaluated *in vitro*, the use of in vitro methods for determining antinutritional effects of tannins should be further explored. In addition, the possible natural function of individual polyphenolic compounds and other substances involved in plant and seed protection should be evaluated. This information could form the basis for the development of new varieties of faba beans with a low tannin content, a low susceptibility to infestation by micro-organisms and to diseases, and a high nutritive value.

Samenvatting

In de EG bestaat het streven om meer in Europa verbouwde eiwit-houdende grondstoffen in veevoeders te verwerken. Dit vermindert enerzijds de afhankelijkheid van geïmporteerde eiwitbronnen als soja. Anderzijds wordt hierdoor een bijdrage geleverd aan de beperking van import van grote hoeveelheden mineralen via veevoedergrondstoffen. Het mineralenoverschot leidt in toenemende mate tot milieuproblemen in de intensieve veehouderij.

Als alternatieve eiwitbronnen in veevoeders staan het laatste decennium onder andere erwten (Pisum sativum L.) en veldbonen (Vicia faba L.) in de belangstelling. De opname van deze grondstoffen in voeders voor éénmagige landbouwhuisdieren wordt echter beperkt door antinutritionele factoren (ANF's) in deze peulvruchten. ANF's zijn van nature aanwezige stoffen die onder meer een belangrijke rol spelen bij de bescherming van het zaad tegen vraat en aantasting door schimmels en micro-organismen en bij de resistentie tegen ziekten. Zij worden daarom ook wel als biopesticiden aangeduid. ANF's in voeders hebben echter negatieve effecten op de groei, produktie en/of gezondheid van dieren. In veldbonen komen onder andere de volgende ANF's voor: trypsineremmers, lectinen, vicine/convicine en gecondenseerde tanninen. De laatstgenoemde worden vooral in verband gebracht met de nutritionele waarde van veldbonen. Tanninen zijn wateroplosbare polyfenolen met een molecuulgewicht tussen 500 en 3000 daltons, die in staat zijn eiwitten neer te slaan in waterige oplossingen. Het tanninegehalte in veldbonen varieert aanzienlijk tussen variëteiten. Traditionele bontbloeiende rassen hebben veelal een relatief hoog tanninegehalte (0.5 - 1.5% catechine equivalenten bij analyse volgens de vanilline-zwavelzuur methode). Via veredeling heeft men witbloeiende variëteiten ontwikkeld, die een zeer laag gehalte aan tanninen hebben (<0.1% catechine equivalenten). Sommige van de laatstgenoemde variëteiten blijken echter gevoeliger voor verschillende gewasziekten te zijn. Bij de teelt van veldbonen lijkt het dus wenselijk rassen te gebruiken met een tanninegehalte dat hoger is dan het gehalte in de zaden van witbloeiende variëteiten.

Tanninen in voeders voor éénmagige diersoorten kunnen echter antinutritionele effecten hebben. In een literatuuronderzoek (Hoofdstuk 2) zijn de belangrijkste eigenschappen en antinutritionele effecten van tanninen in plantaardige veevoedergrondstoffen beschreven. Tanninen worden onderverdeeld in hydrolyseerbare en gecondenseerde tanninen. De eerstgenoemde bestaan uit esters van koolhydraten (o.a. glucose) met galluszuur en oligomeren ervan. Looizuur is een bekend voorbeeld van een hydrolyseerbare tannine. Gecondenseerde tanninen bestaan veelal uit polymeren van catechine, die via covalente bindingen zijn gekoppeld. Tanninen komen voor in een zeer groot aantal plantesoorten en worden aangetroffen in verschillende morfologische delen van de plant, zoals bladeren, stengels, vruchten, zaden en de bast van bomen en struiken. Gecondenseerde tanninen worden onder andere aangetroffen in sorghum en in zaden van verschillende vlinderbloemigen, zoals erwten en veldbonen. De exacte chemische structuur van tanninen en het voorkomen ervan in verschillende grondstoffen is nog niet volledig duidelijk als gevolg van het ontbreken van adequate analyse- en identificatietechnieken. Analyse van tanninen vindt veelal plaats met behulp van colorimetrische methoden. Deze methoden houden geen rekening met het feit dat tanninen in grondstoffen bestaan uit een groot aantal verwante stoffen, met

bepaalde overeenkomstige eigenschappen. Deze kunnen echter verschillen in schadelijkheid voor het dier en in gevoeligheid in de analyse. Tanninen kunnen als gevolg van hun adstringerende werking negatieve effecten hebben op de voeropname, de schijnbare verteerbaarheid van nutriënten, de activiteit van verteringsenzymen en het functioneren van de darmwandmucosa. Tevens kunnen volgens de literatuur bepaalde tanninen aanleiding geven tot systemische/toxische effecten en kan de benutting van geabsorbeerde nutriënten worden beïnvloed. Uit het literatuuronderzoek blijkt dat de nutritionele effecten van gecondenseerde tanninen in veldbonen op éénmagige landbouwhuisdieren (varkens en pluimvee) onvoldoende bekend zijn. Daarom zijn ook de consequenties van tanninen voor de voederwaarde van veldbonen niet volledig duidelijk. In het in dit proefschrift beschreven onderzoek zijn daarom de antinutritionele effecten van tanninen in veldbonen bij enkele diersoorten (ratten, kuikens en varkens) nader onderzocht. Ratten werden in het onderzoek betrokken omdat deze vaak als modeldier in het onderzoek naar antinutritionele effecten van tanninen worden gebruikt. Varkens en pluimvee kunnen in het kader van het onderzoek als doeldier worden aangemerkt.

Gecondenseerde tanninen uit sorghum bleken de eiwitsecretie van de oorspeekselklieren (glandula parotis) te beïnvloeden (Hoofdstuk 2). Daarom werden in het onderzoek met ratten de effecten van geïsoleerde tanninen uit veldbonen en veldboonschillen met een hoog tanninegehalte op de eiwitsecretieactiviteit van de oorspeekselklieren en op de verteerbaarheid van nutriënten (aminozuren) onderzocht. Opname in de rantsoenen van veldboonschillen met een hoog tanninegehalte (400 g/kg) en van een tannine-extract uit veldbonen (60 g/kg) leidde binnen zeven dagen tot een twee- tot vijfvoudige vergroting van de oorspeekselklieren. Tevens werd de secretie van bepaalde prolinerijke eiwitten (PRP's) sterk gestimuleerd. De schijnbare verteerbaarheid van het eiwit, met name van proline, glycine en glutaminezuur, werd sterk verlaagd. Juist deze aminozuren bleken in belangrijke mate voor te komen in PRP's die door de oorspeekselklier worden uitgescheiden. Er werd daarom geconcludeerd dat tanninen in veldbonen de eiwitsecretie door de oorspeekselklieren van ratten stimuleren. Waarschijnlijk hebben deze eiwitten een zeer hoge bindingsaffiniteit voor tanninen. Binding van tanninen met PRP's voorkomt schadelijke effecten van tanninen in hun verdere gang door het maagdarmkanaal. Tanninen in rantsoenen vergroten dus met name de faecale uitscheiding van enkele niet-essentiële aminozuren. De effecten van tanninen in de rantsoenen op de groei van ratten bleven als gevolg hiervan beperkt.

In twee experimenten met slachtkuikens werd de groei en voederconversie gemeten van dieren op rantsoenen met 300 g/kg veldbonen met een hoog of laag gehalte aan tanninen. Noch de groei noch de voederconversie werd negatief beïnvloed door opname van veldbonen met een hoog tanninegehalte in het rantsoen (Hoofdstuk 4). In een verteringsproef met jonge biggen werd de ileale en faecale verteerbaarheid van veldbonen met een laag en hoog tanninegehalte bepaald (Hoofdstuk 5). De verteerbaarheid van eiwit in veldbonen van een variëteit met een laag tanninegehalte (<0.1% catechine equivalenten) enerzijds en een variëteit met een matig (0.4%) en met een hoog tanninegehalte (1.0%) anderzijds was respektievelijk 13 en 7 eenheden hoger op ileaal en faecaal nivo. Deze uitkomsten suggereren dat tanninen in veldbonen

een belangrijk negatief effect op de verteerbaarheid van met name eiwit hebben. Er kunnen echter ook andere factoren hebben bijgedragen aan de verschillen in schijnbare verteerbaarheid, zoals verschillen in gehalten aan andere antinutritionele factoren en verschillen in de ware verteerbaarheid van het eiwit.

In het vervolgonderzoek werden de effecten van tanninen in veldbonen in varkens daarom nader onderzocht. Aangezien tanninen in veldbonen zich uitsluitend in de zaadhuid bevinden, werden in dit onderzoek alleen de zaadhuiden (schilfractie) van de veldboon als bron van tanninen gebruikt. Het aandeel zaadhuid in veldbonen bedraagt tussen 100 en 150 g/kg. Zaadhuiden van veldbonen zijn tevens vezelrijk. Het gehalte aan 'neutral detergent fibre' (NDF) varieert tussen 700 en 800 g/kg. Door vergelijking van opname in het voer van 200 g/kg veldboonschillen met een hoog tanninegehalte (3.3% catechine equivalenten) en met een laag tanninegehalte (<0.1% catechine equivalenten) werden effecten van tanninen gemeten. In een balansproef met jonge biggen werden effecten op de faecale verteerbaarheid van nutriënten, de N-retentie, de activiteit van enkele verteringsenzymen in chymus uit de dunne darm en het gewicht van enkele organen bestudeerd (Hoofdstuk 6). Veldboontanninen reduceerden de schijnbare faecale verteerbaarheid van eiwit (8 eenheden) en de verschillende aminozuren (5-19 eenheden). De verteerbaarheid van de overige koolhydraatfractie (4 eenheden) werd in mindere mate beïnvloed. De groei, de N-retentie en de activiteit van trypsine en chymotrypsine in de inhoud van de dunne darm van dieren op het rantsoen met veldboonschillen met een hoog tanninegehalte was verlaagd. Tanninen bleken geen effect te hebben op het gewicht van de lever, nieren, milt en de oor- en onderkaakspeekselklieren. Uit dit experiment werd geconcludeerd dat tanninen in veldbonen bij varkens met name invloed hebben op de vertering van nutriënten, in het bijzonder van eiwit en aminozuren. Deze resultaten suggereren tevens dat tanninen in veldbonen geen ernstig toxische effecten veroorzaken. In een volgende studie werden de effecten van tanninen uit veldbonen op de activiteit van trypsine en chymotrypsine in de dunne darm van varkens nader onderzocht (Hoofdstuk 7). Beide enzymen spelen een belangrijke rol bij de vertering van eiwit. De in het onderzoek betrokken varkens waren voorzien van een enkelvoudige T-canule vooraan in het duodenum en van een PVTC canule aan het einde van het ileum. Rantsoenen bevatten 200 g/kg schillen met een laag of een hoog tanninegehalte. De activiteit van trypsine en chymotrypsine in chymus uit het duodenum werd niet door de samenstelling van het rantsoen beïnvloed. In chymus verzameld op het einde van de dunne darm werd echter een lagere activiteit van trypsine gemeten bij verstrekking van het rantsoen met een hoog tanninegehalte. Het effect van tanninen op de activiteit van enzymen kan worden verklaard door de vorming van tanninen-enzymcomplexen, waardoor de activiteit van het enzym vermindert of verloren gaat. De activiteit van chymotrypsine werd niet significant verlaagd. De verschillen in resultaten ten aanzien van enzymactiviteit van duodenale en ileale chymus kunnen worden verklaard uit verschillen in samenstelling (verhouding tussen endogeen en exogeen eiwit) en door verschillen in fysische/chemische condities (o.a. pH) van beide soorten chymus. Resultaten ten aanzien van de activiteit van enzymen in ileale chymus duiden op een grotere affiniteit van tanninen uit veldbonen voor trypsine dan voor chymotrypsine. In de literatuur wordt vermeld dat varkens verteringsenzymen in een grote overmaat produceren. Gebleken is dat aan het einde van de dunne darm nog een aanzienlijke activiteit van verteringsenzymen resteert bij

verstrekking van een rantsoen met een hoog tanninegehalte. Daarom werd geconcludeerd dat het effect van tanninen uit veldbonen op de schijnbare verteerbaarheid van het eiwit niet kan worden verklaard uit een reductie van de verteringscapaciteit van het dier zelf.

Vervolgens werden de effecten van tanninen op de schijnbare ileale verteerbaarheid van nutriënten, in het bijzonder van eiwit en aminozuren, in jonge biggen vastgesteld (Hoofdstuk 8). Bovendien werd het effect van tanninen op de ware verteerbaarheid van het voereiwit en de endogene uitscheiding van eiwit in ileale chymus en faeces bestudeerd. Voor het laatstgenoemde werd gebruik gemaakt van de zogenaamde ^{15}N-verdunningstechniek. Hierbij wordt het lichaamseiwit (endogeen eiwit) van het varken gemarkeerd middels continue infusie in het bloed van ^{15}N gemerkt leucine. Op deze wijze is stikstof van endogene oorsprong in chymus en faeces te onderscheiden van niet verteerd stikstof dat afkomstig is van het voer.

Opname van 200 g/kg veldboonschillen met een hoog tanninegehalte reduceert de schijnbare ileale verteerbaarheid van eiwit met 7-10 eenheden en van verschillende aminozuren met 4-29 eenheden. Van het extra eiwit dat in chymus en faeces wordt aangetroffen, blijkt de helft te bestaan uit endogeen eiwit. De andere helft bestaat uit een toename van niet verteerd eiwit uit het voer. Tanninen uit veldbonen reduceren dus de ware verteerbaarheid van het voereiwit en vergroten de verliezen aan endogeen eiwit. De uitkomsten van het onderzoek suggereren dat het laatste niet zozeer een gevolg is van extra secretie van endogeen eiwit in het maagdarmkanaal, maar van een verminderde terugresorptie van endogeen uitgescheiden eiwitten. Deze laatste kunnen bestaan uit speekseleiwitten, verteringsenzymen en eiwit afkomstig van de gal en van afgestoten darmwandcellen. De reductie in schijnbare verteerbaarheid van eiwit door tanninen lijkt een gevolg te zijn van complexvorming van tanninen met zowel voereiwitten als eiwitten van endogene oorsprong. Hierdoor worden deze eiwitten minder toegankelijk voor verteringsenzymen, waardoor een groter deel van het eiwit het lichaam via de faeces verlaat. Ook duiden de resultaten op een zekere selectiviteit van tanninen voor binding aan bepaalde eiwitten. Eiwitten die rijk zijn aan proline en histidine worden met een zekere voorkeur door tanninen gebonden. Dit kan worden afgeleid uit het effect van tanninen op de schijnbare verteerbaarheid van individuele aminozuren. De selectiviteit van tanninen ten aanzien van binding met eiwitten kan worden verklaard door hydrofobe interacties die een belangrijke rol spelen bij de complexvorming tussen tanninen en eiwitten.

In de slotdiscussie (Hoofdstuk 9) wordt een concept voor de nutritionele effecten van gecondenseerde tanninen in veldbonen in éénmagige diersoorten besproken. Er wordt verondersteld dat tanninen na orale opname voor een deel tijdens hun gang door het verteringskanaal in oplossing gaan. De opgeloste tanninen kunnen zich vervolgens binden aan nutriënten uit het voer en/of aan endogeen uitgescheiden componenten. In ratten vindt een sterke stimulering van uitscheiding van prolinerijke speekseleiwitten met een hoge affiniteit voor tanninen plaats. In onderzoek met varkens, zoals beschreven in dit proefschrift en in de literatuur zijn geen duidelijke aanwijzingen gevonden voor het bestaan van een soortgelijke respons bij varkens en pluimvee.

Tijdens de verdere passage van het voer door het verteringskanaal lossen steeds meer tanninen op. Deze kunnen vervolgens nutriënten en endogene componenten

binden. Volgens de literatuur hangen de interacties tussen tanninen en eiwitten onder andere samen met de pH van het milieu waarin deze zich bevinden. De pH van digesta varieert sterk binnen het verteringskanaal. Hierdoor is het mogelijk dat tannine-eiwitcomplexen dissociëren bij een lage pH in de maag. Als gevolg hiervan kunnen tanninen die reeds in een eerder stadium waren gecomplexeerd, interacties aangaan met verteringsenzymen en de mucosa van de dunne darm. Beschadiging van het dunne-darmslijmvlies van varkens bij opname van hoge gehalten aan veldboontanninen werd echter niet waargenomen. Als verklaring hiervoor kan het grote aantal alternatieve bindingsplaatsen voor taninnen *in vivo* worden aangegeven. De negatieve effecten van tanninen op de groei en produktie van éénmagigen zijn met name het gevolg van effecten op de schijnbare verteerbaarheid van eiwit en aminozuren. Wanneer de essentiële aminozuren in marginale hoeveelheden in het voer voorkomen kunnen tanninen de N-aanzet en produktie reduceren.

Na bestudering van de effecten van tanninen in veldbonen in de literatuur en in onderzoek beschreven in dit proefschrift wordt verondersteld dat de antinutritionele effecten van tanninen uit veldbonen niet sterk verschillen bij varkens en pluimvee. Het feit dat er geen effecten werden waargenomen ten aanzien van de groei en voederconversie van slachtkuikens bij studies beschreven in Hoofdstuk 3, moet waarschijnlijk worden toegeschreven aan de relatief lage tanninegehalten (<0.3% catechine equivalenten) in het voer bij opname van 300 g/kg veldbonen met een hoog tanninegehalte. Bij hogere tanninenconcentraties in de rantsoenen voor pluimvee werden in de literatuur vergelijkbare negatieve effecten op onder andere de verteerbaarheid van nutriënten en de groei van pluimvee waargenomen als bij varkens.

Geconcludeerd kan worden dat gecondenseerde tanninen in veldbonen de voederwaarde van deze grondstof negatief beïnvloeden. De negatieve effecten zijn met name toe te schrijven aan een verlaging van de schijnbare verteerbaarheid van eiwit en aminozuren. De *in vivo* affiniteit van tanninen voor andere nutriënten, zoals koolhydraten en mineralen, blijkt relatief laag te zijn. Afname van de eiwit- en aminozuurverteerbaarheid kan leiden tot een lagere groei en produktie van dieren, met name wanneer essentiële aminozuren niet in overmaat in het voer voorkomen. Bovendien leidt de aanwezigheid van tanninen in rantsoenen voor éénmagige landbouwhuisdieren tot een verhoogde uitscheiding van stikstof via de mest. Dit kan vanuit milieuoogpunt als ongewenst worden beschouwd.

In toekomstig onderzoek zal de nadruk moeten worden gelegd op het analyseren en karakteriseren van de te onderscheiden componenten in de groep van verbindingen die door de thans gangbare analysemethoden als tanninen worden aangeduid. De antinutritionele effecten van elk van deze componenten zal moeten worden onderzocht. Dit geldt met name voor de bindingsaffiniteit en -capaciteit van deze polyfenolen. Aangezien de antinutritionele effecten van tanninen voor een deel ook in *in vitro* verteringsstudies kunnen worden vastgesteld, zal verdere ontwikkeling van deze technieken voldoende aandacht moeten krijgen. Tevens zal het belang van deze individuele (poly)fenolen en andere factoren voor de bescherming van het gewas tegen microbiële aantasting en voor ziekteresistentie moeten worden vastgesteld. Dit kan leiden tot de ontwikkeling van nieuwe variëteiten veldbonen met een laag tanninegehalte, een goede ziekteresistentie en een hoge nutritionele kwaliteit.

CURRICULUM VITAE

Alfonsus Johannes Maria Jansman werd op 11 september 1962 geboren te Oosterbeek. In 1980 behaalde hij het diploma Gymnasium B aan het Katholiek Gelders Lyceum te Arnhem. In datzelfde jaar begon hij met de studie Zoötechniek aan de toenmalige Landbouwhogeschool te Wageningen.
Het doctoraal examen omvatte Gezondheids- en ziekteleer der huisdieren en De leer van het grasland als hoofdvakken en Veevoeding en Agrarische bedrijfseconomie als bijvakken.
Na het afstuderen in april 1987 werd hij aangesteld als universitair docent in tijdelijke dienst bij de Landbouwuniversiteit, vakgroep Veevoeding met een gedeeltelijke detachering bij TNO als wetenschappelijk medewerker.
In 1988 werd gestart met het onderzoek naar de antinutritionele effekten van tanninen in veldbonen, hetgeen resulteerde in dit proefschrift.
Per 1 januari 1993 is hij als veevoedkundig onderzoeker in vaste dienst getreden van TNO, Instituut voor Toxicologie en Voeding, afdeling ILOB te Wageningen.