ANTINUTRITIONAL EFFECTS OF LEGUME SEEDS IN PIGLETS, RATS AND CHICKENS

Promotoren: Dr Ir M. W. A. Verstegen
Buitengewoon hoogleraar op het vakgebied van de veevoeding in het bijzonder de voeding van eenmagigen.

Dr J. M. V. M. Mouwen
Hoogleraar in de algemene en bijzondere ziektekunde der dieren aan de Rijksuniversiteit Utrecht.

Co-promotor: Dr Ir E. J. van Weerden
Voormalig hoofd van het TNO-Instituut voor diervoeding en fysiologie (ILOB-TNO) te Wageningen.

J. Huisman

ANTINUTRITIONAL EFFECTS OF LEGUME SEEDS IN PIGLETS, RATS AND CHICKENS

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 vrijdag 26 oktober 1990
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

The studies described in this thesis were carried out at the TNO Institute for Animal Nutrition and Physiology (ILOB), Wageningen and financially supported by TNO Nutrition and Food Research, Zeist, Netherlands.

STELLINGEN

I

Effecten van antinutritionele factoren (ANFs) dienen in het doeldier zelf onderzocht te worden.
(Dit proefschrift).

II

Het is aan te bevelen voor verteringsfysiologisch onderzoek niet de rat maar het varken als model voor de mens te gebruiken.
(Dit proefschrift).

III

Een deel van de in de literatuur beschreven effecten van tanninen kunnen voor praktische toepassing tot verkeerde conclusies leiden doordat te hoge doseringen in de proefvoeders werden gebruikt.
(Jansman et al., 1989. In: Recent advances of research in antinutritional factors in legume seeds. J. Huisman, A.F.B. van der Poel and I.E. Liener (Eds.), 1989. Pudoc, Wageningen, 176-180).

IV

Het is juister de dierbehoeften aan aminozuren te baseren op ileaal verteerde aminozuren dan op in het bloed geabsorbeerde aminozuren.
(Rerat et al., 1988. British Journal of Nutrition, 60, 91-104).

V

Het effect van verlies aan endogeen eiwit op de eiwitvertering is tot nu toe onderschat.
(Dit proefschrift).

VI

Bepaling van de ware eiwitverteerbaarheid geeft nieuwe inzichten waarop technologische en enzymatische behandelingen gericht dienen te zijn voor verbetering van de schijnbare eiwitverteerbaarheid.
(Dit proefschrift).

VII

Het ontbreken van drempelwaarden voor ANFs in voeders belemmert adequaat onderzoek naar mogelijkheden ANFs te reduceren.

VIII

Plantenveredelaars houden in hun kweekprogramma's te weinig rekening met ANFs.

IX

Samenwerking met als basis koppeling van het toegepaste TNO-onderzoek en het fundamenteel onderzoek van de landbouwuniversiteit geeft voor beiden kansen op vergroting van kennis en onderzoekscapaciteiten. Het slagen hiervan is echter afhankelijk van de inzet van personen en faciliteiten van beide kanten.

X

Eén van de zorgen van de overheid is iedereen een kans op een menswaardig bestaan te geven. De overheidszorg voor de zwakzinnigen is de laatste jaren echter eerder zwak dan zinnig te noemen.

XI

Prinsessen en jonge biggen zijn beiden overgevoelig voor erwten.

XII

Archeologen en varkens hebben gemeen dat ze beiden in de grond wroeten.
De vondsten waarnaar gezocht wordt zijn echter verschillend van aard.

XIII

Eigenheimers zijn goede aardappelen maar geen goede amateur-archeologen.

Stellingen behorend bij het proefschrift van J. Huisman:
Antinutritional effects of legume seeds in piglets, rats and chickens.

Wageningen, 26 oktober 1990.

Aan mijn lieve Hilly, mijn grote steun.
Aan mijn fijne kinderen.
Aan mijn moeder, die samen met mijn overleden vader de basis voor mijn vorming gelegd heeft.

VOORWOORD

Het in dit proefschrift beschreven onderzoek is een onderdeel van een omvangrijk internationaal onderzoeksproject waarin Thomas van der Poel en ik in 1986 een promotieonderzoek startten. Vrij snel kwam Marja Kik erbij als promovenda. Weer later sloot Alfons Jansman er bij aan. Thomas, hartelijk dank voor de fijne samenwerking en steun bij de afronding van het proefschrift, Marja en Alfons hartelijk dank voor jullie plezierige samenwerking. Vanwege de omvangrijkheid van het onderwerp werd een stuurgroep gevormd die mede richting gaf aan de verschillende promotieonderzoeken. In alfabetische volgorde zijn dit: Dr. R.J. Hamer, Prof.Dr.Ir. J.M.V.M. Mouwen, Prof.Dr.Ir. S. Tamminga, Dr.Ir. E.J. van Weerden, Prof.Dr.Ir. M.W.A. Verstegen en Ir. D.J. van Zuilichem. Heel hartelijk dank voor de ondersteunende en leerzame discussies. Het verheugt me dat we met dezelfde stuurgroep promotieonderzoeken die als vervolg hierop gestart zijn zullen blijven begeleiden. Het onderzoek werd sterk gestimuleerd door Drs. J.W. van der Kamp, direkteur van TNO-Wageningen. Hartelijk dank voor alle steun en stimulering die ik van jou kreeg. Vanaf de start kreeg het promotieonderzoek belangstelling en ondersteuning van Prof.Ir. B. Krol, hoofddirekteur van de Hoofdgroep Voeding en Voedingsmiddelen. Heel hartelijk dank voor je persoonlijke inzet het promotieonderzoek succesvol te laten verlopen. Sinds twee jaar wordt het ILOB geleid door Dr.Ir. G.J.M. van Kempen. Ik heb de stimulering die ik van jou kreeg om het geheel vrij snel af te ronden op prijs gesteld.

De twee promotoren en de co-promotor vervulden een dubbele rol, ze waren lid van de stuurgroep en tevens direct begeleider. Het was voor mij een ervaring te ondervinden dat hun visie op het promotieonderzoek niet steeds gelijk was. Prof.Dr.Ir. M.W.A. Verstegen, jou wil ik bijzonder bedanken voor je intensieve en stimulerende begeleiding. Altijd stond je klaar en was je bereid mij snel advies en steun te geven. Prof.Dr. J.M.V.M. Mouwen, hartelijk dank voor jouw aandeel in de begeleiding. Dr.Ir. E.J. van Weerden, jouw rol als co-promotor in de begeleiding was bijzonder omdat we al 30 jaar samenwerken. In feite heb je me al een groot aantal jaren begeleid, als hoofd van het wetenschappelijke onderzoek van ILOB. Mijn wetenschappelijke vorming heb ik voor een belangrijk deel aan jou te danken. De begeleiding van het proefschrift was als van ouds: kritisch met een scherp gevoel voor zin en onzin in het geheel. Heel hartelijk dank voor alles. Aan het samenstellen van het proefschrift hebben verschillende personen hun steentje bijgedragen. Veel medewerkers van het ILOB hebben op de één of andere manier met het onderzoek te maken gehad. Allen die er bij betrokken waren wil ik bedanken. Enkelen die een grote bijdrage aan het onderzoek geleverd hebben wil ik met name noemen. Piet van Leeuwen, jouw chirurgische kwaliteiten en jouw betrokkenheid bij het onderzoek zijn van groot belang geweest voor het slagen van de verschillende proeven. Om dit te laten blijken heb ik jouw gevraagd als paranimf mij terzijde te staan bij de afronding van de promotie. Het deed me erg veel plezier dat je dit aanvaardde. Johan de Jong en Gerard Beelen, jullie beiden waren mijn steun en toeverlaat bij de organisatie en het goed laten verlopen van de proeven. Jullie grote inzet heb ik zeer gewaardeerd. De proeven verliepen goed dank zij de inzet van verschillende dierexperimentele medewerkers. Dick van Kleef, Anne Hoek, Kasper Deuring, Jan van Harn, Jan van de Broek, Rik van de Heuvel en Johan de Zeeuw hartelijk dank. In het onderzoek

werden vele analyses uitgevoerd. De analytische groep onder leiding van Martin van Baak wil ik hierbij danken omdat zij vaak onder tijddruk kans zagen de resultaten op tijd af te leveren. Some parts of the research could only be carried out in the framework of cooperation with other institutes and scientists. Dr J. Delort-Laval, director at INRA-Nantes (France), thank you for all you did to make the research with peas a succes. Dr W.B. Souffrant from the Oskar Kellner Institute in the DDR, thank you very much for your stimulation and the effort you made to come to cooperative research with the 15N-dilution technique. I was impressed that inspite of the difficult political situation you could accomplish much. Marie-Pierre Le Guen thank you very much for all the work you did in the research programme with peas. Dr. Thomas Heinz, thank you for all the work you did in our cooperative ^{15}N research. Bij de onderzoekingen waren verschillende studenten betrokken. Vrijwel allen zijn inmiddels afgestudeerd. Drie wil ik met name noemen omdat zij een meer dan gemiddelde bijdrage hebben geleverd. Ir. Lambert ten Haaf, Ir. Coen Smits en Ir. Peter Mollee heel hartelijk dank. Het afronden van het proefschrift gaf de nodige activiteiten voor het secretariaat. Gretha van de Berg wil ik danken voor de organisatie ervan en Vicky Kok voor het steeds blijmoedig (her)typen van de hoofdstukken. Gerrit Haak wil ik danken voor de steun bij het rekenwerk. Dr. Martje Fentener van Vlissingen wil ik danken voor de fraaie anatomische tekening van een mono-gastrisch dier. Het Centrum voor Automatisering van TNO-Voeding onder leiding van Ir. J.J.H. Donders, en Dienst Tekstverwerking onder leiding van H.J. de Vetter en Lyda Mayntz dank ik voor de afwerking en typografische verzorging van dit proefschrift. Ann Chadwick wil ik danken voor de correcties op Engels van een aantal hoofdstukken.

Tenslotte wil ik mijn vrouw danken. Lieve Hilly, bedankt voor de steun en stimulering die ik van jou kreeg. Jouw begrip voor mijn afwezigheid tijdens mijn aanwezigheid heb ik zeer gewaardeerd.

Het onderzoek was niet tot stand gekomen zonder financiële ondersteuning. Verschillende fondsen hebben een bijdrage geleverd. Naast TNO Nutrition and Food Research wil ik noemen het Produktschap voor Veevoeder, het Fonds Mestonderzoek, de Eureka organisatie en Euretec in Frankrijk.

CONTENTS

There is a growing interest in the EC-countries to use protein sources grown in Europe for animal diets. One class of these crops are legume seeds. The use of these seeds is, however, hampered due to the presence of antinutritional factors (ANFs) (Liener and Kakade, 1980). In order to gain some insight into which ANFs are important in relation to farm animal nutrition, a literature review has been prepared. In this thesis the literature review is focussed on ANFs in peas (Pisum sativum), common beans (Phaseolus vulgaris) and soybeans (Glycine Max). In the review it has been concluded that ANFs often act as biopesticides in plants and seeds. The defensive effect of ANFs in plants and seeds seems to be related to disturbances in digestive processes in the predating organisms like microorganisms and insects. It is assumed on the basis of similarity in metabolic pathways that ANFs can disturb the digestive processes in animals in more or less the same way they do in microorganisms and insects. A study was therefore started into the antinutritional effects of peas (Pisum sativum) and commmon beans (Phaseolus vulgaris) in monogastric animals. The reason for the choice of these legume seeds was:

- when raw common beans are fed to animals, weight gain can be markedly reduced in pigs (Myer et al., 1982) and rats (Grant et al., 1985).
- when raw peas are fed to young piglets, reduced weight gain is observed (Grosjean and Gatel, 1986; Huisman, 1989; Savage and Deo, 1989). These effects however, are less marked than with common beans.
- both seeds are important for human and animal nutrition (Liener, 1980; Savage and Deo, 1989; Sgarbieri and Whitaker, 1982).

The literature review (Chapter 1) shows that it can be assumed that the main ANFs in peas and common beans are trypsin inhibitors and lectins. The primary effect of trypsin inhibitors is related to the inactivation of (chymo)trypsin activity in the small intestine. As a secondary effect, the secretion of pancreatic enzymes trypsin and chymo trypsin is enhanced when these enzymes are inhibited by trypsin inhibitors. The effect on the pancreas is regulated by a negative feedback mechanism. The extra secretion of pancreatic enzymes causes an increased loss of endogenous protein for the animal. Thus, the negative effect of trypsin inhibitors may be related to two modes of action: inactivation of the digestive enzymes trypsin and chymotrypsin, and an extra loss of endogenous protein by hypersecretion of the pancreas. Research into effects of trypsin inhibitors in relation to nutritional aspects are mainly concerned with soybean trypsin inhibitors and hardly at all concerned with those present in peas and beans. Lectins can bind to the epithelial cells of the intestinal mucosa. Due to this binding endocytosis and disturbances in digestive and absorptive processes may occur. Also, the production of mucins is increased and cells of the mucosa are disrupted (Pusztai, 1989). Due the damage of the intestinal mucosa the permeability of the epithelium may be increased. As a result, lectins and other peptides can permeate and may induce immune reaction. Most studies with lectins are carried out with common bean lectins in small laboratory animals. Little attention is paid to lectins present in peas and soya. Most studies with lectins refer to effects on the intestinal mucosa and on performance, and less to effects on the digestive processes. The literature review (Chapter 1) demonstrates that many

aspects related to the mode of action of trypsin inhibitors and lectins in the animal remain unclear. Major points directed to the line of research in this thesis are:

- Much of the present knowledge related to ANFs is based on results obtained with small laboratory animals such as rats, mice and chickens. Research using larger animals such as the pig is scarce. Not only the use of small laboratory animals as a model for the pig is questionable, it is also uncertain as to whether often used parameters such as Protein Efficiency Ratio (PER), Net Protein Utilization (NPU) and N balance can be extrapolated to the pig. It is, therefore, important to know whether results obtained with these small laboratory animals can be extrapolated to larger monogastric animals such as pigs. In some publications it has been suggested that there are differences in response between rats and pigs to the feeding of raw soybeans (Combs et al., 1967; Yen et al., 1977) and to feeding of chick peas. (Visitpanich et al., 1985). However, this information is too scarce to allow conclusions to be drawn as to whether or not rats and chickens can be used as an alternative for the pig. Because of its importance, three experiments were carried out to find out whether there are species differences between piglets, rats and chickens in effects on performance and digestion when common beans or peas are fed. The results of these studies are described in Chapter 2. In the study described in Paragraph 2.1 a comparison was made between piglets and rats fed common beans. In a second experiment it was studied whether the marked differences in performances and organ weights between the two animal species could be related to the marked difference in protein digestibility observed in the first study. These results are described in Paragraph 2.2. Chickens were also included in this experiment. In the third experiment (Paragraph 2.3.), peas were included in the diets fed to piglets, rats and chickens.
  In these three studies the following criteria were investigated.
  - performance was studied as a general criterion.
  - apparent digestibility was measured in the studies with common beans because in the literature it is shown that these beans can disturb the digestibility. In the study with peas (Paragraph 2.3) no digestibility determinations were carried out because digestibility of peas have been studied in detail in the experiments of Chapter 3.
  - In all three studies organ weights were measured. The pancreas because of its relation to the digestive process and because of the effects of trypsin inhibitors on the secretion of pancreatic enzymes. Spleen and thymus weights were measured because of their relation to the immune system. Liver and kidneys were weighed because they are involved in detoxification.

- There is no clear information about the mode of action of ANFs in peas and common beans, because these legume seeds always contain more than one ANF (Chubb, 1982; Liener, 1980, 1981; Savage and Deo, 1989). In most studies whole seed is fed to animals. Therefore, the negative effects are always related to more than one factor. Also, the possibility that characteristics of the protein per sé and the presence of carbohydrates have played a role in the negative effect cannot be excluded. The only way to understand the mode of action of ANFs and possibly other factors, is to isolate and purify the ANFs and other fractions such as protein and carbohydrates, and test them in animal studies. The literature concerning studies with isolated ANFs is scarce and small laboratory animals were used in

nearly all these studies. For the purpose of this thesis, a study was made to discover whether ANFs or carbohydrates are responsible for the negative effects with peas or common beans in piglets. One problem in the piglet studies was that large amounts of the isolated fraction need to be produced. The costs of producing these large amounts are so high that it was impossible to do this research with both peas and common beans. Because in Europe peas are a more important feedstuff for pigs than common beans, the different fractions were isolated from peas. The high cost of producing large amounts of isolated fractions was a second reason for studying whether small laboratory animals could be used as an alternative for the piglets. From the animal species difference studies, clear indications were obtained that piglets are more sensitive to ANFs than either rats or chickens. It was concluded that rats and chickens were no good alternatives for studying the effects of different fractions of peas on digestive physiological parameters in piglets. The studies focussed on this question were therefore carried out with piglets. The following fractions were prepared to study which ones are responsible for the negative effects:

- a protein isolate from which ANFs and carbohydrates were removed
- a protein fraction with very high levels of ANFs (ANF-concentrate)
- a fraction consisting exclusively of a mix of soluble and insoluble carbohydrates.

Two pea varieties were used: the summer variety FINALE with low levels of trypsin inhibitors, and the winter variety FRIJAUNE with high levels of trypsin inhibitors. There was not much difference in the lectin contents in either variety. The objective was to study whether removing of ANFs and carbohydrates from peas has a positive effect on digestion. Two experiments were done using isolated and purified pea fractions and are described in Chapter 3. In the first experiment, the faecal and ileal digestibility of raw peas and pea protein isolate from which the ANFs and carbohydrates had been removed, was studied. The sensitivity of faecal or ileal digestibility as a criterion was also checked. The results are described in Paragraph 3.1. The second experiment was carried out to study whether pea ANFs or pea carbohydrates are responsible for the differences in ileal digestibility between raw peas and pea protein isolate. The results are described in Paragraph 3.2.

- It is unclear whether low apparent ileal protein digestibility is exclusively related to ANFs or whether the raw native protein has a high resistance to the hydrolysis of digestive enzymes. Measurement of apparent digestibility is based on the ingested feed protein minus the excreted protein with ileal chyme or with faeces. This excreted protein consists of undigested feed protein and endogenous protein. The true digestibility of the feed protein can be determined by correcting the apparent protein digestibility for the excreted endogenous protein. One of the most advanced methods of measuring the excretion of endogenous protein is the ^{15}N dilution technique, in which the body protein of the animal is labeled by infusion of ^{15}N amino acids into the blood (Souffrant et al., 1986). This technique enables a distinction to be made between the excretion of undigested, unlabeled feed protein and labeled endogenous protein. In this thesis, the ^{15}N dilution technique was used to study the true digestibility of protein from peas and common beans. The results of this experiment are described in Chapter 4. In the General Discussion, various aspects of the results relating to animal species differences and to the effects of isolated fractions from peas on digestive physiological

parameters in piglets are discussed. Special attention is paid to the observation that pancreas activity is decreased with lower protein digestibility.

REFERENCES

Chubb, L.G. (1982). Anti-nutritive factors in animal feedstuffs. In: W. Haresign. (editor). Recent Advances in Animal Nutrition. Butterworths, London, UK, 21-37.

Combs, G.E., Connes, R.G., Berry, T.H. and Wallace, H.D. (1967). Effect of raw and heated soyabean meal on gain, nutrient digestibility, plasma amino acids and other blood constituents of growing swine. Journal of Animal Science, 26, 1067-1071.

Grant, G., Greer, F., McKenzie, N. and Pusztai, A. (1985). Nutritional response of mature rats to kidney bean (Phaseolus vulgaris) lectins. Journal of the Science of Food and Agriculturel, 36, 409-414.

Grosjean, F. and Gatel, F. (1986). Peas for pigs. Pig news and information, vol 7, 443-448.

Huisman, J. (1989). Antinutritional factors (ANFs) in the nutrition of monogastric farm animals. In: Nutrition and digestive physiology in monogastric farm animals. [E.J. van Weerden and J. Huisman, editors]. Pudoc, Wageningen, The Netherlands, 17-35.

Liener, I.E. (1980). Book: Toxic constituents of plant foodstuffs. Academic Press, New York, US, 502 pp.

Liener, I.E. (1981). Factors affecting the nutritional quality of soya products. Journal of the American Oil Chemists' Society, 58, 3, 406-415.

Liener, I.E. and Kakade, M.L. (1980). Protease inhibitors. In: Toxic Constituents of Plant Foodstuffs. [I.E. Liener, editor]. New York, Academic press, 7-71.

Myer, O.M., Froseth, J.A. and Coon, C.N. (1982). Protein utilization and toxic effects of raw beans (Phaseolus vulgaris) for young pigs. Journal of Animal Science 55, 1087 -1098.

Pusztai, A. (1989). Biological effects of dietary lectins. In: Recent advances of research in antinutritional factors in legume seeds. [J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. Pudoc, Wageningen, The Netherlands, 17-29.

Savage, G.P. and Deo, S. (1989). The nutritional value of peas (Pisum sativum). A literature review. Nutrition Abstracts and Reviews (Series A), 59, 2, 66-88.

Sgarbieri, V.C. and Whitaker, J.R. (1982). Physical, chemical and nutritional properties of common bean proteins. Advances Food Research, 28, 93-166.

Souffrant, W.B., Darcy-Vrillon, B., Corring, T., Laplace, J.P., Köhler, R., Gebhardt, G. and Rerat, A. (1986). Recycling of endogenous nitrogen in the pig. Archiv für Tierernährung, 36, 269-274.

Visitpanich, T., Batterham, E.S. and Norton, B.W. (1985). Nutritional value of chickpea (Cicer arietinum) and pigeon pea (Cajanus cajan) meals for growing, pigs and rats. II. Effects of autoclaving and alkali treatment. Australian Journal of Agricultural Research, 36, 327-335.

Yen, J.T., Jensen, A.H. and Simon, J. (1977). Effect of dietary raw soyabean trypsin inhibitor on trypsin and chymotrypsin activities in the pancreas and the small intestinal juice of growing swine. Journal of Nutrition, 107, 156-165.

CHAPTER 1

DIETARY EFFECTS AND SOME ANALYTICAL ASPECTS OF ANTINUTRITIONAL FACTORS IN PEAS (PISUM SATIVUM), COMMON BEANS (PHASEOLUS VULGARIS) AND SOYBEANS (GLYCINE MAX) IN MONOGASTRIC FARM ANIMALS

A LITERATURE REVIEW

J. Huisman[1)] and A.J.M. Jansman[1,2)]

1) TNO-Institute of Animal Nutrition and Physiology (IGMB-dept. ILOB), P.O. Box 15, 6700 AA Wageningen, The Netherlands.
2) Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen. The Netherlands.

Submitted for publication.

CONTENTS

I ABSTRACT

A survey is made on the occurrence and role of antinutritional factors (ANFs) in peas (Pisum sativum) and common beans (Phaseolus vulgaris), the state of knowledge concerning the mode of action of ANFs in monogastric animals, the effects of ANFs on nutritional value and some aspects of analytical methods for determining ANFs. Because these seeds contain the same classes of ANFs as those found in soybeans (Glycine max) and most research into the mode of action of ANFs in animals is done with soya, also information about soya ANFs is included. ANFs protect the seed against attacks of moulds, bacteria, insects and birds. This defensive effect of ANFs seems to be related to disturbances in digestive processes in insects and micro-organisms. Ingested ANFs seem to disturb the digestion processes in animals in the same way as they do in micro-organisms and insects. The main ANFs in peas, common beans and soya are trypsin inhibitors and lectins. The effects of these ANFs in monogastric animals are described in detail. The effects of other ANFs are briefly discussed. Different points and recommendations for improvement of analytical methods are considered. Discussion and recommendations for future research are made about various points related to nutritional effects of ANFs in the animal, animal species differences, need for studies with isolated ANFs in target animals, and studies on threshold levels of ANFs.

II INTRODUCTION

Grain legumes became edible as food for man during prehistoric times when the cooking process was discovered. Cooked beans, peas, lentils and other seeds have been recognized since then as foods of high nutritional value. However, the cooking or heating of beans and peas may be considered to be too expensive for animal nutrition. For soybeans this is different because first oil, with a high nutritional and economical value, can be extracted. The protein-rich fraction which remains after extraction, contains high levels of protein and antinutritional factors (ANFs). After steam-heating (toasting) the ANFs in this soybean meal are reduced. Soybean meal also has a high nutritional and economic value. The protein content in extracted and toasted soybean meal is higher than in other raw beans and peas (40-50% in toasted soybean meal and 20-35% in peas and beans). There is a growing interest in Europe to become more self-supporting regarding the protein supply for animal diets. Other legume seeds such as peas and beans may provide alternatives for soya. Peas and beans grow well under European climatic conditions. However, these legume seeds contain the same classes of ANFs as those found in soybeans. Thus, the use of these seeds in a raw form is seriously hampered due to the presence of these ANFs. It is important, therefore, to find economically feasible inactivation processes which will reduce the ANFs. To achieve this, more knowledge is required about the ways in which ANFs affect the metabolic processes in animals and to establish the levels in the seeds that can be tolerated. In the present paper, a survey is made on the occurrence and role of ANFs in peas (Pisum sativum) and common beans (Phaseolus vulgaris), the state of knowledge concerning the action of ANFs in monogastric animals, the effect of ANFs on nutritional value and to consider aspects of analytical methods for determining ANFs. Lack of information and recommendations for future research are also discussed. Most research into the

action of ANFs in animals is done with soybean (Glycine max). Therefore, in this review information from soya ANFs is also included.

III DEFINITION OF ANFs

In this review ANFs are described as non-fibrous natural substances causing negative effects on growth or health in man and animals. In this definition fibre is excluded because in human food it may be classified as a positive factor and may also have a certain energy value when digested in the large intestine. Also excluded in this definition are the contaminants such as mycotoxins and factors originating due to processing which can also have antinutritional effects (Yannai, 1980).

IV OCCURRENCE AND ROLE OF ANFs IN PEAS, COMMON BEANS AND SOYBEANS

Recent reviews, reports and books about the presence and distribution of ANFs in various seeds are given by Cheeke and Shull (1985), Friedman (1986), Huisman et al. (1990), Huisman (1989), Liener (1980, 1981, 1989a), Rackis et al. (1986), Pusztai et al. (1983) and Savage and Deo (1989). Most legume seeds contain different ANFs. (Chubb 1982, Liener, 1981; Savage and Deo, 1989). When these seeds are fed to animals the effects of ANFs on performance may be mainly due to a combination of different ANFs. ANFs often act in plants and seeds as biopesticides, protecting the seed against attacks of moulds, bacteria, insects and birds (Birk, 1987, 1989; Bond and Smith, 1989; Broadway et al., 1986; Ryan, 1978, 1979, 1983; Liener and Kakade, 1980). One class of these ANFs are the protease inhibitors (PIs). The majority of PIs are proteins with a molecular weight ranging from 4000 to 10000. The PIs from different plant species differ in their molecular weight, amino acid composition and physical structure (Rackis and Gumbmann, 1986). PIs are able to inhibit the activity of proteolytic enzymes. Different studies show that plant PIs play a role in the defence system of the plant against predators (Ryan, 1978, 1979; Ryan et al., 1985). The role of PI in the seed against attacks of micro-organisms, insects and other predators is not completely clear. It has been shown (Birk, 1987, 1989; Ryan, 1978, 1983; Broadway et al., 1986) that soybeans and wheat grains contain substances which inhibit gut proteases of the insects species Tribolium and Tenebrio. Gatehouse et al. (1979) showed that PIs have a function in deterring insect pests. A rapid accumulation of PI has been found in tomato and potato leaves in reaction to signals initiated by the wounding of plant leaves or due to attack by insects. These studies also demonstrated that insects become starved because of inhibition of digestive enzymes after eating leaves containing plant PIs (Birk, 1987; Broadway et al., 1986; Brown et al., 1986 and Ryan, 1978, 1979).

Another important class of ANFs are lectins. Lectins are proteins predominantly in the form of glycoproteins (Jaffé, 1980). They are characterized by their unique capability of binding sugar components. Many proteins in plants and animals are glycoproteins to which lectins may bind. The structure and chemical properties have been studied in many lectins (reviewed by Goldstein and Poretz, 1986). Lectins are mainly located in the seeds of plants, but are also found in leaves, stems and roots (Etzler, 1986). In

seeds they are usually located in the cells of the cotelydons and in embryonic tissue. Several physiological functions of lectins in plants have been suggested (Jaffé, 1980; Etzler, 1986 and Pusztai et al. 1983). Jaffé (1980) reported the following functions: counteracting soil bacteria, protecting plants against fungal attacks, playing a role in the development and differentiation of embryonic cells and in the transportation and storage of sugars. Interactions of plant lectins with various micro-organisms have been found (reviewed by Pistole, 1981). A defensive function of plant lectins against fungi, bacteria and virusses has been postulated in many studies (reviewed by Etzler, 1986). Resistance of some plants to bacterial infection has been related to the presence of lectins (Bogers, 1972, Pistole, 1981). Janzen et al. (1976) found that lectins from Phaseolus vulgaris kill the larvae of beetles, indicating that lectins may also play a role in defence against insects. The counteraction of soil bacteria by lectins has been discussed by Etzler (1986). Lectins appear to play a role in Rhizobium recognition and in the symbiosis of plant roots and nitrogen-fixing bacteria (Etzler, 1986).

An important class are the tannins. Tannins are defined as water-soluble phenolic compounds having a molecular weight between 500 and 3000. Based on their structural features they are classified in two distinct groups: hydrolyzable and condensed tannins. Tannins form complexes with proteins, carbohydrates and other polymers (Rao and Prabhavati, 1982). Tannins have been determined in many seeds (Singleton, 1981). They play a role in the defence against fungi, bacteria and herbivorous insects (Bond and Smith, 1989; Swain, 1979). In this respect, Doggett (1976) showed that more damage was done by birds in low tannin sorghum compared with high tannin sorghum. The resistance to high tannin grain sorghum to birds may be attributed to the astringent effect of tannins (Bullard and Elias, 1980). Tannin contents are related to the colour of flower. In seeds from legume cultivars with white flowers tannin contents are low. However, these seeds are less resistant to attacks of soil bacteria than high tannin seeds (Bond and Smith, 1989; Bond et al., 1986).

Various plants contain alpha-amylase inhibitors. These inhibitors are proteins with a molecular weight ranging from 13 000 to 50 000. These inhibitors are able to reduce the activity of alpha-amylases. Buonocore and Silano (1986) point out that most alpha-amylase inhibitors show an inhibiting capacity against amylases in bacteria, fungi and insects.

Summarizing, it can be stated that the defensive effect of ANFs in plants and seeds seems to be related to disturbances in digestive processes of micro-organisms and insects by ANFs. There are similarities in digestive processes in animals, micro-organisms and insects. It can be expected, therefore, that ANFs disturb the digestive processes in animals in the same way as they do in micro-organisms and insects.

V DESCRIPTION, SOME ANALYTICAL ASPECTS AND DIETARY EFFECTS OF ANFs IN MONOGASTRIC FARM ANIMALS

Known ANFs present in peas, common beans and soybeans are protease inhibitors, haemagglutinins or lectins, tannins, alpha-amylase inhibitors, allergens or antigenic

proteins, phytates, flatulence factors, goitrogens, antivitamins, saponins, oxalic acid and oestrogens (Cheek and Shull, 1985; Chubb, 1982; Liener, 1980 and Savage and Deo, 1989). Some of these ANFs such as goitrogens, antivitamins, saponins, oxalic acid and oestrogens are present in very low levels in peas, common beans and soybeans. It may be assumed therefore, that they are of minor importance from a nutritional point of view. In other seeds they may be important. In peas and common beans trypsin inhibitors and lectins are present at such levels that they may be classified as important factors. Tannins are important in coloured flowering varieties. In this review trypsin inhibitors and lectins are detailed discussed in chapters V.1 and V.2. The other ANFs are briefly discussed in chapter V.3. ANFs effect the digestion and metabolic processes at different sites in the animal. In Figure 1 (see page 28) an overview is given where the ANFs act in monogastric farm animals.

1 TRYPSIN INHIBITORS

1.1 General characteristics

Research into protease inhibitors (PIs) began about fifty years ago with the discovery by Read and Haas (1938) that aqueous extracts of defatted soybean flour inhibited the ability of trypsin to break down gelatin. Kunitz (1945, 1946) isolated a crystalline trypsin inhibitor from the soya fraction responsible for the inhibiting effect observed by Read and Haas (1938). Since then knowledge has increased considerably on PIs. Hundreds of PIs spread over different botanical families have been characterized, as reviewed by Liener and Kakade (1980). Those characterized the best are PIs from seeds and tubers of agriculturally important crops. Among them are the seeds of Leguminosae (Rackis et al., 1986). The majority of the PIs differ in specificity and inhibiting capacity. Many are able to inhibit one or two enzymes. In one particular seed different types of inhibitors may be present. Most of the inhibitors inhibit trypsin and many also inhibit chymotrypsin. There are various families of plant PIs (Birk, 1989; Laskowski, 1986; Liener and Kakade, 1980). Among the PIs the trypsin inhibitors (TIs) are the most important for animal nutrition because they inhibit digestive enzymes. Two families of TIs are important in peas, common beans and soybeans: the Kunitz (soybean) trypsin inhibitor family (STI) and the Bowman-Birk trypsin inhibitor family (BBI).

1.1.1 Kunitz soybean inhibitor (STI) family

STI was the first plant protease inhibitor to be isolated and characterized (Kunitz 1945, 1946, 1947a,b,). It has a molecular weight of about 21000 daltons. It is a peptide consisting of 181 amino acids and contains two disulphide bridges. It primarily inhibits trypsin and weakly inhibits chymotrypsin (Birk 1987, 1989; Liener and Kakade, 1980). STI is inactivated by heat and gastric juices. STI is predominantly found in soybeans, only a few homologous inhibitors have been found in legume and other edible seeds. Numerous studies on STI have been carried out; they are compiled and reviewed by Birk (1976, 1987), Kassell (1970) and Liener and Kakade (1980).

The structure of STI is shown in Figure 2.

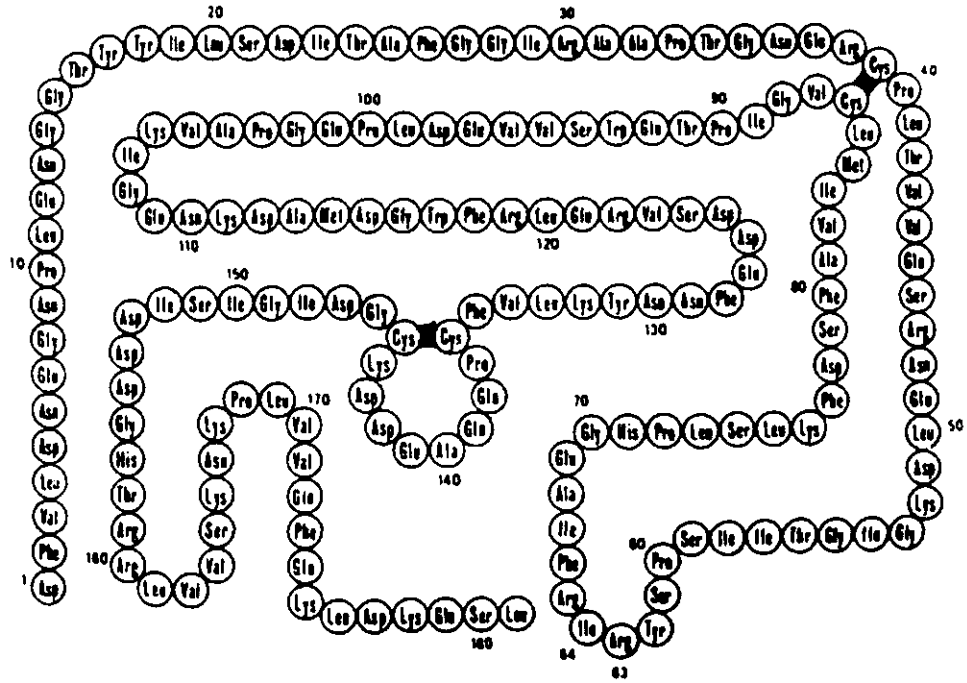


Figure 2 Amino acid sequence of the Kunitz soybean inhibitor. (From Koide et al., 1973, reproduced with the permission of the European Journal of Biochemistry.)

1.1.2 Bowman-Birk protease inhibitor (BBI) family

The BBI was first recognized by Bowman (1944) and purified from soybeans and characterized by Birk (1961). The BB type inhibitors are not only present in soybeans but in most legume seeds. Most of the members of the BBI family are present in numerous iso-inhibitor forms (Birk 1989, Liener and Kakade, 1980). The BBI is smaller than the STI. BBI is a peptide consisting of 71 amino acids. The molecular weight is about 8000 daltons. It contains high contents of cystine and has seven disulphide bridges. BBI is a double-headed molecule which inhibits trypsin and chymotrypsin at two independent binding sites. It is resistant to gastric juices and to various proteolytic enzymes including pepsin and pronase and acids. There are conflicting reports about stability of BBI to heat. Birk (1989), Liener and Kakade (1980) and Tan-Wilson and Wilson (1986) report that the BBI is relatively stable to

heat. They discussed that the high degree of cross-linked disulphide bridges may be responsible for the heat stability. However, the paper of Di Pietro and Liener (1989) showed that purified BBI was inactivated more quickly than purified STI by heat.

The structure of BBI is shown in Figure 3.

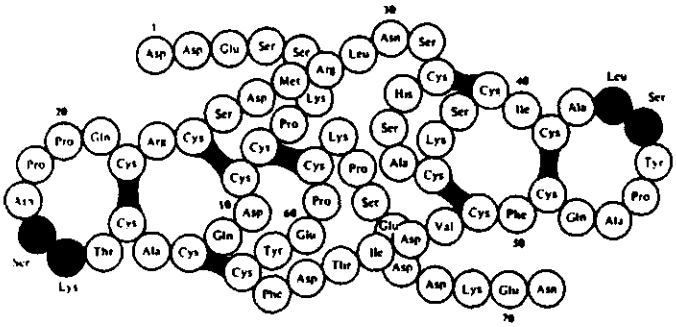


Figure 3 Amino acid sequence of the Bowman-Birk inhibitor. The reactive sites responsible for the inhibition of trypsin (Lys 16-Ser 17) and chymotrypsin (Leu 44-Ser 45), are shown in black. (From Odani and Ikenaka, 1973, reproduced with the permission of the Journal of Biochemistry (Tokyo).

1.2 Analysis of trypsin inhibitors

The standard assay for the determination of trypsin inhibitor activity is based on the decrease (inhibition) of enzymatic hydrolysis by trypsin of various proteins or synthetic substrates which is measured spectrophotometrically (Rackis et al., 1986). Most assays are based on the method developed by Kakade et al. (1974). In the Kakade method, the trypsin inhibitor activity is determined by measuring the decrease in activity of added (bovine) trypsin to a soybean extract, using the synthetic substrate Benzoyl-d1-Arginine-P-NitroAnilide (BAPNA). In the course of time, different modifications have been applied to this original concept of analysis (Rackis et al, 1986; Van Oort et al, 1989). In the Kakade method and its modifications the inhibiting activity of other substances such as phytate, tannins, fatty acids or saponins present in the extracts of seeds prepared for the assay is also measured (Liener, 1989b; Rackis et al., 1986). For determining specific proteinaceous trypsin inhibitor activity, the immuno assay techniques (ELISA) (Brandon et al. 1988, 1989; Freed and Ryan, 1978a, b) and affinity chromatography (Roozen and de Groot, 1987) may be suitable. A point of consideration may be that bovine trypsin is mainly used for the determination of trypsin inhibitor activity. Boisen (1989) and Liu and Markakis (1989) showed that the use of trypsin from different animal species in the

assay gives different results. Thus, the use of bovine trypsin can give misleading results unless correlated with biological effects determined in the target species. The use of trypsin from target animals is therefore recommendable. Rackis et al. (1986) discussed that no general TI assay can be used for all feedstuffs. Adjustments of buffers, pH and removing compounds by ultra centrifugation may still remain necessary. (Rackis et al., 1986; Liu and Markakis, 1989).

When soya meal is used in animal diets it should be checked for trypsin inhibitor activity. In practice not each batch is controlled but procedures for the testing of soya are standardized in such a way that trypsin inhibitor activities are sufficiently reduced. However, when other legume seeds, such as peas and beans, are included in animal diets measurements of trypsin inhibitor activities are not usually carried out. It may not be excluded that the trypsin inhibitor activity in some raw legume seeds are higher than in toasted soya. Therefore, a comparison of trypsin inhibitor activities in soya, peas and common beans should be made. A practical problem is that various laboratories use different units to express trypsin inhibitor activity, hampering comparison of analytical results reported in literature (Rackis et al., 1986). For comparison of data in literature Rackis et al. (1986) made an attempt to recalculate different units, such as trypsin units (TU), trypsin inhibitor units (TIU) and trypsin units inhibited (TUI) to trypsin inhibitor activity (TIA) expressed as mg inhibited trypsin per g sample or g protein. In these calculations one TU was arbitrarily defined as an increase of 0.01 absorbance units at 410 nm, under conditions of the assay. Activity expressed as TIU or TUI were based on the assumption that 1 µg of pure trypsin increased the absorbance with 1.9 TU. Thus in this assumption 1.9 TUI of TIU is equivalent to 1 µg inhibited trypsin. Using this calculation method some points have to be mentioned. It may be that a TIU obtained with different substrates such as BAPA or TAME (p-Toluenesulfonyl Arginine Methyl Ester) are not equivalent. Another point is that trypsin from commercial sources contains different levels of pure trypsin (Kakade et al., 1969; Hamerstrand et al., 1981; van Oort et al., 1989).

As pointed out by Rackis et al. (1986) different forms of compounds with trypsin inhibitor activity can be present in feedstuffs, each with different properties, binding stoichiometry and molecular weights, the equivalence of 1 µg TI to 1 µg trypsin, would only be of value for comparative purposes between different feedstuffs. The converted values would therefore not necessarily indicate the absolute activity of TI. Using the factor 1.9 to calculate TIU and TUI levels to mg TI per gram sample or protein, a comparison was made between TIA contents of a number of samples of peas, commonbeans and soybeans (Table 1).
The TIA levels in common beans and peas are distinctly lower compared with levels found in raw soybeans. Relative to raw soybeans, TIA levels in peas are 12% and in common beans 38%. For toasted soybean meal, a maximal TIA level of between 2 and 5 mg inhibited TIA/gram product (about 1 to 3 mg TIA per g protein) is often recommended in the Netherlands. The values of TIA per g protein in peas are between about 0.4 and 34.6 mg and in common beans between 15.6 and 92.6 mg. Related to the TIA levels in protein, the majority of the pea and common bean samples are higher than those of toasted soybean meal.

Table 1 Comparison of trypsin inhibitor activity in soybeans, peas and common beans

Product	TI activity [a]		Reference
	mg per g product	mg per g protein	
Glycine Max			
raw soybeans	18.7	49.6	Doell et al. (1981)
raw soybeans (108)	--	35-123[b]	Kakade et al. (1972)
raw soybeans	26.2	--	Hove and King (1979)
raw soybeans	32.6c	85.4	Valdebouze et al.(1980)
raw soybeans (Brazil) (3)	20.1	56.5	Slump and Dukel (1979)
raw soybeans (US)(3)	24.4	65.6	Slump and Dukel (1979)
defatted soybeans	30.2	57.8	Rackis et al. (1985)
defatted soybeans	31.3[b,c]	--	Valdebouze et al. (1980)
raw soya isolate	24.1	29.4	Rackis et al. (1985)
soya concentrate	13.9	20.7	Rackis et al. (1979)
toasted defatted soybeans	8.5	16.0	Rackis et al. (1985)
heated soya isolate	4.7	6.1	Rackis et al. (1985)
toasted soybean meal (Brazil) (5)	2.6	5.6	Slump and Dukel (1979)
toasted soybean meal (US) (5)	2.8	6.8	Slump and Dukel (1979)
toasted soybean meal (The Netherlands) (5)	3.7	8.2	Slump and Dukel (1979)
Pisum sativum			
spring peas (8)	1.5-5.7bc	6.0-22.8*	Valdebouze et al. (1980)
spring peas (33)	0.9-3.9bc	3.9-17.1*	Leterme et al. (1989)
spring peas (2)	1.7-7.9bc	7.5-34.6*	Carré and Conan (1989)
peas (4)	0.7-3.5	3.1-15.4*	Hove and King (1979)
peas (9)	0.1-2.4b	0.4-10.5*	Griffiths (1984)
winter peas (6)	3.8-5.9bc	13.2-25.9*	Leterme et al. (1989)
Phaseolus vulgaris			
common beans (4)	3.4-20.2	15.6-92.6*	Hove and King (1979)
common beans	--	43.6-61.8	del Rosario et al.(1981)
common beans (1)	8.7c	39.9*	Leterme et al. (1989)
common beans (12)	11.0	46.1	Van der Poel (1990)

* Levels in protein calculated from mean protein levels according to the Dutch CVB-table (1988): peas 22.8% and common beans 21.8%.

[a] TIA expressed as mg pure trypsin inhibited per gram.

[b] originally expressed in TIU, recalculated assuming that 1.9 TIU is equivalent to 1 µg of pure trypsin inhibited.

[c] expressed in mg trypsin inhibited per gram dry matter

( ) in parentheses: number of varieties

1.3 Effect of trypsin inhibitors (TIs) on digestive metabolism in animals

In 1917, Osborne and Mendel observed that soybeans did not support growth in rats unless they were cooked. With this observation in mind, research began and was extended to many other animal species (Liener 1958). After the identification of protease inhibitors in raw soybeans (Kunitz 1945, 1947a,b; Bowman 1944), a direct relationship between antitryptic fractions and growth inhibition was observed. The history of the development of knowledge concerning trypsin inhibitors has been reviewed extensively by Liener and Kakade (1980). It was observed that there was a growth reduction in rats when predigested proteins or free amino acids were fed together with a high antitryptic fraction prepared from soybeans (Liener and Kakade, 1980). This result indicates that the antinutritional effect of TI cannot only be explained by the inhibition of trypsin activity in the gut. In other studies with rats it was shown that TI also influenced the secretion of pancreatic enzymes (Schneemann et al., 1977). The secretion of pancreatic enzymes is mediated by the humoral cholecystokinin-pancreomyzin (CCK-PZ) hormone which stimulates the acinar cells of the pancreas to produce digestive enzymes. The CCK-PZ hormone is produced by endocrine cells in the small intestinal mucosa. The CCK-PZ hormone production is negatively related to levels of free trypsin in intestinal chyme. When trypsin is inhibited by TI, the CCK-PZ hormone production is enhanced resulting in an increased production of pancreatic digestive enzymes. According to Fushiki and Iwai (1989) trypsin and trypsin inhibitors do not interact directly with the luminal surface of the small intestine, but their actions are mediated by a trypsin-sensitive, cholecystokinin-releasing peptide. Due to enhanced enzyme production, hypertrophy and hyperplasia of the pancreas may occur. Chernick et al., (1948) discovered pancreatic enlargement in chicks caused by feeding raw soybeans. This finding was confirmed in several other studies, not only in chicks but also in rats, mice and young guinea pigs (Liener and Kakade, 1980). Pancreatic hypertrophy is not observed in large animal species such as growing pigs, dogs, calves and presumably man (reviewed by Liener and Kakade, 1980; Gallaher and Schneemann, 1986). Hypertrophy of the pancreas was also observed in rats after feeding purified soya Kunitz inhibitor (Rackis, 1965; Sambeth et al. 1967). A significant correlation between TI content in the diets and pancreatic hypertrophy in rats was found by Struthers et al. (1983) and Liener et al. (1985). Histological examination of enlarged pancreas of rats after feeding TI indicates that the number of cells was increased (Kakade et al, 1967; Spangler et al., 1985). Hypertrophy of the pancreas is not only found in relation to soya TI but also in rats and chicks fed Vicia faba L. or purified TI from faba beans. (Kardivel and Clandinin, 1974; Abbey et al., 1979) and in chicks fed Pisum sativum (Johns, 1987). The negative feedback mechanism regulating the secretion of pancreatic enzymes found in rats also exists in pigs and calves, but without causing pancreatic hypertrophy (reviewed by Gallaher and Schneeman, 1986). According to Liener and Kakade (1980) the relative weight of the pancreas of various species is related with the occurrence of pancreatic hypertrophy due to soya TI (Table 2). They observed that those animal species in which the relative pancreatic weight exceeded 0.3% of the body weight the pancreas became hypertrophic. Those animals whose relative pancreas weight was below this value did not respond in terms of pancreas hypertrophy.

Table 2 Relationship between size of pancreas of various species of animals and response of pancreas to raw soybeans or trypsin inhibitor.

Species	Size of pancreas (% of body weight)	Pancreatic hypertrophy
Mouse	0.6 - 0.8	+
Rat	0.5 - 0.6	+
Chick	0.4 - 0.6	+
Guinea pig	0.29	±[a]
Dog	0.21-0.24	-
Pig	0.10-0.12	-
Human being	0.09-0.12	(-)[b]
Calf	0.06-0.08	-

[a]Observed in young guinea pigs but not in adults.
[b]Predicted response.
Adapted from Liener and Kakade (1980).

It is not clear whether CCK-PZ is the only factor causing hypertrophy of the pancreas, because Struthers et al. (1983) found distinct differences in the response of the pancreas between a group of rats fed raw soybeans and a group infused with CCK. Naim et al. (1982) demonstrated that TI may not be the only factor in soybeans causing hypertrophy in rats. Green et al. (1986) found that when amino acid availability is inadequate, pancreatic growth and adaptive response to CCK is impaired and protease secretion is insufficient to normalize CCK secretion, resulting in sustained elevation of the plasma CCK level. Indications that pancreatic hypertrophic effects and enzyme secretion are also influenced by protein quality and levels of protein in the diets can be found in studies by Green et al. (1986); Green and Nasset (1983) and Hasdai et al. (1989). Most of the studies on the effects of TIs on weight gain, protein efficiency ratio (PER), digestibility and pancreas have been carried out using small animal species such as rats, mice and chickens. The way TI acts in animals is mainly based on data from small animals and not on data from larger monogastric farm animals such as pigs.

2 LECTINS

2.1 General characteristics

The first description of a lectin, also called (phyto)haemagglutinin, was made by Stillmark in 1889. He studied the toxicity of press-cake from castor beans (Ricinus communis), and found a protein which was toxic and called it ricin. Ricin was able to agglutinate erythrocytes from man and animal. The term lectins was first used by Boyd and Shapleigh (1954). The word originates from the Latin verb "legere", meaning "to select" or "to choose", and was used to indicate the specificity towards human erythrocytes of specific blood groups and towards red blood cells of different

animal species. Because of the capability of lectins to agglutinate erythrocytes they were often called (haem)agglutinins. As already stated before lectins are proteins, most in the form of glycoproteins (Jaffé, 1980). They are characterized by their unique capability to bind to sugar components. The affinity to sugar components may differ among the various lectins. The structure and chemical properties have been studied in many lectins (reviewed by Goldstein and Poretz, 1986). In general, lectins have a dimeric or tetrameric structure. The lectin molecule consists of one or more subunits. The lectins in Phaseolus vulgaris beans are present in tetrameric form. These lectins can be divided into two different subunits (E and L subunits). L-subunits have a strong affinity for receptors on the membrane of lymphocytes and have low affinity for erythrocyte receptors. R-subunits have a strong affinity for receptors on erythrocytes but low affinity for those on lymphocytes. Each subunit has about the same molecular size of 34 000 - 38 000 daltons (Weber et al. (1972); Yachnin and Svenson, 1972 and Yachnin et al., 1972). Differences in biological activities of lectins are explained by small differences in the structure of the subunits. Pea (Pisum sativum) lectins in mature seeds usually consist of two tetrameric subunits, a heavy (B) chain (MW 18 000) and a light (A) chain (MW 10 500) (Masson et al., 1986). Immature peas contain also a γ subunit (Marik et al., 1974; Van Driessche et al., 1989). Soybean (Glycine max) lectins are also tetrameric glycoproteins consisting of equal amounts of two slightly different subunits (Goldstein and Poretz, 1986).

2.2 Analysis of lectins

In vitro agglutination of red blood cells is mainly used as a method of detecting and quantifying lectins. This haemagglutination reaction is related to the binding of lectins to glycoconjugates of the walls of red blood cells. Agglutination occurs because of the binding of two erythrocytes to the same lectin molecule. Agglutination of cells is used as a measure of lectin activity. Jaffé et al. (1972); Jaffé, 1980; Marquardt et al. (1975), and Newton and Hill (1983) reported differences in agglutination activity as measured with red blood cells from different animal species. Huisman et al. (1990) also demonstrated differences in agglutination activity between cells from different animal species with the same feed samples (Table 3).

These results show that the haemagglutination method is not adequate. In our example, horse red blood cells seem most appropiate for measuring Phaseolus vulgaris lectin activity, but rabbit cells are more appropiate for soya lectins. Some other discussion points are:

- L-lectins of Phaseolus vulgaris agglutinate lymphocytes but display only weak activity towards red blood cells. Thus, using red blood cells L-lectins will not be readily detected. (Jaffé, 1980).
- The aim of the lectin assay is to predict the amount of lectins having affinity for glycoconjugates in the gut wall. One may discuss whether the glycoproteins of red blood cells are identical to those in the gut wall.
- From the point of view of nutritionists, technologists and plant breeders it is important to have an assay which makes it possible to distinguish toxic lectins from non-toxic ones. Jaffé et al. (1972) and Jaffé (1980) attempted to classify lectins in pathogenic and non- pathogenic groups using red blood cells from different blood

Table 3 Comparison of haemagglutination activity of Phaseolus vulgaris using red blood cells from rabbit, pig and horse.

	Haemagglutinin activity (units* per gram sample)		
	Rabbit	Pig	Horse
Ph. vulgaris, cv processor			
- raw	80	20	640
- 20 minutes steam-heated	5	1	100
Ph. vulgaris, cv procol	20	5	not determined
Raw soybean meal	20	1	0.1

* one haemagglutinin activity unit is defined as the smallest amount of sample required for visual agglutination under test conditions. Haemagglutinin activity is expressed in haemagglutinin units per milligram sample.

groups or from different animals pretreated with various proteases. But, as discussed, blood cells do not detect all lectins and may not be adequate for detecting lectins affecting the gut wall.

An improved lectin assay is the ELISA method (Gabius, 1989). However, no separation between toxic and non-toxic lectins can be made using this method. A new approach in ELISA technique was developed by Hendriks et al. (1987) who coated microtitre plates with brush border membranes. This method enables measurement of a direct binding of lectins to the brush border. A very promising assay for determining different types of lectins and isolectins is the FLIA (Funtional Lectin Immuno Assay), developed by Hamer et al. (1989). This method is based on the ability of lectins to bind to microtitre plates which have been coated with either different carbohydrate matrices or brush border membranes. This method enables the measurement of different isolectins in a particular sample.

2.3 Effect of lectins on digestive metabolism in animals

2.3.1 General

Most research has been carried out with lectins from Phaseolus vulgaris beans since they are highly toxic for man and animal. Information about the mode of action of lectins from peas and soya in animals is scarce. The majority of the research on lectins is carried out using rats and other small laboratory animals. Only a few studies have been carried out on monogastric farm animals. Information about the nutritional significance of lectins in animal and man recently has been reviewed by Kik et al.

(1989), Liener (1986) and Pusztai (1989). Many studies have been made to explain the reduction in weight gain of growing animals due to dietary lectins. It became clear in these studies that lectins interfere with digestive and absorptive processes in the digestive tract and, in addition, affect the immune system. These different aspects are discussed in the following section

2.3.2 Effects of lectins on the intestinal wall

Most lectins are resistant to proteolytic breakdown in the gut and are excreted with the faeces (Jaffé, 1980; Nakata and Kimura, 1985). Because of this resistance, lectins remain active in the gut and are able to bind to surface receptors of the intestinal epithelium. Constituents of the epithelial cells of the intestine are the carbohydrates of the glycocalyx, providing a large surface for interactions between dietary lectins and the gut wall (recently reviewed by Pusztai, 1989 and Kik et al. 1989a). Since lectins have different sugar affinities, the binding of lectins will depend on the type of sugars present. The carbohydrate composition of the glycocalyx changes with maturation. The less differentiated crypt cells contain mainly polymannose type oligosaccharide side chains, whereas the more differentiated mature cells move up the villus and contain more complex structured carbohydrates. Thus, binding of lectins with these cells may depend on the degree of maturation. It has been shown that lectins react with different parts of the intestinal villi (Etzler and Branstrator, 1974). Lectins with D-mannose or D-glucose specificity such as pea and lentil lectins bind preferentially to the lower part of the small intestinal villi. Lectins with a preference for binding with more complex sugar matrices (oligosaccharide type), such as Phaseolus vulgaris and soya lectins, bind to the upper part of the small intestinal villi, consisting of more mature cells. Binding of Phaseolus vulgaris lectins to the upper part of the villi was shown in rats (King et al., 1986) and in pigs (King et al., 1983). Binding to the upper part of the villi leads to disruption of the brush border and interferes with the digestion and absorption of nutrients (Liener, 1986; Pusztai, 1989). Damage of the brush border in rats has been reported by various authors (King et al., 1980 a,b, 1982; Greer, 1983; Pusztai, 1987; Rouanet et al., 1985) but in this respect reports about studies in pigs are scarce (Myer et al., 1982; Kik et al., 1989b; King et al., 1983, van der Poel et al., 1990).

Binding of lectins does not always cause damage to the brush border. No effects on the brush border of piglets fed a diet containing 1.25% purified pea lectins were found (Bertrand et al., 1988). Histological observations showed that the intestinal wall was not altered in this experiment. Kilpatrick et al. (1985) found that tomato lectins were bound to the villus epithelium, but no toxic effect was observed. Interference of lectins with the digestive and absorptive processes may occur when the binding of lectins to the membrane receptors of the epithelial cells causes endocytosis.

2.3.3 Effect of lectins on brush border enzyme activity

In many studies effects of lectins on brush border enzyme activities were observed. Rouanet et al. (1985) reported reduced enzyme activities of enterokinase, alkaline phosphatase, leucylnaphtylamidase and glutamyl transpeptidase in the intestinal

mucosa of rats after feeding 0.25% pure phytoagglutinins (PHA). They suggested that this observation may be related to a reduced number of enzyme-producing enterocytes. This results in a lower capacity for enzyme synthesis. This results in a lower capacity for enzyme synthesis. Nakata and Kimura (1985) showed that activity of the brush border enzymes sucrase, alkaline phosphatase and leucine aminopeptidase was decreased due to feeding of the Phaseolus lectin and Concanavalin A. They concluded that the toxicity of ingested bean lectins involves their binding to the luminal surface of the small intestine, where they disturb the function of the brush border membrane. Contrasting results have been reported with lectins from peas. Bertrand et al. (1988) studied the effect of pea lectins on the activity of maltase and amylase in the mucosa of the small intestine of piglets. They found no effects on these parameters when 1.25% purified lectin was included in the diet. Jindal et al. (1982) found decreased activities of mucosal disaccharides and total proteolytic activity in rats when purified lectins from peas and lentils were fed.

2.3.4 Effects of lectins on cellular metabolism in the small intestinal mucosa

Cellular metabolism of the small intestinal mucosa was found to be affected by lectins, indicating that endocytosis may be an essential process in explaining the effects of lectins (King et al., 1986; Pusztai, 1989). They claimed that the uptake of lectins by the gut epithelial cells was very rapid and much faster than the uptake of antigens from other dietary origins. One of the consequences of the uptake of lectins by gut wall epithelium is a rapid increase in the cell protein synthesis in the mucosa (Oliveira et al., 1988). Increases in protein synthesis in the jejunal mucosa of 70% (Palmer et al. 1987) and a doubling of the weight of the small intestinal wall has been reported (Greer et al., 1985). Also the sugar concentration of the small intestinal wall doubled, which can be related to an often observed increased mucin secretion after feeding PHA from Phaseolus to rats (Greer et al., 1985). Bardocz et al. (1989) found that the stimulated growth of the small intestine due to PHA feeding was accompanied by an increase in the polyamine content in the intestinal tissue, especially in the jejunum. The polyamine uptake with feed intake is normally restricted and its content in the blood is low. It has been suggested that the increased need for polyamines for tissue growth may result in a catabolic breakdown of body protein.

2.3.5 Effects of lectins on bacterial ecology in the gut

Higher counts for E. coli were found in the intestinal lumen of rats after feeding diets containing Phaseolus vulgaris lectins compared with rats fed other diets (Banwell et al., 1983; Wilson et al., 1980). Higher amounts of coliform bacteria in the gut lumen of pigs after feeding PHA were found by King et al. (1983). Various explanations have been given for this observation. One explanation is that due to a lower nutrient digestibility more substrate for the bacteria becomes available in the intestine. This may lead to multiplication of bacteria. Pusztai (1989) discussed that lectins binding to the gut wall could act as new receptors for E. coli bacteria. Toxic effects of PHA are more severe in conventional rats than in germ-free (gnotobiotic) rats (Jayne-Williams and Hewitt, 1972; Rattray et al., 1974). This is probably because E. coli facilitates

endocytosis by PHA, and may thus increase the effects of lectins on the gut cellular metabolism and may also increase systemic effects (Pusztai, 1989). Another possibility is that due to the effects of lectins on the gut epithelium, bacteria are able to enter the body and organs more easily, causing toxic effects within the body (Jayne-William and Burgess, 1974).

2.3.6 *Effect of lectins on protein and fat metabolism*

Studies by Pusztai et al. (1981) showed that, when feeding Phaseolus beans with high lectin content or when feeding pure PHA, N excretion in the urine was markedly increased. Two different options for explaining this observation were postulated. It is possible that lectins may block protein synthesis in the body or may cause an increase in tissue (protein) catabolism. Evidence for tissue breakdown was found by Palmer et al. (1987) in rats fed increasing amounts of Phaseolus beans. Bardocz et al.(1989) also found indications of a catabolic effect of PHA when Phaseolus beans were fed. Oliveira et al. (1988) showed muscle loss due to the lectins in Phaseolus beans. Pusztai (1989) discussed that the catabolic breakdown of protein in muscles is a particular effect of Phaseolus beans since soya lectins did not produce such effects (Grant et al., 1987). Fat metabolism also seems to be affected by lectins. Grant et al.(1987) observed an increased loss of body fat after feeding PHA to rats. At the same time, glycogen stores in the body were depleted, while the blood insulin level was lowered. The underlying mechanism for these changes in systemic metabolism is not clear (Pusztai, 1989).

2.4 *Effects of dietary lectins on the immune system*

Hardly any information is available on the effects of dietary lectins on the local gut immune system. There are some indications that lectins of Phaseolus vulgaris can cause mast cell degranulation in the gut wall, increased vasicular permeability and consequently an increased leakage of serum proteins into the gut lumen (Greer and Pusztai, 1985). Pusztai (1989) suggested that IgA, produced locally in the gut, plays only a minor role in the neutralization of dietary lectins. With ^{125}I labelled lectins, it was shown that 10% of the toxic Phaseolus lectins applied intragastrically reached the blood within three hours (discussed in Pusztai, 1989). In contrast, the amount of non-toxic tomato lectins which was absorbed was only 0.1% of the initial dose (Kilpatrick et al. 1985). Thus toxic lectins, by affecting the brush border seem to be transported into the systemic circulation. These lectins can then bind to blood cells, leading to the production of humoral anti-lectins of the IgG-class. (Pusztai, 1980; Pusztai et al., 1981, 1983; Grant et al., 1985; Begbie and King, 1985).

3 OTHER ANFs

Tannins are mainly present in coloured-flowering varieties of peas and Phaseolus beans. Tannins form complexes with proteins, carbohydrates and other polymers in foods (Rao and Prabhavathi, 1982). The greater tendency to form complexes with protein than with carbohydrates is attributed to the strong hydrogen-bond affinity of

the carboxyl oxygen of the peptide group in proteins. Due to this complexation digestibility of protein is reduced (Jansman et al., 1989; Liebert and Gebhardt, 1983). The way dietary tannins act in the animal is not entirely clear. After consumption, tannins may also form complexes with digestive enzymes. Due to this complex formation the activity of digestive enzymes is decreased which may cause a lower digestibility of nutrients (Griffiths and Mosely, 1980; Marquardt, 1989). Other antinutritional effects which are thought to be due to tannins, are damage of the gut wall, toxicity of tannins absorbed from the gut and interference with the absorption of some minerals (Mitjavila et al., 1977). Generally, tannins interfere with different aspects of the digestive process resulting in reduced growth, increased feed conversion efficiency and lower egg production in laying hens. Tannins in peas and beans are mainly located in the seed coats (Reddy et al., 1985). White coloured seeds contain low levels of tannins and coloured varieties in general high levels (Bond and Smith, 1989; Bond et al., 1986; Bressani and Elias, 1980; Papadopoulos et al., 1985). Some of the effects of tannins on nutrition can be reduced by removing the seed coats (dehulling) (Edwards and Duthie, 1973; Henry and Bourdon, 1973). Soya beans and the majority of pea varieties contain only low levels of tannins.
Alpha-amylase inhibitors are widely distributed throughout the plant kingdom. They are detected in Phaseolus vulgaris (Buonocore and Silano, 1986). Alpha-amylase inhibitors have been indicated as factors responsible for the impaired digestibility of starch in red kidney beans (Jaffé and Vega Letta, 1968). However in studies of Savaiano et al. (1977) it was found that alpha-amylase inhibitors from red kidney beans did not affect the rate of growth of weanling rats, nor did it alter the availability of energy from dietary starch. The latter results indicate that this factor seems to be of minor importance from a nutritional point of view.

Flatulence factors are related to oligosaccharides which are fermented by intestinal bacteria in the large intestine. These oligosaccharides are not broken down in the small intestine because of the lack of appropriate enzymes and flow into the large intestine. There they are degraded by the action of bacterial alpha-1,6-galactosidases. The cleavage products are converted into volatile fatty acids, carbon-dioxide, hydrogen and methane, resulting in flatulence, diarrhoea, nausea, cramps and discomfort in the animals (Rackis, 1975; Saini, 1989).
Proteins from soya and peas may act as antigens, causing gut wall damage and immunological reactions, linked with disorders in gut function in piglets and veal calves (Kilshaw and Sissons, 1979; Miller et al., 1984; Seegraber and Morril, 1982, 1986; Sisson et al., 1982; Sissons and Smith, 1976; Toullec and Guilloteau, 1989). Phytic acid is present in legume seeds to the extent of 1% to 5% of the dry weight. Phytic acid forms complexes with anions resulting in a reduced availability of the minerals Ca, P, Mg, Zn, Cu and Fe. (Reddy et al., 1982; Forbes and Erdman, 1983).

VI DISCUSSION

1 Nutritional effects of ANFs in the animal

Trypsin inhibitors and lectins cause serious disturbances in physiological processes including digestion and absorption and may also cause immunological reactions. As a result, weight gain is reduced and feed conversion is less efficient. The role of ANFs

in animal nutrition may become more important in the future. This is related to the general expectation among zootechnicians that the future farm animal will grow faster and deposit more protein in the body because of advances in animal breeding, health care and housing (Webb, 1989). These animals will need more highly digestible protein (thus low in ANF) in their diet. In pigs this may become more important because the feed intake capacity has not yet been increased in the modern breeds (Brandt et al., 1985; Webb and Curran, 1986). As reported by Huisman and van der Poel (1987) and Huisman (1989), most protein-rich seeds contain ANFs. The results discussed in this review show that there are gaps in our knowledge about the mechanism by which ANFs act in farm animals. The negative effects of trypsin inhibitors on animals growth may be explained in at least two ways. One is related to the fact that in the gut, activity of the digestive enzymes trypsin and chymotrypsin is reduced by the TIs (Holm, 1989; Liener and Kakade, 1980). This may result in a lower protein digestibility. Due to this lower protein digestibility, less amino acids will be available, which will result in a lower weight gain. It may be questioned whether the inhibition of trypsin has an important effect on the protein digestibility because in the literature (Liener and Kakade, 1980; Gallaher and Schneemann, 1986) it is reported that when trypsin is inhibited the pancreas is stimulated to produce more (chymo) trypsin. Moreover, Solomon (1987) reported that normally there is a surplus of digestive enzymes produced by the pancreas. The other way in which negative effects on performance may be caused is related to the fact that inhibition of trypsin and chymotrypsin activity may result in enhanced secretion of pancreas enzymes. Liener and Kakade, 1980 Gallaher and Scheemann, 1986). Lyman and Lepkowsky (1957) were the first to find indications that the growth depression in rats may be related to endogenous loss of protein and amino acids due to a hyper active pancreas resulting in an increased secretion of pancreas enzymes. With extra excretion of pancreatic enzymes there is an increased loss of endogenous protein. The pancreatic enzymes trypsin and chymotrypsin are rich in cystine. Because cystine is derived from methionine, the extra secretion of cystine may lead to a situation in which less methionine is available for the synthesis of body tissues. In rats and chicks, it was demonstrated that the negative effect due to soya TI could be reduced significantly by including extra amino acids (methionine, threonine, valine and lysine) in the diet (Borchers, 1961, 1962; Khayamhashi and Lyman, 1966). Summarizing, it is not clear which effect of trypsin inhibitor is most important from nutritional point of view: the possible decreased protein digestibility due to reduced (chymo) trypsin activity or the increased secretion of endogenous protein. The best way to obtain insight in this is to measure the true protein digestibility in proteins containing trypsin in hibitors. This point is further discussed in paragraph 5 of this chapter. The possibility of partly abolish ANF effects by supplementing with amino acids has only been demonstrated in rats and chickens and not in pigs. It is questionable whether in pigs pancreatic secretion is affected to the same extent and whether the negative effect of soya TI can also be reduced by addition of amino acids. There is insufficient information about the reaction of the pancreas of pigs in response to TIs.

Cannulation of the pancreas seems to be a promising method for studying the effect of TIs on the secretion of pancreatic enzymes in pigs. Different methods for cannulating the pancreas have already been published (Corring, 1980; Corring et al., 1972; Hee, 1985; and Zebrowska et al.,1983). In order to study the effects of trypsin

inhibitors in animals in relation to their contents in the diets, it is essential to know to which family the trypsin inhibitors belong. Because of the stability to gastric juices and likely heat, the BBI may be more important than the STI in this respect. This information is also important for inactivation studies and plant breeding.

As stated before after oral intake, lectins can reach the small intestine where they can bind to the epithelial cells of the intestinal mucosa. This binding can result in a damage of the intestinal epithelial cells which results in a decreased absorption of nutrients, a change in the activity of brushborder enzymes and a hyper secretion of endogenous protein due to shedding of damaged cells, increased production of mucus, and a loss of plasma proteins to the intestinal lumen. Toxic lectins, affecting the brushborder, seem to be transported in to the systemic circulation. This may lead to the production of humoral anti-lectins of the IgG-class. Overall, these effects lead to a decreased nutriënt digestibility, decreased nitrogen retention, and sometimes scouring resulting in a reduced weight gain and less efficient feed conversion. There are marked differences in toxicity of lectins. Lectins from common beans are very toxic (Liener, 1986; Pusztai, 1989). Pea lectins were found to be non-toxic by Bertrand et al. (1988) and toxic by Jindal et al. (1982). One example of a non-toxic lectin is the tomato lectin (Kilpatrick, 1985). These results demonstrate that for a nutritional evaluation it is important to study the toxicity of each type of lectin separately. Another discussion point is that most investigations concerning how lectins act in animals are carried out with diets containing high levels of lectins. These levels are generally distinctly higher than the levels present in practial diets. It is likely that the marked effects with common bean lectins will be less pronounced when practical levels are used. For practical nutrition, it is important to have more data about the effects of ANFs at practical inclusion levels. As discussed, common bean lectins affect the intestinal wall severely. These effects are so marked that they generally overrule the effects of other ANFs. It is difficult to obtain information about the effects of other ANFs present in these seeds. The effects of trypsin inhibitors and lectins on digestion can be summarized as follows:

- both can effect the apparent protein digestibility directly. Trypsin inhibitors by inactivation of (chymo) trypsin. Lectins due to a decreased production of brushborder enzymes and due to a reduced absorption of nutrients. Reduced absorption may also effect the digestibility of other nutrients.
- both can have an indirect effect on the apparent protein digestibility by stimulation of the secretion of endogenous protein. Trypsin inhibitors by stimulation of the secretion of pancreatic enzymes and lectins by stimulation of mucin proteins.

2 Animal species differences

Most research into the mode of action of ANFs in animals and studies on the possibilities for reducing ANF activity by technological treatments is carried out small animal species such as rats, chickens and mice. The choice for small animals is related to high costs and the large amounts of purified ANFs needed in studies with larger animal species. Rats have been used in most of the studies dealing with technologically treated products. Criteria in these studies were often parameters such as Protein Efficiency Ratio (PER), Net Protein Utilization (NPU) and N balance. Not only the use of the rat as a model for other animals is questionable in this respect, but it is also uncertain whether parameters such as PER, NPU and N balance

obtained in rats can be extrapolated to pigs. There are indications that animal species react differently to ANFs. As already discusssed, there is a difference in reaction to trypsin inhibitors on the pancreas between small and larger animal species (Chapter V.1.3. and Table 2 of this chapter). Differences in response between rats and piglets to ANFs present in raw soybeans are suggested by Combs et al. (1967) and Yen et al. (1977). Visitpanich et al. (1985) found a similar relative growth depression in rats and piglets fed pigeon pea (Cajanus cajan), but differences in response to feeding chick peas (Cicer arietinum). The results suggest that the rat reacts differently to ANFs in legume seeds than the pig. However, information about animals species differences are too scarce to conclude whether or not small laboratory animals such as rats and chickens can be used as an alternative for pigs.

3 Need for studies with isolated ANFs

Many seeds contain more than one ANF (Chubb, 1982.; Liener, 1980, 1981; Savage and Deo, 1989). For checking in animals the effects of technological treatments and determination of maximum inclusion levels (threshold levels), it is essential to study the effects of each particular ANF separately. For example the effects of lectins in common beans are so dominant that there is hardly any information about the effect and importance of trypsin inhibitors in these beans. When peas are fed to young piglets there may be a negative effect on weight gain. But there is hardly any indication in the literature about which factor may be responsible. The use of isolated and purified ANFs is the most advanced method for studying the effects of the individual ANFs. However, producing sufficient amounts of ANFs is very time-consuming and expensive, especially when studies with larger animals such as pigs are concerned. Because of the high costs, studies with purified ANFs have so far only been carried out on small animals. Masson et al. (1986) demonstrated that it is possible to isolate relatively large quantities of ANFs, although it is still expensive. In this respect the development of special cannulation techniques is important. These techniques should make it possible to study with small amounts the effect of ANFs at the site of reaction e.g: TI and pancreas, lectins and gut wall.

4 Threshold levels

So far, hardly any attention has been given to threshold levels for the above mentioned reasons. Threshold levels are the levels of ANFs which can be tolerated without causing negative effects in the animals. Most research focussing on the mode of action of ANFs in animals is mainly done using high inclusion levels of ANF-containing seeds. However, these results give no information about the threshold levels. For nutritionists, technologists and plant breeders it is important to have information on threshold levels. It is questionable whether ANF contents need to be reduced to zero. There are different factors that may influence threshold levels for ANFs. It is known that in diets for young chickens and older pigs more heated soya or raw peas can be included, without causing negative effects, than in diets for piglets. This indicates that it may be possible for different threshold levels to be used depending on animal species and age or live weight range.

Adequate nutritional evaluation of ANF-containing seeds and the determination of threshold levels are hampered because of the lack of adequate analytical techniques. The trypsin inhibitor assay was based on determining TIs in soya. It is uncertain whether this assay can be used without adaptation to determine TI in other seeds. As already stated Boisen (1989) and Liu and Markakis (1989) mentioned the influence of pH and the type of trypsin used on the TI analysis. It may, therefore, be recommended that trypsin from the target animal to be used rather than bovine trypsin in the assay. As mentioned, different analytical methods have been employed and the units in which the trypsin inhibitor activity is expressed are often different. Therefore, it is very difficult to compare results obtained in different laboratories. In this respect standardization of analytical methods for determining of ANFs is important. The development of the FLIA (Functional Lectin Immuno Assay) method offers possibilities to analyse (iso)lectins which react directly with the gut wall. This opens up the possibility for studying the toxicity of different lectins and to determine threshold levels for individual toxic lectins, as well as for different target animals. This assay is also very important for technologists performing lectin inactivation studies and for plant breeders to select new varieties based on the presence of toxic and non-toxic lectins.

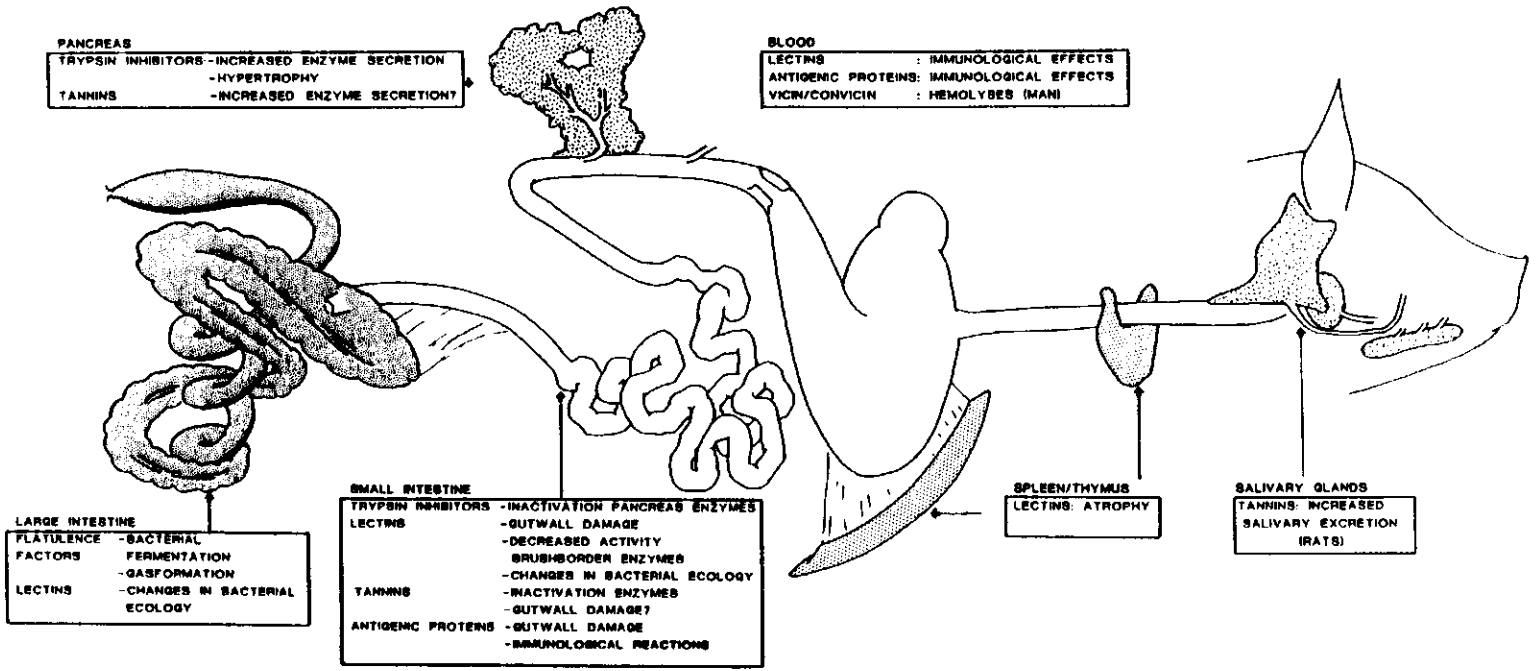
5 Recommendations for future research

The various points discussed make it clear that there is a need for more research on various aspects of ANFs. Some of these points are discussed briefly in the following section. Most research focussing on how ANFs act in animals is done on small animals. There are indications that animal species react differently to ANFs. It is, therefore, worthwhile to study when, and under which circumstances, data from one animal species may be extrapolated to another. From the nutritional point of view, it is important to carry out more research on target animals. In this respect it is also important to do these studies with practical ANF inclusion levels in the diets. So far, most research has been carried out using high inclusion levels of ANFs or ANF-containing seeds. Many studies on chickens and rats have shown that the pancreas reacts to the dietary inclusion of TI-containing seeds or to the addition of TIs (Gallaher and Schneemann, 1986; Liener and Kakade, 1980). But none of these studies are focussed on the question of which level of TIs are important in this respect. Chickens can tolerate high levels of toasted soya in their diets while piglets do not. Therefore, chickens seem to be less sensitive to ANFs in soya than pig(let)s. However, information about the effect of TIs on the secretion of pancreatic enzymes in pigs is scarce. It is important to investigate these effects and obtain more data about the threshold levels for TIs. The development of pancreas cannulation techniques create the possibility to study effects of TIs on the pancreas of pigs and to determine threshold levels for TIs. It may be important in these studies to take the age of the animals into account.

More studies dealing with the toxicity of lectins in seeds of different plant species need to be carried out. So far, most research has been done on lectins from Phaseolus vulgaris. Information about lectins present in other seeds is scarce. It has been shown that there are differences in toxicity between the various lectins.

Therefore, it is important to know more about the toxicity of lectins from the various plant species.
There is also no information about threshold levels for lectins originating from the different plant species. The development of brush border cell cultures (Kik et al., publications in preparation) in combination with the development of the FLIA lectin assay (Hamer et al., 1989), opens up possibilities to study threshold levels for toxic lectins. There is insufficient information about which part of a low "apparent digestibility" of a feed ingredient can be attributed to the increased secretion of endogenous protein caused by ANFs, and which part can be related to a low digestibility of the protein as such. In a classic digestibility experiment the "apparent digestibility" of protein is based on the excretion of undigested protein from the ingredient and the excretion of endogenous protein from the animal. It is important to make a distinction between the two ways in which protein may excreted. This makes it possible to calculate which part of the lower protein digestibility is related to an increased endogenous secretion caused by ANFs. This information is important for studies into the possibilities of increasing the protein digestibility of feed ingredients. If the lower digestibility is caused by an increased secretion of endogenous protein due to ANFs, the activities of technologists or plant breeders must be focussed on reducing the ANFs. If the feed protein has a "low digestibility", than attention should also be focussed on the protein per se. The "true" digestibility of the protein per se can be calculated by correcting the "apparent" protein digestibility for the part of endogenous protein. Corrections, using data of endogenous protein obtained by feeding protein-free diets, are inadequate in this respect, because specific effects of ANFs on endogenous secretion are not included in this method. (Krawielitzki et al., 1977; de Lange, 1989). The ^{15}N technique, in which all proteins in the animal are labelled, seems therefore to be the most relevant method (de Lange et al., 1990). This technique enables a distinction to be made between the labelled endogenous protein from the animal and the unlabelled undigested protein from the feed.

SITES OF EFFECTS OF ANFs PRESENT IN BEANS AND PEAS
IN MONOGASTRIC ANIMALS



Legend of Figure 1

Lectins	can bind to receptors of epithelial cells of the intestinal mucosa and subsequent endocytosis, resulting in disturbances of the digestion and absorption processes. Due to the damage of the intestinal mucosa, the permeability can be increased and as a result lectins and other peptides may be absorbed, and may cause effects on immunity and on some organs.
Antigenic proteins	cause damage of the intestinal mucosa, resulting in effects on the digestion and absorption processes, and effects on local and humoral immunity.
Trypsin inhibitors	primarily inactivate (chymo) trypsin activity. As a secondary effect, regulated by a negative feed back mechanism, the secretion of pancreas enzymes is enhanced.
Tannins	form complexes with proteins, carbohydrates and other polymers. Protein complexation can occur with all kinds of proteins, such as digestive enzymes, mucosal proteins and glycoproteins in saliva.
Flatulence factors	are related which polysaccharides broken down by bacterial fermentation in the large intestine.
Vicin and convicin	are hydrolyzed by intestinal microflora into compounds, which may cause haemolysis of blood.

VII REFERENCES

Abbey, B.W., Norton, G. and Neale, R.J. (1979). Effects of dietary proteinase inhibitors from field bean (Vicia faba L.) and field bean meal on pancreatic function in rats. Britisch Journal of Nutrition, 41, 39-45.

Banwell, J.G., Boldt, D.H., Meyers, J. and Weber, F.L. (1983). Phytohemagglutinin derived from red kidney bean (Phaseolus vulgaris): a cause for intestinal malabsorption with bacterial overgrowth in rats. Gastroenterology 84, 506-515.

Bardocz, S., Grant, G., Brown, D.S. and Pusztai, A. (1989). Stimulation of polyamine synthesis and growth of the small intestine by dietary kidney bean lectin. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 39-42.

Begbie, R. and King, T.P. (1985). The interaction of dietary lectin with porcine small intestine and the production of lectin-specific antibodies. In: T.C. Bog-Hansen and J. Breborowicz (Eds). Lectins, Biology, Biochemistry, Clinical Biochemistry. Walter de Gruyter, Berlin, 15-27.

Bertrand, G., Sève, B., Gallant, D.J. and Tomé, R. (1988). Absence d`effets antinutritionel des lectines de pois, sous forme native ou purifee chez porcelet. Sciences des Aliments, 8, 187-212.

Birk, Y. (1961). Purification and some properties of a highly active inhibitor of trypsin and chymotrypsin from soybeans. Biochimica et Biophysica Acta. 54, 378-381.

Birk, Y. (1976). Proteinase inhibitors from plant sources. Methods in Enzymology, 45, 695-739.

Birk, Y. (1987). Proteinase inhibitors. In: A. Neuberger and K. Brocklehurst (Eds). Hydrolytic enzymes. Elsevier, Amsterdam, 257-305.

Birk, Y. (1989). Protein protease inhibitors of plant origin and their significance in nutrition. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 83-94.

Bogers, R.J. (1972). In: H.P. Maas Geesteranus (Ed). Plant pathogenic bacteria. Pudoc, Wageningen, The Netherlands, 239-250.

Boisen, S. (1989). Comparative studies on trypsin inhibitors in legumes and cereals. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 118-120.

Bond, D.A. and Smith, D.B. (1989). Possibilities for the reduction of antinutritional factors in grain legumes by breeding. In: J. Huisman, A.F.B. van der Poel and I.E. Liener (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 285-296.

Bond, D.A., Toynbee-Clarke, G., Pope, M. and Hall, J.A. (1986). Comparison between white- and coloured-flower plants and between near-isogenic tannin-free and tannin containing lines of Vicia faba. Vorträge für Pflanzenzuchtung, 11, 137-150.

Borchers, R. (1961). Counteraction of the growth depression of raw soybean oil meal by amino acid supplements in weanling rats. Journal of Nutrition, 75, 330-334.

Borchers, R. (1962). Supplementary methionine requirement of weanling rats fed soybean oil meal rations. Journal of Nutrition, 77, 309-311.

Bowman, D. (1944). Fractions derived from soybeans and navybeans which retard tryptic digestion of casein. Proceedings Society Experimental Biology Medicine, 57, 139-140.

Boyd, W.C. and Shapleigh, E. (1954). Specific Precipitating Activity of Plant Agglutinins (lectins) Science, 119, 419.

Brandon, D.L., Bates, A.H. and Friedman, M.(1988). Enzyme-linked Immuno Assay of Soybean Kunitz Trypsin Inhibitor using monoclonal antibodies. Journal of Food science, 53, 102-110.

Brandon, D.L., Bates, A.H. and Friedman, M. (1989). Monoclonal antibody Based Enzyme Immuno assay of the Bowman-Birk Protease Inhibitor of soybeans. Journal of Agricultural and Food Chemistry, 37, 1192-1196.

Brandt, H., Hong, K.Ch. and Glodek, P. (1985). Das Zuchtziel in der Deutschen Schweinzucht. 2. Mitteilung: Die Berücksichtigung der Futtteraufnahme bei der Zuchtwertschätzung. Züchtungskunde, 57, 92-98.

Bressani, R. and Elias, L.C.(1980). In: J.H. Hulse.(Ed). Polyphenols in cereals and legumes. International Development Research Centre, Ottawa, Canada, 61.

Broadway, R.M., Duffey, S.S., Pearce, G. and Ryan, C.A. (1986). Plant proteinase inhibitors: A defence against herbivorous insects? Entomologia Experimentalis et applicata. 41, 33-38.

Brown, W.E., Graham, J.S., Lee, J.S. and Ryan, C.A. (1986). Regulation of proteinase inhibitor genes in food plants. In: M. Friedman. (Ed). Nutritional and toxicological significance of enzyme inhibitors in foods. Plenum Press, New York, US, 281-290.

Bullard, R.W. and Elias, D.J. (1980). Sorghum polyphenols and bird resistance. In: J.H. Hulse. (Ed). Polyphenols in cereals and legumes. International Development Research Centre, Ottawa, Canada., 43-49.

Buonocore, V. and Silano, V. (1986). Biochemical, nutritional, and toxicological aspects of alpha-amylase inhibitors from plant foods. In: M. Friedman. (Ed). Nutritional and toxicological significance of enzyme inhibitors in foods. Plenum Press, New York, US, 483-507.

Carré, B. and Conan, L. (1989). Relationship between the trypsin inhibitor content of pea seeds and pea protein digestibility in poultry. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances in research of antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 103-106.

Cheeke, P.R. and Shull, L.R. (1985). Natural toxicants in feeds and poisonous plants. AVI, Westport, Connecticut, US.

Chernick, S.S., Lepkovsky, S. and Chaikoff, I.L. (1948). A dietary factor regulating the enzyme content of the pancreas: changes induced in size proteolytic activity by the ingestion of raw soybean meal. American Journal Physiology, 155, 33-41.

Chubb, L.G. (1982). Anti-nutritive factors in animal feedstuffs. In: W. Haresign. (Ed). Recent Advances in Animal Nutrition. Butterworths, London, UK, 21-37.

Combs, G.E., Connes, R.G., Berry, T.H. and Wallace, H.D. (1967). Effect of raw and heated soyabean meal on gain, nutrient digestibility, plasma amino acids and other blood constituents of growing swine. Journal of Animal Science, 26, 1067-1071.

Corring, T., Aumaitre, A. and Rerat, A. (1972). Fistulation permanente du pancreas exocrine chez le porc application: response de la secretion pancreatique au repas. Annales de Biologie Animale Biochimie et Biophysique, 12, 109-124.

Corring, T. (1980). Endogenous secretion in the pigs. In: A.G. Low and I.G. Partridge. (Eds). Current concepts of digestion and absorption in pigs. Technical Bulletin 3. National Institute for Research in Dairying, Reading, England.

De Lange, C.F.M. (1989). Endogenous protein in digestibility studies in pigs. Ph.D. Thesis University of Alberta, Edmonton, Canada.

De Lange, C.F.M., Souffrant, W.B. and Sauer, W.C. (1990). Real ileal protein amino acid digestibilities in feedstuffs for growing pigs as determined with the ^{15}N-isotope dilution technique. Journal of Animal Science, 68, 409-418.

Del Rosario, R.R., Lozano, S., Pamorasamit, M.G. and Noél, M.G. (1980). The trypsin inhibitor activity of legume seeds. Philippine Agriculturist, 63, 339.

DiPietro, C.M. and Liener, I.E. (1989). Heat inactivation of the Kunitz and Bowman-Birk soybean protease inhibitors. Journal of Agricultural and Food Chemistry, 37, 39-44.

Doell, B.H., Ebden, C.J. and Smith, C.A. (1981). Trypsin inhibitor activity of conventional foods which are part of the British diet and some soya products. Qualitas Plantarum. Foods Human Nutrition, 31, 139.

Doggett, H. (1976). Sorghum (Sorghum bicolor). In: N.W. Simmonds (Ed). Evolution of crop plants, Longman, 112-116.

Dutch CVB table. (1988). Veevoedertabel: Gegevens over voederwaarde, verteerbaarheid en samenstelling. Centraal veevoederbureau in Nederland, Lelystad, The Netherlands.

Edwards, D.G. and Duthie, I.E. (1973). Processing to improve the nutritive value of field beans. Journal of the Science of Food and Agriculture, 24, 496-498.

Etzler, M. and Branstrator, M.L. (1974). Differential localization of cell surface and secretory components in rat intestinal epithelium by use of lectins. The Journal of Cell Biology, 62, 329-343.

Etzler, M.E. (1986). Distribution and function of plant lectins. In: I.E. Liener, N. Sharon and I.J. Goldstein (Eds). The Lectins. Properties, functions, and applications in biology and medicine. Academic Press, New York, US, 371-435.

Forbes, R.M. and Erdman, J.W. (1983). Bioavailability of trace mineral elements. Annual Review of Nutrition, 3, 213-231.

Freed, R.C. and Ryan, D.S. (1978a). Changes in Kunitz trypsin inhibitor during germination of soybeans: An immunoelectrophoresis assay system. Journal of Food Science, 43, 1316.

Freed, R.C. and Ryan, D.S. (1978b). Note on modification of the Kunitz soybean trypsin inhibitor during seed germination. Cereal Chemistry, 55, 534.

Friedman, M. (1986). Nutritional and toxicological significance of enzyme inhibitors in foods. Plenum Press. New York, 572 pp.

Fushiki, T. and Iwai, K. (1989). Two hypotheses on the feedback regulation of pancreatic enzyme secretion. The FASEB Journal, 3, 121-126.

Gabius, H.J., Engelhardt, R., Hellmann, K.P., Hellman, T and Ochsenfarht, A. (1987). Preparation of neoglycoprotein-enzyme conjugate using a heterobifunctional reagent and its use in solid-phase assays and histochemistry. Analytical Biochemistry 165, 349-355.

Gallaher, D. and Schneemann, B.O. (1986) Nutritional and metabolic response to plant inhibitors of digestive enzymes. In: M. Friedman (Ed). Nutritional and toxicological significance of enzyme inhibitors in foods. Plenum Press, New York, US, 167-185.

Gatehouse, A.M.R., Gatehouse, J.A., Dobie, P., Kilminster, A.M. and Boulter, D. (1979). Biochemical basis of insect resistance in Vigna unguiculata. Journal of the Science of Food and Agriculture, 30, 948-958.

Goldstein, I.J. and Poretz, R.D. (1986). Isolation, Physicochemical Characterization, and Carbohydrate-binding specificity of lectins. In: I.E. Liener, N. Sharon and I.J. Goldstein. (Eds). The Lectins. Properties, functions, and applications in biology and medicine. Academic Press, New York, US, 35-247.

Grant, G., Greer, F., McKenzie, N.H. and Pusztai, A. (1985). Nutritional response of mature rats to kidney bean (Phaseolus vulgaris) lectins. Journal of Science of Food and Agriculture, 36, 409-414.

Grant, G., Watt, W.B., Stewart, J.C. and Pusztai, A. (1987). Changes in the small intestine and hind leg muscles of rats induced by dietary soyabean (Glycine max) proteins. Medical Science Research, 15, 1355-1356.

Green, G.M., Olds, B.A., Matthews, G. and Lyman, R.L. (1973). Protein as a regulator of pancreatic enzyme secretion in the rat. Proceedings of the society for experimental biology and medicine, 142, 1162-1167.

Green, G.M. and Nasset, E.S. (1983). Role of dietary protein in the rat pancreatic enzyme response to a meal. Journal of Nutrition, 113, 2245-2252.

Green, G.M., Levan, V.H. and Liddle, R.A. (1986) Interaction of dietary protein and trypsin inhibitor on plasma cholecystokinin and pancreatic growth in rats. In: M. Friedman. (Ed). Nutritional and toxicological significance of enzyme inhibitors in foods. Plenum Press, New York, US, 123-132.

Greer, F. (1983). Local (intestinal) and systemic responses of animals to ingested Phaseolus vulgaris lectins. Mechanism of lectin toxicity. Ph.D. Thesis university of Aberdeen, Scotland, 167 pp.

Greer, F. and Pusztai, A. (1985). Toxicity of kidney bean (Phaseolus vulgaris) in rats: changes in intestinal permeability. Digestion, 32, 42-46.

Greer, F., Brewer, A.C. and Pusztai, A. (1985). Effect of kidney bean (Phaseolus vulgaris) toxin on tissue weight and composition and some metabolic functions of rats. British Journal of Nutrition, 54, 95-103.

Griffiths, W. (1984). The trypsin and chymotrypsin inhibitor activities of various pea (Pisum spp.) and Field bean (Vicia faba) cultivars. Journal of the Science of Food and Agricultural, 35, 481-486.

Griffiths, D.W. and Mosely, G. (1980). The effects of diets containing field beans of high or low polyphenolic content on the activity of digestive enzymes in the intestine of rats. Journal of the Science of Food and Agriculture, 31, 255-259.

Hamer, R.J., Koninkx, J., van Oort, M.G., Mouwen, J. and Huisman, J. (1989). New developments in lectin analysis. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 30-33.

Hamerstrand, G.E., Black, L.T. and Glover, J.D. (1981). Trypsin inhibitors in soy products: Modification of the standard analytical procedure. Cereal Chemistry, 58, 42.

Hee, J.H., Sauer, W.Z., Berzin, R. and Ozimek, L. (1985). Permanent re-entrant diversion of porcine pancreatic secretions. Canadian Journal of Animal Science, 65, 451-457.

Hendriks, H.G.C.J.M., Koninkx, M., Draaijer, M., van Dijk, J.E., Raaijmakers, J.A.M. and Mouwen, J.M.V.M. (1987). Quantitative determination of the lectin binding capacity of small intestinal brush-border membrane. An enzyme-linked lectin sorbent assay. Biochimica et Biophysica Acta 905, 371-375.

Henry, Y. and Bourdon, D. (1973). Apparent digestibility of energy and protein in horse bean, with and without dehulling, as compared to soybean oil meal. Journées Recherche Porcine en France, 105-114.

Holm, H. (1989). Inhibitor resistant trypsin and chymotrypsin due to feeding raw soy. The Bowman-Birk inhibitor and the Kunitz trypsin inhibitor in the rat. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 125-128.

Hove, E.L. and King, S. (1979). trypsin inhibitor contents of lupin seeds and other grain legumes. New Zealand Journal of Agricultural Research, 22, 41-42.

Huisman, J. and van der Poel, A.F.B. (1987). Effects of antinutritional factors (ANF) in pig nutrition. 38th Annual meeting of the E.A.A.P., Lissabon, Portugal.

Huisman, J. (1989). Antinutritional factors (ANFs) in the nutrition of monogastric farm animals. *In: E.J. van Weerden and J. Huisman. (Eds). Nutrition and digestive physiology in* monogastric animals. Pudoc, Wageningen, The Netherlands, 17-35.

Huisman, J., van der Poel, A.F.B., Verstegen, M.W.A. and van Weerden, E.J. (1990). Antinutritional factors in pig nutrition. World Review of Animal Production. In press.

Jaffé, W.G. and Vega Letta, C.V. (1968). Heat labile growth inhibiting fractions in beans (Phaseolus vulgaris). Journal of Nutrition, 94, 203-210.

Jaffé, W.G., Brücher, O. and Palozzo, A. (1972). Zeitschrift Immunitätsforschung, 142, 439-447.

Jaffé, W.G. (1980). Hemagglutinins (lectins). In: I.E. Liener (Ed). Toxic constituents of plant foodstuffs. Academic Press, New York, US, 73-102.

Jansman, A.J.M., Huisman, J. and van der Poel, A.F.B. (1989). Faba beans with different tannin contents: ileal and faecal digestibility in piglets and growth in chicks. In: J. Huisman, A.F.B. van der Poel and I.E. Liener (Eds). Recent advances in research of antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 176-180.

Janzen, D.H., Juster, H.B. and Liener, I.E. (1976) Insecticidal action of the Phytohemagglutinin in Black Beans on bruchid Beetle. Sience 192, 795-796.

Jayne-Williams, D.J. and Burgess, D.J. (1974). Further observations on the toxicity of navy beans (Phaseolus vulgaris) for Japanese quail (Coturnix Coturnix Japonica). Journal of Applied Bactoriology, 37, 149-169.

Jayne-Williams, D.J. and Hewitt, D. (1972). The relationship between intestinal microflora and the effects of diets containing raw navy beans (Phaseolus vulgaris) on the growth of Japanees quail (Coturnix Coturnix Japonica). Journal of Applied Bacteriology, 35, 331-334.

Jindal, S., Soni, G.L. and Singh, R. (1982). Effect of feeding of lectins from lentils and peas on the intestinal and hepatic enzymes of albino rats. Journal of Plant Foods, 4, 95-103.

Johns, D.C. (1987). Influence of trypsin inhibitors in four varieties of peas (Pisum sativum) and growth of chickens. New Zealand Journal of Agricultural Research, 30, 169-175.

Kakade, M.L., Barton, T.L. and Schaibel, P.J. (1967). Biochemical changes in the pancreas of chicks fed raw soybeans and soybean meal. Poultry Science, 46, 1578-1580.

Kakade, M.L., Simons, N. and Liener, I.E. (1969). An evaluation of natural vs synthetic substrates for measuring the antitryptic activity of soybean samples. Cereals Chemistry, 46, 518.

Kakade, M.L., Simons, N.R., Liener, I.E. and Lamberts, J.W. (1972). Biochemical and nutritional assesment of different varieties of soybeans. Journal of Science Food & Chemistry, 20, 87.

Kakade, M.L., Rackis, J.J., McGhee, J.E. and Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: collaborative analysis of an improved procedure. Cereal Chemistry, 51, 376-382.

Kardirvel, R. and Clandinin, D.R. (1974). The effect of faba bean (Vicia faba L.) on the performance of turkey poults and broiler chicks from 0-4 weeks of age. Poultry Science, 53, 1810-1816.

Kassell, B. (1970). Naturally-occuring inhibitors of proteolytic enzymes. Methods in Enzymology, 19, 839-906.

Khayamhashi, H. and Lyman, R.L. (1966). Growth depression and pancreatic and intestinal changes in rats force-fed amino acid diets containing soybean trypsin inhibitor. Journal of Nutrition, 89, 455-464.

Kik, M.J.L., Rojer, J.M., Mouwen, J.M.V.M., Koninkx, J.F.J.G., van Dijk, J.E. and van der Hage, M.H. (1989a). The interaction between plant lectins and the small intestinal epithelium: a primary cause of intestinal disturbance. The Veterinary Quaterly, 11, 2, 108-115.

Kik, M.J.L., Huisman, J, van der Poel, A.F.B. and Mouwen, J.M.V.M. (1989b). Pathological changes of the small intestinal mucosa of piglets after feeding Phaseolus vulgaris beans. In: J. Huisman, A.F.B. van der Poel and I.E. Liener (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 49-53.

Kilpatrick, D.C., Pusztai, A, Grant, G., Graham, C. and Ewen, S.W.B. (1985). Tomato lectin resists digestion in the mammalian alimentary canal and binds to intestinal villi without deleterious effects. FEBS Letters, 185, 299-305.

Kilshaw, P.J. and Sissons, J.W. (1979). Gastrointestinal allergy to soyabean protein in preruminant calves. Antibody production and digestive disturbances in calves fed heated soyabean flour. Research in Veterinary Science, 27, 361-365.

King, T.P., Pusztai, A. and Clarke, E.M.W. (1980a). Immunocytochemical localization of ingested kidney bean (Phaseolus vulgaris) lectins in rat gut. Histochemical Journal, 12, 201-208.

King, T.P., Pusztai, A. and Clarke, E.M.W. (1980b). Kidney bean (Phaseolus vulgaris) lectin-induced lesions in the rat small intestine: 1. Light microscope studies. Journal of Comparative Pathology, 90, 585-595.

King, T.P., Pusztai, A. and Clarke, E.M.W. (1982). Kidney bean (Phaseolus vulgaris) lectin-induced lesions in rat small intestine: 3. Ultrastructural studies. Journal of Comparative Pathology, 92, 357-373.

King, T.P., Begbie, R. and Cadenhead, A. (1983). Nutritional toxicity of raw kidney beans in pigs. Immunocytochemical and cytopathological studies on the gut and the pancreas. Journal of the Science of Food and Agriculture, 34, 1404-1412.

King, T.P., Pusztai, A., Grant, G. and Slater, D. (1986). Immunogold localization of ingested kidney bean (Phaseolus vulgaris) lectins in epithelial cells of rat small intestine. Histochemical Journal, 18, 413-420.

Koide, T., Tsunasawa, S. and Ikenaka, T. (1973). Studies on soybean trypsin inhibitors. European Journal of Biochemistry, 32, 408-416.

Krawielitzki, K., Volker,. T., Smulikowska, S., Bock, H.D. and Wünsche, J. (1977). Weitere Untersuchungen zun Multikompartment-Modell des Proteinstoffwechsels. Archiv für Tierernährung, 27, 609.

Kunitz, M. (1945). Crystallization of a trypsin inhibitor from soybeans. Science, 101, 668-669.

Kunitz, M. (1946). Crystalline soybean trypsin inhibitor. Journal Genetic Physiology, 29, 149-154.

Kunitz, M. (1947a). Crystalline soybean trypsin inhibitor. Journal Genetic Physiology, 30, 291-310.

Kunitz, M. (1947b). Isolation of a crystalline protein compound of trypsin and of soybean trypsin inhibitor. Journal Genetic Physiology, 30, 311-320.

Laskowski, M. Jr. (1986). Protein inhibitors of serine proteinases - mechanism and classification. In: M. Friedman (Ed). Nutritional and toxicological significance of enzyme inhibitors in foods. Plenum Press, New York, US, 1-17.

Leterme, P., Beckers, Y. and Thewis, A. (1989). Inter- and intravarietal variability of the trypsin inhibitor content of peas and his influence on apparent digestibility of crude protein in

growing pigs. In: J. Huisman, A.F.B. van der Poel and I.E. Liener (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 121-124.

Liebert, F. and Gebhardt, G. (1983) Ergebnisse der Vergleichen ernährungsphysiologischen Prüfung verschiedene Ackerbohnenherkünfte am Mastschweine unter besonderer Beachtung einer weissblühenden Neuzucht. Archiv für Tierernärung, 33, 47-56.

Liener, I.E. (1958). In: A.M. Altschul (Ed). Processed Plant Protein Foodstuffs. Academic Press, New York, US, 79-129.

Liener, I.E. (1980). Toxic constituents of plant foodstuffs. Academic Press, New York, US, 502 pp.

Liener I.E. and Kakade, M.L. (1980). Protease inhibitors. In: I.E. Liener. (Ed). Toxic constituents of plant foodstuffs. Academic Press, New york, US., 7-71.

Liener, I.E. (1981). Factors affecting the nutritional quality of soya products. Journal of the American Oil Chemists' Society, 58, 3, 406-415.

Liener, I.E., Nitsan, Z., Srisangnam, C., Rackis, J.J., and Gumbmann, M.R. (1985). The USDA trypsin inhibitor study. II. Time related biochemical changes in the pancreas of rats. Qualitas Plantarum. Plant Foods Human Nutrition, 35, 243-257.

Liener, I.E. (1986). Nutritional significance of lectins in the diet. In: I.E. Liener, N. Sharon and I.J. Goldstein. (Eds). The lectins. Academic Press, New York, US, 527-552.

Liener, I.E. (1989a). Antinutritional factors in legume seeds: State of the Art. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 6-13.

Liener, I.E. (1989b). Summary of group discussions and recommendations for future research. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 381-384.

Liu, K. and Markakis, P. (1989). An improved colorimetric method for determining antitryptic activity in soybean products. Cereal Chemistry, 66, 415-422.

Marik, R., Entlicher, G. and Kocourek, J. (1974). Studies on phytohemagglutinins. XVI. Subunit structure of the pea isophytohemagglutinins. Biochemica et Biophysica Acta, 336, 53-61.

Marquardt, R.R., McKirdy, J.A., Ward, T. and Campbell, L.D. (1975). Amino acid, hemagglutinins and trypsin inhibitor levels, and proximate analyses of faba beans (Vicia faba) and faba bean fractions. Canadian Journal of Animal Science, 55, 421-429.

Marquardt, R.R. (1989). Dietary effects of tannins, vicine and convicine. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional research in legume seeds. Pudoc, Wageningen, The Netherlands, 141-155.

Masson, P., Tome, D. and Gaborit, T. (1986). Large-scale preparation and characterization of pea seed lectins. (Pisum sativum L.). Lebensmittel Wissenschaft und Technologie, 19, 138-143.

Myer, O.M., Froseth, J.A. and Coon, C.N. (1982). Protein utilization and toxic effects of raw beans (Phaseolus vulgaris) for young pigs. Journal of Animal Science, 55, 1087-1098.

Miller, B.G., Newby, T.J., Stokes, C.R., Hampson, D.J., Brown, P.J. and Bourne, F.J. (1984). The importance of dietary antigen in the case of postweaning diarrhoea in pigs. American Journal Veterinary Research, 45, 1730-1733.

Mitjavila, S., Lacombe, C. Carrera, G. and Derache, R. (1977). Tannic acid and oxidized tannic acid on the functional state of rat intestinal epithelium. Journal of Nutrition, 107, 2113-2121.

Naim, M., Gertler, A. and Birk, Y. (1982). The effect of dietary raw and autoclaved soya-bean protein fractions on growth, pancreatic enzymes in rats. British Journal of Nutrition, 47, 281-288.

Nakata, S. and Kimura, T. (1985). Effect of ingested toxic bean lectins on the gastrointestinal tract in the rat. Journal of Nutrition 115, 1621-1629.

Newton, S.D. and Hill, G.D. (1983). The composition and nutritive value of field beans. Nutrition Abstracts and Reviews, series B, 53, 99-115.

Odani, S. and Ikenaka (1973). Studies on soybean trypsin inhibitors. VIII Disulphides bridges in soybean Bowman-Birk proteinase inhibitor. Journal of Biochemistry, 74, 697-715.

Oliveira de, J.T.A., Pusztai, A. and Grant, G. (1988). Changes in organs and tissues induced by feeding of purified kidney bean (Phaseolus vulgaris) lectins. Nutrition Research, 8, 943-947.

Osborn, T.B. and Mendel, L.B. (1917). The use of soybean as food. Journal of Biological Chemistry, 32, 369-387.

Palmer, R.M., Pusztai, A., Bain, P. and Grant, G. (1987). Changes in rates of tissue protein synthesis in rats induced in vivo by consumption of kidney bean (Phaseolus vulgaris) lectins. Comparative Biochemistry and Physiology. 88C, 179-183.

Papadopoulos, C.H., Tsaftaris, A.S. and Ronparkias, D.G. (1985). Correlation between tannin content and testa colour in faba beans (Vicia faba L.). FABIS 13, 38-40.

Pistole, T.G. (1981). Interaction of bacteria and fungi with lectins and lectin-like substances. Annual Review Microbiology, 35, 85-112.

Pusztai, A. (1980). Nutritional toxicity of the kidney bean (Phaseolus vulgaris). Report of Rowett Institute. 36, 110-118.

Pusztai, A., Clarke, E.M.W., Grant, G. and King, T.P. (1981). The toxicity of Phaseolus vulgaris lectins. Nitrogen balance and immunochemical studies. Journal of the Science of Food and Agriculture, 32, 1037-1046.

Pusztai, A. Greer, F., Silva Lima, G.M., Prouvost-Danon, A. and King, T.P. (1983). Local and systemic responses to dietary lectins. In: I.J. Goldstein and M.E. Etzler. (Eds). Chemical Taxonomy, Molecular Biology and Function of Plant Lectins. Alan R. Liss, New York, US, 272-273.

Pusztai, A., Croy, R.R.D., Grant, G. and Stewart, J.C. (1983). Seed lectins: Distribution, Location and Biological role. In: J. Daussant, J. Mosse and J. Vaughan. (Eds). Academic Press. New York, US, 53-82.

Pusztai, A. (1987). Plant lectins-biological functions. Acta Biochemica et Biophysica, Hungary, 99, 355-375.

Pusztai, A. (1989). Biological effects of dietary lectins. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 17-29.

Rackis, J.J. (1965). Physiological properties of soybean trypsin inhibitor and their relationship to pancreatic hypertrophy and growth inhibition of rats. Proceedings Federal American Society Experimental Biology, 24, 1488-1493.

Rackis, J.J. (1975). Oligosaccharides of food legumes: Alpha-galactosidases actvity and flatus problems. In: J. Allen and J. Heilge. (Eds). Physiological effects of food carbohydrates. American Chemists Society, Washington, DC, US, 207-222.

Rackis, J.J., McGhee, J.E., Gumbmann, M.R., Booth A.N. (1979). Effect of soy proteins containing trypsin inhibitors in long term studies in rats. Journal of American Oil Chemists' Society. 56, 162.

Rackis, J.J., Gumbmann, M.R. and Liener, I.E. (1985). The USDA trypsin inhibitor study. I. Backgrounds, objectives and procedural details. Qualitas Plantarum. Plant Foods Human Nutrition. 35, 213-242.

Rackis, J.J. and Gumbmann, M.R. (1986). Protease inhibitors: Physiological properties and nutritional significance. In: R. L. Ory (Ed). Antinutrients and natural toxicants in foods. Food and Nutrition press, Inc., Westport, US, 203-237.

Rackis, J.J., Wolf, W.J. and Baker, E.C. (1986). Protease inhibitors in plant foods: content and inactivation. In: M. Friedman. (Ed). Nutritional and toxicological significance of enzyme inhibitors in foods. Plenum Press, New York. US, 299-347.

Rao, B.S.N. and Prabhavathi, J. (1982), The tannin content in foods commonly consumed in India and its influence on ionisable iron. Journal of the Science of Food and Agriculture, 33, 89.

Rattry, E.A.S., Palmer, R, and Pusztai, A. (1974). Toxicity of kidney bean (Phaseolus vulgaris L.) to conventional and gnotobiotic rats. Journal of the Science of Food and Agriculture, 25, 1035-1040.

Read, J.W. and Haas, L.W. (1938). Studies on the baking quality of flour as affected by certain enzyme actions. V. Further studies concerning potassium bromate and enzyme activity. Cereal Chemistry 15, 59-68.

Reddy, N.R., Sathe, S.K. and Salunkhe, D.K. (1982). Phytates in cereals and legumes. Advances Food Research, 28, 1-92.

Reddy, N.R., Pierson, H.D., Sathe, S.K. and Salunkhe, D.K. (1985). Dry beans: a review of nutritional implications. Journal of American Oil Chemists Society, 62, 541-549.

Roozen, J.P. and de Groot, J. (1987). Analysis of low levels of trypsin inhibitor activity in food. Lebensmittel Wissenschaft und Technologie, 20, 305-308.

Rouanet, J.M., Lafont, J., Creppy, A. and Besancon, P. (1985). Effects of dietary kidney bean (Phaseolus vulgaris) lectins in growing rats. Nutrition Reports International, 31, 237-244.

Ryan, C.A. (1978). Proteinase inhibitors in plant leaves: A biochemical model for natural plant protection. Trends biochemical science. 5 : 148-150.

Ryan, C.A. (1979). Proteinase inhibitors. In: G.A. Rosenthal and D.A. Janszen. (Eds). Herbivores: Their interaction with secondary plant metabolites. Academic Press, New York, US.

Ryan, C.A. (1983). Insect-induced chemical signals regulating natural plant protection responses. In: R.F. Denno and M.S. McClure. (Eds). Variable plant and herbivores in natural and managed systems. Academic Press, New York, US. 43-60.

Ryan C.A., Bishop, P., Pearce, G. Walker-Simmons, M. (1985). Pectic fragments regulate the expression of proteinase inhibitor genes in plants. In: J.L. Key and T. Kosuge. (Eds). Cellular and molecular biology of plant stress. Alan R. Liss Inc. US.

Saini, H.S. (1989). Legume seed oligosaccharides. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 329-341.

Sambeth, W., Nesheim, M.C. and Serafin, J.A. (1967). Separation of soybean whey into fractions with different biological activities for chicks and rats. Journal of Nutrition, 92, 479-490.

Savage, G.P. and Deo, S. (1989). The nutritional value of peas (Pisum sativum). A literature review. Nutriton Abstracts and Reviews (Series A), Vol. 59, No 2, 66-88.

Savaiano, D.A., Powers, J.R., Costello, M.J., Whitaker, J.R. and Clifford, A.J. (1977). The effect of an alpha-amylase inhibitor on the growth rate of weanling rats. Nutrition Reports International, 15, 443-449.

Schneemann, B.O., Chang, I., Smith, L and Lyman, L.R. (1977). Effect of dietary amino acids, casein and soybean trypsin inhibitor on pancreatic protein secretion in rats. Journal of Nutrition, 107, 281-288.

Seegraber, F.J. and Morril, J.L. (1982). Effect of soy protein on calves' intestinal absorptive ability and morphology determined by scanning electron microscopy. Journal of Dairy Science, 65, 1962-1970.

Seegraber, F.J. and Morril, J.L. (1986). Effect of protein source in calf milkreplacers on morhology and absorptive ability of the small intestine. Journal of Dairy Science, 69, 460-469.

Singleton, V.L. (1981). Naturally occuring food toxicants: phenolic substances of plant origin common in foods. Advances in Food research, 27, 149-242.

Sissons, J.W. and Smith, R.H. (1976). The effect of different diets including those containing soya-bean products, on the passage of digesta movement and water and nitrogen absorption on the small intestine of the pre-ruminant calf. British Journal of Nutrition, 36, 421-438.

Sissons, J.W., Smith, R.H., Hewitt, D. and Nyrup, A. (1982). Prediction of the suitability of soy-bean products for feeding to preruminant calves by an in vitro immunochemical method. British Journal of Nutrition, 47, 311-318.

Slump, P. and Dukel, F. (1979). Aminozuurgehalten en andere parameters van sojaschroot en sojabonen van verschillende herkomst. TNO-report. 6022.

Spangler, W.L., Gumbmann, M.R., Liener, I.E. and Rackis, J.J. (1985). The USDA trypsin inhibitor study. III. Sequential development of pancreatic pathology in rats. Qualitas Plantarum. Plant Foods Human Nutrition, 35, 259-274.

Solomon, T.E. (1987). Control of exocrine pancreatic secretion. In: L.R. Johnson (Ed). Physiology of the Gastrointestinal Tract, second edition. Raven Press, New York, 1173-1207.

Stillmark, H. (1889). In: R. Kobert. (Ed). Arbeiten des pharmakologischen Institutes zu Dorpat, Enke, Stutgart, vol 3, 57-62.

Struthers, B.J., MacDonald, J.R., Dahlgren, R.R. and Hopkins, D.T. (1983). Effects on the monkey, pig and rat pancreas of soyproducts with varying levels of trypsin inhibitors and comparison with the administration of cholecystokinin. Journal of Nutrition, 113, 86-97.

Swain, T. (1979). Tannins and lignins. In: G.A. Rosenthal and D.H. Janszen (Eds). Herbivores, their interaction with secondary plant metabolites. Academic Press, New York, US, 657-682.

Tan-Wilson, A.L. and Wilson, K.A. (1986). Relevance of multiple soybean trypsin inhibitor forms to nutritional quality. In: M. Friedman. (Ed). Nutritional and toxicological significance of enzyme inhibitors in foods. Plenum Press, New York, US, 391-411.

Toullec, R. and Guilloteau, P. (1989). Research into the digestive physiology of the milk-fed calf. In: E.J. van Weerden and J. Huisman. (Eds). Nutrition and digestive physiology in monogastric farm animals. Pudoc, Wageningen, The Netherlands, 37-55.

Valdebouze, P., Bergeron, E., Gaborit, T. and Delort-Laval, J. (1980). Content and distribution of trypsin inhibitors and haemagglutinins in some legume seeds. Canadian Journal of Plant Science, 60, 695-701.

Van der Poel., A.F.B. (1990). Effects of processing on bean (Phaseolus vulgaris L.). Protein quality. Ph.D. Thesis Agricultural University Wageningen, Netherlands.

Van der Poel, A.F.B., Aarts, H.L.M. and Kik, M.J.L. (1990). Air classification of Bean Flour - effects on Protein, Antinutritional Factors and the Effect of a Fines Fraction on Cultured Explants of Small Intestinal Mucosa. Journal of the Science of Food and Agriculture, In press.

Van Driessche, E., Charlier, G., Schoup, J., Beeckmans, S., Pohl, P., Lintermans, P. and Kanarek, L. (1989). Maturation of pea lectin; a comparison with other leguminosae lectins. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 67-72.

Van Oort, M.G., Hamer, R.J. and Slager, E.A. (1989). The trypsin inhibitor assay: improvement of an existing method. In: J. Huisman, A.F.B. van der Poel and I.E. Liener. (Eds). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 110-113.

Visitpanich, T., Batterham, E.S. and Norton, B.W. (1985). Nutritional value of chickpea (Cicer arietinum) and pigeon pea (Cajanus cajan) meals for growing pigs and rats. I. Energy content and protein quality. Australian Journal of Agricultural Research, 36, 327-335.

Webb, J. (1989). Trends in breeding high quality pigs. Pigs, 5, 20-25.

Webb, A.J. and Curran, M.K. (1986). Selection regime by production system interaction in pig improvement: a review of possible causes and solutions. Livestock Production Science, 14, 415-423.

Weber, T.H., Aro, H. and Norman, C.T. (1972). Characterization of lymphoside stimulating blood cell agglutinating glycoproteins from red kidney beans (Phaseolus vulgaris). Biochimica et Biophysica Acta, 263, 94-105.

Wilson, A.B., King, T.P., Clarke, E.M.W. and Pusztai, A. (1980). Kidney bean (Phaseolus vulgaris) lectin induced lesions in the rat small intestine. II. Microbiological studies. Journal of Comparative Pathology, 90, 597-602.

Yachnin, S. and Svenson, R.H. (1972). Protein structure. Immunology, 22, 871-883.

Yachnin, S., Allen, L.W., Baron, J.M. and Svenson, R.H.. (1972). The potentiation of phytohemagglutinin in used lymphoside transformation by cell-cell interaction: a matrix hypothesis. Cellular Immunology, 3, 569-589.

Yannai, S. (1980). Toxic factors induced by processing. In: Liener, I.E. (Ed.). Toxic constituents of plant foodstuffs. Academic Press. New York., US, 371-418.

Yen, J.T., Jensen, A.H. and Simon, J. (1977). Effect of dietary raw soybean and soybean trypsin inhibitor on trypsin and chymotrypsin activities in the pancreas and in the small intestinal juice of growing swine. Journal of Nutrition, 107, 156-165.

Zebrowska, T., Low, A.G. and Zebrowska, H. (1983). Studies on gastric digestion of protein and carbohydrate, gastric secretion and exocrine pancreatic secretion in the growing pigs. British Journal of Nutrition, 29, 401-410.

CHAPTER 2

ANIMAL SPECIES DIFFERENCE RESEARCH WITH PIGLETS, RATS AND CHICKENS FED PEAS AND COMMON BEANS

2.1 Comparison of growth, nitrogen metabolism and organ weights in piglets and rats fed on diets containing Phaseolus vulgaris beans

J. Huisman[1)], A.F.B. van der Poel[2)], M.W.A. Verstegen[2)] and P. van Leeuwen [1)]

1) TNO-Institute of Animal Nutrition and Physiology (IGMB-Dept. ILOB), P.O. Box 15, 6700 AA Wageningen, The Netherlands.
2) Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen.

British Journal of Nutrition. In press

I ABSTRACT

The effects of lectins have mainly been studied in rats. An important question is whether results obtained in rats can be extrapolated to larger animals like the pig. Phaseolus vulgaris beans are rich in toxic lectins. Therefore, a study was carried out to compare the effects of diets containing 20% Phaseolus vulgaris (raw or toasted) in rats and young piglets. Live weight gain, N digestibility and N balance were much lower in piglets fed diets containing raw beans than in rats. Live weight gain and N balance were even slightly negative in the piglets. When toasted beans were fed, live weight gain and N balance values were reduced in piglets but hardly at all in rats. Feeding raw beans caused hypertrophy of the pancreas in the rats but in piglets the weight of the pancreas was depressed. Spleen weight was depressed in the piglets but not in the rats. Weight of liver was not affected in both animal species. When toasted beans were fed no effects on the weights of pancreas, spleen or liver were found in either piglets or rats. In a second experiment was studied whether the negative effects in piglets were age related. Diets containing 20% Phaseolus beans were fed to pigs of about 18 kg, 35 kg and 55 kg, respectively. At all three ages live weight was distinctly reduced. It was concluded that the piglet is much more sensitive to ANFs in Phaseolus vulgaris beans than the rat. The differences in sensitivity could not be explained by possible differences in physiological ages between piglets and rats.

II INTRODUCTION

Many seeds contain substances which are referred as to antinutritional factors (Cheeke and Shull, 1985; Chubb, 1983; Friedman, 1986; Huisman, 1989; Huisman et al., 1989; Liener, 1980; Liener, et al., 1986). This term is used because these factors can disturb metabolic processes and reduce the utilization of nutrients in the animal. The mode of action of ANFs has been studied mainly in rats and chickens. Only a few studies have been carried out with pigs. In view of this an important question is whether the results obtained in rats can be extrapolated to the pig. Studies by Combs et al. (1967) and Yen et al. (1977) suggest that the rat and piglet respond differently to ANF in raw soya beans. Visitpanich et al. (1985) found different effects when chickpea (Cicer arietinum) were fed. There is not enough information about the sensitivity of various animal species to lectins. Therefore, this study was carried out to compare the effects of diets containing 20% Phaseolus vulgaris beans in rats and piglets. The most important ANF in these beans are the lectins (Bond and Smith, 1989). Since young animals would probably show the greatest effects, it was decided to use early weaned piglets and young rats. The object of the present study was to compare the effects of ANFs in untreated and treated Phaseolus vulgaris beans on N digestibilty, N utilization, weight gain and organ weights in piglets and rats. In the young piglets used in our study much more marked negative effects on performance were measured than in rats. Although the piglets were closely matched with the rats in terms of actual age, it could be that there are differences with respect to their physiological ages. It may be possible that the physiological age of young rats is closer to older pigs than to young pigs. In this case the older pig may be less sensitive to ANFs in the Phaseolus beans and more comparable with young rats. In a second experiment was studied whether the negative effects in piglets are age related.

III MATERIAL AND METHODS

1 Diets

In experiment 1 three diets were formulated, a control diet containing no beans and two test diets containing 20% Phaseolus vulgaris beans. In one of the test diets raw Phaseolus beans were included, while the other test diet contained Phaseolus beans which had been toasted for 40 minutes. The composition of these diets is given in Table 1. A batch of beans with a medium high lectin content was selected.

Table 1 Composition of the diets of experiment 1 (g/kg)

Ingredients	Control diet	Test diets
Phaseolus vulgaris beans	---	200
Skimmilkpowder	50	50
Fishmeal	12	35
Meatmeal	39	39
Soybeanmeal (44% crude protein)	126	---
Barley	150	150
Wheat starch	264	218
Corn starch	264	218
Wheat bran	51	51
Beet molasses	19	17.5
Vitamin/mineral mixture*	10	10
$CaCO_3$	5	3
$CaHPO_4.2H_2O$	6	5
$CaCl_2$	3	3
L-Lysine	0.8	-
Dl-Methionine	2	5
Contents (calculated)		
Crude protein	184	182 +
Metabolizable Energy (kcal/kg)	3420	3420
Ash	56	55
Crude fat	30	31
Crude fibre	28	28
Lysine	10	10
Methionine + cystine	6.5	6.6
Ca	9.9	9.7
Phosphorus	6.9	7.0

* Contributed the following nutrients/kg diet: Retinol 2.7 mg, cholecalciferol 45 µg, Dl-α-tocopherol 40 mg, menadione 3 mg, riboflavine 5 mg, nicotinic acid 30 mg, D-pantothenic acid 15 mg, choline chloride 120 mg, cyanocobalamin 40 µg, ascorbic acid 50 mg, $CuSO_4.5H_2O$ 20 mg, $ZnSO_4.H_2O$ 200 mg, MnO 70 mg, $FeSO_4.7H_2O$ 400 mg, $CoSO_4.7H_2O$ 2.5 mg, $Na_2SeO_3.5H_2O$ 0.2 mg, KJ 0.5 mg.

\+ About 45 grams originates from the beans and 137 grams from other sources.

A part of these beans were steam heated for 40 minutes at 104°C and 19% moisture in the bean. Before autoclaving, the beans were cracked in a hammermill. The chemical composition and the contents of ANF are given in Table 3. The diets were balanced for total protein, lysine, methionine + cystine, net energy, Ca and P (see Table 1). The diets were pelleted at a low temperature (50-55°C). The pellet diameter for the piglets was 3 mm and for the rats 9 mm.

In experiment 2 two diets were formulated: a control diet containing no Phaseolus vulgaris and a diet containing raw 20% Phaseolus beans (Table 2). The beans were from another practical mixed batch than those used in experiment 1.

Table 2 Composition of the diets of experiment 2 (g/kg)

Ingredients	Control diet	Test diet
Phaseolus vulgaris	---	200
Casein	40	40
Fish meal	50	50
Maize	435	240
Maize starch	50	50
Tapioca	201.5	242.7
Wheat bran	80	80
Maize gluten feed	45	---
Cellulose powder (Akufloc)	13.8	8
Sugar cane molasses	40	40
Vitamin mineral mixture	10	10
$CaCO_3$	4.6	3.5
$CaHPO_4.H_2O$	12.4	12.6
NaCl	3	3
$NaHCO_3$	11.2	7.1
L-Lysine HCL	2	0.4
DI-Methionine	0.3	1.3
L-Threonine	1	1
L-Tryptophan	0.55	0.4
Calculated contents (g/kg)		
Crude protein	158	162
Digestible protein	140	140
Metabolisable energy (kcal/kg)	3285	3285
Digestible lysine	9	9
Digestible methionine + cystine	5.5	5.5
Digestible threonine	6.5	6.5
Digestible tryptophan	1.9	1.9

The vitamin and mineral mixture supplied per kg feed: Contributed the following nutrients/kg diet: Retinol 2.7 mg, cholecalciferol 45 µg, DL-α-tocopherol 40 mg, menadione 3 mg, riboflavine 5 mg, nicotinic acid 30 mg, D-pantothenic acid 15 mg, choline chloride 120 mg, cyanocobalamin 40 µg, ascorbic acid 50 mg, $CuSO_4.5H_2O$ 20 mg, $ZnSO_4.H_2O$ 200 mg, MnO 70 mg, $FeSO_4.7H_2O$ 400 mg, $CoSO_4.7H_2O$ 2.5 mg, $Na_2SeO_3.5H_2O$ 0.2 mg, KJ 0.5 mg.

Table 3 Chemical composition of the beans (g/kg)

Criteria	Raw beans*		Steam heated
	Exp. 1	Exp. 2	Exp. 1
Dry matter	891.5	888.6	893.6
Ash	48.0	48.0	48.2
Crude protein (Nx6.25)	226.6	231.0	230.2
Crude fat	20.0	21.0	20.5
Nitrogen Free Extract (crude fibre included)	596.9	588.6	594.7
Contents of ANFs			
Haemagglutinins (HA) **	30	42	1.92
Trypsin inhibitor activity +	4.7	5.2	0.3
Protein Dispersibility Index	36	n.d.	22
Urease activity#	0.02	n.d.	0.0

* The beans of experiments 1 and 2 were from different batches.
** Haemagglutination of rabbit red bloodcells. 1HA = 1: 1000 dilution step.
\+ mg inhibited trypsin per g product.
\# mg released ammonia N/minute/g product.
n.d. = not determined.

The composition of the beans is given in Table 3. The main protein sources were casein and fish meal to be sure that no ANFs were present in the other ingredients. In a preliminary experiment with pigs of about 20 kg the digistibility of N was measured to be 79% when these beans were toasted for 40 minutes at 104°C and 19% moisture in the bean. This digestibility coefficient was used to balance the control and test diets for digestible protein. To balance the diets with regard to energy and minerals the CVB-table (1988) was used. To calculate the amount of digestible amino acids in the diets, ILOB-tables (not published) were used. Synthetic lysine, methionine, tryptophan and threonine were included to balance the diets with respect to the levels of digestible amino acids. The diets were fed as dry meal and water was freely available from nipple drinkers.

2 Animals and experimental procedure

Experiment 1 was carried out with 45 male piglets and 45 male rats; 15 rats or piglets received each diet. The piglets (15 litters of 3 piglets each) were of the crossbred Dutch landrace x Dutch Yorkshire. The piglets were weaned at 2 weeks of age and allow to adapt to battery cages for one week. During this period they received a starter diet consisting mainly of barley, maize, whey powder, herring meal and meat meal, enriched with vitamins and minerals. Fourteen piglets of each treatment were pair housed and one was single housed. After the adaptation period they were weighed and assigned to the treatments in such a way that each treatment comprised one piglet of each litter and the treatments were further balanced for mean

body weight and animal variation per treatment. In the following week the control piglets were allowed to adapt to the control diet and the test piglets to a diet containing 20% of a commercial batch of toasted beans. The piglets destined for the bean diets were adapted as follows: days 1 and 2, 100% practical diet; day 3, 75% practical diet + 25% commercial toasted bean diet; day 4, 50% practical diet + 50% commercial toasted bean diet; day 5, 25% practical diet + 75% commercial toasted bean diet; and days 6 and 7, 100% commercial toasted bean diet. On day 8 the piglets on the diet with the commercial toasted beans were changed to their test bean diets, while the control piglets remained on the control diet. Growth, feed intake and feed conversion efficiency were measured weekly during the three weeks of study.

The rats were Wistar animals; 45 rats were weaned at 4 weeks of age and placed in cages each containing 3 rats. In the first week they were allowed to adapt to the diets according to the same scheme used for the piglets. On day 7 they were distributed among the treatments according to the same criteria used for the piglets. From day 8 onwards growth, feed intake and feed conversion efficiency (g feed/g weight gain) were measured each week for three weeks. The daily amount of feed to the piglets was restricted to about 4% of the body weight, which is about 2.2. times their maintenance requirement for energy. The feed was given daily and adapted twice weekly to body weight. Water was freely available from nipple drinkers. The feed consumption of the rats was restricted to 80% of the ad libitum amount consumed by a separate group of 7 rats fed the control diet. The feed was offered once daily. Water was continuously available from nipple drinkers. The piglets and rats were housed in two separate rooms with a continuous artificial lighting at a low intensity. The room temperature for the piglets was between 25°C and 28°C and for the rats 21°C.

At the termination of the growth experiment 7 piglets and 7 rats from each treatment were chosen at random and placed individually in metabolic cages. After one week of adaptation, faeces and urine were collected separately. The faeces of the piglets were collected twice daily for 5 days using special bags attached around the anus. Urine flowed through a screen in the bottom of the cage and through a funnel into bottles containing 30 ml of a mixture of 25% H_2SO_4 (18M) and 75% water. Faeces and urine of the rats were collected for 4 days quantitatively and separately using metabolic cages. The urine was collected in bottles containing 1 ml of a mixture of 25% H_2SO_4 (18M) and 75% water. The faeces were frozen immediately after collection. The collection periods were 5 days for the piglets and 4 days for the rats. The age of the animals at the start of the collection period was 56 days for the piglets and 63 days for the rats. The piglets and rats were fed restrictedly according to the same formula as in the growth period. The daily amount of feed was higher because of increased live weights.

Experiment 2 was carried out with 144 pigs of the cross Dutch Landrace x Dutch Yorkshire. The pigs were housed in 36 pens of 4 animals each. At three periods (P1, P2 and P3) 12 pens were grouped in two treatments of 6 pens of 4 pigs each in such a way that the mean live weight of each group was as close as possible. Until grouping, both treatments were fed the control diet. Immediately after grouping one of the two treatments was changed from the control diet to the test diet containing the raw Phaseolus beans. The other group remained on the control diet. In the P1 the live

weight was about 18 kgs (= about 8 weeks old), in P2 about 35 kgs (= about 12 weeks old) and in P3 about 55 kgs (= about 16 weeks old).
Each test period consisted of 10 days adaptation to the diets and a 2 week period in which the live weight was measured weekly. The daily allotment of feed to the pigs was based on 3.2 times maintenance for energy (assumed to be 100 kcal metabolizable energy per kg metabolic weight). Weight gain was measured over only 2 weeks because marked negative effects on performance were observed.

3 Collection of organs

In experiment 1 the organs of the piglets and rats were collected on the day following the termination of the growth period 8 animals were taken at random from each group of piglets and rats for dissection of the liver, pancreas and spleen. After killing the abdomen was opened, and the organs were removed quickly and weighed immediately. Just before dissection the animals were weighed; the organs weights were calculated as a percentage of the body weight. In experiment 2 no organs were collected.

3.1 Chemical analyses

The dry matter content was determined by drying the samples to constant weight at 101°C. Ash was determined by incineration at 550°C for 4 hours. Nitrogen was analysed in fresh material using a Technicon Auto-Analyser. After wet digestion with 2.0M potassium sulphate solution in 18M sulphuric acid and selenium as catalyst, the nitrogen was bound by hypochlorite and phenol. The nitrogen complex was measured at 630 nm. Crude fat was analysed by treating for one hour with 3M hydrochloric acid and drying for 3 hours under vacuum at 100°C, followed by 8 hours extraction with diethylether. Nitrogen free extract was calculated as DM - (ash + (N x 6.25) + crude fat). Lectins were measured by haemagglutination of red blood cells according to Valdebouze et al. (1980). The content of trypsin inhibitors was analysed according to the method described by Kakade et al.(1974). The protein dispersibility index (PDI) was measured according to method Ba-10-65 of the American Oil Chemists Society. The urease activity was measured as the release of ammonia-N (mg) in one minute from an urea solution at 30°C caused by the addition of 1 gram product, EC method for feed L155/36 and L155/37.

3.2 Statistical analysis

The values for the various criteria are given as means with their standard deviations. The differences between treatments are analysed by the student's t test.

IV RESULTS

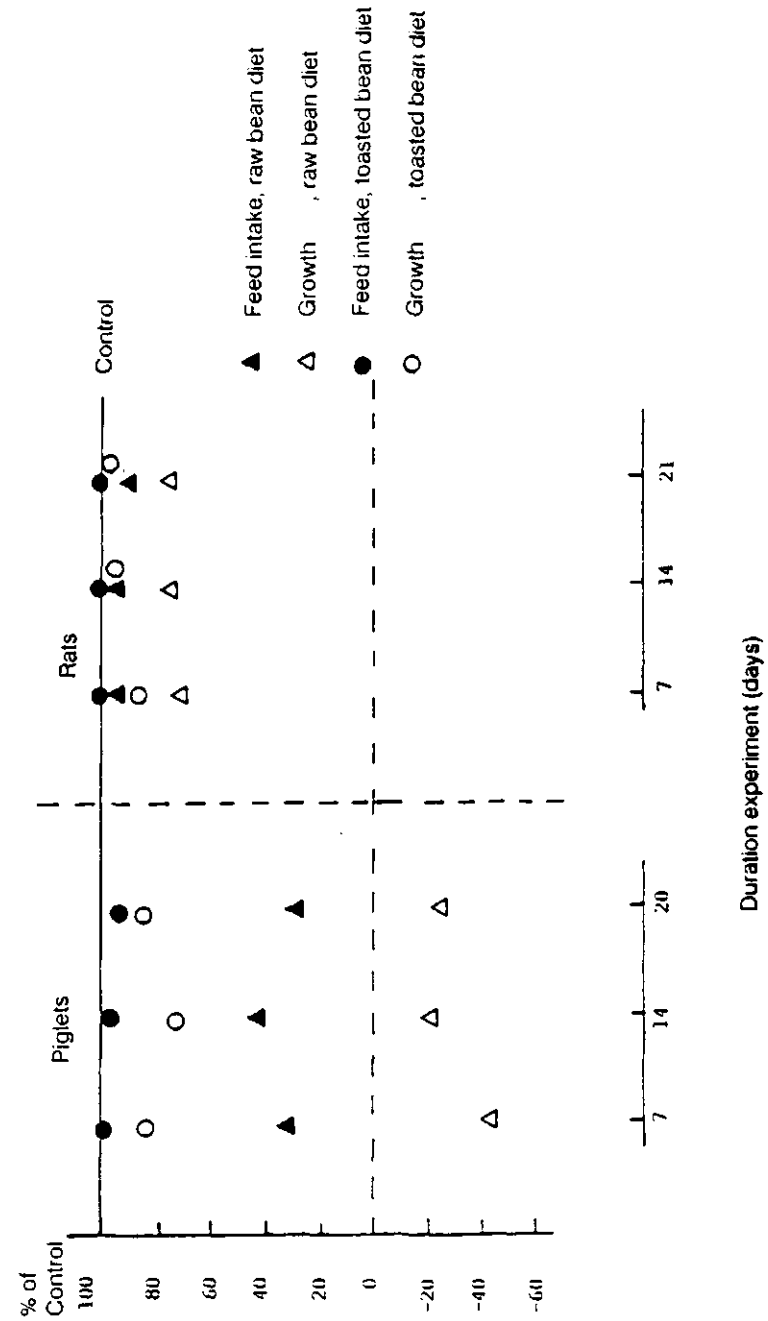
Experiment 1 Weight gain, feed intake and feed conversion ratio of the piglets and rats are given in Table 4 and Figure 1.

Table 4 Growth, feed intake and feed conversion ratio (g feed per g weight gain) with SD in piglets during 20 days

Diet		Growth (g/day) mean	SD	%	Feed intake (g/day) mean	SD	%	Feed conversion ratio mean	SD	%
					Piglets					
I,	control	137.7a	19.3	100	239.5a	33.9	100	1.74a	0.12	100
II,	20% unheated beans	-36.0b	5.8	-266	96.5b	24.4	40	negative		
III,	20% beans, 40 min. heated	111.8c	20.7	81	231.6a	34.4	97	2.09c	0.11	120
					Rats					
I,	control	6.68a	0.24	100	19.33a	1.38	100	2.99a	0.21	100
II,	20% unheated beans	4.86b	0.20	73	17.54b	1.98	91	3.72b	0.32	124
III,	20% beans, 40 min. heated	6.32c	0.46	95	19.56a	1.40	101	3.10a	0.18	104

Means in the same column of piglets or rats respectively, that do not have a common letter differ ($P < 0.05$).

Fig. 1 Feed intake and growth in piglets and rats fed the bean diets in experiment 1



In both species inclusion of beans in the diet reduced growth and increased the feed conversion ratio. However, in piglets the negative effects were much greater than in rats. Piglets fed the raw beans even lost weight. In rats fed the raw beans growth was significantly ($P < 0.05$) reduced by 27% compared with the control. Effects of the same magnitude were found for the feed conversion ratio. When the diet containing the toasted beans was fed, growth was reduced by 19% ($P < 0.05$) in piglets; in rats the difference compared with the control was 5% ($P < 0.05$). Feed conversion ratio in the piglets was 20% above the control value ($P < 0.05$) and in the rats 4% (not significant). Feed intake in piglets was much more depressed than in rats. Reduction in the feed intake when raw beans were fed was 60% in piglets and 9% ($P < 0.05$) in rats. When the toasted beans were fed, feed intake in the piglets was reduced by 3% (not significant), while in rats feed intake was not reduced. In both species the faecal N digestibilities of the bean diets were significantly ($P < 0.05$) lower than those of the control diet. The N digestibilities of the diet containing raw beans were 37 and 13 units below control values in the piglets and the rats, respectively. Thus N digestibility was more depressed in piglets than in rats (Table 5). The differences between the diets containing toasted beans and the control diet were 8 units ($P < 0.05$) and 6 units ($P < 0.05$) for the piglets and the rats, respectively.

Table 5 Feed intake, apparent N digestibility of the diets and N balance of piglets and rats of feeding diets containing raw and heated Phaseolus vulgaris beans

Diets	Feed intake	N intake	Apparent N digestibility		N balance			Retained N	
	g/day	g/day	mean	SD	g/day	SD	% of control	% of total N intake	% of digested N
				Piglets					
control	320a	9.35a	84.7a	2.2	4.72a	0.90	100	50.5	59.7
200 g unheated beans/kg	174b	5.06b	47.6b	13.2	-0.33b	0.38	-7	-6.5	-14.3
200 g heated bean/kg (40 min. heated)	299a	8.74c	76.5c	3.6	3.80c	0.42	57	43.5	56.7
				Rats					
control	30.1a	0.89a	77.8a	1.0	0.25a	0.034	100	28.1	36.1
200 g unheated beans/kg	24.8b	0.72b	64.5b	1.9	0.21b	0.042	84	29.0	45.0
200 g heated beans/kg (40 min. heated)	28.9a	0.84a	71.6c	1.3	0.24a	0.031	96	28.5	39.8

Mean values in the same column of each animal species that do not have a common letter differ significantly ($P < 0.05$).

Results of the N balance (Table 5), expressed in g/day, showed the same pattern as observed for weight gain. In rats the N balance was positive and about 16% ($P < 0.05$) lower than for the control animals. In piglets fed the toasted beans the N balance was about 19% ($P < 0.05$) below the control; in rats the difference was not significant and only about 4%.

Table 6 Mean weigth of organs with SD in piglets and rats (% of live weight)

Treatment		Liver		Pancreas		Spleen	
		mean	SD	mean	SD	mean	SD
				Piglets			
I,	control	2.52a	0.35	0.21a	0.04	0.34a	0.09
II,	20% unheated beans	2.35a	0.24	0.10b	0.03	0.14b	0.05
III,	20% beans, 40 min. heated	2.37a	0.22	0.20a	0.03	0.32a	0.10
				Rats			
I,	control	4.05a	0.43	0.34a	0.07	0.24a	0.07
II,	20% unheated beans	4.13a	0.35	0.61b	0.07	0.23a	0.07
III,	20% beans 40 min. heated	4.17a	0.59	0.36a	0.06	0.25a	0.06

Means in the same column of piglets or rats respectively that do not have a common letter differ ($P < 0.05$).

The results of the organ weights are given in Table 6. The organ weights are given as a percentage of live weight. No significant differences for the weights of the liver between treatments were observed in either species. The weight of the pancreas and the spleen of the piglets fed the raw beans was significantly ($P < 0.05$) lower than in the control piglets and in the piglets fed the toasted beans. The weights of the pancreas and the spleen of the piglets fed the toasted beans were not different from the control animals. The weight of the pancreas of rats fed the raw beans was significantly ($P < 0.05$) higher than for the controls and the rats fed the toasted beans. There were no differences in pancreas weight between the rats of the control group and those fed the toasted beans. The relative weight of the spleen of the rats was not significantly different between the treatments.

Experiment 2 The values for feed intake, weight gain and feed conversion ratio are given in Table 7. Feed intake and weight gain were markedly reduced ($P < .001$) in the raw bean fed pigs. Weight gain of the pigs fed the control diet was normal, but the pigs fed the raw beans even lost weight at all three ages. Feed conversion ratio was

negative during all three age periods. Although the test period was rather short (about three weeks), the physical appearance of the bean fed pig deteriorated. The

Table 7 Mean feed intake, weight gain and feed conversion ratio (kg feed per kg weight gain) with SD measured in experiment 2 during 14 days

Treatment	Period P1 Mean	SD	Period P2 Mean	SD	Period P3 Mean	SD
			Feed intake (g/day)			
control diet	836a	13.4	1437a	6.2	2074a	20.3
raw bean diet	325b	37.7	621b	51.3	907b	127.1
			Weight gain (g/day)			
control diet	448a	32	626a	36	801a	53
raw bean diet	-65b	22	-154b	54	-128b	94
			Feed conversion ratio			
control diet	1.87	0.11	2.30	0.13	2.60	0.17
raw bean diet	negative		negative		negative	

Means in the same column that do not have a common letter differ ($P < 0.05$).

skin was scaly and covered with long hair. Commonly bloated abdomen was observed in the bean fed pigs. The faeces of the bean fed pigs were loose, indicating a disturbed digestion process.

V DISCUSSION

In most studies reported in the literature very high levels of Phaseolus vulgaris are used. In our study we used lower levels because in practical pig diets the bean levels are generally not higher than 10-20%. In experiment 1, the inclusion of 20% Phaseolus Vulgaris in the diet of the piglets markedly reduced feed intake (60%) while in the rats the reduction was only 9% (Table 4). It is important to know whether the effect on feed intake can be attributed to the presence of a toxic factor in the bean or to palatibility. In a study with pigs of approximately 16 weeks old, King et al. (1983) found a marked reduction in feed intake and also weight loss when a diet containing 40% Phaseolus beans was fed. In their experiment, the control pigs were fed at the same low level of feed intake as the beanfed pigs. Both the control pigs and the beanfed pigs lost weight, but in the beanfed pigs the weight loss was 3 times greater. This result indicates that a "toxic" factor in the bean has played a role in addition to the effect of the reduced feed intake.

In rats, Pusztai et al. (1981) found a reduced feed intake when purified lectins from the Phaseolus bean were included in the diet. This indicates that the reduction in feed intake may be specifically related to the lectins present in the Phaseolus bean. The negative N balance (Table 5) in the piglets, indicates that some body protein was lost due to the feeding of raw Phaseolus beans. The negative N balance was caused by an increased N excretion with urine. This indicates that in the piglets not only the N absorption from the gut was depressed, but also its deposition of digested N in the body. The mechanism responsible for the disturbed N deposition in the piglets is not entirely clear. Pusztai et al. (1981) showed in a study with rats that lectins of the Phaseolus bean are responsible for negative N balance. In accordance with the results of feed intake, N digestibility and N balance, the weight gain in the piglets fed the raw Phaseolus beans was distinctly more depressed than in the rats fed the same diet. These piglets could not even maintain their body weight, whereas the rats were still gaining weight, although at a lower level (-27%) compared with the control rats. In rats pancreas hypertrophy was observed (Table 6). Pancreas hypertrophy is mainly related to trypsin inhibitors (Liener and Kakade, 1980), but Oliveira et al. (1988) demonstrated that Phaseolus lectins may also cause pancreas hypertrophy. The observation that the pancreas weight in piglets fed the raw beans was reduced (Table 6) seems to agree with the results of King et al. (1983) who observed degenerative changes in the pancreas cells of pigs fed raw Phaseolus beans. A direct comparison with our study could not be made because the pancreas was not weighed in their study. Myer et al. (1982) also found a tendency for relatively lower pancreas weight in pigs fed raw kidney beans. Green et al. (1986) demonstrated that growth of the pancreas can be inhibited if insufficient protein and amino acids are available. In our study the protein digestibility of the raw bean diet was markedly depressed in the piglets (Table 5). This may explain the reduced pancreas weights in the piglets. On the other hand, we also found severe damage of the small intestinal mucosa in the piglets fed the raw Phaseolus beans, while in rats the gut wall damage was less severe (Kik et al. 1989).

One can speculate that the lower pancreas weight of the piglets in our study may also be related to damage of the CCK-PZ hormone producing intestinal endocrine cells, resulting in a depressed CCK-PZ hormone production and hence a decrease in pancreas mass. In the piglets fed the raw beans the spleen weight was significantly lower compared with the piglets fed the control diet and the diet containing the toasted beans. Slight atrophy of the spleen was also reported by Myer et al. (1982) in pigs of about 25 kg fed 15% raw Phaseolus beans in the diet. The weight of the spleen in the rats did not differ between the treatments. Feed intake in experiment 2 was reduced at all three ages when raw Phaseolus beans were included in the diet (table 6). In all three periods the pigs fed the raw beans lost weight. This clearly demonstrates that there is no age dependency in pigs regarding the effects on weight gain when raw Phaseolus beans are fed, in the period up to an age of 4 months. This means that pigs up to 4 months are also sensitive to ANFs in Phaseolus bean. The differences in sensitivety to ANFs found in experiment 1 between piglets and rats when Phaseolus beans were fed, can therefore, not be explained by differences in physiological age. Summarizing, it can be concluded that piglets are more sensitive to ANFs in the Phaseolus vulgaris bean than rats. The reason for this species difference is not known. One point that may have played a role is that in the present study the diets were balanced on the basis of the content of total protein and

not on the content of digestible protein. The results in Table 5 show that the protein digestibility of the bean diets was lower than that of the control diet. The difference in N digestibility between the rats and piglets fed the diets containing the raw beans were marked (64.5 vs 47.6). It may be possible that the difference in digestibility may also explain some of the differences in ANF effects between rats and piglets observed in this study.

VI REFERENCES

Bond, D.A. and D.B. Smith, 1989. Possibilities for the reduction of antinutritional factors in grain legumes by breeding. In: Recent advances of research in antinutritional factors in legume seeds, pp 285 - 296.[J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. PUDOC, Wageningen, The Netherlands.

Cheeke, P.R. and L.R. Shull. 1985. Natural toxicants in feeds and poisonous plants. AVI Publishing Company, Inc., Westport, Connecticut, U.S.

Chubb, L.G. 1983. Anti-nutritive factors in animals feedstuffs. In: Recent advances in animal nutrition, pp. 21-37. [W. Haresign, editor]. Butterworth, London.

Combs, G.E., R.G. Connes, T.H. Berry and H.D. Wallace. 1967. Effect of raw and heated soybean meal on gain, nutrient digestibility, plasma amino acids and other blood constituents of growing swine. Journal of Animal of Science 26, 1067-1071.

CVB. 1988. Veevoedertabel: Gegevens over voederwaarde, verteerbaarheid en samenstelling. Centraal Veevoederbureau in Nederland, Lelystad, The Netherlands.

Friedman, M. 1986. Nutritional and Toxicological significance of enzyme inhibitors in foods. Plenum Press, New York, U.S.

Green, G.M., Levan, V.H. and R.A. Liddle, 1986. Interaction of dietary protein and trypsin inhibitor on plasma cholecystokinin and pancreatic growth in rats. In: Nutritional and toxicological significance of enzyme inhibitors in foods. pp 123-132. [M. Friedmann, editor]. Plenum Press, New York.

Huisman, J. 1989. Antinutritional factors (ANF) in the nutrition of monogastric farm animals. In: Nutrition and digestive physiology in monogastric farm animals [E.J. van Weerden and J. Huisman, editors]. PUDOC, Wageningen, The Netherlands.

Huisman, J., A.F.B. van der Poel, M.W.A. Verstegen and E.J. van Weerden. 1989. Antinutritional factors (ANF) in pig nutrition. World Review of Animal Science. In press.

Kakade, M.L., J.J. Rackis, J.E. McGhee and G. Puski. 1974. Determination of Trypsin Inhibitor Activity of Soy Products: A collaborative Analysis of an Improved Procedure. Cereal Chemistry, 51, 376-382.

Kik, M.J.L., J. Huisman, A.F.B. van der Poel and J.M.V.M. Mouwen 1989. Pathological changes of the small intestinal mucosa of piglets after feeding Phaseolus vulgaris beans. In: Recent advances of research in antinutritional factors in legume seeds. pp 49-53. [J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. PUDOC, Wageningen, The Netherlands.

King, T.P., R. Begbie, R. and A. Cadenhead. 1983. Nutritional toxicity of raw kidney beans in pigs. Immunocytochemical and cytopathological studies on the gut and the pancreas. Journal of the Science of Food and Agriculture, 34, 1404-1412.

Liener, I.E. 1980. Toxic constituents of plant foodstuffs, Academic Press, Inc. New York, U.S.

Liener, I.E. and M.L. Kakade 1980. Protease inhibitors. In: Toxic Constituents of Plant Foodstuffs. pp 7 -71. [I.E. Liener, editor]. New York, Academic press.

Liener, I.E., N. Sharon, I.J. Goldstein. 1986. The lectins. Properties, functions, and applications in biology and medicine. Academic Press, Inc. New York, U.S.

Myer, O.M., J.A. Froseth and C.N. Coon. 1982. Protein utilization and toxic effects of raw beans (Phaseolus vulgaris) for young pigs. Journal of Animal Science, 55, 1087-1098.

Oliveira, J.T.A., A. Pusztai and G. Grant. 1988. Changes in organs and tissues induced by feeding of purified kidney bean (Phaseolus vulgaris) lectins. Nutrition Research, 8, 943-947.

Pusztai, A., E.M.W., Clarke, G. Grant and T.P. King. 1981. The toxicity of Phaseolus vulgaris lectins. Nitrogen and immunochemical studies. Journal of the Science of Food and Agriculture, 32, 1037-1046.

Valdebouze, P. Bergezon, E., Garborit, T. and Delort-Laval. J. 1980. Content and distribution of trypsin inhibitors and haemagglutimins in some legume seeds. Canadian Journal of Animal Science, 60, 695-701.

Visitpanich, T., E.S. Batterham and B.W. Norton. 1985. Nutritional Value of Chickpea (Cicer arietinum) and Pigeonpea (Cajanus cajan) Meals for Growing Pigs and Rats. II. Effect of Autoclaving and Alkali Treatment. Australian Journal of Agricultural Research, 36, 327-335.

Yen, J.T., Jensen, A.H. and Simon, J. 1977. Effect of dietary raw soybean and soybean trypsin inhibitor on trypsin and chymotrypsin activities in the pancreas and in the small intestinal juice of growing swine. Journal of Nutrition, 107, 156-165.

CHAPTER 2

ANIMAL SPECIES DIFFERENCE RESEARCH WITH PIGLETS, RATS AND CHICKENS FED PEAS AND COMMON BEANS

2.2 Effects of variable protein contents in diets containing Phaseolus vulgaris beans on performance, organ weights and blood parameters in piglets, rats and chickens

J. Huisman[1], A.F.B. van der Poel[2], J.M.V.M. Mouwen[3], E.J. van Weerden[1] and M.J.L. Kik[3]

1) TNO-Institute of Animal Nutrition and Physiology (IGMB-dept. ILOB), PO Box 15, 6700 AA Wageningen, The Netherlands
2) Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen, The Netherlands
3) Department of Pathology, Faculty of Veterinary Medicine, State University of Utrecht, P.O. Box 80158, 3508 TD Utrecht, The Netherlands.

British Journal of Nutrition. In press

I ABSTRACT

A comparison was made of the effects of ANFs present in Phaseolus vulgaris between piglets, rats and chickens. Also the hypothesis whether the negative effect on weight gain due to the inclusion of raw Phaseolus vulgaris can be attributed to an insufficient supply of amino acids was tested. Test diets containing 20% raw Phaseolus beans were balanced for digestible protein and amino acids, in one diet extra casein was incorporated. The main response criteria were live weight gain and the weight of various organs including the intestine. Live weight gain in piglets was markedly reduced during feeding 20% raw Phaseolus vulgaris in the diet, but not in rats and chickens. Addition of casein did not improve the weight gain of the piglets, indicating that a toxic factor was responsible for the reduced weight gain and not an insufficient supply of amino acids. The weight of the spleen and thymus was markedly reduced in the piglets when the diets with raw Phaseolus beans were fed, but not in the rats and chickens. Extra supply of casein did not change this effect. The pancreas weight was not effected in piglets and rats. In chickens the pancreas was enlarged with raw beans. The weight of the intestine was increased in all three species due to feeding raw Phaseolus vulgaris.

II INTRODUCTION

Phaseolus vulgaris beans contain various antinutritional factors (ANFs) (Bressani, 1983). The main ANFs in these beans are lectins, trypsin inhibitors and in the coloured flowering varieties tannins. It is known that the lectins in Phaseolus vulgaris are highly toxic and are the main factor responsible for the negative effects when fed to livestock (Pusztai, 1985). Huisman et al. (1990) found that piglets were more sensitive to ANF in Phaseolus vulgaris than rats. Differences between both animal species were noted in effects on live weight gain and feed conversion efficiency. Weights of pancreas and spleen in these piglets were markedly reduced when raw Phaseolus beans were fed, but in rats there were no such effects. In that study protein digestibility of the diets containing 20% raw Phaseolus beans was 48% in piglets and 65% in rats. As a result less digestible amino acids were available for absorption in piglets than in rats. A hypothesis can therefore be that the former marked negative effects in piglets compared to the rats, may be related to an inadequacy of protein and amino acids. This hypothesis was checked in the present study. A control and three bean containing diets were balanced for digestible protein and amino acids using digestibility coefficients for piglets. In one diet extra casein was included to increase the protein and amino acid supply. These diets were fed to piglets, rats and also to chickens. Chickens were incorporated in the study because they are important farm animals.

The objectives of this study were:

- comparison of effects of ANF present in Phaseolus vulgaris in piglets, rats and chickens.
- testing the hypothesis that the negative effects of inclusion of raw Phaseolus vulgaris on live weight gain is due to an insufficient protein and amino acid supply.
- testing whether the weight of the pancreas in piglets is affected by inclusion of raw Phaseolus vulgaris in diets balanced for digestible protein and amino acids.

III MATERIALS AND METHODS

Four diets were formulated (table 1 and 2); a control diet (C), two diets containing 20% raw Phaseolus vulgaris (R) and one diet containing 20% toasted Phaseolus vulgaris (T). The main protein source in the control diet was casein. Casein and fish were chosen as protein source to be sure that no ANFs were present in the control diet. In the test diets a part of the casein was replaced by the Phaseolus beans. A part of this batch beans was steam heated for 40 minutes. Before heating, the beans were broken. The composition of the beans is given in Table 3.

In a separate digestibility trial with piglets the faecal digestibility of N of the 40 minutes heated batch beans (T) was measured to be approximately 60%. This digestibility coefficient was used to balance the bean diets on the basis of their content of digestible protein (Nx6.25). The amounts of amino acids in the piglet diets were calculated using the CVB table (CVB, 1988): Ileal digestible amino acids of feedstuffs for pigs (in press). The amounts of digestible amino acids in the beans were calculated from the same table with a correction for the lower protein digestibility assuming that the digestibility of the amino acids was reduced in proportion to the reduction for the protein. The rats were fed the piglet diets. The amounts of amino acids in the chicken diets were based on the data published in the CVB table (1988). As indicated in table 1 an extra treatment (R0) was incorporated in which the digestibility of the raw bean protein was assumed to be 0%. This means that in this diet as much casein was included as in the control diet. Free lysine, methionine, threonine and tryptophan were included for balancing the diets for these amino acids. In the chicken diet extra methionine and arginine was included. The diets were pelleted without steam, the pellet size was 3 mm for all three animal species. The composition of the diets is given in Table 2.

Table 1 Treatments

I	, Control (C).
II	, Test diet containing 200 g/kg untoasted Phaseolus vulgaris beans (R0).*
III	, Test diet containing 200 g/kg untoasted Phaseolus vulgaris beans (R60).**
IV	, Test diet containing 200 g/kg Phaseolus vulgaris beans, 40 minutes toasted (T).**

* The digestibility of the protein of the beans was assumed to be 0% in the diet formulation

** The digestibility of the protein of the beans was assumed to be 60% in the diet formulation. In piglets it was measured that the protein digestibility of the 40 minutes toasted Phaseolus Vulgaris beans was approximately 60%.
This figure was used for balancing the diets for digestible protein.

Table 2 Composition of the diets (g/kg)

Ingredients	Treatment C	Treatment R0	Treatment R60 and T
Phaseolus vulgaris beans	-	200	200
Casein	131	130	98
Fish meal	50	50	50
Corn starch	556	351	393
Wheat bran	50	50	50
Cellulose	21	6	6
Soya oil	29	58	46
Dextrose	60	60	60
Sugar cane molasses	40	40	40
Vitamin/mineral mixture	10	10	10
$CaCO_3$	2	4	3
$CaHPO_4.2H_2O$	29	25	26
$CaCl_2$	3	3	3
$NaHCO_3$	12	12	12
$KHCO_3$	6	-	0.1
Amino acids added to the diets of the piglets and rats			
L-Lysine	-	-	1
DI-Methionine	1.1	1.1	1.6
L-Threonine	0.6	0.6	0.8
L-Tryptophan	0.2	0.2	0.3
L-Arginine			
Amino acid added to the diets of the chickens			
L-Lysine	0.2	0.1	1.1
DI-Methionine	3.7	2.7	3.6
L-Threonine	0.6	0.6	0.8
L-Tryptophan	0.2	0.2	0.3
L-Arginine	6.2	6.0	5.5
The extra amino acids were substituted for corn starch			
Calculated contents, g/kg			
Metabolizable energy (kcal/kg):			
piglets	3640	3600	3600
chickens	3350	3350	3270
Crude protein	168	210	184
Digestible crude protein	155	155	155
Ash	60	64	64
Crude fat	41	74	60
Crude fibre	25	25	25
Calcium	96	96	96
Phosphorus	80	80	80
Digestible lysine:			
- piglets and rats*	127	127	127
- chickens	126	147	128
Digestible methionine and cystine:			
- piglets and rats*	65	65	65
- chickens	87	84	85
Digestible threonine:			
- piglets and rats*	73	73	73
- chickens	72	84	73
Digestible tryptophan:			
- piglets and rats*	21	21	21
- chickens	20	24	21

The vitamin/mineral mixture supplied per kg feed:
Retinol 2.7 mg, cholecalciferol 45 µg, DI-α-tocopherol 40 mg, menadione 3 mg, riboflavine 5 mg, nicotinic acid 30 mg, D-pantothenic acid 15 mg, choline chloride 120 mg, cyanocobalamin 40 µg, ascorbic acid 50 mg, $CuSO_4.5H_2O$ 20 mg, $ZnSO_4.H_2O$ 200 mg, MnO 70 mg, $FeSO_4.7H_2O$ 400 mg, $CoSO_4.7H_2O$ 2.5 mg, $Na_2SeO_3.5H_2O$ 0.2 mg, KJ 0.5 mg.

* based on digestibility data from piglets

Table 3 Chemical composition g/kg and ANF contents in the Phaseolus vulgaris bean

	unheated	heated
Dry matter	894	897
Ash	49	50
Crude protein (Nx6.25)	224	232
Crude fat	20	20
Crude fibre	71	65
Nitrogen free extract	530	529
Haemaglutinins (HA)*	40	0.8
Trypsin inhibitor activity +	4.7 mg	< 0.3 mg

* Haemagglutination of rabbit red blood cells. Haeagglutination activity (HA) is expressed in units/mg sample. One unit is defined as the smallest amount of sample necessary for agglutination under test conditions (Valdebouze et al., 1980).
+ mg inhibited trypsin per g product.

1 Animals and experimental procedure

Twelve piglets, 15 rats and 60 chickens were each given each diet. The piglets were of the crossbred Dutch Landrace x Dutch Yorkshire type. They were housed in metabolism cages with two in one cage. The piglets were assigned to the treatments in such a way that the mean live weight and the variation per treatment were about the same for each treatment. The rats were Wistar type; they were weaned at 4 weeks of age and immediately placed in the metabolism cages. The way the piglets and rats were adapted to the diets, the housing conditions and the way of assigment to the treatments have been described previously (Huisman et al., 1990). The chickens were one day old Hybro birds and placed in battery cages with 10 birds in each cage. The first two days they received a normal commercial starter diet. During the next four days the control chickens were adapted to the C diet and the test chickens to a diet containing 20% of a commercial toasted batch Phaseolus beans. During adaptation the chickens were fed according to the following scheme: day 1: 75% practical diet and 25% commercial bean diet; day 2: 50% practical diet and 50% commercial bean diet; day 3: 25% practical diet and 75% commercial diet and day 4: 100% commercial bean diet. At day 5 the chickens were changed to the specially prepared test diets, the control chickens remained on the control diet. All three species were fed on a restricted base according to a scheme based on 2.2 times maintenance requirement for energy. Body weight was measured weekly and the feeding schedule was adjusted twice weekly based on the expected growth. Weight gain of the piglets was measured over a period of 2 weeks. This time was chosen arbitrarily because in a previous experiment piglets lost weight after feeding raw Phaseolus beans during 3 weeks. The weight gain of the rats and the chickens was measured over 3 weeks.

2 Collection of organs and blood samples

On the day following the end of the growth period, from each of the treatments C, R60 and T, 7 piglets, 7 rats and 12 chickens were taken at random for dissection and collection of the various organs and the intestine. From the piglets of treatment R0 all 12 animals were dissected. Just before dissection the animals were weighed. All animals were then anaestesized using Fluothane^{R}, nitrous oxide and oxygen. After anaesthesia the abdomen was opened and the organs and intestine were removed quickly and weighed immediately. The content of the intestine was removed by hand by stripping. The weight of the organs and intestine are expressed as a percentage of live weight.

2.1 Chemical analyses

The dry matter content was determined by drying the samples to constant weight at 101°C. Ash was determined by incineration at 550°C during 4 hours. Nitrogen was analysed in fresh material using a Technicon Auto-Analyser. After wet digestion with 2.0M potassium sulphate solution in 18M sulphyric acid and selenium as catalyst, the nitrogen was bound by hypochlorite and phenol. This nitrogen complex was measured at 630 nm. Crude fat was analysed by treating for one hour with 3M hydrochloric acid and drying for 3 hours under vacuum at 100°C, followed by 8 hours extraction with diethylether. Crude fibre was determined according to NEN 3326. After boiling the sample with a sulphuric solution of standard concentration the residue was boiled with sodium hydroxide solution of a standard concentration, then separation, washing, drying and weighing of the insoluble residue, and determination of the loss in mass on incineration. Urea content in blood plasma was measured according to the patented two-step American Moniter urea assay (U.S. patent 4.074.972). The primary reaction occurs between the phthalaldehyde compound and urea resulting in the formation of isoindoline derivate. The isoindoline derivate then reacts with 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline to form an intensely coloured product which can be quantitated by photometric measurement at 510 nm. Nitrogen free extract was calculated as DM-(ash + (Nx6.25) + crude fat + crude fibre). The content of trypsin inhibitors were analysed according to Kakade et al. (1974). The content of haemagglutinins was measured according to Valdebouze et al. (1980).

2.2 Statistical analysis

The values for the different criteria are given as means with their standard errors. The differences between treatments were analysed by Students t test.

IV RESULTS

4.1 Weight gain, feed intake and feed conversion efficiency

The results of weight gain, feed intake and feed conversion efficiency ratio of the three animal species are presented in Table 4. In piglets there was weight loss with both R bean diets.

Table 4 Mean weight gain*, feed intake* and feed conversion ratio (g feed per g weight gain)* with SDs in piglets, rats and chickens

Treatments	Weight gain		Feed intake		Feed conversion ratio	
	g/day	SD	g/day	SD	mean	SD
			Piglets			
C	151.6a	32.9	233.9a	53.9	1.55a	0.09
R0	- 2.1b	26.3	146.0b	52.8	negative	
R60	- 3.5b	23.7	134.8b	103.0	negative	
T	145.8a	37.0	225.4a	43.3	1.56a	0.17
			Rats			
C	3.0a	0.6	12.3a	1.5	4.19a	0.47
R0	3.1a	0.4	12.5a	1.3	4.03a	0.30
R60	2.9a	0.5	12.3a	1.2	4.26a	0.37
T	3.1a	0.4	12.4a	1.2	3.97a	0.33
			Chickens			
C	11.7a	0.7	20.8a	0.4	1.78a	0.09
R0	12.1b	0.3	20.8a	0.4	1.72b	0.05
R60	10.6c	0.4	20.0a	0.2	1.89c	0.05
T	11.9a	0.5	20.9a	0.3	1.76ab	0.05

C, control; RO, unheated beans + extra casein; R60, unheated beans, T, heated beans.
Means in the same column that do not have a common letter differ ($P < .05$).
* In piglets measured over 14 days and in rats and chickens over 21 days.

The weight loss of treatment R0, in which extra casein was incorporated, was similar to that of the treatment without casein. In rats weight gain with the R diets were almost similar to the C diet. In chickens the weight gain with the R60 diet was significantly reduced (9%) compared to the control. When the R0 diet (containing extra casein) was fed, weight gain in chickens was ($P < .05$) above that of the control animals. When diet T was fed, the weight gain in the piglets was nearly at the control level and in rats and chickens slightly above the controls. Feed intake was reduced drastically in the piglets when both R diets were fed. The extra casein in the R0 diet had no positive effect on feed intake. In rats and chickens the feed intake was either

slightly or not reduced during feeding the R diets. When diet T was fed, feed intake in piglets was slightly reduced compared to the control diet and in the rats and chickens feed intake was not reduced. Effects of the same magnitude were found for feed conversion ratio. The feed conversion ratio in rats was rather high. This may be related to the restricted feeding level (Huisman, unpublished data).

4.2 Organs and blood parameters

Data on organ weights are presented in Tables 5, 6 and 7, respectively. The data of the weight of the small intestine are given in table 8 and those on plasma contents of urea in Table 9.

Table 5 Weight of organs in piglets (% of live weight) with SDs

reat-ment	Liver		Pancreas		Kidney		Spleen		Thymus	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
C	2.44a	0.25	0.18a	0.03	0.48a	0.08	0.27a	0.09	0.37a	0.12
RO	2.86bc	0.36	0.16a	0.02	0.50a	0.05	0.15b	0.04	0.18b	0.07
R60	2.48a	0.55	0.16a	0.04	0.46a	0.07	0.15b	0.04	0.18b	0.10
T	2.77ac	0.30	0.16a	0.02	0.49a	0.04	0.21c	0.09	0.30a	0.07

C, control; RO, unheated beans + extra casein; R60, unheated beans, T, heated beans.
Means in the same column that do not have a common letter differ ($P < .05$).

Table 6 Mean weight of organs in rats (% of live weight) with SDs

Treat-ment	Liver		Pancreas		Kidney		Spleen		Thymus	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
C	4.23a	0.72	0.51a	0.15	0.87a	0.05	0.24a	0.03	0.35a	0.11
R60	4.26a	0.79	0.69a	0.23	0.84a	0.03	0.23a	0.03	0.33a	0.09
T	4.47a	1.01	0.65a	0.22	0.85a	0.03	0.25a	0.03	0.37a	0.03

C, control; RO, unheated beans + extra casein; R60, unheated beans, T, heated beans.
Means in the same column that do not have a common letter differ ($P < .05$).

Table 7 Mean weight of organs in chickens (% of live weight) with SDs

Treat-ment	Liver		Pancreas		Kidney		Spleen		Thymus		Bursa of fabricius	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
C	3.03a	0.29	0.29a	0.04	1.02a	0.13	0.10a	0.02	0.55a	0.09	0.34a	0.09
R60	2.83ab	0.31	0.39b	0.05	1.17b	0.07	0.10a	0.03	0.51ab	0.09	0.37ab	0.10
T	2.64b	0.33	0.30a	0.03	1.02a	0.10	0.11a	0.03	0.47b	0.07	0.43b	0.08

C, control; RO, unheated beans + extra casein; R60, unheated beans, T, heated beans.
Means in the same column that do not have a common letter differ ($P < .05$).

The liver weight of the piglets fed the R0 diet was significantly ($P < .05$) higher compared to the C and R 60 diet. The liver weights were not significantly different between the treatments in the rats. The liver weight of the chickens fed the R and T bean diets were lower compared to the control animals, the difference between the C diet and the toasted bean (T) diet was significant ($P < .05$). The pancreas weights of piglets did not differ significantly between treatments. The pancreas weights of the rats of both bean diets (R60 and T) were slightly increased. In chickens fed the raw beans the pancreas weight was significantly ($P < .05$) higher compared to the control group. The pancreas weight of the chickens fed the T diet did not differ from control. In piglets and rats there were no significant differences between the treatments in kidney weight. In chickens the kidney weight of the R60 diet fed animals was significantly higher than the control animals ($P < .05$). The spleen weight of the piglets fed the R diet was significantly lower ($P < .05$) than the control and the T treatment. In the rats and chickens there were no significant differences between the treatments. In the piglets the weight of the thymus was markedly ($P < .05$) reduced when both raw bean diets R0 and R60 were fed. In rats no significant differences between the treatments were found ($P < .05$). In chickens the weight of the thymus of the T diet treatment was significantly lower compared to the control. The Bursa Fabricius of the chickens fed the T diet were heavier ($P < .05$) compared to the control animals. In all three animal species the weight of the small intestine in the R60 treatment were higher ($P < .05$) than in the C and the T animals. In chickens also the weight of the large intestine was determined, the weight in the R60 treatment was doubled ($P < .05$) compared to the C and the T treatment.

Table 8 Mean weight of intestine in piglets, rats and chickens (% of live weight) with SDs

	Small intestine						Large intestine	
Treatment	Piglets		Rats		Chickens		Chickens	
	mean	SD	mean	SD	mean	SD	mean	SD
C	3.52a	0.50	2.43a	0.42	2.24a	0.22	0.57a	0.19
R60	4.70b	0.56	4.00b	0.42	3.48b	0.49	1.12b	0.44
T	3.92a	0.39	2.40a	0.2	1.95c	0.24	0.55a	0.27

C, control; RO, unheated beans + extra casein; R60, unheated beans, T, heated beans.
Means in the same column that do not have a common letter differ ($P < .05$).

Table 9 Mean urea contents (mg/l) in plasma of piglets and chickens with SDs

Treatment	Piglets		Chickens	
	mg/l	SD	mg/l	SD
C	80a	25	12a	1.88
R0	473b	207	-	-
R60	455b	101	10a	2.7
T	126a	58	10a	2.7

C, control; RO, unheated beans + extra casein; R60, unheated beans, T, heated beans.
Means in the same column that do not have a common letter differ ($P < .05$).

From piglets and chickens blood samples were taken for the determination of the content of urea. In the R piglets the plasma urea content was about five times higher than the C animals, while the content in the samples from the T piglets was not significantly increased. In chickens there were no significant differences between the treatments (Table 9).

V DISCUSSION

Inclusion of 20% Phaseolus beans caused a markedly reduced feed intake and weight gain in piglets, but little or no effect in rats and chickens. Also the weight of the spleen and the thymus was distinctly reduced in piglets, but not in rats and chickens. These results indicate that the piglet was much more sensitive to ANFs present in the Phaseolus bean than the rat and the chicken. The rat and the chicken seem similar in sensitivity. The fact that the spleen and thymus weights were only affected in the piglet may be an indication, that the immune system of the piglet is more burdened to factors present in Phaseolus beans than the rat and the chicken. It is difficult to explain which factors are responsible for effects on the immune system although it is known that lectins (Pusztai, 1989) affect the immune system. In all three animal species the weight of the small intestine fed the R60 diet was significantly increased compared to the controls, indicating that the small intestinal tissue of all three animal species is sensitive to the ANFs present in the this bean. De Oliviera et al. (1988) found that the enhanced weight of the small intestine in rats when feeding raw Phaseolus beans, is associated with lectins. As described before the diets were balanced for digestible protein and amino acids, and moreover in diet R0 extra casein was incorporated. In spite of balancing the diets the piglets fed the raw Phaseolus beans did not gain body weight. The results of the plasma urea contents in the piglets indicate that protein deposition was seriously reduced. This disturbance of protein deposition cannot be attributed to an insufficient amino acid supply and therefore another factor must be responsible for the negative effects.

Similar indications were found by King et al. (1983). They showed that when pigs were fed the same amount of a control diet or a Phaseolus bean containing diet, respectively, the weight gain in the bean fed pigs was markedly reduced. In a study with rats by Pusztai et al. (1981) was demonstrated that lectins are responsible for the reduction in feed intake and weight gain when Phaseolus beans are fed. Huisman et al. (1990) found a reduced weight of the pancreas in piglets when diets contained 20% raw Phaseolus beans. The protein digestibility of the raw bean diet in that study was low compared to the control diet (48% vs 85%) and as a result the supply of the amino acids was inadequate from the bean diet. In the studies of Green et al. (1986), Gumbmann et al. (1986) and Liener et al. (1985) it was shown that pancreas weight relative to body weight was positively correlated with the protein content in the diet. Moreover Green et al. (1986) demonstrated that pancreas growth was inhibited by insufficiency of essential amino acids. In the present experiment the diets balanced for digestible protein and amino acids did not result in a difference in the weight of the pancreas of the piglets. These results indicate that when the supply of protein is adequate there is no reducing effect on the weight of the pancreas of piglets due to inclusion of Phaseolus beans in the diet. A striking observation is that due to feeding raw Phaseolus beans, pancreas hypertrophy and increased weight of the small intestine was found in rats and chickens with little or no effects on weight gain. It seems that under conditions of this study (diets balanced for digestible protein and

amino acids, energy, vitamins, minerals and low feeding level) these animals are able to compensate for effects on the pancreas and the gutwall. Hypertrophy is an indication for increased activity of the pancreas resulting in increased enzyme secretion (Liener and Kakade, 1980). These enzymes are rich in S-containing amino acids (Liener and Kakade, 1980). Reports by Barnes et al. (1962), Borchers (1961, 1962), Khayambashi and Lyman (1966), show that addition of extra methionine, threonine and valine to the diet can eliminate the negative effect of trypsin inhibitors on weight gain. Under the conditions of present study the amount of amino acids in the diet should have been sufficient to avoid the effect of stimulated pancreatic activity on weight gain of rats and chickens. The results demonstrate that biological responses like pancreatic hypertrophy and increased weight of the small intestine are not always reflected in weight gain.

From this experiment it can be concluded that the piglet is distinctly more sensitive to ANFs in Phaseolus beans than rats and chickens. The marked effects in piglets cannot be explained by inadequacy of amino acid supply.

VI REFERENCES

Barnes, R.H., Falia, G. & Kwong, E. (1962). Methionine supplementation of processed soybeans in the rat. Journal of Nutrition 77, 278-284.

Borchers, R. (1961). Counteraction of the growth depression of raw soybean oil meal by amino acid supplements in weanling rats. Journal of Nutrition 75, 330-334.

Borchers, R. (1962). Supplementary methionine requirement of weanling rats fed soybean oil meal rations. Journal of Nutrition 77, 309-311.

Bressani, R. (1983). Research needs to up-grade the nutritional quality of common beans (Phaseolus vulgaris). Qualitas Plantarum. Plant Foods for Human Nutrition 32, 101-110.

CVB (1988), Veevoeder tabel: Gegevens over voederwaarde, verteerbaarheid en samenstelling: Lelystad, The Netherlands. Centraal Veevoederbureau in Nederland.

De Oliviera, J.T.A., Pusztai, A. & Grant, G. (1988). Changes in organs and tissues induced by feeding of purified kidney bean (Phaseolus vulgaris) lectins. Nutrition Research 8, 943-947.

Green, G.M., Levan, V.H. & Liddle, R.A. (1986). Interaction of dietary protein and trypsin inhibitor on plasma cholecystokinin and pancreatic growth in rats. In Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods. pp. 123-132 [M. Friedmann, editor]. New York: Plenum Press.

Gumbmann, R.M., Spangler, W.L., Dugan, G.M. & Rackis, J.J. (1986). Safety of trypsin inhibitors in the diet: Effects on the rat pancreas on the long-term feeding of soy flour and soy protein isolate. In Nutritional and Toxicological Significance of Enzyme Inhibitors in the Foods. pp. 33-80 [M. Friedmann, editor]. New York: Plenum Press.

Huisman, J., van der Poel, A.F.B., Verstegen M.W.A. and van Leeuwen, P. (1990). Comparison of zootechnical characteristics in piglets and rats fed diets containing Phaseolus vulgaris. British Journal of Nutrition. In press.

Kakade, M.L., Rackis, J.J., McGhee, J.E. & Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chemistry 51, 376-382.

Khayambashi, H. & Lyman, R.L. (1966). Growth depression and pancreatic and intestinal changes in rats force-fed amino acid diets containing soybean trypsin inhibitor. Journal of Nutrition 89, 455-464.

King, T.P., Begbie, R. & Cadenhead, A. (1983). Nutritional toxicity of raw kidney beans in pigs. Immunocytochemical and cytopathological studies on the gut and the pancreas. Journal of the Science of Food and Agriculture 34, 1404-1412.

Liener, I.E. and Kakade, M.L. (1980). Protease inhibitors. In: Toxic Constituents of Plant Foodstuffs, pp. 7-71 [I.E. Liener, editor]. New York: Academic Press.

Liener, I.E., Nitsan, Z., Srisangnam. C., Rackis, J.J. & Gumbmann, M.R. (1985). The USDA trypsin inhibitor study. II. Timed related biochemical changes in the pancreas of rats. Qualitas Plantarum. Plant Foods for Human Nutrition 35, 243-257.

NEN 3326 (1966). *Onderzoeksmethoden voor veevoeders. Bepaling van het gehalte aan ruwe* celstof volgens de verkorte methode.

Pusztai, A., Clarke, E.M.W., Grant, G. & King, T.P. (1981). The toxicity of Phaseolus vulgaris lectins. Nitrogen and immunochemical studies. Journal of the Science of Food and Agriculture 32, 1037-1046.

Pusztai, A. (1985). Constraints on the Nutritional Utilization of Plant Proteins. Nutrition Abstracts and Reviews, series B, 55, 363-369.

Pusztai, A. (1989). Biological effects of dietary lectins. In Recent advances of research in antinutritional factors in legume seeds. pp. 17-29 [J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. Pudoc Wageningen, The Netherlands.

Valdebouze, P., Bergezon, E., Gaborit, T. & Delort-Laval, J. (1980). Content and distribution of trypsin inhibitors and haemagglutinins in some legume seeds. Canadian Journal of Animal Science 60, 695-701.

CHAPTER 2

ANIMAL SPECIES DIFFERENCE RESEARCH WITH PIGLETS, RATS AND CHICKENS FED PEAS AND COMMON BEANS

2.3 Performance and organ weights of piglets, rats and chickens fed diets containing Pisum sativum

J. Huisman[1], A.F.B. van der Poel[2], M.J.L. Kik[3] and J.M.V.M. Mouwen[3]

1) TNO- Institute of Animal Nutrition and Physiology (IGMB-dept. ILOB), P.O. Box 15, 6700 AA Wageningen, The Netherlands.
2) Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen.
3) Department of Pathology, Faculty of Veterinary Medicine, State University of Utrecht, P.O. Box 80158, 3508 TD Utrecht, The Netherlands.

Journal of Animal Nutrition and Animal Physiology (1990, 63, 273-279)

I ABSTRACT

In diets fed to piglets, rats and chickens 30% Pisum sativum were included and balanced for contents of amino acids and energy. These diets were fed at a low and high feeding level, respectively. In all three animal species the following criteria were studied: feed intake, weight gain, feed conversion efficiency and the weight of various organs. The main objective was to study whether rats and chicks can serve as a model for piglets to predict performance and biological effects. Weight gain was reduced in piglets, but not in rats and chickens. In piglets relative weight of the thymus was affected. In rats the pancreas and kidney and in chickens the pancreas, kidney and small intestine were affected. The results demonstrate that rats and chickens are not adequate models for prediction of performance and biological responses in piglets when Pisum sativum is fed.

II INTRODUCTION

Various studies have demonstrated that inclusion of Pisum sativum in diets of young pigs may have depressive effects on performance (Castaing and Grosjean, 1985; Fekete et al., 1984; Bengala-Freire et al., 1989; Grosjean and Castaing, 1983; Grosjean et al., 1986; Grosjean and Gatel 1989). This effect is possibly related with antinutritional factors (ANFs) present in peas, such as trypsin inhibitors (Griffiths, 1984; Grosjean and Gatel, 1989; Hove and King, 1979; Leterme et al., 1989; Valdebouze et al., 1980), haemagglutinins (Grant et al., 1983, Valdebouze, et al., 1980) and tannins (Griffiths, 1981; Stickland, 1984). It is unknown which of these factors is responsible for the negative effects. To study this question it is necessary to isolate the different components from the pea and purify ANFs and test them separately. Isolation of the various components and purification of ANFs in quantities enough for experiments with pigs is very laborious and expensive. Therefore a study was carried out to investigate the possibility to use small animals like the rat or chicken as a representative model for the pig. Another motive for this study was that in previous studies (Huisman at al., 1990a,b), it was found that piglets are much more sensitive to ANFs in Phaseolus beans than rats and chickens. Phaseolus beans contain lectins which cause severe damage of the gutwall (Jaffe, 1980; Pusztai, 1987, 1989; Torres-Pinedo, 1983). Bertrand (1988) found that the pea lectins seem not to have damaging effects to the gutwall. It is worthwhile to study whether there are animal species differences when a product is fed containing lectins having no or minor damaging effects on the gutwall. The objectives of present study were:

- comparison of the effects of a practical dietary inclusion of 30% Pisum sativum on performance and weight of various organs in piglets, rats and chickens.
- testing whether the rat or chicken can serve as a model for studying the biological effects of Pisum sativum in piglets.

III MATERIALS AND METHODS

1 Diets

Two diets were formulated; a control diet containing no Pisum sativum and a test diet containing 30 % Pisum sativum (Table 1).

Table 1 Composition of the diets

	Piglets and rats		Chickens	
Ingredients (g/kg)	Control diet	Test diet	Control diet	Test diet
Pisum sativum	-	300	-	300
Casein	131	64	129	63
Fish meal	50	50	50	50
Corn starch	556	346	545	320
Wheat bran	50	50	50	50
Cellulose	21	2	21	2
Soya oil	29	40	32	50
Dextrose	60	60	60	60
Sugar cane molasses	40	30	40	40
Vitamin/mineral mixture	10	10	10	10
$CaCO_3$	2	4	2	4
$CaHPO_4.H_2O$	29	24	29	25
$CaCl_2$	3	3	3	3
L-Lysine HCL	-	1.5	0.3	0.2
Dl-Methionine	1.2	1.8	3.7	4.0
L-Threonine	0.6	1.2	0.7	0.1
L-Tryptophan	0.2	0.4	0.2	0.4
L-Arginine	-	-	6.5	3.4
Calculated contents (g/kg)				
Crude protein	168	175	168	176
Digestible protein	155	155	155	155
Net energy (kJ/kg)	10538	10542	--	--
Metabolizable energy (kJ/kg)	--	--	14057	13480
Digestible lysine	12.7	12.7	12.5	12.5
Digestible methionine + cystine	6.5	6.5	8.7	8.2
Digestible threonine	7.3	7.3	7.0	7.0
Digestible tryptophan	2.1	2.1	2.0	2.0

The vitamin/mineral mixture supplied per kg feed:
Retinol 2.7 mg, cholecalciferol 45 µg, Dl-α-tocopherol 40 mg, menadione 3 mg, riboflavine 5 mg, nicotinic acid 30 mg, D-pantothenic acid 15 mg, choline chloride 120 mg, cyanocobalamin 40 µg, ascorbic acid 50 mg, $CuSO_4.5H_2O$ 20 mg, $ZnSO_4.H_2O$ 200 mg, MnO 70 mg, $FeSO_4.7H_20$ 400 mg, $CoSO_4.7H_2O$ 2.5 mg, $Na_2SeO_3.5H_2O$ 0.2 mg, KJ 0.5 mg.

The main protein source in the control diet was casein and some fish. These protein sources were chosen to be sure that no ANFs were present in the control diet. In the test diet a part of the casein was replaced by Pisum sativum. In a separate digestibility experiment with piglets the faecal digestibility of N was measured to be approximately 85%. This digestibility coefficient was used to balance the diets of piglets and rats on the content of digestible protein. For the other ingredients protein digestibility coefficients from the CVB-table (1988) were used. The amounts of digestible amino acids were also based on the CVB-table (1988). The chicken diets were balanced on digestible protein and free amino acids using the CVB-table (1988). Lysine, methionine, threonine and tryptophan were included for balancing the diets on these amino acids. In the chicken diet extra arginine and methionine were included. The composition of the diets is given in Table 1. The ANF contents in the pea were: haemagglutination: 20 activity units (measured according to Valdebouze, et al. (1980); trypsin inhibitor activity 1.1 mg inhibited trypsin/gram product (measured according to Kakade et al., (1974) and tannins 0.4 % (Folin Denis method).
The diets were pelleted without steam, the pellet size was 3 mm for the piglets and the chickens and 9 mm for the rats.

2 Animals, experimental scheme and management of the experiment

The experiment was carried out with two treatments of 12 piglets, 12 Wistar rats and 60 Hybro chickens each, respectively. The piglets were of the crossbred Dutch Landrace x Dutch Yorkshire. The piglets were assigned to the treatments in such a way that the mean live weight and the standard variation were comparable as good as possible. The rats were weaned at 4 weeks and immediately placed in the metabolism cages. The chickens were one day old when placed in the metabolism cages. The way of adaptation to the diets, the housing conditions, way of assignment to the treatments have been described previously (Huisman et al., 1990a, b). All three animal species were fed at two feeding levels: 2.2 times and 3.2 times maintenance for energy, respectively. The daily offered feed for the rats and the piglets was calculated according to the following formula: 420 x 2.2 or 3.2/ 13560, in which 420 is the metabolizable energy for maintenance (kJ) per unit metabolic weight (kg3/4) and 13560 the metabolizable energy (kJ) per kg feed. For the chickens the following formula was used: 520 x 2.2 or 3.2/13800, in which 520 is the metabolizable energy (kJ) for maintenance (kg3/4) and 13800 metabolizable energy (kJ) per kg feed. Body weight was measured weekly and adjusted twice weekly. Weight gain of the piglets was measured in the period 4-6 weeks of age, in the rats in the period 5-8 weeks of age and in the chickens in the period of 1-4 weeks of age.

3 Collection of organs and blood samples

On the day following the termination of the growth period from each treatment 7 piglets, 7 rats and 12 chickens were taken at random for dissection and collection of various organs and the small intestine. Just before dissection the animals were weighed. All animals were anaestesized using Fluothane^{R}, nitrous oxide and oxygen. After anaesthesia the abdomen was opened and the organs and intestine were removed quickly and weight immediately. The content of the intestine was removed by hand stripping. The weight of the organs and intestine are expressed as a percentage of live weight.

3.1 Statistical analysis

The values for the different criteria are given as means with their standard errors. The differences between treatments are analysed by the Student t test.

IV RESULTS

4.1 Feed intake, weight gain and feed conversion efficiency

In piglets and rats there were no significant differences in feed intake between the control group and the test group. Feed intake of the chickens fed the pea diet was significantly higher compared to the controls ($P < 0.05$). Weight gain in the piglets fed the pea diet was reduced compared to the control animals. At the low feeding level the difference was significant ($P < 0.05$), but not at the higher feeding level. In the rats there were no significant differences in weight gain between both groups at both feeding levels. Weight gain of the pea-fed chickens was higher than in the controls. At the high feeding level this difference was significant but not at the low feeding level. Feed conversion efficiency in the pea fed piglets was higher than in the control animals. The differences were not significant. In the rats and chickens there were only small differences in feed conversion efficiency between both diets. In the chickens these small differences were significant.

Table 2 Feed intake (g/day), weight gain (g/day) and feed conversion efficiency in piglets, rats and chickens

Criteria	Piglets				Rats				Chickens			
	control diet		test diet		control diet		test diet		control diet		test diet	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
	Feeding level 2.2. Maintenance											
Feed intake	202.9a	31.0	198.2a	25.3	9.4a	1.1	9.7a	1.3	35.7a	0.4	37.7b	0.4
Weight gain	124.4a	27.1	98.1b	22.3	3.0a	0.5	3.3a	0.5	20.9a	0.4	21.3a	0.9
Feed conversion efficiency	1.63a	0.13	2.02a	0.27	3.13a	0.35	2.94a	0.10	1.71a	0.03	1.77b	0.06
	Feeding level 3.2. Maintenance											
Feed intake	274.5a	56.8	282.3a	34.8	13.6a	1.5	12.9a	1.4	64.8a	0.9	69.7b	0.8
Weight gain	201.7a	52.7	187.2a	34.7	5.8a	0.7	5.8a	0.5	41.7a	1.6	43.4b	0.6
Feed conversion efficiency	1.36a	0.15	1.51a	0.15	2.34a	0.07	2.22a	0.06	1.58a	0.02	1.60b	0.01

Data of each animal species in the same row not having the same letter differ significantly ($P < 0.05$).

4.2 Weight of organs and small intestine

In all three animal species there were no significant differences between both treatments for weights of the liver, the spleen and the small intestine. The liver weight of the rats fed at the higher feeding level was significantly ($P < 0.05$) higher compared to those fed at the lower feeding level. In piglets there was also a tendency for a higher liver weight at the high feeding level, but the difference with the low feeding level were small and not significant. The weight of the pancreas of the piglets was similar with both diets. In rats fed the pea diet the pancreas weight was higher than in the control animals. At the low feeding level the difference was significant ($P < 0.05$) but just not at the higher feeding level. In chickens fed the pea diet the relative pancreas weight was higher ($P < 0.05$) compared to the control. The kidney weight in the piglets and chickens was not different between both treatments. In rats fed the pea diet at the low feeding level the kidney weight was lower ($P < 0.05$) compared to the controls, but at the high feeding level there were no significant differences. The weight of the thymus of the pea fed piglets at the low feeding level was significantly ($P < 0.05$) lower compared to the control animals. No significant differences in thymus weights were in the piglets fed at the higher feeding level, the rats at both feeding levels and the chickens at the higher feeding level. The weight of the small intestine was not affected in all three animal species.

V DISCUSSION

Inclusion of 30% peas in the diet caused in piglets a reduced weight gain, while feed intake was not distinctly lower, but not in rats and chickens. The reduction of weight gain in the piglets was more marked at the low feeding level (21%) than at the higher feeding level (7%). For feed conversion efficiency about the same differences were found. These results indicate that the piglet is more sensitive to factors causing impaired performance than rats and chickens. Therefore it can be concluded that the rat and chicken are not good representatives for the piglets for the determination of the effect of peas on performance. A similar result was found by Visitpanich et al. (1985) who found that the rat is not an adequate model for the prediction of pig performance when chick peas are fed. The relative organ weights in piglets were differently affected compared to rats and chickens. In piglets only the thymus weight was different between the two treatments on the low feeding level. The relevance of this observation is not clear because at the higher feeding level there were no differences. In rats pancreas and kidney were different between both treatments and in chickens the weights of pancreas. These results show that rats and chickens are also not adequate models for prediction of biological responses in piglets. It is difficult to indicate which factors are responsible for the reduced weight gain in piglets, because peas contain various ANFs. In our study an increased pancreas weight was found in rats and chickens, but not in piglets. In many studies it has been shown that trypsin inhibitors can cause an hypertrophy of the pancreas in small animals like rats and chickens, but not in larger animals like pigs and calves (Liener and Kakade, 1980; Gallaher and Schneeman, 1986). The hypertrophic effect of trypsin inhibitor on the pancreas is explained by a negative feedback mechanism mediated by the humoral cholecystokinin-pancreozymin (CCK-PZ) hormone which is produced by

Table 3 Weight of organs and intestine (% of live weight) of piglets and chickens

Criteria	Piglets				Rats				Chickens			
	control diet		test diet		control diet		test diet		control diet		test diet	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
					Feeding level 2.2. Maintenance							
Liver	2.77a	0.60	2.92a	0.23	3.67a	0.26	3.42a	0.23	-	-	-	-
Pancreas	0.19a	0.02	0.18a	0.04	0.46a	0.06	0.60b	0.08	-	-	-	-
Kidney	0.56a	0.12	0.57a	0.07	0.89a	0.07	0.83b	0.02	-	-	-	-
Spleen	0.27a	0.08	0.22a	0.04	0.23a	0.03	0.25a	0.02	-	-	-	-
Thymus	0.38a	0.14	0.23b	0.06	0.28a	0.03	0.30a	0.05	-	-	-	-
Small intestine	4.67a	0.70	5.01a	0.44	2.14a	0.25	2.56a	0.55	-	-	-	-
					Feeding level 3.2. maintenance							
Liver	3.08a	0.40	3.17a	0.28	5.26a	0.53	5.02a	0.24	3.18a	0.53	3.05a	0.37
Pancreas	0.17a	0.06	0.18a	0.02	0.49a	0.11	0.53a	0.08	0.18a	0.02	0.21b	0.03
Kidney	0.55a	0.06	0.58a	0.06	0.84a	0.04	0.83a	0.03	0.85a	0.11	0.90a	0.16
Spleen	0.24a	0.05	0.21a	0.04	0.22a	0.02	0.25a	0.04	0.14a	0.06	0.13a	0.06
Thymus	0.24a	0.06	0.26a	0.06	0.26a	0.04	0.28a	0.05	0.49a	0.09	0.50a	0.15
Bursa fabricius	-	-	-	-	-	-	-	-	0.32a	0.10	0.25a	0.04
Small intestine	5.13a	0.84	4.81a	0.70	2.24a	0.33	2.19a	0.23	2.19a	0.16	2.29a	0.17

Data of each animal species in the same row not having the same letter differ significantly ($P < 0.05$).

endocrine cells in the gut wall. Free trypsin and chymotrypsin in the intestinal fluid depress the secretion of CCK-PZ hormone. When trypsin and chymo trypsin are inhibited more CCK-PZ hormone will be produced which stimulates the pancreas to increase the production of digestive enzymes. The stimulated secretion of pancreas enzymes leads in small animals to enlargement of the pancreas but not in larger animals (Gallaher and Schneeman, 1986). In our study the enlargement of the pancreas in rats and chickens indicates that the trypsin inhibitor present in the peas did affect pancreas enzym secretion. Based on these results one may speculate that in piglets also the secretion of pancreas enzymes was increased. Therefore it seems plausible that the trypsin inhibitor has played a role in the negative effect in piglets. In conformity with these speculations are the results of Leterme et al. (1989) and Savage (1989) who found indications that trypsin inhibitors have a depressive effect on protein digestibility in peas. It is not clear whether or not lectins do play a role in the depressive effect on growth when peas are fed to young piglets. Bertrand (1988) found that pea lectins do not cause gut wall damage in piglets. However, Jindal et al. (1982) found that inclusion of purified pea lectins in diets caused reduced growth in young rats. Huisman et al., (1990a, b), showed in previous studies that the relative weight of the small intestine in piglets, rats and chickens was distinctly increased when Phaseolus vulgaris beans containing high levels of toxic lectins, were fed. In present study no or only small effects on the relative weight of the small intestine were found. These results, however, do not allow to conclude that the gutwall was not affected. In conclusion, this study demonstrated that the piglet responds differently compared to rats and chickens to inclusion of 30% peas in diet. But it is not clear which factor is responsible for the differences in response.

IV REFERENCES

Bengala-Freire, J.P., Hulin, J.C., Peiniau, J. and Aumaitre, A. (1989). Effet de la cuisson-extrusion du pois de printemps sur la digestibilité des aliments de sevrage precoce du porcelet et consequences sur les performances jusqu'a l'abattage. Journées Recherche Porcine en France, 21, 75-82.

Bertrand, G., Sève, B., Gallant, D.J. and Tomé, R. (1988). Absence d'effets antinutritionel des lectines de pois, sous forme native ou purifée chez le porcelet. Sciences des Aliments, 8, 187-212.

Castaing, J. and Grosjean, F. (1985). Effet de forts pourcentages de pois de printemps, dans des régimes pour porcs charcutiers, à base de maïs ou d'orge et en complément de tourteau de colza. Journées Recherce Porcine en France, 17, 407-418.

CVB. (1988). Veevoeder tabel: Gegevens over voederwaarde, verteerbaarheid en samenstelling, Centraal Veevoederbureau in Nederland, Lelystad, The Netherlands.

Fekete, J., Castaing, J., Lavorel, O. Leuillet, M. and Quemere, P.(1984) Utilisation des pois protéagineux par le porcelet sevré. Journées Recherche Porcine en France, 16, 393-400.

Gallaher, D. and Schneeman, B.O. (1986). Nutritional and metabolic response to plant inhibitors of digestive enzymes. In: Nutritional and toxicological significance of enzyme inhibiotors in foods. pp. 167-184. [M. Friedman, editor]. Plenum, New York, U.S.

Grant, G., More, L.J., McKenzie N.H., Stewart, J.C. and Pusztai, A. (1983). A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK. British Journal of Nutrition, 50, 207-214.

Griffiths, D.W. (1981). The polyphenolic content and enzyme inhibitory activity of testas from bean (Vicia faba) and pea (Pisum sativum) varieties. Journal of the Science of Food and Agriculture. 32, 797-804.

Griffiths, D.W. (1984). The trypsin and chymotrypsin inhibitor activities of various pea (Pisum spp) and Field bean (Vicia faba) cultivars. Journal of the Science of Food and Agriculture, 35, 481-486.

Grosjean, F. and Castaing, J. (1983). Recherche d'amélioration de la valeur alimentaire du pois d'hiver pour le porc charcutier. Journees recherche porcine en France, 15, 335-346.

Grosjean, F., Castaing, J. and Gatel, F. (1986). Utilisation comparée de différences variétés de pois et pois et d'une association pois de printemps-feverole par le porc charcutier. Journées Recherche Porcine en France, 18, 47-56.

Grosjean, F. and Gatel, F. (1989). Feeding value of Pisum sativum for pigs: -influence of technology, -influence of genotype (trypsin inhibitor activity). In: Recent advances of research in antinutritional factors in legume seeds. pp. 239-242. [J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. Pudoc, Wageningen, The Netherlands.

Hove, E.I. and King, S. (1979). Trypsin inhibitor contents of lupin seeds and other grain legumes. New Zealand Journal of Agricultural Research, 22, 41-42.

Huisman, J., Van der Poel, A.F.B., Verstegen, M.W.A. and van Leeuwen, P. (1990a). Comparison of zootechnical characteristics in piglets and ratio fed diets containing Phaseolus vulgaris. British Journal of Nutrition, In press.

Huisman, J., A.F.B. van der Poel, Mouwen, J.M.V.M. and van Weerden, E.J. (1990b). Effect of variable protein contents in diets containing Phaseolus vulgaris beans on performance, organ weights and blood parameters in piglets, rats and chickens. British Journal of Nutrition, In press.

Jaffé, W. (1980). Hemagglutinins (lectins). In: Toxic constituents of Plant Foodstuffs. pp 73-102. [I.E. Liener, editor], Academic Press, New York, U.S.

Jindal, S., Soni, G.L. and Singh, R. (1982). Effect of feeding of lectins from lentils and peas on the intestinal and hepatic enzymes of albino rats. Journal of Plant Foods, 4, 95-103.

Kakade, M.L., Rackis, J.J., McGhee, J.E. and Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chemistry 51, 376-382.

Leterme, P, Beckers, Y and Théwis. (1989). Inter- and intravarietal variability of the trypsin inhibitors content of peas and his influence on apparent digestibility of crude proteins by growing pigs. In: Recent advances of research in antinutritional factors in legume seeds. [J.Huisman, A.F.B. van der Poel and I.E. Liener, editors]. Pudoc, Wageningen, The Netherlands.

Liener, I.E. and Kakade, M.L. (1980). Protease inhibitors. In: Toxic constituents of Plant Foodstuffs. (I.E. Liener, editor). New York, Academic Press., 7-71.

Pusztai, A. (1987). Plant Lectins-Biological/Functions. Acta Biochemica et Biophysica. Hungary, pp. 355-375.

Savage, G.P. (1989). Antinutritive factors in peas. In: Recent advances of research in antinutritional factors in legume seeds. pp. 342-350. [J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. Pudoc, Wageningen, The Netherlands.

Stickland, R.G. (1984). Condensed tannins of pea seeds. Plant Science Letters, 34, 403-410.

Torres-Pinedo, R. (1983). Lectins and the intestine, Journal of the Pediatric Gastroenterology and Nutrition 2, 588-594.

Valdebouze, P., Bergeron, P., Gaborit, T. and Delort-Laval, J. (1980). Content and distribution of trypsin inhibitors and hemagglutinins in some legume seeds. Canadian Journal of Plant Science, 60, 695-701.

Visitpanich, T., Batterham, E.S. and Norton, B.W. (1985). Nutritional value of chickpea (Cicer arietinum) and pigeonpea (Cajanus cajan) meals for growing pigs and rats. I. Energy content and protein quality. Australian Journal of Agricultural Research, 36, 327-335.

CHAPTER 3

DIGESTIVE RESPONSE OF PIGLETS TO ISOLATED FRACTIONS FROM PEAS

3.1 Apparent faecal and ileal digestibility of pea proteins in early-weaned piglets: Comparison of raw peas and pea protein isolate

J. Huisman[1)], M-P. Le Guen[2)], J. Gueguen[3)], G.M. Beelen [1)], A.F.B. van der Poel[4)]

1) TNO-Institute of Animal Nutrition and Physiology (IGMB-dept. ILOB), PO Box 15, 6700 AA Wageningen, The Netherlands.
2) EURETEC GIE, 12, Avenue George V, 75008 Paris, France.
3) INRA, Institut National de la Recherche Agronomique, F 44072, Nantes Cedex, France.
4) Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen, The Netherlands.

Submitted for publication

I ABSTRACT

Various reports have demonstrated that inclusion of raw peas in diets for piglets may cause a negative effect on performance. To study whether the depressive effect is related to the pea protein itself or to other factors, a pea protein isolate was prepared from which antinutritional factors (ANFs) and carbohydrates were removed. Two ileal and two faecal digestibility experiments, comprising a control group and two test groups of six piglets each, were carried out. Apparent protein and amino acid digestibility was measured of raw FINALE and FRIJAUNE peas and pea protein isolates prepared from both varieties. The FINALE peas are characterized by low ANF contents and FRIJAUNE peas by relatively high ANF contents. The apparent protein digestibility coefficients for FINALE and FRIJAUNE were at faecal level 89% and 86%, respectively, and at ileal level 72% for both varieties. For pea protein isolate the faecal values were 94% for both varieties, and at ileal level 86% for FINALE and 87% for FRIJAUNE. For raw peas the apparent ileal amino acid digestibilities ranged between 34% and 87%, and for the pea protein isolate ranged between 61% and 94%. The lowest values were obtained for methionine, cystine, tryptophan, threonine, glycine and proline. The results show that factors other than the pea protein itself are responsible for the low apparent ileal protein digestibility of raw peas.

II INTRODUCTION

Peas are recognized to be a valuable protein source for both animal and man. The protein content of peas varies distinctly between varieties but in general the mean protein content is about 25%. This is intermediate between soybean meal and cereal grains (Gatel and Grosjean, 1990; Savage and Deo, 1989; Wiseman and Cole, 1988). There are many reports demonstrating that the inclusion of raw peas above 15 to 20% in diets of piglets may cause a depressive effect on performances (Castaing and Grosjean, 1985; Fekete et al., 1984; Bengala-Freire et al., 1989; Grosjean and Castaing, 1983; Grosjean et al., 1986; Grosjean and Gatel, 1986, 1989). Different factors may explain the reduced performance. Many authors discuss the possible relation with antinutritional factors (ANFs) in peas, such as trypsin inhibitors (Gatel and Grosjean, 1990; Griffiths, 1984; Grosjean and Gatel, 1989; Hove and King, 1979; Leterme et al., 1989; Valdebouze et al, 1980) and haemagglutinins or lectins (Grant et al., 1983; Valdebouze et al., 1980). In coloured flowering varieties tannins may also play a role (Griffiths, 1981; Stickland, 1984). Peas contain high levels of carbohydrates (60 to 63%, Grosjean and Gatel, 1986), mainly consisting of starch (48 to 50% in the seed, Grosjean and Gatel, 1986) and some alpha-galactosides (5 to 10%, Savage and Deo, 1989). Alpha-galactosides are not broken down in the small intestine because of the lack of appropriate digestive enzymes. These components are digested in the large intestine by bacterial fermentation. This fermentation results in energy losses and flatulence and diarrhoea may also occur (Saini, 1989). Therefore, it cannot be ruled out that these carbohydrates also play a role in the negative effect on performance. It is important to investigate which factor(s) are responsible for the reduced performance when peas are fed to piglets. As a first step, the apparent ileal and faecal digestibility of protein and amino acids was measured for raw peas and in pea protein isolate containing low levels of ANFs and no

carbohydrates in order to check whether protein or carbohydrates + ANFs were responsible.

III Materials and methods

1 Diets

Five semi-purified diets were formulated (Table 1) containing crude protein contents of between 16 and 17.5% (Table 3).

Control	: protein source only casein and fish meal
RPAP FINALE	: protein source only raw peas and air-classified peas from FINALE
RPAP FRIJAUNE	: protein source only raw peas and air-classified peas from FRIJAUNE
PPI FINALE	: protein source only pea protein isolate from FINALE
PPI FRIJAUNE	: protein source only pea protein isolate from FRIJAUNE

With only raw peas in the diet, the level of peas was approximately 60%. With this high dietary inclusion level of peas the carbohydrate level may become so high that diarrhoea and flatulence may occur. To avoid too high dietary pea carbohydrate levels, some part of the raw peas were replaced by raw pea fractions obtained by airclassification of raw peas. Air classification of peas was carried out according to van der Poel et al. (1989). An Alpine 132 MP classifier was used and the cut point was approximately 15 µm. With air classification the content of carbohydrates was decreased and the content of protein increased in the obtained fractions.
The diets differed in levels of the ANFs trypsin inhibitors and lectins: (none in the control diet, high in the RPAP diets and low in the PPI diets, Table 3). The carbohydrate composition was also different: mainly corn starch + dextrose in the control and PPI diets and corn starch + dextrose + pea carbohydrates in the RPAP diets. Two varieties of peas were tested separately: the spring variety FINALE and the winter variety FRIJAUNE. FRIJAUNE contained higher levels of trypsin inhibitors than FINALE. The differences in lectin contents between both varieties were relatively small (Table 2). The FINALE protein isolate was from the same batch of raw peas as that tested in the digestibility study. The FRIJAUNE protein isolate was from a different batch. The pea protein isolates were prepared at INRA-Nantes (France) according to Guéguen (1983). The chemical composition of the raw peas and the pea products is presented in Table 2. Synthetic amino acids methionine, threonine and tryptophan were added to the pea diets. Cellulose powder was added to balance the crude fibre content. Chromic oxid was included as a digestibility marker. The diets were pelleted without steam, the pellet temperature was between 50 and 55°C. The pellet size was 3 mm. Four experiments were carried out, two faecal digestibility trials (experiments 1 and 3) and two ileal digestibility trials (experiments 2 and 4). In experiments 1 and 2 the FINALE pea and its protein isolate were tested and in experiments 3 and 4 the FRIJAUNE pea and its protein isolate. In each trial, the digestibility of dry matter and protein was measured. At ileal level the digestibility of amino acids was also determined.

Table 1 Percentage composition of the control and pea protein-based diets for piglets

	Finale			Frijaune		
	control	RPAP*	PPI**	control	RPAP*	PPI**
Casein	12.5	-	-	12.5	-	-
Herring meal	6.9	-	-	6.9	-	-
Raw pea	-	25.0	-	-	25.0	-
Air-classified pea	-	18.6	-	-	17.8	-
Pea protein isolate	-	-	18.4	-	-	17.9
Corn starch	51.8	30.0	52.2	51.8	30.7	52.7
Dextrose	15.0	15.0	15.0	15.0	15.0	15.0
Sunflower oil	2.0	2.0	2.0	2.0	2.0	2.0
Cellulose	5.0	3.0	4.8	5.0	3.0	4.8
Vitamin/mineral mix	1.0	1.0	1.0	1.0	1.0	1.0
Monocalcium phosphate	1.95	2.0	2.4	1.95	2.0	2.4
Iodized NaCl	.5	.5	.5	.5	.5	.5
$NaHCO_3$	.35	.4	.4	.35	.4	.4
$KHCO_3$	1.65	.5	1.1	1.65	.5	1.1
DI-methionine	.06	.28	.37	.06	.29	.38
L-threonine	-	.06	.14	-	.06	.16
L-tryptophan	-	.06	.06	-	.06	.06
Chromic oxyde	.1	.1	.1	.1	.1	.1

The vitamin and mineral mixture supplied per kg feed:
Retinol 2.7 mg, cholecalciferol 45 µg, DL-α-tocopherol 40 mg, menadione 3 mg, riboflavine 5 mg, nicotinic acid 30 mg, D-pantothenic acid 15 mg, choline chloride 120 mg, cyanocobalamin 40 µg, ascorbic acid 50 mg, $CuSO_4.5H_2O$ 20 mg, $ZnSO_4.H_2O$ 200 mg, MnO 70 mg, $FeSO_4.7H_2O$ 400 mg, $CoSO_4.7H_2O$ 2.5 mg, $Na_2SeO_3.5H_2O$ 0.2 mg, KI 0.5 mg.
* RPAP = Raw Pea and Air classified Pea diet.
** PPI = Pea Protein Isolate diet.

Table 2 Chemical composition (%) of the pea-protein sources

	Finale			Frijaune		
	raw pea	air-classified pea	protein isolate	raw pea	air-classified pea	protein isolate
Dry matter	87.1	92.0	96.5	87.1	91.4	95.9
Crude protein	23.7	55.2	93.1	21.9	53.4	88.3
Crude fat	1.6	3.2	8.0	0.9	3.4	8.0
Ash	2.9	5.6	2.3	3.1	5.7	2.8
Crude fibre	6.2	2.4	1.0	7.1	3.2	< 1.0
NFE[a]	52.7	25.6	-	54.1	25.7	-
Starch (Ewers)	41.8	8.4	nd	40.2	7.6	nd
R+S+V[b]	3.3	4.2	nd	3.9	4.9	nd
Arginine	2.09	4.75	7.95	1.98	5.20	7.60
Histidine	.60	1.32	2.25	.55	1.39	2.17
Isoleucine	1.03	2.44	4.25	.97	2.49	4.25
Leucine	1.69	3.85	7.30	1.57	4.10	7.15
Lysine	1.71	3.85	6.30	1.64	4.15	6.40
Methionine	.24	.50	.81	.20	.49	.85
Cystine	.34	.62	.74	.32	.70	.72
Phenylalanine	1.10	2.53	4.50	1.01	2.65	4.35
Threonine	.93	2.02	3.20	.85	2.13	3.15
Tryptophan	.22	.49	.80	.19	.50	.77
Valine	1.19	2.73	4.75	1.11	2.80	4.65
Tyrosine	.81	1.82	3.20	.75	1.92	3.05
Tannins[c]	< .1	nd	< .1	< .1	< .1	< .1
TIA[d]	1.19	2.24	.62	5.44	2.10	1.60
Lectins[e]	3536	12310	1394	3657	15148	1604

a Nitrogen Free Extract, calculated from: dry matter - (crude protein + crude fat + ash + crude fibre).
b raffinose + stachyose + verbascose
c % catechins, measured according to Kuhla and Ebmeier (1981)
d TIA = Trypsin Inhibitor Activity expressed as mg inhibited trypsin/g product
e ELISA: µg lectins/g product
nd: not determined

Table 3 Chemical composition of the diets (%)

	Finale control	RPAP*	PPI**	Frijaune control	RPAP*	PPI**
Analysed content:						
Dry matter	89.3	88.3	90.5	90.4	88.2	90.1
Crude protein	17.0	16.6	17.5	16.5	16.0	16.6
Essential amino acids						
Arginine	.68	1.38	1.46	.70	1.44	1.42
Histidine	.46	.45	.45	.45	.40	.42
Isoleucine	.86	.71	.82	.86	.70	.79
Leucine	1.51	1.19	1.34	1.51	1.17	1.36
Lysine	1.19	.94	1.05	1.21	.93	1.03
Methionine***	.52	.41	.50	.50	.40	.50
Cystine	.11	.22	.16	.10	.21	.14
Meth. + cystine	.63	.63	.66	.60	.61	.64
Phenylalanine	.77	.76	.85	.77	.74	.83
Threonine***	.72	.67	.73	.70	.64	.72
Tryptophan***	.19	.18	.19	.19	.18	.19
Valine	1.07	.80	.89	1.07	.77	.86
Non-essential amino acids						
Alanine	.66	.68	.70	.66	.67	.67
Aspartic acid	1.30	1.84	1.90	1.26	1.74	1.82
Glutamic acid	3.35	2.70	3.05	3.25	2.66	2.87
Glycine	.54	.68	.68	.52	.64	.63
Proline	1.43	.69	.73	1.43	.61	.65
Serine	.93	.83	.92	.91	.81	.87
Tyrosine	.72	.53	.56	.72	.52	.54
Tannins a)	nd	< .10	< .10	nd	< .10	< .10
TIA b)	nd	.69	.10	nd	1.89	.36
Lectins c)	nd	2301	405	nd	1915	501
Calculated content	(%)					
Net energy (MJ/kg)	10.2	9.8	10.0	10.2	9.8	10.0
Crude fibre	5.0	5.0	5.0	5.0	5.0	5.0
Crude fat	2.7	2.9	3.5	2.7	2.9	3.5
Calcium	1.00	1.00	1.00	1.00	.99	1.01
Phosphorus	.58	.72	.62	.58	.71	.62
Available phosphorus	.58	.57	.57	.58	.57	.57
Base excess (meq/100 g)[d]	20.4	19.7	20.4	19.7	19.6	
R+S+V [e]	nd	1.59	nd	nd	1.84	nd

** PPI = Pea Protein Isolate diet.
a) % catechins, measured according to Kuhla and Ebmeier (1981)
b) TIA = Trypsin Inhibitor Activity, expressed as mg inhibited trypsin/g product
c) ELISA: µg lectins
d) calculated from (Na + K) - Cl.
e) R+S+V: raffinose + stachose + verbascose
nd: not determined.
* RPAP = Raw Pea and Air classified Pea diet
** PPI = Pea Protein Isolate diet.
*** Added synthetic amino acids are included in this analysis.

2 Animals

In each experiment male piglets of the crossbred Dutch Landrace x Dutch Yorkshire were used. Initial live weight was 8 to 9 kg and initial age was about 4 weeks. The piglets were housed individually in metabolism cages. The experimental design of each trial was a randomized complete block with initial weight and litter used as blocking factors. Each treatment comprised six animals. The piglets used for testing the FINALE products (experiments 1 and 2) were bought at the same time from one breeding farm and were all the same age. Half of the animals were used for the ileal digestibility trial and the other half for the faecal digestibility trial. A similar procedure was used for the FRIJAUNE pea products (experiments 3 and 4).

3 Experimental procedure

After a cage adaptation period of one week, the piglets used in the ileal digestibility trials were surgically fitted with a post-valvular T caecum cannula (PVTC), according to van Leeuwen et al.(1988) and allowed to recover for 8 to 10 days. Following a feed adaptation period of ten days, ileal digesta were collected 12 hours a day (0800 h - 2000 h), for two periods (P1 and P2) of 5 d each, with an interval of 48 h between P1 and P2. The digesta were collected in plastic bags fixed to the cannula, the bags were collected every hour, weighed and immediately frozen (-20°C) until analysis. The ileal digesta collection is not completely total (van Leeuwen et al., 1988), so chromic oxide was used to correct the collected ileal digesta to total collection. The age of the piglets during the collection periods was 8 to 9 weeks and the mean live weight about 15 kg. In the faecal digestibility trials, faeces collection was performed during two periods of 5 d each (P1 and P2), after allowing seven days for cage adaptation and ten days for feed adaptation. An interval of 48 h was kept between the faeces collection periods P1 and P2. The faeces were collected totally by using bags fixed around the anus. The faeces were removed from the bags twice daily and immediately frozen (-20°C) until analysis. The mean live weight and age during the faeces collection periods were about 13 kg and 7 to 8 weeks, respectively. In each experiment the piglets were fed restrictedly at 2.6 times their maintenance energy requirement (ARC, 1981). The feed was offered in dry form, twice daily. Water was freely available from nipple drinkers. Room temperature was kept between 22 and 25°C.

3.1 Chemical analysis

The dry matter content was determined by drying the samples to constant weight at 101°C. Ash was determined according to NEN 3329 (1969). Nitrogen was analysed in fresh material according to AOAC (1980). Crude fat was analysed by treating for one hour with 3M hydrochloric acid and drying for 3 hours under vacuum at 100°C, followed by 8 hours extraction with diethyl ether. The content of trypsin inhibitors was analysed according to the method described by van Oort et al. (1989). Lectins were analysed according to the ELISA principle, reported by Hamer et al. (1990). A 96 well flat bottom microtiterplate was coated with anti pea lectin IgG solution. After incubation at room temperature for 4 hours the anti body solution was removed and

tween-20 was added to all wells to block the residual binding sites. After two hours at room temperature the plates were washed with washing buffers. Serial dilutions of samples were added to the wells. After incubation overnight at 4°C the plates were washed again and anti pea lectin peroxidase conjugate was added to the wells. After 2 hours incubation at 37°C and again washing ortho-phenylenediamine solution was added. The color forming reaction was stopped by sulfuric acid. The absorbances on the plates were measured at 492 nm. Tannins were measured as catechins using the vanillin sulphuric method described by Kuhla and Ebmeier (1981). The content of crude fibre was determined according to NEN standard 3326, and the starch content according to NEN standard 3572 (Ewers method). The contents of raffinose, stachyose and verbascose were determined by separation on an HPLC column and detection by a refractive index detector (ERMA Inc. 7510). The amounts of sugars were calculated by calibration with purified sugars. Separation of the carbohydrates in the sample was achieved by a Waters M-45 solvent delivery system and a Bio-rad HPX-87p HPLC column using acetonitril/water (78/20, v/v) as eluent. Amino acids, with the exception of methionine, cysteine and tryptophan, were determined automatically after acid hydrolysis (6 N HCL during 22 h at 100°C) according to Slump (1969). For methionine and cysteine prior to this hydrolysis, the samples were subjected to formic acid oxidation. Tryptophan was determined after hydrolysis with 2.7 N Ba(OH)2, 8 h at 130°C, according to Slump and Schreuder (1969). Chromic analyses were performed by hydrolysing the samples in a mixture of perchloric and nitric acids and measuring the Cr6+ by flame atomic absorption spectrophotometry.

3.2 Calculations and statistical procedures

Digestibilities were determined from the nutrient concentrations of the diets and in faeces and digesta samples. Ileal digesta collection was corrected to total using the Cr recovery factor. As mentioned the diets were enriched with the synthetic acids, methionine, threonine and tryptophan. When calculating the digestibility of protein and these amino acids, corrections were made assuming that the added amino acids were completely absorbed in the small intestine (Huisman et al., 1986). The values for the various criteria are given as means with their standard deviations. Analysis of variance was carried out according to procedures described by Steel and Torrie (1960). Differences between treatments are analysed by the Student' t test.

IV RESULTS

The daily feed intake ranged from 450 to 498 g during the faecal trial and from 530 to 577 g during the ileal trial without refusal of any feed. The apparent N and amino acid digestibilities calculated from the experimental diets (Tables 4 and 5) are the N and amino acid digestibilities of their respective protein sources: casein + fish, raw peas and pea protein isolate. The apparent ileal N digestibilities of all diets (Table 4) were significantly ($P < 0.05$) lower than determined in faeces. Thus an important part of the undigested protein from casein + fish and the pea sources passed the terminal ileum and was broken down and absorbed in the large intestine. A significant ($P < .05$) period effect was found for the apparent ileal digestibilities of protein and dry matter of the RPAP and PPI diets. The increase in N ileal digestibility of P2 vs P1 was with the

Table 4 Apparent ileal and faecal digestibility coefficient of the control and the pea-protein-based diets for piglets

Variety		Finale				Frijaune		
Diet	control	RPAP**	PPI***	Differences RPAP vs PPI	control	RPAP**	PPI***	Differences RPAP vs PPI
				ileal level				
Nitrogen								
P1*	82.4	69.1	83.7	14.6	83.1	69.5	85.4	15.9
P2*	83.8	73.7	87.4	13.7	84.2	73.5	88.2	14.7
mean	83.2[a]	71.5[b]	85.6[a]	14.1	83.7[a]	71.6[b]	86.9[c]	15.3
sd.	(1.5)	(3.9)	(2.1)		(1.2)	(2.1)	(1.5)	
Dry matter								
P1*	85.8	73.9	84.2	10.3	85.5	74.5	86.2	11.7
P2*	86.3	75.3	85.7	10.4	87.1	76.5	86.7	10.2
mean	86.1[a]	74.6[b]	85.0[c]	10.4	86.3[ac]	75.5[b]	86.5[a]	11.0
sd.	(.5)	(1.0)	(1.2)		(1.0)	(1.0)	(1.2)	
Number of animals	5	6	6		6	6	6	
				faecal level				
Nitrogen								
P1	94.2	88.2	94.4	6.2	94.6	85.2	93.6	8.4
P2	94.8	89.0	94.4	5.4	94.6	86.0	94.4	8.4
mean	94.5[a]	88.6[b]	94.4[a]	5.8	94.6[a]	85.6[b]	94.0[c]	8.4
sd.	(.7)	(2.5)	(.8)		(.7)	(3.9)	(1.0)	
Dry matter								
P1	91.9	88.9	90.7	1.8	92.5	89.0	92.1	3.1
P2	92.3	89.8	91.8	2.0	93.0	89.2	92.1	2.9
mean	92.1[a]	89.4[b]	91.3[a]	1.9	92.8[a]	89.1[b]	92.1[c]	3.0
sd.	(.8)	(1.5)	(1.0)		(.9)	(1.7)	(.05)	
Number of animals	6	6	6		6	5	5	

sd : standard deviation
a,b,c : means per pea variety in the same row that do not have a common letter differ significantly ($P < .05$).
* : significant effect between the periods of the RPAP and PPI diets ($P < .05$).
** : RPAP = Raw Pea and Air classified Pea
*** : PPI = Pea Protein Isolate

Table 5 Apparent ileal digestibility coefficients of amino acids of the control and the pea protein-based diets for piglets (mean and standard deviation)

	Finale						Frijaune					
	control		RPAP*		PPI**		control		RPAP*		PPI**	
Essential amino acids												
Arginine	88.9^{a}	(1.3)	87.4^{a}	(2.5)	93.9^{b}	(1.1)	90.2^{a}	(1.4)	86.3^{b}	(1.2)	94.4^{c}	(0.4)
Histidine	89.4^{a}	(1.6)	81.8^{b}	(3.5)	90.5^{a}	(1.7)	90.3	(.9)	78.7^{b}	(3.1)	90.8^{a}	(1.1)
Isoleucine	88.7^{a}	(1.3)	75.9^{b}	(5.8)	88.8^{a}	(1.5)	88.7^{a}	(1.0)	73.7^{b}	(1.8)	89.6^{a}	(.8)
Leucine	91.0^{a}	(1.3)	76.8^{b}	(5.8)	89.6^{c}	(1.5)	91.9^{a}	(1.0)	75.3^{b}	(1.9)	90.5^{a}	(.8)
Lysine	88.2^{a}	(2.1)	72.2^{b}	(3.0)	86.9^{c}	(1.7)	89.8^{a}	(1.1)	74.0^{b}	(2.1)	89.6^{a}	(.8)
Methionine	91.2^{a}	(1.3)	48.5^{b}	(7.5)	64.5^{c}	(2.9)	91.6^{a}	(.8)	34.3^{b}	(3.6)	74.8^{c}	(1.4)
Cystine	55.3^{ab}	(7.3)	52.6^{a}	(7.8)	61.3^{b}	(5.0)	54.9^{a}	(5.3)	47.0^{b}	(3.7)	60.5^{c}	(4.4)
Methionine + Cystine	84.3^{a}	(2.4)	51.0^{b}	(7.5)	62.8^{c}	(3.3)	84.8^{a}	(1.6)	42.6^{b}	(3.3)	67.1^{c}	(2.9)
Phenylalanine	90.0^{a}	(1.5)	77.4^{b}	(5.6)	89.3^{a}	(1.6)	91.4^{a}	(1.2)	76.0^{b}	(1.7)	90.4^{a}	(.6)
Threonine	79.6^{a}	(2.3)	63.5^{b}	(5.6)	76.1^{a}	(3.1)	80.8^{a}	(1.3)	63.4^{b}	(2.0)	78.6^{c}	(1.4)
Tryptophan	80.7^{a}	(3.4)	48.1^{b}	(7.8)	67.9^{c}	(5.3)	83.2^{a}	(2.4)	42.3^{b}	(3.9)	71.3^{c}	(2.1)
Valine	88.2^{a}	(1.5)	72.0^{b}	(5.7)	86.3^{a}	(1.9)	88.7^{a}	(1.1)	70.7^{b}	(3.2)	87.2^{a}	(.9)
Non essential amino acids												
Alanine	82.2	(1.4)	71.0	(5.8)	84.9	(2.5)	83.5^{a}	(1.4)	70.0^{b}	(2.6)	86.1^{c}	(1.1)
Aspartic acid	78.1	(1.8)	79.3	(3.6)	90.0	(1.6)	79.3^{a}	(1.4)	77.6^{b}	(1.6)	90.5^{c}	(.8)
Glutamic acid	91.0	(.8)	81.7	(3.4)	92.6	(1.6)	90.8^{a}	(1.0)	82.8^{b}	(1.8)	92.8^{c}	(1.6)
Glycine	70.7	(3.2)	64.6	(4.4)	80.1	(4.2)	72.8^{a}	(1.9)	66.8^{b}	(3.0)	82.0^{c}	(2.5)
Proline	86.4	(1.6)	54.0	(14.7)	73.2	(15.2)	86.6^{a}	(4.0)	61.8^{b}	(10.8)	72.7^{b}	(13.4)
Serine	82.4	(1.9)	71.8	(4.5)	85.6	(1.8)	81.8^{a}	(2.4)	71.5^{b}	(2.3)	86.1^{c}	(1.4)
Tyrosine	91.2^{a}	(1.4)	75.5^{b}	(5.7)	87.9^{a}	(1.7)	92.2^{a}	(1.1)	74.1^{b}	(1.7)	88.3^{c}	(1.0)

a b c d : means per pea variety in the same row that do not have a common letter differ significantly ($P < .05$). The digestibility coefficients of MET, THR and TRP have been corrected for the synthetic amino acids added to the feed.

* RPAP = Raw Pea and Air classified Pea

** PPI = Pea Protein Isolate

RPAP and PPI diets higher than for the control diet. At faecal level there was no significant effect between the periods. At ileal level the difference between apparent digestibility of protein in raw peas and in pea protein isolate was about 14 and 15 units for FINALE and FRIJAUNE, respectively. At faecal level these differences were only 6 and 8 units, respectively. The apparent ileal and faecal protein digestibilities of the PPI diets were almost similar as those of the control diets. There were only small ($P > .05$) differences in ileal and faecal digestibilities between the pea varieties FINALE and FRIJAUNE. The apparent ileal amino acid digestibilities are reported in Table 5. For raw peas, the coëfficients were in the range of 34 to 87% for the essential amino acids (EAA), and 61 to 82% for the non-essential amino acids (NEAA). For EAA, the lowest coefficients were obtained for the S-containing amino acids and tryptophan. For NEAA, the lowest values were found for glycine and proline. The exclusion of ANFs and carbohydrates (PPI-diet) led to an increase in digestibility of all amino acids: the values were between 61 to 91% for the EAA and between 73 to 93% for NEAA. The increase for the amino acid methionine from FRIJAUNE was very large: 41 units. However, the amino acids methionine, cystine, tryptophan, glycine and proline of PPI were still less digestible than the other amino acids. In Table 6 a comparison between the amino acid composition of feed protein and ileal digesta protein is made. The protein in digesta is in all cases richer in cystine, threonine, tryptophan, alanine, proline, glycine and serine than the feed proteins. The composition of digesta with the control diets of both experiments were almost similar. The composition of the digesta-protein obtained with the RPAP and PPI diets had a good similarity for most amino acids. Exceptions are the amino acids arginine (FRIJAUNE RPAP higher than with other pea protein), threonine, glycine and proline (with RPAP lower than with PPI), aspartic acid and glutamic acid (with RPAP lower than with PPI).

V DISCUSSION

As already stated, the main objective of this study was to investigate which fraction in peas is responsible for reduced performance when high levels of peas are fed to piglets. It can be seen from Table 2 that the main difference in the chemical composition between raw peas and pea protein isolate are contents of protein, trypsin inhibitors and carbohydrates. The ileal digestibility of protein and dry matter of the control diet was significantly lower than the faecal digestibility. It is difficult to indicate what the reason is for the low ileal protein digestibility because no data are available about the ileal digestibility of casein and fish protein in piglets of this age. In pigs older than those used in the present study, relatively low ileal protein digestibilities for fish meal have been mentioned. In a publication by Jörgensen et al. (1984); Van Leeuwen et al. (1989) and Rudolph et al. (1983) values of between 73 and 79% are reported. The low ileal protein and dry matter digestibillity of the control diet is, therefore, most likely to be attributed to the fish meal. The dry matter contents of digesta were lower with the RPAP diets by 2 percentage units at ileum level and by 9 to 12 units at faeces level, compared with the PPI and control diets. The lower dry matter contents in faeces could possibly be related to the bacterial fermentation of pea carbohydrates in the large intestine. Pea carbohydrates are only partly digested in the small intestine of piglets, a substantial part is digested in the large intestine (Le Guen and Huisman, 1990, publication in preparation). The apparent faecal

Table 6 Mean analysed amino acid (A.A.) composition of feed (F) and ileal digesta (D). Each A.A. is expressed as a percentage of the total A.A. assayed

	Endogenous protein***	Finale control		RPAP*		PPI**		Frijaune control		RPAP*		PPI**	
		F	D	F	D	F	D	F	D	F	D	F	D
Essential amino acids													
Arginine	4.4	4.0	3.3	8.9	4.5	8.6	4.3	4.2	3.4	9.3	5.3	8.5	4.4
Histidine	1.8	2.7	2.1	2.9	2.1	2.6	2.1	4.0	2.1	2.6	2.3	2.5	2.1
Isoleucine	2.4	5.1	4.3	4.5	4.5	4.8	4.4	5.0	4.7	4.5	4.9	4.7	4.5
Leucine	5.0	8.9	6.0	7.6	7.2	8.4	7.2	8.8	5.9	7.5	7.7	8.1	7.1
Lysine	3.8	7.0	6.2	6.0	6.8	6.2	6.6	7.1	5.9	6.0	6.5	6.1	5.9
Methionine	1.0	3.1	1.8	2.6	1.7	2.9	2.2	3.2	1.8	3.9	1.9	4.6	1.7
Cystine	2.0	0.6	2.2	1.8	2.7	1.0	3.0	0.6	2.2	1.3	3.0	0.8	3.0
Phenylalanine	3.2	4.5	3.4	4.8	4.5	5.0	4.4	4.5	3.2	4.8	4.7	5.0	4.4
Threonine	5.1	4.2	6.5	4.3	5.8	4.3	6.8	4.1	6.5	4.4	5.7	5.0	6.6
Tryptophan	1.8	1.1	1.6	1.1	1.8	1.1	2.0	1.1	1.5	1.4	1.8	1.4	2.0
Valine	4.0	6.3	5.6	5.1	5.9	5.2	5.9	6.3	5.8	5.0	6.0	5.1	6.0
Non essential amino acids													
Alanine	5.1	3.9	5.2	4.3	5.1	4.1	5.1	3.9	5.2	4.3	5.4	4.0	5.1
Aspartic acid	7.8	7.6	12.6	11.7	10.0	11.1	9.2	7.4	12.5	11.2	10.5	10.9	9.6
Glutamic acid	8.3	19.7	13.4	17.2	12.9	17.9	10.9	19.0	14.4	17.1	12.3	17.1	11.4
Glycine	11.8	3.2	7.0	4.3	6.3	4.0	6.6	3.0	6.8	4.1	5.7	3.8	6.3
Proline	25.1	8.4	8.6	4.4	8.3	4.3	9.5	8.4	9.2	3.9	6.3	3.9	9.7
Serine	4.5	5.5	7.2	5.3	6.1	5.4	6.4	5.3	8.0	5.2	6.2	5.2	6.6
Tyrosine	2.5	4.2	2.8	3.4	3.4	3.3	3.3	4.2	2.7	3.3	3.6	3.2	3.5

* RPAP = Raw Pea and Airclassified Pea diet
** PPI = Pea Protein Isolate diet
*** Comparison of endogenous protein at the distal ileum: mean of 18 publications summarized by Wünsche et al. (1987).
F = Feed
D = Digesta

digestibility of protein of peas has been studied in many experiments. As summarized by Gatel and Grosjean (1990), protein digestibility in white coloured pea varieties ranged from 80 to 91%. The values of the present experiment are in the same range: 89% for FINALE and 86% for FRIJAUNE. The apparent faecal digestibilty coefficients of dry matter and protein of all three diets (Table 4) were distinctly higher compared with those measured at ileal level. This indicates that a substantial part of the diets are digested in the large intestine. Carbohydrates, which are digested in the large intestine, lose about 30 to 40% of the energy compared which those digested in the small intestine, which does not contribute to the animals' energy balance (ARC, 1981; Müller et al., 1989). For protein this is different. The part of protein that has passed the distal ileum and disappears in the large intestine, does not benefit the animals protein deposition (Zebrowska, 1973; Zebrowska et al., 1975, Wünsche et al., 1982). Thus, for protein it can be postulated that ileal digestibility will be a much more adequate measure than faecal digestibility. In our study the differences in protein digestibility between raw peas and pea protein isolate were at ileal level distinctly higher than at faecal level. This indicates that in present study the ileal digestibility is a more sensitive measure than faecal digestibility. There are different reports in the literature which show also that ileal digestibility, compared with faecal digestibility, is a more sensitive method for measuring differences in protein and amino acid digestibilities (reviewed by Sauer and Ozimek, 1986). Values concerning apparent ileal protein digestibility of peas are scarce in literature. The results of Buraczewska et al. (1989) and Green (1988) indicate a considerable difference in protein digestibility between varieties. In the study made by Buraczewska et al.(1989), using pigs weighing 40-70 kg, the apparent ileal protein digestibility of Pisum sativum ranged between 72 and 83. In the present study, the apparent ileal protein digestibility was 72 for both varieties. The apparent ileal protein digestibility for raw peas was distinctly lower than for pea protein isolate in which ANFs and carbohydrates were removed (72% vs 86-87%, Table 4). These results show that the pea protein in the isolated form is highly digestible. The low ileal digestibility with raw peas must likely be related to ANFs or to carbohydrates or to a combination of both. A striking result is that for both raw pea varieties, which were mainly different in trypsin inhibitor activity, about the same low ileal apparent protein digestibility was measured. Trypsin inhibitors reduce the activity of trypsin and chymotrypsin and stimulate the secretion of pancreatic enzymes (Liener and Kakade, 1980). The pea protein levels in the diets used in the present experiment were comparable with levels obtained with the inclusion of 60% peas. One may speculate that with a dietary inclusion level of 60% FINALE peas, the trypsin inhibitor activity is already so high that maximal inhibition of digestive enzymes and hypersecretion of pancreatic enzymes is already reached. The apparent ileal digestibility of amino acids with raw peas is distinctly lower than for the PPI or the control diet. With raw peas some amino acids are relatively low in ileal digestibility: the S-containing amino acids methionine and cystine, threonine, tryptophan, glycine and proline. Buraczewska et al. (1989) also measured very low apparent ileal digestibilities of protein, S-containing amino acids, threonine and tryptophan in some pea varieties, with 40-70 kg pigs. Only tannins were analysed in the diets used in this study. Therefore, conclusions about relationships of ileal digestibilities and other ANF activity cannot be made. The reason for the apparent ileal low digestibility of protein and amino acids of raw peas in the present experiment is not known. A low digestibility of protein and amino acids can be caused by different factors:

- Native pea protein can be highly stable to the digestive enzymes. Especially the globulins (70% of the pea protein) whose tertiary structure is globular with the hydrophobic amino acids as tryptophan located deep in the center.
- The digestibility of protein can be low due to reduction of activity of digestive enzymes by inhibitors.
- Also the secretion of endogenous protein may be increased, for example due to trypsin inhibitors, lectins and antigenicity of protein.

The data of Table 6 show that the concentration of cystine, threonine, tryptophan, glycine and proline are higher in digesta-protein than in feed protein. Endogenous protein is relatively rich in these amino acids (Table 6). This indicates that the low ileal digestibility of protein may at least partly be related with the secretion of endogenous protein. Pancreatic enzymes, compared with other proteins, are relatively rich in cystine (Corring and Jung, 1972; Juste, 1982). The lower ileal digestibility of cystine may, therefore, possibly be related to the increased secretion of pancreatic enzymes due to trypsin inhibitors (Liener and Kakade, 1980). Concerning the S-containing amino acids, ileal digestibility of methionine is generally high and cystine low (van Leeuwen et al., 1989). In line with this, in the present study a low cystine digestibility was found for all diets. The methionine digestibility was high in the control diet, as is normally observed, but was low with the pea diets. The reason for this low methionine digestibility is uncertain. It could be that the methionine digestibility in native pea protein is low. On the other hand, Liener and Kakade (1980) discussed a possible interference of digestibility with the enzymatic release of methionine when raw soya beans were fed. In raw soya, the main ANFs are also trypsin inhibitors and lectins. Because the same ANFs are present in soya and peas, this effect may also play a role when raw peas are fed. The low digestibilities for threonine, proline and glycine could probably be related to the contribution of endogenous protein originating from glycoproteins in saliva (rich in proline), in bile (rich in glycine) and in mucins (rich in threonine, proline, glycine and aspartic acid) to endogenous protein (Buraczewska, 1979; Degand et al., 1972; Horowitz, 1967; Low, 1982, Wünsche et al., 1987). In conclusion, the results of the present study show that the ileal protein and amino acid digestibility of raw peas are distinctly lower compared with pea protein isolates from which the carbohydrates and ANFs have been removed. The low ileal digestibility of important S-containing amino acids, tryptophan and threonine especially, could at least partly explain why the performance of piglets is reduced if more than 15% of peas are included in their diets. The extent to which the carbohydrates, the ANFs and the physical structure of raw pea are related to the reduced ileal digestibility remains to be investigated.

VI REFERENCES

Agricultural Research Council (ARC) (1981). The nutrient requirement of pigs. Commonwealth Agricultural Bureaux, England.

AOAC: Association of Official Analytical Chemists (1980). Thirteenth edition. Benjamin Franklin Station, Washington, DC 20044.

Bengala-Freire, J.P., Hulin, J.C., Peiniau, J. and Aumaitre, A. (1989). Effet de la cuisson-extrusion du pois de printemps sur la digestibilité des aliments de sevrage precoce du porcelet et consequences sur les performances jusqu'a l'abattage. Journées Recherche Porcine en France, 21, 75-82.

Buraczewska, L. (1979). Secretion of nitrogenous compounds in the small intestine of pigs. Acta Physiologica Polonica, 39, 2, 319-326.

Buraczewska, L., Gdala, J. and Grala, W. (1989). Ileal digestibility of protein in pigs fed diets with peas of variable content of protein and tannins. In: Recent advances of research in antinutritional factors in legume seeds. [J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. Pudoc, Wageningen, The Netherlands, 181-184.

Castaing, J. and Grosjean, F. (1985). Effet de forts pourcentages de pois de printemps, dans des régimes pour porcs charcutiers, a bàse de mais ou d'orge et en complément de tourteau de colza. Journées Recherce Porcine en France, 17, 407-418.

Corring, T. and Jung, J. (1972). The amino acid composition of pig pancreatic juice. Nutrition Reports International, 6, 4, 187-190.

Degand, P., Gaueriaux, M. and Havez, R. (1972). Définition biochimique des sulfomucines et des sialomucines de l'intestin grêle du porc. Compte rendu Lebdomadaire des seances de l' Académie des Sciences, 166, 622-627.

Fekete, J., Castaing, J., Lavorel, O. Leuillet, M. and Quemere, P. (1984). Utilisation des pois protéagineux par le porcelet sevré. Journées Recherche Porcine en France, 16, 393-400.

Gatel, F. and Grosjean, F. (1990). Composition and nutritive value of peas for pigs: A review of European results. Livestock Production Science, In Press.

Grant, G., More, L.J., McKenzie N.H., Stewart, J.C. and Pusztai, A. (1983). A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK. British Journal of Nutrition, 50, 207-214.

Green, S. (1988) A note on amino acid digestibility measured in pigs with pre- or post valve ileo-rectal anastomoses, fed soya-bean, pea and meat meals. Animal Production, 49, 330-332.

Griffiths, D.W. (1981). The polyphenolic content and enzyme inhibitory activity of testas from bean (Vicia faba) and pea (Pisum spp) varieties. Journal of the Science of Food and Agriculture. 32, 797-804.

Griffiths, D.W. (1984). The trypsin and chymotrypsin inhibitor activities of various pea (Pisum spp) and Field bean (Vicia faba) cultivars. Journal of the Science of Food and Agriculture, 35, 481-486.

Grosjean, F. and Castaing, J. (1983). Recherche d'amélioration de la valeur alimentaire du pois d'hiver pour le porc charcutier. Journées Recherche Porcine en France, 15, 335-346.

Grosjean, F. and Gatel. (1986). Peas for Pigs. Pig News and Information, 7, 443-448.

Grosjean, F. and Gatel, F. (1989). Feeding value of Pisum sativum for pigs: -influence of technology, -influence of genotype (trypsin inhibitor activity). In: Recent advances of research in antinutritional factors in legume seeds. [J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. PUDOC, Wageningen, The Netherlands., 239-242.

Grosjean, F., Castaing, J. and Gatel, F. (1986). Utilisation comparée de différentes variétés de pois et d'une association pois de printemps-féverole par le porc charcutier. Journées Recherce Porcine en France, 18, 47-56.

Gueguen, J. (1983). Legume seeds protein extraction, processing and end product characteristics. Qualitas Plantarum. Plant Food for Human Nutrition., 32, 267-303.

Hove, E.I. and King, S. (1979). Trypsin inhibitor contents of lupin seeds and other grain legumes. New Zealand Journal of Agricultural Research, 22, 41-42.

Huisman, J. De Vries, P.H., van Weerden, E.J. and Bertram, H.L. (1986). The availability of synthetic methionine in pigs. Journal of Animal Nutrition and Animal physiology, 55, 267-272.

Horowitz, M.I. (1967). Section 6, Alimentary canal. In: Handbook of Physiology. [C.F. Code, editor]. American Physiological Society, Washington, D.C.

Jörgensen, H. Sauer, W.C. and Tacker, P.D. (1984). Amino acid availabilities in soybean meal, sunflower meal, fish meal and meat and bone meal to growing pigs. Journal of Animal Science, 58, 4, 926-934.

Juste, C. (1982). Apports endogènes par les secrétions digestives chez le porc. In: Physiologie digestive chez le porc. Jouy- en -Josas. Les colloques de l'INRA, 12, 155-173.

Kuhla, S. and Ebmeier, C. (1981). Untersuchungen zum Tanningehalt in Ackerbohnen. Archiv für Tierernährung, 31, 573-588.

Leterme, P, Beckers, Y and Thewis. (1989). Inter- and intravarietal variability of the trypsin inhibitors content of peas and his influence on apparent digestibility of crude proteins by growing pigs. In: Recent advances of research in antinutritional factors in legume seeds. [J.Huisman, A.F.B. van der Poel and I.E. Liener, editors]. PUDOC, Wageningen, The Netherlands.

Liener, I.E. and Kakade, M.L. (1980). Protease inhibitors. In: Toxic constituents of plant foodstuffs. [I.E. Liener, editor). Academic Press, New York, 7-71.

Low, A.G. (1982). Endogenous nitrogen evaluation from absorption studies. In: Physiology digestive chez le porc, Jouy-en -Josas. Les Colloques de l'INRA, 12, 189-198.

Müller, H.L., Kirchgessner, M. and Roth, F.X. (1989). Energy utilization of intracaecally infused carbohydrates and casein in sows. In: Energy metabolism of farm animals. Proceedings of the 11th symposium. [Y. van der Honing and W.H. Close, compilers]. Pudoc, Wageningen, The Netherlands, 123-126.

NEN Standards of the Netherlands Normalization Institute: Nr. 3326: Determination of the crude fibre content by the abbreviated method in feed stuffs (1966). Nr. 3329: Determination of crude ash in feed stuffs (1969). Nr. 3572. Polarimetric determination of the starch content in feed stuffs. Ewers method (1985).

Rudolph, B.C., Boggs, L.S., Knabe, D.A., Tanksley, T.D. Jr. and Anderson, S.A. (1983). Digestibility of nitrogen and amino acids in soybean products for young pigs. Journal of Animal Science, 57, 373-386.

Saini, H.S. (1989). Legume seed oligosaccharides. In: Recent advances of research in antinutritional factors in legume seeds. [J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. Pudoc, Wageningen, The Netherlands, 329-341.

Sauer, W.C. and Ozimek, L. (1986). Digestibility of amino acids in swine: results and their practical applications. A review. Livestock Production Science, 15, 367-388.

Savage, G.P. and Deo, S. (1989). The nutritional value of peas (Pisum sativum). A literature review. Nutrition Abstracts and Reviews (Series A), 59, 2, 66 - 88.

Slump, P. (1969).Karakterisering van de voedingswaarde van eiwitten in voedingsmiddelen door hun aminozurensamenstelling en de invloed van verhitting en loogbehandeling op de benutbaarheid van aminozuren. Thesis Free University, Amsterdam, 131 pages.

Slump, P. and Schreuder, H.A. (1969). Determination of tryptophan in foods. Analytical Biochemistry, 27, 181-186.

Steel, R.G. and Torrie, J.H. (1960). Principles and procedures of statistics: A biochemical approach (2nd Ed.). McGraw-Hill Book Co., New York.

Stickland, R.G. (1984). Condensed tannins of pea seeds. Plant Science Letters, 34, 403-410.

Valdebouze, P., Bergeron, P., Gaborit, T. and Delort-Laval, J. (1980). Content and distribution of trypsin inhibitors and hemagglutinins in some legume seeds. Canadian Journal of Plant Science, 60, 695-701.

Van Leeuwen, P., Huisman, J., Verstegen, M.W.A., Baak, M.J., van Kleef, D.J., van Weerden, E.J. and den Hartog, L.A. (1988). A new technique for collection of ileal chyme in pigs. Proceedings of the 4th International Seminar on Digestive Physiology in the pig, Jablonna, Poland.,289-296.

Van Leeuwen, P., Slump, P., Van Weerden, E.J., Huisman, J., Tolman, G.H. and van Kempen, G.J.M.. (1989). Tabel darmverteerbare aminozuren voor varkens, ILOB-rapport nr. I 89-3641.

Van Oort, M.G., Hamer, R.J. and Slager, E.A. (1989). The trypsin inhibitor assay: Improvement of an existing method. In: Recent advances of research in antinutritional factors in legume seeds. [J.Huisman, A.F.B. van der Poel and I.E. Liener, editors]. Pudoc, Wageningen, The Netherlands, 110-113.

Van der Poel, A.F.B., Aarts, H.L.M. and Stolp, W. (1989). Milling and air-classification of two different types of peas. - effect on the distribution of antinutritional factors. Netherlands Journal of Agricultural Science, 37, 273-278.

Wiseman, J. and Cole, D.J.A., 1988. European legumes in diets for non-ruminants. In: Recent Advances in Animal Nutrition. [W. Haresign and D.J.A. Cole, editors]. Butterworths, London, 13-37.

Wünsche, I., Hennig, U., Meinl. M., Kreienbring, F. and Bock, H.D. (1982). Untersuchungen über Resorption und Verwertung von in Zäkum wachsender Schweine infundierten Aminosauren. Archiv für Tierernährung. 32, 337-348.

Wünsche, J., Herrmann, U., Meinl, M., Hennig, U., Kreienbring, F. und Zwierz, P. (1987). Einfluss exogener faktoren auf die präzäkale Närhstoff- und Aminosäurenresorption, ermittelt an Schweinen mit Ileo-Rektal-Anastomosen. Archiv Animalischen Nutrition, 37, 9, 745-764.

Zebrowska, T. (1973). The apparent digestibility of nitrogen and individual amino acids in the large intestine of the pig. Roczniki Nauk Rolniczych, Seria B., Zootechniczna 997, 117-123.

Zebrowska, T., Buraczewska, L. and Buraczewski, S. (1975). The apparent digestibilty of amino acids in the small intestine and the whole digestive tract of pigs fed diets containing different sources of protein. Roczniki Nauk Rolniczych, Seria B., Zootechniczna 99, 87-98.

CHAPTER 3

DIGESTIVE RESPONSE OF PIGLETS TO ISOLATED FRACTIONS FROM PEAS

3.2 Investigation into factors responsible for negative effects on performance of piglets fed high levels of peas: carbohydrates and antinutritional factors

J. Huisman[1], M.P Le Guen[2], S. Berot[3], J. Gueguen[3] and E.J. van Weerden[1]

1) TNO-Institute of Animal Nutrition and Physiology (IGMB-Dept. ILOB), PO Box 15, 6700 AA Wageningen, The Netherlands.
2) EURETEC GIE, 12, Avenue George V, 75008 Paris, France.
3) INRA Institut National de la Recherche Agronomique, F44072, Nantes, Cedex, France

Submitted for publication

I ABSTRACT

A study was made to investigate whether the low apparent ileal protein digestibility of raw peas is related to antinutritional factors (ANFs; mainly trypsin inhibitors and lectins) or to carbohydrates or to both fractions. To study this the following preparations were made from two pea varieties (FINALE and FRIJAUNE):
1. pea protein isolate from which the ANFs and carbohydrates were removed;
2. pea ANF-concentrate containing high levels of trypsin inhibitors and lectins;
3. fraction consisting exclusively of pea carbohydrates.
Ileal digestibility of these three fractions was measured in experiments with young piglets. In the first experiment pea carbohydrates were included in diets differing in protein sources: casein + fish or protein isolate from FINALE peas or protein isolate from FRIJAUNE peas. There was no reduction in apparent ileal protein digestibility due to the addition of pea carbohydrates. The small intestinal chyme flow, however, was increased when pea carbohydrates were included in the diets. In the second experiment, ANF concentrate (consisting mainly of trypsin inhibitors and lectins) was included in a diet with pea protein isolate from FINALE. There was a significant reduction in apparent ileal protein digestibility and weight gain when ANF-concentrate was included in the diet.

II INTRODUCTION

Reduction of performance in piglets fed a high level of Pisum sativum in diets has been reported by various authors (Castaing and Grosjean, 1985; Fekete et al., 1984; Bengala-Freire et al., 1989; Grosjean and Castaing, 1983; Grosjean et al., 1986; Grosjean and Gatel, 1986, 1989). Huisman et al. (1990b) studied the ileal and faecal digestibility of raw peas in comparison with pea protein isolate from which ANFs (mainly trypsin inhibitors and lectins) and carbohydrates were removed. It was shown that the apparent ileal protein digestibility of pea protein isolate was 14 units higher than in raw peas. The aim of the present investigation was to study whether ANFs or carbohydrates are responsible for these marked differences in ileal protein digestibility. To study this the following fractions were prepared: pea protein fraction containing only low contents of ANFs and no carbohydrates, an pea ANF-concentrate containing high levels of trypsin inhibitors and lectins, and a fraction consisting of a mixture of soluble and insoluble pea carbohydrates. These fractions were used in experiments with piglets to study which factor is responsible for the differences in ileal protein digestibility between raw pea and pea protein isolate. Two ileal digestibility experiments with piglets were carried out. In the first experiment pea carbohydrates were included in diets differing in protein sources. The objectives of this study were: to investigate whether pea carbohydrates do affect ileal protein digestibility and to test whether there is an interaction between effects of the carbohydrates and the protein source. In the second experiment, an ANF-concentrate was added to a diet with pea protein isolate from FINALE as the protein source. The objective was to test whether pea ANFs affect the apparent ileal protein digestibility.

III MATERIAL AND METHODS

1 Preparation of pea protein isolate, ANF concentrate and the carbohydrate fraction

The pea protein isolate was prepared at INRA-Nantes (France) according to Guéguen (1983). By this process, two by-products were obtained: a whey fraction containing the soluble part of the flour at pH 4.5, and an insoluble carbohydrate fraction. The ANF concentrates were prepared from the whey fraction by ultrafiltration. The spray-dried whey was dissolved in water at 50°C (volume of water equal to 15 times the weight of dried whey) and ultrafiltered on mineral membranes TECH-SEP M5 (cut size: 10000). The aim of this ultrafiltration procedure was to remove the low molecular weight components of the whey. It comprised a first concentration, a washing step by diafiltration with a volume of water equivalent to 25 times the weight of dried whey) and a final concentration until a weight of retentate equal to 6 times the weight of dried whey. During the ultrafiltration steps, a protein fraction was precipitated in the retentate. This fraction with a very low content of ANF, was separated by centrifugation (5000 g 20 min) and discarded. The supernatant, enriched in ANF was kept and lyophilized. The carbohydrates concentrates consisted of a mixture of the insoluble carbohydrates fraction produced during the isolate process and the soluble carbohydrates prepared by 50°GL aqueous ethanol extraction (temperature 70°C) of the raw pea four in the ratio 3/1 v/w, the mixture being then spray-dried. The quantities of the two components of the mixture were chosen to give about the same proportions of insoluble/soluble carbohydrates as in raw peas. The composition of the protein fractions are presented in Table 1 and those of the carbohydrate fractions in Table 2.

Table 1 Chemical composition (%) of the pea protein isolates and ANF-concentrate

Nutrient	FINALE	FRIJAUNE	ANF CONCENTRATE 4)
Dry matter	96.5	95.9	96.0.
Ash	2.3	2.8	4.0.
Crude protein	89.5	88.3	61.9
Crude fat	8.0	8.0	n.d.
TIA 1)	0.6	1.6	49.1
Lectins 2)	1394	1604	101944
Tannins 3)	< 0.1	< 0.1	< 0.1

1) TIA: mg inhibited trypsin per g product
2) ELISA: µg/ g product
3) % catechins, measured with the vanillin sulphuric acid method.
4) Mix of ANF concentrates from the varieties FINALE and FRIJAUNE
n.d. = not determined.

Table 2 Chemical composition (%) of pea carbohydrate (insoluble and soluble (CIS)) fractions

	CIS FINALE	CIS FRIJAUNE
Dry matter	96.6	96.8
Crude ash	1.8	1.3
Crude protein	4.6	2.5
Crude fat	0.2	0.3
Nitrogen Free Extract	82.0	81.3
Crude fibre	8.0	11.4
ADF	9.3	13.7
NDF	12.6	17.5
Saccharose	2.5	1.0
Raffinose	0.2	0.1
Stachyose	1.2	0.8
Lectins 1)	6.3	8.5
TIA 2)	0.2	0.3
Tannins 3)	< 0.1	< 0.1

1) ELISA: µg lectins/ g product
2) TIA: mg inhibited trypsin per g product
3) % catechins, measured with the vanillin sulphuric acid method.

2 Diets

Two experiments were carried out. In experiment 1 the effect of the carbohydrates on ileal protein digestion was studied, and in experiment 2 the effect of pea ANFs.
In **experiment 1**, three protein sources were used: casein + fish, pea protein isolate from the spring pea FINALE and pea protein isolate from the winter pea FRIJAUNE. Casein + fish were used as a control. The FINALE and FRIJAUNE protein isolates were from the same batches of raw peas from which the low ileal protein digestibility had been measured in the previous experiment (Huisman et al., 1990b). With each protein source, two diets differing in carbohydrate composition were formulated: a control diet with corn starch + dextrose and a test diet with corn starch + dextrose + pea carbohydrates. In this way, three control (I-III) and three experimental diets (IV-VI) were formulated for experiment 1.

I : protein source: casein and fish; carbohydrate source: mix of corn starch + dextrose
II : protein source: casein and fish; carbohydrate source: mix of corn starch + dextrose + pea carbohydrates
III : protein source: pea protein isolate FINALE; carbohydrate source: mix of corn starch + dextrose
IV : protein source: pea protein isolate FINALE; carbohydrate source: mix of corn starch + dextrose + pea carbohydrates

V : protein source: pea protein isolate FRIJAUNE; carbohydrate source: mix of cornstarch + dextrose
VI : protein source: pea protein isolate FRIJAUNE; carbohydrate source: mix of cornstarch + dextrose + pea carbohydrates.

The carbohydrate composition of test diets II, IV and VI was made up as close as possible to the carbohydrate composition in the raw pea diets used in Huisman et al. (1990b). The pea carbohydrate fraction added to the diets, consisted of a mix of soluble and insoluble carbohydrates. The levels of alpha-galactosides in this fraction, however, were somewhat lower than in the carbohydrate fraction present in raw peas. The test diets were made therefore, as similar as possible by addition of extra free alpha- galactosides.
In **experiment 2**, two diets were formulated; a control diet with low levels of the ANFs trypsin inhibitors and lectins, and a test diet with high levels of these ANFs. The protein source in both diets was pea protein isolate from FINALE, which was low in ANF activity. High levels of ANFs in the test diet were obtained by the inclusion of ANF concentrate (mix of ANF concentrates from the varieties FINALE and FRIJAUNE). The carbohydrate sources in the diets were corn starch and dextrose. The diets in both experiments were balanced for contents of protein, amino acids, energy and minerals. Chromic oxide was included as a digestibility marker. The percentage and chemical compositions of the diets of both experiments are presented in Tables 3 and 4, respectively. The diets were pelleted without steam, the pellet size was 3 mm. The pelleting temperature was about 50°C.

Table 3 Percentage composition of the diets (%)

Ingredients	Experiment 1 I	II	III	IV	V	VI	Experiment 2 I	II
Casein	12.50	12.50	-.-	-.-	-.-	-.-	-.-	-.-
Herring meal	6.90	6.90	-.-	-.-	-.-	-.-	-.-	-.-
Protein isolate FINALE	-.-	-.-	18.40	18.40	-.-	-.-	18.40	16.39
Protein isolate FRIJAUNE	-.-	-.-	-.-	-.-	17.90	17.90	-.-	-.-
ANF concentrate	-.-	-.-	-.-	-.-	-.-	-.-		2.90
Corn starch	42.03	30.01	42.03	30.01	42.15	30.74	52.23	51.34
CIS FINALE	-.-	18.82	-.-	18.82	-.-	-.-	-.-	-.-
CIS FRIJAUNE	-.-	-.-	-.-	-.-	-.-	16.93	-.-	-.-
Pea hulls	-.-	3.41	-.-	3.41	-.-	2.80	-.-	-.-
Saccharose	-.-	2.06	-.-	2.06	-.-	1.68	-.-	-.-
Raffinose	-.-	1.18	-.-	1.18	-.-	1.57	-.-	-.-
Arabinose	-.-	0.62	-.-	0.62	-.-	0.92	-.-	-.-
Dextrose	22.76	12.69	23.05	12.98	23.40	14.91	15.00	15.00
Sunflower oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cellulose (Arbocel 3800)	7.00	3.00	7.00	3.00	7.00	3.00	4.85	4.85
Vitamin and mineral mix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ground limestone	1.20	1.20	1.45	1.45	1.45	1.45	1.45	1.45
Mono calcium phosphate	1.95	1.95	2.40	2.40	2.40	2.40	2.40	2.40
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
$NaHCO_3$	0.35	0.35	0.40	0.40	0.40	0.40	0.40	0.40
$KHCO_3$	1.65	1.65	1.10	1.10	1.10	1.10	1.10	1.10
DI-Methionine	0.06	0.06	0.37	0.37	0.38	0.38	0.37	0.37
L-Threonine	-.-	-.-	0.14	0.14	0.16	0.16	0.14	0.14
L-Tryptophan	-.-	-.-	0.06	0.06	0.06	0.06	0.06	0.06
Cr_2O_3	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

The vitamin and mineral mixture supplied per kg diet: Retinol 2.7 mg, cholecalciferol 45 µg, DI-α-tocopherol 40 mg, menadione 3 mg, riboflavine 5 mg, nicotinic acid 30 mg, D-pantothenic acid 15 mg, choline chloride 120 mg, cyanocobalamin 40 µg, ascorbic acid 50 mg, $CuSO4.5H_20$ 20 mg, $ZnSO_4.H_2O$ 200 mg, MnO 70 mg, $FeSO_4.7H_2O$ 400 mg, $CoSO_4.7H_2O$ 2.5 mg, $Na_2SeO_3.5H_2O$ 0.2 mg, KI 0.5 mg.

Table 4 Chemical composition (%) of the diets

Ingredients	Experiment 1 I	II	III	IV	V	VI	Experiment 2 I	II
Analysed contents								
Crude protein	17.2	18.2	18.1	18.9	16.9	17.6	17.4	17.6
Glucose	16.3	10.6	16.9	10.3	17.23	13.2	-.-	-.-
Saccharose	< dl	1.5	< dl	1.3	< dl	0.8	-.-	-.-
Raffinose	< dl	1.1	< dl	1.1	< dl	1.3	-.-	-.-
Stachyose	< dl	0.3	< dl	0.3	< dl	0.2	-.-	-.-
Arabinose	< dl	1.7	< dl	1.4	< dl	1.8	-.-	-.-
Xylose	0.3	0.6	0.3	0.7	0.5	0.8	-.-	-.-
Lectins 1)	-.-	-.-	-.-	-.-	-.-	-.-	507	2732
TIA 2)	-.-	-.-	-.-	-.-	-.-	-.-	0.12	1.24
Calculated contents								
Net Energy (MJ/kg)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
ADF	6.9	6.9	6.9	6.9	6.9	7.5	-.-	-.-
NDF	6.9	7.7	6.9	7.7	6.9	8.3	-.-	-.-
Base excess (meq/100g)	20.4	20.4	19.7	19.7	19.6	19.6	19.6	19.6
Lysine	1.30	1.33	1.16	1.18	1.10	1.12	1.05	1.05
Methionine	0.56	0.57	0.53	0.53	0.53	0.54	0.53	0.53
Methionine + cystine	0.67	0.68	0.67	0.67	0.67	0.67	0.67	0.67
Threonine	0.71	0.73	0.71	0.72	0.71	0.72	0.73	0.73
Tryptophan	0.25	0.25	0.20	0.20	0.20	0.20	0.19	0.19

1) ELISA: µg lectins/ g product
2) TIA: mg inhibited trypsin per gram product
< dl: below detection level.

3 Animals and experimental procedure

The piglets in both experiments were of the crossbred Dutch Landrace x Dutch Yorkshire. The piglets arrived at the Institute at an age of 4 weeks. Their mean live weight on arrival was approximately 8 kg in both experiments. After an adaptation period of 6 days, the piglets were surgically fitted with a post-valve T cannula (PVTC) according to van Leeuwen et al. (1988). After surgery, the piglets were allowed to recover and adapt to the experimental diets for 19 days. Following this period, the ileal chyme was collected on 8 consecutive days, 12 hours per day, in experiment 1, and during 5 consecutive days, also 12 hours per day, in experiment 2. The collection period in experiment 2 was shorter than that of experiment 1, because only a limited amount of ANF concentrate was available. The chyme was collected in plastic bags attached to the cannula, and weighed every hour before being immediately stored in a freezer (-20°C). In experiment 1, Merthiolate dissolved in an alcohol solution was added into the plastic collection bags (1 ml in each bag) as a preservative to inhibit both bacteria and enzyme activity. In experiment 2, no Merthiolate was added to the chyme because also enzymactivity and other observations were carried out in the chyme (results not included in this paragraph). Before analysis, the total collected chyme was pooled per animal homogenized and sampled. The age of the piglets during the ileal chyme collection period, was about 8 to 9 weeks. Their mean live weight was about 15 kg. Originally, four piglets were assigned to each treatment group of experiment 1. Unfortunately, some piglets did not recover after surgery. Hence, treatment groups I to III consisted of 4 piglets each and treatment groups IV to VI of only 3 piglets each. In experiment 2, each experimental group consisted of 5 piglets. The piglets were individually housed in metabolism cages, which were placed in a room with artificial lighting. The room temperature was maintained between 22 and 25°C. In both experiments, the piglets were fed restrictedly at 2.6 times their maintenance energy requirement. Feed was administered twice daily. Water was freely available from nipple drinkers.

3.1 Chemical analyses

The procedures for analysing dry matter, nitrogen, crude fibre, starch, fat, raffinose, stachyose and verbascose have been described previously by Huisman et al. (1990b). The content of trypsin inhibitors were analysed according to the method described by van Oort et al. (1989). Lectins were analysed following the ELISA method described previously in Huisman et al. (1990b). Tannins were measured as catechins using the vanillin sulphuric method of Kuhla and Ebmeier (1981). For the NDF determination 5 gram of material was suspended in 20 ml water, boiled for 1 minute before adding 100 mg pancreatin. After 1 hour of incubation at 37°C, 80 ml NDF reagent (sodium lauryl sulphate in borate buffer of pH 7 containing EDTA) was added and the solution was again boiled for 1 hour. After filtration the residu was washed, dried at 130°C and heated at 550°C for 3 hours. The weight loss during heating gives the amount of NDF. For the ADF determination, 5 g sample was suspended in 10 ml water, 90 ml ADF reagent (cetyltrimethylammoniumbromide in diluted sulphuric acid) was added and the mixture was boiled for 1 hour. After washing and drying, the residue is the crude amount of ADF which was corrected for the ash content in this fraction to obtain the amount of ADF. Chromium analyses were

performed by hydrolysing the samples in a mixture of perchloric and nitric acids and measuring the Cr6 + by flame atomic absorption spectrophotometry.

3.2 Statistical analysis

The values for the various parameters in Tables 6 and 7 are given as means with their standard deviations. Analyses of variance were carried out according to procedures described by Steel and Torrie (1960). The differences between treatments are analysed using the Student's t-test. The values in Table 5 were analysed according to a two-way analysis of variance according to Snedecor and Cochran (1967).

IV RESULTS

There were no significant differences in apparent ileal protein digestibility between casein + fish and both pea protein isolates (Table 5). Inclusion of pea carbohydrates in the diets did not affect the apparent ileal protein digestibility (Table 5). Dry matter digestibility of the test diets with pea carbohydrates was significantly lower ($P < .05$) compared with the control diets containing no pea carbohydrates. This effect was observed with each protein source (Table 5). The results of the dry matter digestibility indicate that the ileal digestibility of the pea carbohydrates is lower than that of cornstarch + dextrose. The amounts of ileal chyme, excreted by the piglets fed the three diets containing pea carbohydrates, was significantly ($P < .05$) higher than those fed the control diets without pea carbohydrates (Figure 1). The dry matter content of ileal chyme of the piglets fed the pea carbohydrates, was significantly ($P < .05$) lower compared with the piglets fed the diets containing no pea carbohydrates (Table 5). Inclusion of the pea ANFs, ANF-concentrate, containing high levels of trypsin inhibitors and lectins in the diet caused a significantly ($P < .05$) reduced ileal protein digestibility (Table 6). Live weight gain was also reduced ($P < .05$, Table 6).

V DISCUSSION

Both pea protein isolates and casein + fish used in the present study and in the previous study (Huisman et al., 1990b) were from the same batches. The apparent ileal protein digestibilities of the three protein sources were similar in both studies. In a previous experiment (Huisman et al., 1990b), it was found that apparent ileal protein digestibility in raw peas was distinctly lower than in pea protein isolate from which ANFs and carbohydrates were removed. Therefore, tests were done to find out whether pea carbohydrates or pea ANFs were associated with the lower ileal protein digestibility. The addition of pea carbohydrates did not reduce the ileal protein digestibility (Table 5). A striking observation was that due to the inclusion of pea carbohydrates in the diets, the flow of wet ileal chyme was markedly increased (Figure 1). The increased chyme flow may be related to the fact that the pea carbohydrates were incompletely digested in the small intestine (Le Guen and

Table 5 Mean dry matter contents in ileal chyme and apparent ileal digestibility of dry matter and crude protein of the diets of experiment 1

	Protein source	Number of animals	Carbohydrate source	Dry matter content ileal chyme		Ileal digestibility Crude protein		Dry matter	
				VALUES PER TREATMENT					
				MEAN	SD	MEAN	SD	MEAN	SD
I,	C + F	4	C + D	13.56a	1.19	87.1a	2.1	86.1a	1.2
II,	C + F	4	C + D + Pea	11.10b	1.19	84.8a	2.3	82.7bcde	1.0
III,	FI Is.	4	C + D	12.05ab	0.43	88.2a	2.4	84.1ce	1.1
IV,	FI Is	3	C + D + Pea	10.86b	0.53	86.7a	1.7	80.8d	1.8
V,	FR Is	3	C + D	14.07a	1.95	85.8a	1.9	84.5ae	1.1
VI,	FR Is	3	C + D + Pea	13.23b	1.82	86.0a	1.9	81.9bcd	0.3
				EFFECT OF CARBOHYDRATE SOURCE; COMPOSITE DATA					
				MEAN	SEM	MEAN	SEM	MEAN	SEM
Diets I, III, and V.				13.15x	0.38	87.12x	0.63	84.93x	0.35
Diets II, IV, and VI				11.66y	0.40	85.73x	0.66	81.86y	0.37

Protein source: C + F = Casein + Fish; FI Is = FINALE protein isolate; FR Is = FRIJAUNE pea protein isolate.
Carbohydrate source: C + D = Corn + Dextrose.
a-e: means in the same column that do not have a common letter, differ significantly ($P < 0.05$).
x-y: means in the same column that do not have a common letter, differ significantly ($P < 0.02$).

Figure 1. WET CHYME FLOW (g/12 HOURS)

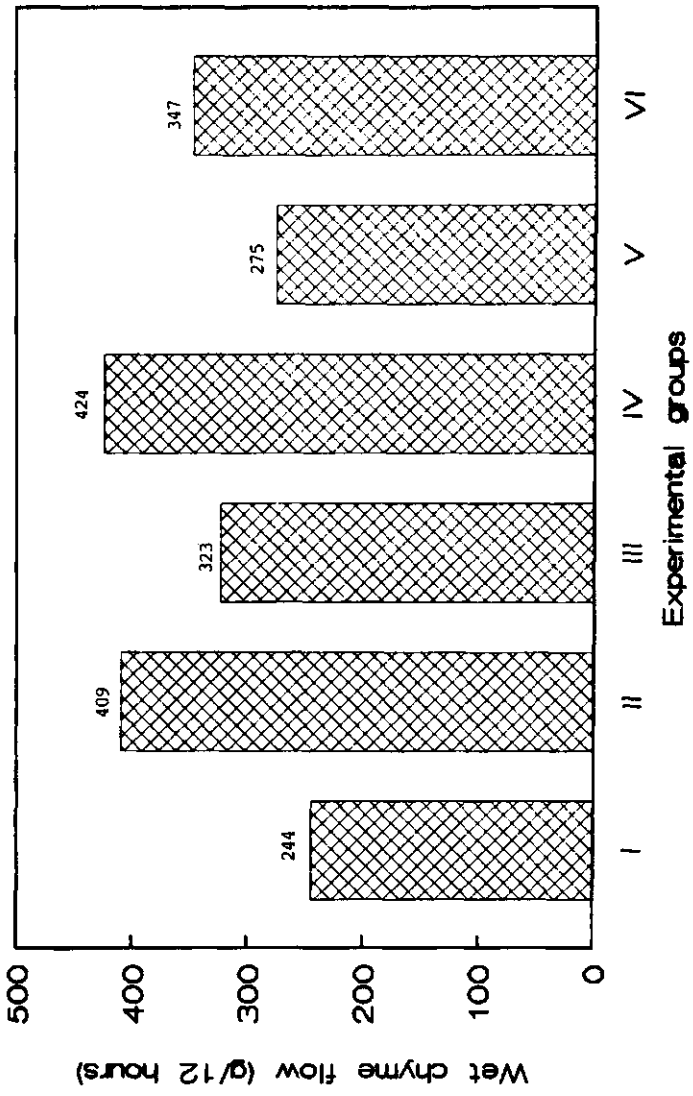


Table 6 Apparent ileal N digestibility, and live weight gain measured in experiment 2

Diet	Number of animals	Ileal N digestibility		Live weight gain	
		mean	SD	g/day	SD
PPI diet	5	86.0a	2.3	277a	29
PPI diet + ANFs	5	78.9b	2.8	229b	25

PPI = Pea protein isolate.
Means with superscripts that do not have a common letter in the same column, differ significantly ($P < 0.05$)

Huisman, 1990, unpublished results). As a result, more osmotic active components will be present in the chyme. The osmotically active carbohydrates in the chyme will cause an inflow of water into the intestinal lumen in order to maintain osmotic equilibrium between blood and lumen content. The results of the present study clearly demonstrate, that with the addition of the pea ANF-concentrate containing high levels of trypsin inhibitors and lectins to diets, the ileal protein digestibility is reduced (Table 6). This reduction of ileal apparent protein digestibility (7 units), was lower than found in the previous study with raw peas, where 14 units difference between raw peas and pea protein isolate were measured (Huisman et al., 1990b). These findings suggest that half the negative effect with raw peas on the apparent protein digestibility is caused by ANFs. The other 7 units difference must be attributed to other factors. The production of pea protein isolate is based on a precipitation at pH 4. This may have caused some changes in the protein structure. In a separate experiment, it was found that antibodies were formed in the blood of piglets due to feeding of the same batches of raw peas (Tolman and Huisman, (unpublished results). This finding shows that the raw pea protein is antigenic. Antigenicity of protein in pea flour is also described by Toullec and Guilloteau (1989). It could be that due to the processes applied in the production of pea protein isolate the structure was altered and the antigenicity of pea protein is decreased. As could be expected with a lower ileal protein digestibility, the daily weight gain in the piglets was significantly lower (17%) with the diet enriched with ANFs (Table 6). Suggestions that trypsin inhibitors in peas may play a role in reduced performance and digestibility have been made by Huisman (1989) and Leterme et al. (1989). In the study of Huisman (1989) the diet with peas containing the highest level of trypsin inhibitor showed lower growth and higher feed conversion efficiency than with peas containing low levels of trypsin inhibitors. Leterme et al. (1989) found with peas lower protein digestibility with higher trypsin inhibitor activity. However, no clear conclusions could be drawn, because raw peas contain also lectins. Grant et al. (1983) concluded from rat experiments, that pea lectins seem to be non-toxic. Bertrand et al. (1988) found, that purified pea lectins did not cause negative effects in

piglets. However, Jindal et al. (1982) found that with purified pea lectins, there was reduced brushborder enzyme activity in rats. Kik et al. (1990, personal communication), found changes in villus/crypt ratio and brushborder enzyme activity (alkaline phosphatase, aminopeptidase and sucrase) in the piglets of the study of Huisman et al. (1990a), which had been fed the raw pea diets. In other in-vitro studies by Kik et al. (personal communication), significant changes in the small intestinal mucosa (villus/crypt ratio and brushborder enzymes) were observed with purified pea lectins in piglets, kept under specific pathogen-free conditions. These results indicate, that pea lectins affect the intestinal wall and therefore, may also play a role in the negative effect on ileal digestibility. Summarizing the results, it can be concluded that ANFs are related to the low apparent ileal protein digestibility with raw peas and pea carbohydrates are not. However, the difference between apparent ileal protein digestibility of pea protein isolate and raw peas could only be partly be explained by ANFs. It can not be excluded that changes in protein structure upon isolation of the protein may also have played a role.

VI REFERENCES

Bengala-Freire, J.P., Hulin, J.C., Peiniau, J. and Aumaitre, A. (1989). Effet de la cuisson-extrusion du pois de printemps sur la digestibilité des aliments de sevrage precoce du porcelet et consequences sur les performances jusqu'a l'abattage. Journées Recherche Porcine en France, 21, 75-82.

Bertrand, G., Sève, B., Gallant, D.J. and Tomé, R. (1988). Absence d`effets antinutritionel des lectines de pois, sous forme native ou purifee chez porcelet. Sciences des Aliments, 8, 187-212.

Castaing, J. and Grosjean, F. (1985). Effet de forts pourcentages de pois de printemps, dans des régimes pour porcs charcutiers, à base de maïs ou d'orge et en complément de tourteau de colza. Journées Recherce Porcine en France, 17, 407-418.

Fekete, J., Castaing, J., Lavorel, O. Leuillet, M. and Quemere, P. (1984). Utilisation des pois protéagineux par le porcelet sevré. Journées Recherche Porcine en France, 16, 393-400.

Grant, G., More, L.J., McKenzie N.H., Stewart, J.C. and Pusztai, A. (1983). A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK. British Journal of Nutrition, 50, 207-214.

Grosjean, F. and Castaing, J. (1983). Recherche d'amélioration de la valeur alimentaire du pois d'hiver pour le porc charcutier. Journées Recherche Porcine en France, 15, 335-346.

Grosjean, F. and Gatel. (1986). Peas for Pigs. Pig News and Information, 7, 4 ,443-448.

Grosjean, F. and Gatel, F. (1989). Feeding value of Pisum sativum for pigs: -influence of technology, -influence of genotype (trypsin inhibitor activity). In: Recent advances of research in antinutritional factors in legume seeds. [J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. PUDOC, Wageningen, The Netherlands., 239-242.

Grosjean, F., Castaing, J. and Gatel, F. (1986). Utilisation comparée de différentes variétés de pois et d'une association pois de printemps-féverole par le porc charcutier. Journées Recherce Porcine en France, 18, 47-56.

Gueguen, J. (1983). Legume seeds protein extraction, processing and end product characteristics. Qualitas Plantarum. Plant Food for Human Nutrition, 32, 267-303.

Hamer, R.J., van Oort, G. and de Jager, M.A. (1990). A Functional Lectin Immunoassay (FLIA). Analytical Biochemistry. Submitted.

Huisman, J. (1989). Antinutritional factors (ANFs) in the nutrition of monogastric farm animals. In: Nutrition and digestive physiology in monogastric farm animals. [E.J. van Weerden and J. Huisman, Eds]. Pudoc, Wageningen, The Netherlands, 17-35.

Huisman, J., van der Poel, A.F.B., Kik, M.J.L. and Mouwen J.M.V.M. (1990a). Performance and organ weights of piglets, rats and chickens fed diets containing Pisum sativum. Journal of Animal Nutrition and Animal Physiology, 63, 273-279.

Huisman, J., Le Guen, M-P., Gueguen, J. Beelen, G. M. and van der Poel, A.F.B. (1990b). Apparent faecal and ileal digestibility of pea proteins in early-weaned piglets: comparison of raw peas and pea protein isolate. In preparation.

Jindal, S., Soni, G.L. and Singh, R. (1982). Effect of feeding of lectins from lentils and peas on the intestinal and hepatic enzymes of albino rats. Journal of Plant Foods, 4, 95-103.

Kuhla, S. and Ebmeier, C. (1981). Untersuchungen zum Tanningehalt in Ackerbohnen. Archiv für Tierernährung, 31, 573-588.

Leterme, P, Beckers, Y. and Thewis. (1989). Inter- and intravarietal variability of the trypsin inhibitors content of peas and his influence on apparent digestibility of crude proteins by growing pigs. In: Recent advances of research in antinutritional factors in legume seeds. [J.Huisman, A.F.B. van der Poel and I.E. Liener, Eds.]. Pudoc, Wageningen, The Netherlands, 121-124.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical methods, 7th edition, Iowa State University Press, Ames IA.

Steel, R.G. and Torrie, J.H. (1960). Principles and procedures of statistics: A biochemical approach (2nd Ed.). McGraw-Hill Book Co., New York.

Toullec, R. and Guilloteau, P. (1989). Research into the digestive physiology of the milk fed calf. In: Nutrition and digestive physiology in monogastric farm animals. [E.J. van Weerden and J. Huisman, editors). Pudoc, Wageningen, The Netherlands, 37-55.

Van Leeuwen, P., Huisman, J., Verstegen, M.W.A., Baak, M.J., van Kleef, D.J., van Weerden, E.J. and den Hartog, L.A. (1988). A new technique for collection of ileal chyme in pigs. Proceedings of the 4th International Seminar on Digestive Physiology in the pig, Jablonna, Poland, 289-296.

Van Oort, M.G., Hamer, R.J. and Slager, E.A. (1989). The trypsin inhibitor assay: Improvement of an existing method. In: Recent advances of research in antinutritional factors in legume seeds. [J.Huisman, A.F.B. van der Poel and I.E. Liener, Eds.]. Pudoc, Wageningen, The Netherlands, 110-113.

CHAPTER 4

TRUE PROTEIN DIGESTIBILITY AND AMOUNTS OF ENDOGENOUS PROTEIN MEASURED WITH THE ^{15}N DILUTION TECHNIQUE IN PIGLETS FED PEAS AND COMMON BEANS

J. Huisman[1], Th. Heinz[2], A.F.B. van der Poel[3], P. van Leeuwen[1], W.B. Souffrant[2] and M.W.A. Verstegen[3]

1) TNO-Institute of Animal Nutrition and Physiology (IGMB-Dept. ILOB), P.O. Box 15, 6700 AA Wageningen, The Netherlands.
2) Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik Forschungszentrum für Tierproduktion Dummerstorf-Rostock Bereich Tierernährung "Oskar Kellner", Justus-von-Liebig-Weg, Rostock 2500, DDR.
3) Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen.

Submitted for publication

I ABSTRACT

The true faecal and ileal digestibility of the raw pea varieties FINALE and FRIJAUNE and the true ileal protein digestibility of steam processed common beans (Phaseolus vulgaris) were measured in piglets using the ^{15}N dilution technique. The true faecal protein digestibility of both pea varieties was about 97%. The true ileal protein digestibility was between 93% and 95% respectively, indicating that the pea protein is almost completely enzymatically digested in the small intestine. The apparent faecal protein digestibility was 85% for both varieties and at ileal level 79% and 74%, respectively. The lower apparent ileal protein digestibility of peas can be attributed completely to the excretion of endogenous protein. The apparent ileal protein digestibility of toasted common beans was about zero (-4%). The true ileal protein digestibility was about 66. This indicates, that the protein of the common beans, although toasted, was highly resistent to enzymatic digestion. It was calculated that per 100 g ingested bean protein, 34 g undigested bean protein and 70 g endogenous protein passed the terminal ileum. The results of the present study explain why in previous experiments a strongly reduced weight gain and even weight loss was observed in piglets fed raw and toasted common beans.

II INTRODUCTION

Considerable differences in apparent ileal digestibility of protein (14 units) have been measured between raw peas (Pisum sativum) and pea protein isolate which contain low levels of antinutritional factors (ANFs) and no carbohydrates (Huisman et al., 1990a). With raw common beans (Phaseolus vulgaris) in diets it was observed that the weight gain was much lower than in control piglets (Huisman et al., 1990b,c). It has also been reported that the apparent faecal and ileal protein digestibility of raw as well as mildly toasted common beans was very low (Huisman et al., 1990c.; van der Poel en Huisman, 1988; van der Poel et al., 1990a). It is important to know which part of the low apparent faecal and ileal protein digestibility of these legume seeds is related to the excretion of endogenous protein. By correcting apparent digestibilities for endogenous protein, data of true digestibilities are obtained. No information was found in literature about the true digestibility of raw peas and common beans in piglets. In the present study, the true digestibility of protein of raw peas and common beans was measured in piglets using the ^{15}N dilution technique. With this technique the body protein, including the excreted endogenous protein, is labeled (Souffrant et al., 1986). With the aid of the labeled endogenous protein, a differentiation between excreted non-digested dietary and non-absorbed endogenous protein can be made. The objective of the present study was to determine the true digestibility of protein of two raw Pisum sativum varieties and of Phaseolus vulgaris beans.

III MATERIAL AND METHODS

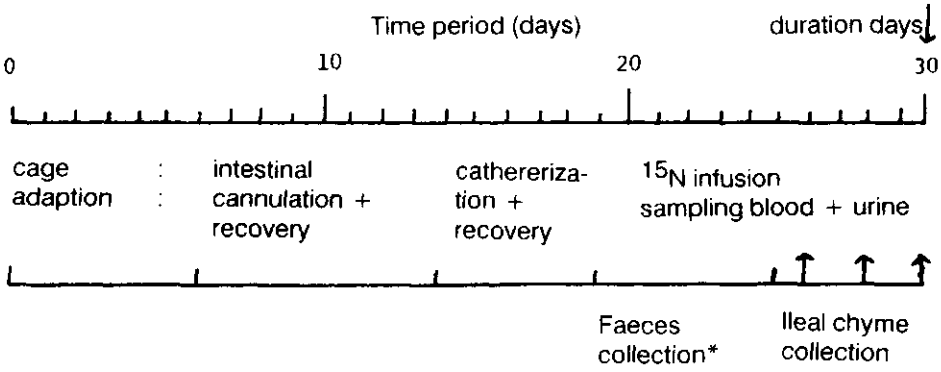
Two experiments were carried out. In experiment 1, two pea varieties were tested. In a separate experiment common beans were tested for apparent and true ileal protein digestibility. In both experiments the design, time schedule (see Figure 1) and body weight of the piglets were the same.

1 Animals and experimental procedure

Each treatment group comprised 3 piglets which were surgically fitted with a PVTC cannula in the caecum at an age of about 4 to 5 weeks (mean live weight between 7.5 and 8.5 kg), according to the method described by van Leeuwen et al. (1988). The size of the cannulas were adapted because the animals of the present experiment were smaller than in the experiment of van Leeuwen et al. (1988). After a period of seven days to allow for recovery from the intestinal cannulation, the piglets were provided with a catheter in the jugular external vein for the continuous infusion of the ^{15}N-L-Leucine solution and a catheter in the carotid artery for blood collection. Each experiment comprised the following consecutive periods:

- adaptation to individual housing in metabolism cages: 5-7 days
- intestinal cannulation and recovery: 7-9 days
- catheterization in blood vessels and recovery: 4-6 days
- infusion of ^{15}N-L-Leucine: 11 days (see Figure 1).

Figure 1 Experimental scheme



* cannulas closed: faeces collected for determination of apparent protein digestibility.

↑day 7, 9 and 11 after start infusion: ileal chyme collection.

Ileal chyme was collected for 24 hours at the days 7, 9 and 11 of the infusion period. The digesta samples were pooled per animal per day. The chyme was collected in small plastic bags attached to the PVTC cannula. Each hour the bags were controlled. When chyme was produced it was weighed and immidiately frozen until -20°C. In the first 6 days of the ^{15}N-infusion period the PVTC cannula was closed. Faeces were collected quantitatively per animal and per day for these 6 days starting at the first ^{15}N-L-Leucine infusion day. In the first 6 days of the ^{15}N-infusion period the PVTC cannula was closed. The apparent faecal digestibility is determined from the ingested feed and the excreted faeces of these 6 days. Blood samples (10 ml) were taken twice daily from the carotis catheter during the feeding at 8.00 h and 20.00 h. After centrifugation (2500 RPM/10 min.) the blood plasma was taken and weighed. Half of this volume was added to 20% trichloroacetic and centrifugated by 5000 RPM for 10 min. The supernatant (TCA-soluble fraction) and the precipitate were stored at -20 °C for further N and ^{15}N analyses. The continuous intravenous ^{15}N-L-Leucine infusion was performed at a rate of approximately 40mg ^{15}N-L-Leucine (95% ^{15}N enrichment) per kg body weight per day with infusion pumps (Perfusor R Dauerinfusionsgeraet, Braun, Melsungen). The ^{15}N L-Leucine was dissolved in a sterile nonpyrogene physiological NaCl solution (0.9% NaC1). About 100 ml of this solution was daily infused in each animal. After N analyses in the TCA soluble fractions of blood plasma and the chyme and faeces samples the ^{15}N analyses were carried out in the remaining ammonium chloride solutions after Kjeldahl N determination. These solutions were evaporated and justified on a N concentration of 300 to 500 µg per ml. These solutions were introduced into emission spectrometers (Isonitromat RFT 5201 or NOI-6 of VEB Statron Fuerstenwalde, GDR.) for ^{15}N analyses. The contribution of endogenous to total N in ileal chyme or faeces could be calculated from the ratio of ^{15}N enrichment excess in ileal chyme or faeces and in the blood TCA soluble fraction, assuming that the 15N excess in the endogenous N and in the blood TCA soluble fraction is similar. The calculations are carried out according to Souffrant et al. (1986) using the following formula:

$$Nen = Ntot \times \frac{Nex\ c/f}{Nex\ bl}$$

where Nen = endogenous N in ileal chyme or faeces (g/day), Ntot = total N in ileal chyme or faeces (g/day), Nex c/f = ^{15}N excess in ileal chyme or faeces, and Nex bl = ^{15}N excess in the TCA-soluble fraction of the blood.

The true protein digestibilities then were calculated from the apparent ileal or faecal protein digestibilities by correcting for the share of endogenous protein. For calculation of the true ileal digestibility the measured ^{15}N excess in the chyme samples of the 12 hours collections at the 7th, 9th and 11th day of infusion and the corresponding ^{15}N excess in the TCA soluble fraction of blood plasma were used for correction of the apparent ileal digestibility of protein. For calculation of true faecal digestibility the measured ^{15}N excess in the faeces samples from the infusion days 7 to 11 and the corresponding ^{15}N excess in the TCA soluble fraction of bloodplasma were used for correction of the apparent to true faecal protein digestibility. Thus the apparent faecal protein digestibility was measured during the days 1 to 6 of the infusion period. The corrections for the share of endogenous protein were based on the analysed ratio of ^{15}N to total N in faeces samples collected in the period of 7 to 11

days of infusion. The piglets were housed individually in metabolism cages. Room temperature was maintained at about 25°C.

2 Diets

Two pea varieties were used, the spring variety FINALE with a relatively low trypsin inhibitor activity, and the winter variety FRIJAUNE with a relatively high trypsin inhibitor activity (Table 1). The main ANFs in the peas of the present study are trypsin inhibitors and lectins. Two pea diets were formulated, each with raw peas as the sole protein source, comprising either FINALE peas (low in trypsin inhibitor content) or FRIJAUNE peas (high in trypsin inhibitor content). To avoid too high levels of pea carbohydrates, which could cause diarrhoea (Saini, 1989), some of the peas were air-classified. Air classification removed some part of the carbohydrates. The used remaining fine fraction contained protein levels of about 50%. The peas were from the same batches as those studied in Huisman et al. (1990a). The chemical composition of the pea sources are given in Table 1.

Table 1 Chemical composition (% as crude product) of the peas sources (varieties FINALE and FRIJAUNE) and toasted common beans

	FINALE		FRIJAUNE		Phaseolus vulgaris
	raw pea	air-classified pea	raw pea	air-classified pea	toasted beans
Dry matter	87.1	92.0	87.1	91.4	92.6
Crude protein	23.7	55.2	21.9	53.4	25.2
Crude fat	1.6	3.2	0.9	3.4	1.7
Ash	2.9	5.6	3.1	5.7	4.4
Crude fibre	6.2	2.4	7.1	3.2	5.5
Nitrogen Free Extract	52.7	25.6	54.7	25.7	55.8
Starch (Ewers)	41.8	8.4	40.2	7.6	nd
Tannins a)	<.1	<.1	<.1	<.1	<.1
Lectins b)	3536	12310	3657	15148	8130
TIA c)	1.19	2.24	5.44	2.10	1.03

a) % catechins measured with the vanillin sulphuric acid method
b) ELISA; µg/g diet.
c) mg inhibited trypsin per g product
nd = not determined.

Table 2 Percentage and chemical composition (%) of the diets

	Finale diet	Frijaune diet	Soya isolate diet	Phaseolus diet	Soya isolate + Phaseolus diet
Raw pea	25.0	25.0	-	-	-
Air-classified pea	18.6	17.85	-	-	-
Soya isolate	-	-	18.2	-	10.9
Phaseolus, toasted	-	-	-	21.7	8.7
Phaseolus air classified, toasted	-	-	-	17.7	7.1
Corn starch	30.0	30.7	52.8	24.9	41.6
Dextrose	15.0	15.0	15.0	26.1	19.4
Sunflower oil	2.0	2.0	2.0	1.7	1.9
Cellulose	3.0	3.0	5.0	3.0	4.2
Vitamin/mineral mixture*	1.0	1.0	1.0	1.0	1.0
Iodized NaCl	0.5	0.5	0.5	0.4	0.5
NaHCO3	0.4	0.4	-	0.2	0.1
KHCO3	0.5	0.5	1.5	0.1	0.9
Monocalciumphosphate	2.0	2.0	2.2	1.5	1.9
Ground limestone	1.5	1.5	1.4	1.3	1.4
Dl-methionine	0.28	0.29	0.17	0.26	0.20
L-lysine-HC1	-	-	0.10	-	0.04
L-threonine	0.06	0.06	0.04	-	0.02
L-tryptophan	0.05	0.06	-	0.03	0.01
Chromic oxyde	0.1	0.1	0.1	0.1	0.1
Analysed contents					
Dry matter	87.34	90.36	90.65	88.93	89.96
Ash	5.2	5.7	5.0	4.6	4.8
Crude protein	16.2	16.2	15.9	14.1	15.2
Crude fat	2.9	2.9	1.7	2.8	2.1
Crude fibre	4.0	4.6	3.8	3.8	3.8
Tannins a)	<.1	<.1	n.d.	<.1	<.1
Lectins b) Pea	2301	1915	n.d.	n.d.	n.d.
Soya	n.d.	n.d.	0.23	n.d.	0.47
Phaseolus	n.d.	n.d.	< 1	8400**	3380
TIA c)	0.69	1.89	0.79	2.04	1.12

a) % catechins measured with the vanillin sulphuric acid method
b) µg lectins/g diet
c) mg inhibited trypsin per g product
n.d. = not determined.

* The vitamin and mineral mixture supplied per kg feed:
Retinol 2.7 mg, cholecalciferol 45 µg, DL-α-tocopherol 40 mg, menadione 3 mg, riboflavine 5 mg, nicotinic acid 30 mg, D-pantothenic acid 15 mg, choline chloride 120 mg, cyanocobalamin 40 µg, ascorbic acid 50 mg, $CuSO_4.5H_2O$ 20 mg, $ZnSO_4.H_2O$ 200 mg, MnO 70 mg, $FeSO_4.7H_2O$ 400 mg, $CoSO_4.7H_2O$ 2.5 mg, $Na_2SeO_3.5H_2O$ 0.2 mg, KI 0.5 mg.

** Analysed lectin content distinctly higher than the calculated level.
Possibly was the lectin activity concentrated in the airclassified fraction included in this diet.

The percentage and chemical composition of the diets are given in Table 2. Each pea diet was fed to 3 piglets. The common beans of the present study were toasted for 40 minutes according to the procedure described by van der Poel et al. (1990b). The reason for toasting was that in previous experiments (Huisman et al., 1990b,c) and in a pre-test it was observed that with raw common beans feed intake is markedly reduced in piglets. To improve the feed intake, the beans were toasted. Using these toasted beans as the sole dietary protein source (air classified fractions from toasted beans were included), feed intake was still reduced. Therefore, the toasted common bean diet was mixed with a diet containing soya isolate as the only protein source, in the ratio of 40 : 60. In this mixed diet about 9.6 % protein originates from soya isolate and about 6.3 % from the toasted common beans. The mixed diet and the soya isolate diet were tested for apparent and true ileal protein digestibility. The true digestibility of the common beans and the amount of endogenous protein in the digesta with these beans were calculated by difference, assuming that the soya isolate protein in the mixed diet had the same digestibility coefficient as in the diet with soya isolate as the sole protein source. The chemical composition of the toasted beans is given in Table 1. Chromic oxide was included in the diets as a marker to determine recovery of protein in ileal chyme and in digesta and faeces. The diets were pelleted without steam, the temperature of the pellets during the pelleting process did not exceed 55°C. The size of the pellets was 3 mm.

3 Feeding

The piglets were fed twice daily at 08.00 h. and 20.00 h. Twelve hours before surgery, feed was withheld. After surgery, the amount of feed was gradually increased until, after 5-6 days, a level of 380 g/day was reached. The piglets were maintained at this feeding level during the whole experiment.

3.1 Chemical analysis

The procedures for analysing dry matter, ash, nitrogen, fat, raffinose, stachyose and verbascose have been reported previously by Huisman et al. (1990a). Glucose, xylose and sacharose contents were analysed by gas liquid chromatography according to Sweeley et al. (1963). The content of trypsin inhibitors was analysed according to the method described by van Oort et al. (1989). Lectins were analysed according to the ELISA method reported previously by Huisman et al. (1990a). For analysis of the lectins in each legume seed specific anti-lectin IgG were used: for Phaseolus vulgaris: anti-Phaseolus lectin IgG and anti-Phaseolus lectin IgG-peroxidase (PO)- conjugate; for peas anti-pea-lectin IgG and anti-pea lectin IgG-PO-conjugate and for soya anti-soya-lectin IgG and anti-soya-IgG-PO-conjugate. Tannins were measured as catechins using the vanillin sulphuric method of Kuhla and Ebmeier (1981). The content of crude fibre was determined according to NEN standard 3326. The starch content in the sample was determined according to the NEN standard 3572. The ^{15}N analyses were carried out according to the procedures described by Souffrant et al. (1986).

3.2 Statistical analysis

Values for the various criteria are given as means with their standard deviations. The differences between treatments were statistically analysed according to Rasch et al. (1978).

IV RESULTS

The results of apparent and true ileal and faecal protein digestibilities of FINALE and FRIJAUNE peas are summarized in Table 3. The results of ileal apparent and true protein digestibility of common beans are given in Table 5.

Table 3 Apparent and true faecal and ileal protein digestibilities of FINALE and FRIJAUNE peas

	FINALE mean	SD	FRIJAUNE mean	SD
Apparent ileal	$79.0^{a,w}$	1.4	$74.1^{b,w}$	0.7
Apparent faecal	$85.1^{a,x}$	2.6	$84.9^{a,x}$	1.4
True ileal	$95.1^{a,y}$	1.0	$92.9^{a,y}$	1.2
True faecal	$96.6^{a,y}$	0.2	$96.5^{a,z}$	0.8

a,b : Values in the same row with different letter, differ significantly ($P < 0.05$)
w,x,y,z : Values in the same column with different letter, differ significantly ($P < 0.05$).

Table 4 Secretion of endogenous protein in ileal chyme and faeces from piglets fed pea diets

	FINALE diet mean	SD	FRIJAUNE diet mean	SD
	g/100 g dry matter intake			
Ileal chyme	3.06^{a}	0.10	3.36^{a}	0.24
Faeces	2.23^{b}	0.52	2.06^{b}	0.22
	g/100 g protein intake			
Ileal chyme	16.6^{x}	0.5	18.8^{x}	1.3
Faeces	12.0^{y}	2.8	11.5^{y}	1.2

The differences between FINALE and FRIJAUNE were not significant.
a,b and x,y: data in the same column with different letter differ significantly ($P < 0.05$).

Table 5 Apparent and true ileal protein digestibilities of Phaseolus vulgaris and excretion of endogenous protein at the distal ileum

	Ileal digestibility	
	mean	SD
Apparent	-3.9[a]	33.3
True	65.8[b]	11.3
	Endogenous secretion	
g/ 100 g dry matter intake	10.7	6.8
g/ 100 g protein intake	67.6	42.6

a,b: Values in the same column with different letter, differ significantly ($P < 0.05$).

Apparent digestibility. The apparent ileal protein digestibility of FINALE was about 5 units ($P < 0.05$) higher than for FRIJAUNE. The apparent faecal protein digestibilities of both pea varieties were almost the same. Apparent faecal protein digestibility was higher ($P < 0.05$) than apparent ileal digestibility. The apparent ileal protein digestibilities for FINALE and FRIJAUNE were 79 and 74%, respectively, the faecal values were 85% for both varieties (Table 3). The apparent ileal protein digestibility of common beans was about zero (-4%) with an extremely high standard deviation (Table 5).

True digestibility. True ileal protein digestibility of FINALE was significantly ($P < 0.05$) higher than for FRIJAUNE (95.1 vs 92.9%). True faecal protein digestibility was identical for the two pea varieties (96.6% for FINALE and 96.5% for FRIJAUNE). True ileal protein digestibility for both varieties was significantly ($P < 0.05$) higher than apparent ileal digestibility, the differences were about 16 units for FINALE and about 19 units for FRIJAUNE. The true faecal protein digestibility was also significantly ($P < 0.05$) higher than apparent faecal digestibility, the differences were about 12 units for both varieties. True ileal digestibility of common beans was significantly ($P < 0.05$) higher than apparent digestibility, the values were 66% and -4%, respectively. The standard deviation was very high, which is often observed with low digestibilities.

Endogenous protein. Data concerning the excretion of endogenous protein obtained with peas are given in Table 4. The amounts of excreted endogenous protein from the distal ileum were significantly ($P < 0.05$) higher than in the faeces. There were no significant differences in endogenous secretion between FINALE and FRIJAUNE peas. In Table 5 the data on endogenous secretion from the distal ileum with toasted common beans are given. Excretion of endogenous protein with common beans was considerably higher compared to those obtained with peas (Tables 3 and 4).

V DISCUSSION

The apparent ileal protein digestibilities of the present experiment were 7 units (FINALE) and 2 units (FRIJAUNE) higher than observed in the previous experiment

(Huisman et al., 1990a). This difference could possibly be related to the differences in feed regime, 2.6 and 2.0 times maintenance for energy in the previous and the present experiment, respectively. Kesting and Bolduan (1989) demonstrated with pigs of 70 to 80 kg live weight, that the apparent ileal digestibility gradually decreased by gradually increasing levels of feed intake. The apparent faecal digestibility of protein of the two pea varieties (about 85%, for both varieties) differend only slightly from that measured in the previous experiment (89 and 86%, respectively) with the same batches of peas (Huisman et al., 1990a).

True and apparent digestibility of peas. The true ileal and faecal digestibilities of protein were distinctly higher than the apparent ileal and faecal digestibilities. The differences between the apparent and true digestibilities are related to the secretion of endogenous protein. At the ileal level, these differences were 16 and 19 units, respectively, and at faecal level 12 units, for FINALE and FRIJAUNE peas, respectively. The high true faecal digestibilities of 96% for both FINALE and FRIJAUNE, indicates that the protein of raw peas is almost completely digested. At ileal level the true digestibilities were 95 and 93%, respectively, for FINALE and FRIJAUNE. These high values show that pea protein is almost completely enzymatically digested in the small intestine. The apparent and true ileal protein digestibilities for FRIJAUNE were significantly (5 and 2 units, respectively) lower compared with FINALE. The lower true protein digestibility of FRIJAUNE indicates that the protein of FRIJAUNE could be slightly more resistent to digestive enzymes than the protein from FINALE.

True and apparent digestibility of common beans. The apparent ileal protein digestibility of the toasted common beans was about zero (-4%). This low digestibility is in accordance with those obtained by van der Poel and Huisman, 1988 and van der Poel et al. (1990c). The true ileal protein digestibility was about 66. Related to 100 g of ingested bean protein, 34 g undigested bean protein and 70 g [4 + (100- 34)] endogenous protein passed the distal ileum. This indicates that the major part of the protein which passed the distal ileum was endogenous. The part of the protein digested in the large intestine does not benefit the animal and is in fact lost protein (Zebrowska, 1973, Zebrowska et al., 1975; Wünsche et al., 1982). The considerable loss of endogenous protein explains the weight losses in piglets observed in the studies of Huisman et al. (1990b,c) when raw Phaseolus vulgaris was fed.

Secretion of endogenous protein. The amounts of endogenous protein excreted with chyme and faeces with both pea varieties were similar (Table 4). The amount of endogenous protein excreted from the distal ileum was between 3.1 and 3.4 g/100g dry matter intake. With the faeces these levels were 2.2 and 2.1 g/100g dry matter intake, respectively. Thus, the net disappearance rate of endogenous protein in the large intestine with these peas is a quarter or more of the amount of endogenous protein flowing from the distal ileum into the large intestine. With the toasted common beans, the ileal excretion of endogenous protein was 10.7 g/100 g dry matter intake. Obviously, with the toasted common beans in present study, the amount of excreted endogenous protein was more than three times higher than with peas. The amount of ileal endogenous protein with peas is about wice the mean measured with protein-free diets (1.4 g/100 g dry matter intake) as summarized by Wünsche et al. (1987) from 18 publications. With the toasted beans, the ileal endogenous secretion was

about 8 times higher than that reported by Wünsche et al. (1987). Also, Santoro et al. (1989) found in rats that the amount of endogenous protein measured with protein-free diets, was inadequate for determining the true protein digestibility of the glycoprotein II (phaseolin, GII) protein fraction of Phaseolus vulgaris. The amount of endogenous protein in faeces with the pea diets was in present study was also higher compared with the mean figure of 15 publications summarized by Wünsche et al. (1987). In our study, the amount of excreted faecal endogenous protein with peas was between 2.2 and 2.1 g/100 g dry matter intake respectively, while the mean value with protein-free diets in the reported by Wünsche et al. (1987) was 0.85 g/100 g dry matter. With protein-free diets the excretion of bile, pancreatic enzymes, brushborder enzymes and mucin protein may be less stimulated than with normal diets containing protein, ANFs, non starch polysaccharides and crude fibre. In conclusion, the results of the present study have clearly demonstrated that the protein from raw peas is almost completely digested in the small intestine. Therefore, the low apparent ileal digestibility of peas can be attributed to the secretion of endogenous protein. Although the common beans were toasted, the true digestibility was only about 66%. This indicates that the protein from this batch of toasted common beans is highly resistant to enzymatic digestion. The reason for the very high secretion of endogenous protein with common beans is not exactly clear. The residual activity of lectins and trypsin inhibitors in the toasted beans (Table 1) may have stimulated the endogenous protein secretion. On the other hand, Santoro et al. (1989) discussed the hypothesis that the GII protein fraction may also have a stimulative effect on the secretion of endogenous protein in the small intestine.

VI REFERENCES

Huisman, J., Le Guen, M-P., Gueguen, J. Beelen, G.M. and van der Poel, A.F.B., (1990a). Apparent faecal and ileal digestibility of pea proteins in early-weaned piglets: comparison of raw peas and pea protein isolate. Submitted.

Huisman, J., van der Poel, A.F.B., Verstegen, M.W.A. and van Leeuwen, P. (1990b). Comparison of zootechnical characteristics in piglets and rats fed diets containing Phaseolus vulgaris. British Journal of Nutrition, In press.

Huisman, J., van der Poel, A.F.B., Mouwen, J.M.V.M., and van Weerden, E.J. (1990c). Effect of variable protein contents in diets containing Phaseolus vulgaris beans on performance, organ weights and blood parameters in piglets, rats and chickens. British Journal of Nutrition, In press.

Kuhla, S. and Ebmeier, C. (1981). Untersuchungen zum Tanningehalt in Ackerbohnen. Archiv für Tierernährung, 31, 573-588.

Kesting, U. and Bolduan, G. (1989). Methodische Einflüsse bei der präzäkalen Verdaulichkeitsbestimmung. Archives of Animal Nutrition, 39, 10, 823-831.

NEN standards of the Netherlands Normalization Institute: Nr. 3326: Determination of the crude fibre content by the abbreviated method in feedstuffs (1966). Nr. 3572. Polarimetric determination of the starch content in feedstuffs. Ewers method (1985).

Rasch, D., Herrendörfer, G., Bock, J. and Busch, K. (1978). Verfahrensbibliothek Versuchsplanung und -auswertung. VEB Deutscher Landwirtschaftsverlag, Berlin.

Saini, H.S. (1990). Legume seed oligosaccharides. In: Recent advances of research in antinutritional factors in legume seeds. [J. Huisman, A.F.B. van der Poel and I.E. Liener, editors]. Pudoc, Wageningen, The Netherlands, 329-341.

Santoro, L.G., Grant, G. and Pusztai, A. (1989). Degradation of glycoprotein II (Phaseolin), the major storage protein of Phaseolus vulgaris seeds. In: Recent advances of research in

antinutritional factors in legume seeds. [J.Huisman, A.F.B. van der Poel and I.E. Liener, editors]. Pudoc, Wageningen, The Netherlands, 363-367.

Souffrant, W.B., Darcy-Vrillon, B, Corring, T., Laplace, J.P., Köhler, R., Gebhardt, G. and Rerat, A. (1986). Reclycing of endogenous nitrogen in the pig. Archiv für Tierernährung, 36, 269 - 274.

Sweeley, C.C., Bentley, R., Makita M. and Wells W.W. (1963). Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. Journal of American Chemical Society 85, 2497-2507.

Van Leeuwen, P., Huisman, J., Verstegen, M.W.A., Baak, M.J., van Kleef, D.J., van Weerden, E.J. and den Hartog, L.A. (1988). A new technique for collection of ileal chyme in pigs. Proceedings of the 4th International Seminar on Digestive Physiology in the pig, Jablonna, Poland, 289-296.

Van der Poel, A.F.B. and Huisman, J. (1988). Effect of steam treatment of a dry bean (Phaseolus vulgaris) with extreme high lectin content on ileal digestibility in pigs. Proceedings of the 4th International Seminar on Digestive Physiology in the pig. Jablonna, Poland, 297-301.

Van der Poel, A.F.B., Mollee, P.W., Huisman, J. and Liener, I.E. (1990). Variations among Species of Animals in Response to the Feeding of Heat Processed Beans (Phaseolus vulgaris). 1. Bean Processing and Effects on Growth, Digestibility and Organ Weights in Piglets. Livestock Production Science, 25, 121 - 135.

Van der Poel, A.F.B. Blonk, J., van Zuilichem, D.J. and van Oort, M.G. (1990b). Thermal inactivation of lectins and trypsin inhibitors activity during steam processing of dry beans (Phaseolus vulgaris L) and effects on protein quality. Journal of the Sience of Food and Agricultural, In press.

Van der Poel, A.F.B., Blonk, J., Huisman, J. and den Hartog, L.A. (1990c). Effect of steam processing temperature and time on the protein nutritional value of Phaseolus vulgaris beans for swine. Livestock Production Science, Accepted for publication.

Van Oort, M.G., Hamer, R.J. and Slager, E.A. (1989). The trypsin inhibitor assay: improvement of an existing method. In: Recent advances of research in antinutritional factors in legume seeds. [J. Huisman, A.F.B van der Poel and I.E. Liener, editors]. Pudoc, Wageningen, The Netherlands, 110-113.

Wünsche, I., Hennig, U., Meinl, K., Kreienbring, F. and Bock, H.D. (1982). Untersuchungen über Resorption und Verwertung von in Zäkum wachsender Schweine infundierten Aminosauren. Archiv für Tierernährung, 32, 337-348.

Wünsche, J., Herrmann, U., Meinl, M., Hennig, U., Kreienbring, F. und Zwierz, P. (1987). Einfluss exogener Faktoren auf die präzäkale Närhstoff- und Aminosäurenresorption, ermittelt an Schweinen mit Ileo-Rektal-Anastomosen. Archives of Animal Nutrition, 37, 9, 745-764.

Zebrowska, T. (1973). The apparent digestibility of nitrogen and individual amino acids in the large intestine of the pig. Roczniki Nauk Rolniczych, Seria B. Zootechniczna, 97, 117-123.

Zebrowska, T., Buraczewska, L. and Buraczewski, S. (1975). The apparent digestibility of amino acids in the small intestine and the whole digestive tract of pigs fed diets containing different sources of protein. Roczniki Nauk Rolniczych, Seria B. Zootechniczna, 99, 87-98.

Introduction

The literature review (Chapter 1) reveals that ANFs often protect plants and seeds against predators such as insects and microorganisms. The protecting effect of ANFs seems to be related to disturbances in digestive processes of these predators. On the basis of a similarity in the metabolic pathways it was assumed that the metabolic processes in monogastric animals would be disturbed in a similar way. The literature study focussed on the mode of action and on some analytical aspects of ANFs present in peas (Pisum sativum), common beans (Phaseolus vulgaris) and soybeans (Glycine Max). It was shown that trypsin inhibitors and lectins are the main ANFs in these seeds. The mode of action of these ANFs is described in the literature review. The primary effect of trypsin inhibitors is associated with the inhibition of (chymo)trypsin in the intestine. Due to this inhibition the activity of these enzymes is reduced. As a result of the lower trypsin activity in the intestinal chyme, the pancreas is stimulated, regulated by a negative feed back mechanism, to produce more (chymo)trypsin. Due to the decreased activity of digestive enzymes and increased production of pancreatic enzymes which lead to an increased loss of endogenous protein, the apparent protein digestibility is decreased. Lectins can bind to the surface of the intestinal epithelial cells which may result in an increased production of mucins and damage of the small intestinal mucosa. Overall, digestion and absorption processes are disturbed. It became clear that ANFs in legume seeds mainly decrease the protein digestibility in animals. Thus, by eliminating ANF activity, an increase in protein digestibility can be expected and associated with this increased protein digestibility, a reduced secretion of N into the environment will be achieved. The literature review demonstrates that there are still many points regarding the antinutritional effects of ANFs in monogastric farm animals which are not clear. Some are studied in this thesis. The following three topics were studied in this thesis:

- Animal species differences between piglets, rats and chickens in response to ANFs in common beans and peas.
- Digestive responses of piglets to isolated fractions from peas.
- The true digestibility in the piglet of protein in raw peas and common beans and amounts of endogenous protein measured with the ^{15}N technique in piglets fed raw peas and toasted common beans.

The experiments carried out in relation to these three topics are discussed in this chapter. In some of the studies indications were found of a relationship between activity of the pancreas and the digestibility of protein. This observation, together with data from the literature is discussed in the last paragraph of this chapter.

Research on animal species difference

The mode of action of ANFs in animals and also possibilities for reducing ANF activity by (bio)technological treatments, have mainly been investigated in small animal species such as rats, chickens and mice (Chapter 1). The choice of small animals is often related to cost and the large amounts of isolated ANF needed in studies using larger animal species. In some reports, however, it has been suggested that there are

differences in response to the feeding of raw soybeans between rats and pigs (Combs et al., 1967; Yen et al., 1977) and to the feeding of chick peas (Visitpanich et al., 1985). Information about animal species differences is too scarce to allow any conclusion to be drawn as to whether rats or chickens could be used as an alternative for piglets in studies with peas and common beans. Therefore, experiments were designed to investigate animal species differences with special regard to common beans and peas. The results of studies with common beans in different animal species (Paragraph 2.1 and 2.2) clearly show that feed intake and weight gain were markedly reduced in piglets and only slightly reduced in rats. The piglets lost weight with raw common beans in both experiments. In the following experiment (Paragraph 2.2) in which higher digestible protein-rich ingredients were included in the diets, no negative effect on weight gain in rats was found with raw common beans. However, the piglets again lost weight. These results clearly indicate that with extra protein in the diet the reduction in performance in rats could be precluded, but not in piglets. The performance of piglets is affected much more than that of rats. The apparent faecal digestibility of the diets with common beans was reduced compared with the control diet in both species. This reduction was clearly more severe in piglets than in rats, indicating that the digestion processes in piglets are more disturbed than in rats. Evidence of a disturbed digestion process was found in an experiment using growing pigs with live weights of between 30 and 40 kg (Van der Poel and Huisman, 1988). In this experiment, the ileal digestibility of three diets including 20% toasted common beans was determined. The digestibility of the common beans was calculated from the difference in digestibility between the control diet and the test diets consisting of 80% control diet + 20% toasted beans. In this calculation it was assumed that the part of the control diet used in the test diet had the same ileal digestibility as measured for the control diet. The results are summarized in Table 1.

Table 1 Apparent ileal digestibility (%) of toasted common beans

Criterion	Beans, 20 min. toasted		Beans, 40 min. toasted		Beans, 80 min. toasted	
	mean	SD	mean	SD	mean	SD
Dry matter	2.0a	37.4	32.6ab	14.9	46.3b	6.6
Crude protein	-36.1a	46.7	8.3ab	23.9	37.3b	18.5

a-b: Means in the same row with different superscript differ significantly ($P < 0.05$)

The apparent ileal protein digestibility of the common beans toasted for 20 minutes was negative and those of the beans which were toasted for 40 and 80 minutes were 8% and 37%, respectively. Apparently, the beans had not been toasted sufficiently to eliminate negative factors. The negative protein digestibility can only be explained by a hypersecretion of endogenous protein. The results show that with common beans the digestion and absorption processes of protein is disturbed in the small intestine of piglets. With raw common beans even more disturbances in the small intestinal digestion process can be expected. Whether the reduced weight gain in piglets due

to common beans could be eliminated by including extra highly digestible protein in the diet was also investigated (Paragraph 2.2). This was done by formulating one test diet to which the common beans were added with a similar amount of non-bean protein (mainly casein and fish) as in the control diet. The addition of common beans was achieved by replacing cornstarch for these beans. The weight gain with the common bean diet containing extra highly digestible protein was similar when compared with the common bean diet without extra protein. Thus, the reduced weight gain with raw common beans could not be related to the low digestibility of the bean protein it self. It must be related to other toxic factors. Kik et al. (personal communication) found severe damage of the intestinal mucosa in the same piglets fed the common bean diets. It seems that the digestion and absorption processes were so severely disturbed that the extra protein in the test diets could not benefit the piglet.

For a full evaluation of the differential sensitivity of animal species the effect on age and live weight needs to be determined. It is possible that a difference in physiological age is associated with a difference in sensitivity between rats and piglets. The piglets used in our studies were 4 to 7 weeks old and the rats were 5 to 8 weeks old. The physiological age of rats of a certain age will be different to that of pigs of the same age. Therefore, effects of the inclusion of raw beans on weight gain were also tested in pigs of other ages: 8, 12 and 16 weeks, respectively (Paragraph 2.1). At all three ages, the pigs lost weight when fed raw common beans. These results show that in the age periods tested, the differences in response between young rats and piglets cannot be explained by a difference in physiological age. Grant et al. (1985) studied the effects of common beans at different ages in rats. They found no effect of age when the rats were fed beans for a period varying from 39 to 123 days. In a further experiment (Paragraph 2.2) a comparison with chickens was included. In chickens, only small effects on weight gain were observed when raw common beans were fed. The effects were similar to those obtained with rats. For comparison of effects between animal species it is important that the design is monofactorial. The diets fed in the experiments were therefore, designed so that the only different factor was the inclusion of the beans. By doing this, factors such as feeding level and protein content were not optimal for each animal species. The feeding level for the three animal species was based on a similar low level related to metabolic weight. Especially for chickens was this feeding level low. It may be possible that with higher feeding levels somewhat more negative effects in rats and chickens would be observed. As discussed in Paragraph 2.1, the fact that in our rat experiments there were less marked negative effects than are often reported in the literature, could possibly be related to the proportion of bean protein in comparison with the total protein in the diet. Although these factors may have influenced the negative effects with rats and chickens, the results of our experiments clearly show that the piglet is more sensitive to common beans than either rats or chickens. With peas, the negative effects on performance (Paragraph 2.3) were distinctly less negative than with common beans. But, growth was also reduced in piglets and not in rats or chickens. Thus, with peas there was also a species difference between piglets on the one hand and rats and chickens on the other.

In addition to assessing performance, body organs of piglets, rats and chickens were weighed in the experiments with common beans and with peas. Special attention was paid to pancreas weight because the pancreas is related to the digestive processes via enzyme production. It has also been pointed out in the literature that trypsin inhibitors in the chyme regulate the secretion of pancreatic enzymes and may induce hypertrophy in small animals (Birk, 1989; Liener and Kakade, 1980; Gallaher and Schneemann, 1986). Raw common beans and the peas used in our animal species studies contained trypsin inhibitors. In line with this, an increased weight of the pancreas was found in rats and chickens when fed peas and common beans. With peas the pancreas weight of the piglets was not affected. This result is in agreement with the literature and shows that there is no hypertrophy of the pancreas due to trypsin inhibitors in larger animals such as pigs (Liener and Kakade, 1980; Gallaher and Schneemann, 1986). The pancreas weight of the piglets fed the raw common beans in the first experiment (Paragraph 2.1), was significantly lower compared with that of the piglets fed the control diet. The reduced pancreas weight in the first experiment with the common beans may be related to the extremely low protein digestibility. A negative relationship between the supply of digestible protein and amino acids, and pancreas weight relative to body weight has been reported (Green et al., 1986; Liener et al., 1985, Solomon, 1987). Therefore, in all the diets in the second experiment, higher digestible protein sources were included (Paragraph 2.2). The apparent faecal protein digestibility of these diets was measured by van der Poel et al. (1990). The apparent faecal digestibilty coefficients of protein for the control diet and the diets with raw and toasted beanswere, respectively, 10, 9 and 5 units higher than the protein digestibilities found in the first experiment. In this study the weight of the pancreas of the piglets was not significantly reduced when raw beans were fed. This observation suggests that with more highly digestible protein in the diet there, is little adverse effect of the ANFs on the growth of the pancreas in the piglets. More details about a possible relationship between protein digestibility and pancreas weight will be discussed in the last paragraph of this chapter.

Spleen and thymus weights were determined in the study because they may be important in relation to the effects of lectins on the immune system. Pusztai (1989) argued that effects of lectins on the immune system are related to damage of the intestinal mucosa which leads to an increase in permeability of the gut wall. Lectins and other peptides may then pass through the gut wall and enter the blood causing immunological reactions. There may be a relationship between the weights of spleen and thymus and these immunological reactions. In our studies, the weights of the spleen and the thymus were reduced in piglets fed the diets with raw common beans. Extra dietary protein supply (Paragraph 2.2) did not eliminate this effect. The weight of neither spleen nor thymus was affected in either rats or chickens. There were no indications that the weights of liver and kidney were affected due to feeding common beans or peas in the three animal species.
The main results are summarized in Table 2.
The results obtained in our studies show that piglets are more sensitive than rats or chickens to factors present in peas and common beans in terms of body weight gain and protein digestibility The reason for the observed differences between animal species remains an interesting field for further studies. Small animals may be valuable models for understanding particular effects of ANFs. Our present results, however,

Table 2 Summary of the effects of ANFs in piglets, rats and chickens given diets containing raw common beans and peas

Diet	Measurement	Effect		
		Piglet	Rat	Chicken
20% raw Phaseolus vulgaris	Weight gain	----	-/0	-/0
	Weight pancreas*	-/0	+	+
	Weight spleen*	-/--	0	0
	Protein digestibility	---	-	N.D.
30% Pisum sativum	Weight gain	-	0	0
	Weight pancreas *	0	+	+
	Weight spleen*	0	0	0

0	= no effect
+	= increase
-/--/---/----	= decrease
N.D.	= not determined
*	= % of live weight

show that for nutritional evaluation, it is important to study the ANF effects in the target animal. This conclusion is relevant, because it will certainly have consequences for future research. Rats are often used as a model for man, but it may be questioned whether they are adequate models for man when studying the effects of ANFs. Because piglets are much more sensitive to ANFs in peas and common beans than rats or chickens, the piglet may be recommended as a model for ANF-research in man instead of the rat. In this respect, Graham and Aman (1987) stated that "of all domesticated animal species, the pig is in gastro-intestinal physiology, diet and size most similar to man".

Research with isolated fractions from peas

The results of the animal species studies clearly indicate that rats and chickens react differently than piglets when fed diets with raw common beans or peas (Paragraphs 2.1, 2.2 and 2.3). Therefore, the following research was carried out using the target animal, the piglet. The first question investigated was which fraction of the pea is associated with the negative effects. The following fractions were prepared from peas:

- a pea protein isolate from which carbohydrates and ANFs were removed. This fraction contained mainly protein and low levels of ANFs (see Paragraph 3.1).
- a pea protein fraction with high concentrations of ANFs. This fraction contained a high level of protein and very high levels of ANFs (Paragraph 3.2).
- a fraction consisting of a mix of soluble and insoluble pea carbohydrates. This fraction consisted mainly of carbohydrates and contained hardly any ANFs.

The results presented in Paragraph 3.1 show that the apparent ileal protein digestibility of raw peas was distinctly lower (about 14 units) than that of pea protein isolate. This means that as a result of removing carbohydrates and ANFs, the protein

digestibility increased considerably. One important question is which of the two factors (ANFs or carbohydrates) is associated with the low apparent ileal digestibility of raw peas. Also, the possiblity that the protein structure in pea protein isolate was different compared with the native protein in raw peas cannot be discounted. The production of pea protein isolate is based on a precipitation at a pH 4. Manipulation of the pH may have altered the protein structure. Ileal digestibility was found to be much more sensitive when testing ANF effects than faecal digestibility (Paragraph 3.1). This result is in line with the conclusions drawn by van Weerden et al. (1985) and Sauer and Ozimek (1986). Based on these results, ileal digestibility was chosen as the main criterion in the following experiment, which was aimed at determining whether the low apparent ileal protein digestibility in raw peas was associated with ANFs or with carbohydrates.

The addition of pea carbohydrates to diets did not alter the apparent ileal protein digestibility (Paragraph 3.2). Pea carbohydrates caused increased chyme flow of the small intestine. This may be related to a lower digestibility of these carbohydrates in the small intestine (Le Guen and Huisman, unpublished results). Due to the incomplete carbohydrate digestibility, more osmotically active components will be present in the ileal chyme. These components will cause an inflow of water into the intestinal lumen, in order to maintain osmotic equilibrium between the blood and lumen contents. With an incomplete ileal digestibility, the remaining part of the pea carbohydrates will be digested in the large intestine. When carbohydrates are digested in the large intestine there is a loss of about 30% to 40% of the energy compared with the fraction digested in the small intestine (ARC, 1981; Müller et al., 1989). The addition of pea-ANFs to a diet containing pea protein isolate with very low levels of ANFs as the sole protein source, caused a reduction in apparent ileal protein digestibility. However, the difference of 14 units in apparent ileal protein digestibility between raw peas and pea protein isolate (Paragraph 3.1) could not be completely simulated by the addition of pea ANFs to pea protein isolate. With the addition of pea-ANFs a reduction in apparent ileal protein digestibility of 7 units was found. The remaining 7 units difference could possibly be attributed to changes in the structure of the pea protein isolate during preparation, which could have resulted in two effects:

- an increase in hydrolyzability of the protein by intestinal digestive enzymes
- a reduction in antigenicity of the pea protein. When piglets were fed the same batches of raw peas used as in the studies described in Paragraph 3.1, immunoglobulins (IgG) against pea protein were detected in the blood (Tolman and Huisman, unpublished results). This indicates that the raw pea protein is antigenic. Antigenicity of pea protein was also demonstrated in veal calves fed raw pea flour (Toullec and Guilloteau, 1989).

The weight gain of the piglets fed the diets containing pea protein isolate enriched with pea ANFs was distinctly (about 17%) lower than that of the piglets given the protein isolate diet without the addition of ANFs. This indicates that the reduction in pig performance with raw peas seems to be associated, for a major part, with the effects of ANFs.

True ileal and faecal digestibility of protein in raw peas and toasted beans

The nutritional value of a protein in a certain feedstuff is not only dependent on the hydrolyzability of the protein and its amino acid content, but also on the amount of endogenous protein secreted as a result of feeding that feedstuff. It is possible that with a highly hydrolyzable protein, a low determined apparent protein digestibility is due to a high secretion of endogenous protein. Secretion of endogenous protein can be stimulated by trypsin inhibitors, lectins and antigenicity of the protein. A common method of measuring the secretion of endogenous protein, is by feeding the animals a protein-free diet. However, with protein-free diets the secretion of endogenous protein may be less stimulated than with normal diets containing protein, ANFs, non-starch-polysaccharides and crude fibre. A recently developed advanced method of measuring the secretion of endogenous protein is the ^{15}N dilution technique (Souffrant, et al., 1986; de Lange, 1989). Over a certain period, the pigs are continuously infused with a ^{15}N amino acid solution into the blood. During this period the pigs are fed the test diets. As discussed in Chapter 4, it has been shown (de Lange et al., 1990) that with protein-free diets, the amounts of secreted endogenous protein are lower than those obtained with the ^{15}N dilution technique. When the secretion of endogenous protein is known, the true digestibility of protein can be calculated by correcting the apparent protein digestibility for the endogenous protein. In our studies, the true protein digestibility was measured for two varieties of peas: FINALE with low levels of trypsin inhibitors and FRIJAUNE with high levels of trypsin inhibitors. The common beans were from a different batch than that reported in the studies discussed earlier (Paragraphs 2.1 and 2.2). The initially aim was to measure the true digestibility of protein in raw common beans. However, feed intake with the raw beans was very low and, therefore, the beans were toasted for 40 minutes at 104°C. The true ileal protein digestibility of both pea varieties was between 93% and 95%. This indicates that the pea protein is almost completely digested in the small intestine. It also indicates that the pea protein it self is highly hydrolyzable by the intestinal digestive enzymes. The low apparent digestibility of raw peas (Paragraph 3.1) must therefore be associated with a high secretion of endogenous protein. This is of importance for studies focussed on increasing protein digestibility by (bio)technological treatment. The present study shows that for peas such treatments need to be focussed especially on factors responsible for an increase in the secretion of endogenous protein and not on the hydrolyzability of protein. The high losses of endogenous protein could be related to different factors such as trypsin inhibitors, lectins and antigenicity of the protein.
In this respect it is important to know to which level the trypsin inhibitors, lectins and possibly the antigenicity of protein need to be reduced without extra stimulating the secretion of endogenous protein.

The true ileal digestibility of the protein from the toasted common beans was about 66%. The apparent ileal protein digestibility was about zero (-4%). The low apparent ileal protein digestibility must therefore be associated with a very high secretion of endogenous protein. With these beans, it was calculated that per 100 g ingested bean protein, 34 g undigested bean protein and 70 g of endogenous protein were excreted with the ileum chyme. These losses of endogenous protein at the distal ileum explain why the piglets given beans in the experiments described in Paragraph 2.1 and 2.2 lost weight. When the secretion of endogenous protein is greater than the

amount of protein ingested, tissue breakdown must occur. Evidence of such tissue breakdown was found by Palmer et al. (1987) in rats fed either common beans as the sole protein source, or lectins added to a lactalbumin diet. Bardocz et al. (1989) and Oliveira et al. (1988) also found evidence of tissue breakdown due to phytohaemagglutinins or lectins from Phaseolus vulgaris. Pusztai (1989) suggested that the breakdown of protein in muscle is a specific effect of lectins in Phaseolus vulgaris since soya lectins do not show these effects (Grant et al., 1987). The increase in secretion of endogenous protein can be related to different factors:

- lectins damage the intestinal mucosa and thereby stimulate the production of endogenous protein (Pusztai, 1989)
- the Phaseolus protein is antigenic and stimulates the secretion of endogenous protein (Santoro et al., 1989)
- common beans contain trypsin inhibitors, which stimulate the secretion of pancreatic enzymes. Evidence of this was found in the studies reported in Paragraphs 2.1 and 2.2 in which hypertrophy of the pancreas was found in rats and chickens. It is not clear whether this pancreatic stimulation was also present in piglets.

Pancreas activity in relation to protein digestibility

In the experiment described in Paragraph 2.1 a markedly reduced relative pancreas weight in piglets was observed when feeding raw common beans. The same piglets also lost live weight. Because the pancreas is a tissue that rapidly responds to starvation (Fauconneau and Michel, 1970), this reduction in weight of the pancreas could be associated with the live weight loss of the piglets. Based on the results of the experiment described in Paragraph 2.2 it was concluded that the lower pancreas weight could also be associated with low protein digestibility. It is pertinent to mention that these raw common beans contained trypsin inhibitors which, in our experiments caused hypertrophy of the pancreas in the rats and chickens (Paragraph 2.1 and 2.2). Thus, whereas hypertrophy was found in rats and chickens due to trypsin inhibitors in the common beans, atrophy with the same beans was observed in piglets. On the pea diets the piglets grew well. Therefore, to gain more insight into the existence of a possible association between protein digestibility and weight of the pancreas or enzyme activity in the pancreas tissue, these criteria were measured in the digestibility experiment using raw FRIJAUNE peas and FRIJAUNE pea protein isolate (Paragraph 3.1). Apparent faecal and ileal protein digestibility of raw FRIJAUNE peas was distinctly lower than that of FRIJAUNE pea protein isolate. The pancreas weight relative to body weight of both groups of piglets was almost similar. The trypsin activity in the pancreatic tissue (Table 3, Huisman et al., 1990b) and the secretion of pancreatic enzymes (Table 3, Le Guen and Huisman) of the piglets fed the raw peas was, however, significantly lower than in the piglets fed the pea protein isolate. Summarizing, it can be stated that, although there were trypsin inhibitors in the raw peas, pancreatic activity of the piglets was reduced. This shows that the pancreas of the piglet seems to react differently to trypsin inhibitors than that of rats or chickens in our previous study (Paragraph 2.3), and is contrary to what is generally stated in the literature (Birk, 1989; Gallaher and Schneeman, 1986; Liener and Kakade, 1980). Reduced pancreas activity was also found with raw soy protein in piglets and guinea pigs (Hasdai et al., 1989; Yen et al., 1977) and in veal calves when

milk protein was replaced by isolated soy protein (Khorasani et al., 1989) or by soya flour (Gorrill et al., 1967 and Gorrill and Thomas, 1967). Table 3 summarizes the findings of our experiments and those reported in the literature. Table 3 shows that pancreas weight relative to body weight in piglets, guinea pigs and veal calves does not relate to pancreas activity. Green et al. (1986) argued that relative values of pancreas weights alone give misinterpretations (see also Table 4). In some of the experiments shown in Table 3, also protein digestibility was measured. Yen et al. (1977) found that the nitrogen content detected in the chyme of the upper and lower parts of the small intestine of the piglets was significantly higher with raw soy protein than with toasted soy protein indicating that, as could be expected, the protein digestibility with the raw soy protein was lower than with the toasted soy protein. In the studies reported by Gorrill et al. (1967) and Gorrill and Thomas (1967) no protein digestibility was measured, but it is known that digestibility of soy protein in veal calves is always lower than that of milk protein. The lower protein digestibility is in line with the lower proteolytic activity in the small intestinal chyme and with the decreased excretion of pancreatic enzymes. The raw peas, raw soy protein and the soybean flour contained trypsin inhibitors. Khorassani et al. (1989) did not report the trypsin inhibitor contents, but most soy protein isolates contain residual trypsin inhibitors. A striking observation is that with these trypsin inhibitor containing proteins there was no increase in the secretion of pancreatic enzymes.
In this respect the pancreas of piglets, veal calves and guinea pigs seems to react differently to trypsin inhibtors than that of rats, mice or chickens. The explanation for this observation merits further study. Different points need to be clarified in this respect. Some suggestions are:

a. Relation of ANFs and decreased pancreas activity
   It has been reviewed that trypsin inhibitors stimulate the pancreatic secretion of enzymes (Birk, 1989; Gallaher and Schneeman, 1986; Liener and Kakade, 1980). The way the pancreas is stimulated to increase enzyme production is explained as follows. The levels of free trypsin and chymotrypsin in the intestinal chyme regulate pancreatic secretions by a negative feedback mechanism regulated by the humoral agent CCK-PZ (see also Chapter 1). When the levels of free trypsin and chymotrypsin are low, then the pancreas is stimulated to produce more enzymes. In the studies summarized in Table 3, a lower trypsin activity in the intestinal chyme was found with diets containing raw peas, raw soy protein, soy flour and soy isolate. Pancreas activity (expressed in terms of trypsin contents in tissues or in amounts in pancreatic juice) decreased, however, when feeding these proteins. Based on what is generally stated in literature (see Chapter 1 and the reviews of Birk, 1989; Gallaher and Schneeman, 1986 and Liener and Kakade, 1980), increased secretion of the pancreas should be expected when the free trypsin levels are decreased in the chyme. The results presented in Table 3 demonstrate that the negative feedback mechanism which regulates the hypersecretion of pancreatic enzymes does not work in piglets, veal calves or guinea pigs when fed raw peas, raw soy protein, soy flour and soy isolate. Gallaher and Schneeman (1986) reviewed that there is evidence that the negative feedback mechanism found in rats also exists in pigs, calves, and possibly in man. Why the negative feedback failed to work in the studies mentioned in Table 3 is not clear. Green et al. (1986) mentioned the hypothesis of Miyasaka and Green (1983) that trypsin

Table 3 Apparent faecal and ileal N digestibility related to pancreas activity

Animal species	Treatment	N digestibility Faecal	N digestibility Ileal	Pancreas weight*	Trypsin activity pancreas	Trypsin activity intestinal chyme	Reference
Piglets	control diet	85a	nd	0.21a	nd	nd	Huisman et al., 1990a
	diet with 20% Ph. beans	48b	nd	0.10b	nd	nd	Paragraph 2.1)
Piglets	control diet	95a	nd	0.18a	nd	nd	Huisman et al., 1990b
	diet with 20% Ph. beans	57b	nd	0.16a	nd	nd	(Paragraph 2.2)
Piglets	16% pea isolate protein**	95a	84a	0.19a	82a 1)	nd	Huisman et al., 1990c
	16% raw pea protein***	86b	72b	0.15a	34b 1)	nd	(Paragraph 3.1)
Piglets	16% pea isolate protein**	see	see	nd	96.6a 2)	nd	Le Guen and Huisman, 1990
	16% raw pea protein***	above	above	nd	79.7b 2)	nd	(unpublished)
Piglets	Trial 1: 2 weeks ad lib. feeding						
	16% toasted soy protein	#	#	0.19	890a 1)	14.43a	Yen et al., 1977
	16% raw soy protein	#	#	0.16	1040a 1)	3.31b	
Piglets	Trial 2: 6 weeks ad lib. feeding						
	16% toasted soy protein	#	#	0.14	1210a 1)	37.25a	
	16% raw soy protein	#	#	0.12	850b 1)	2.96b	
Piglets	Trial 4: 1 week feeding 5% of live weight						
	16% toasted soy protein	#	#	0.12	1170a 1)	41.92a	
	16% raw soy protein	#	#	0.12	670b 1)	2.36b	
Piglets	Trial 5: 2 weeks feeding 5% of live weight						
	16% toasted soy protein	#	#	0.13	760a 1)	45.95a	
	16% raw soy protein	#	#	0.13	470b 1)	6.12b	
Guinea pigs	12% heated soy protein	70a	nd	0.42a	1900 3)	nd	Hasdai et al., 1989.
	12% raw soy protein	50b	nd	0.40a	750 3)	nd	
Veal calves	29.9% skimmed milk protein	90a	80a	nd	44.5a 4)	nd	Khorasani et al., 1989.
	31.5% isolated soy protein	78b	60b	nd	24.9b 4)	nd	
Veal calves	19.2% skimmed milk and whey protein	nd	nd	nd	159a 4)	nd	Gornit et al., 1967.
	14.5% protein of soy flour## + 9.7% protein from skimmed milk and whey	nd	nd	nd	69b 4)	nd	
Veal calves	Whole milk	nd	nd	0.060a	5.69a 5)	3732a 6)	Gornit and Thomas, 1967.
	14.5% protein of soy flour## + 9.7% protein from skimmed milk and whey.	nd	nd	0.057a	3.46b 5)	1764b 6)	
	20.8% protein of soy concentrate## + 3.4% protein from skimmed milk and whey	nd	nd	0.047b	4.93a,b 5)	2160a,b 6)	

1) units trypsin/g wet pancreatic tissue
2) units trypsin x 1000/ 12 hours. Measurements in piglets with cannulas in the pancreas.
3) units trypsin in pancreatic tissue, expressed in units per kg body weight
4) units trypsin/ml/pancreatic juice. Measurements in calves with cannulas in the pancreas.
5) units trypsin in pancreatic tissue/mg dry weight
6) one unit equals hydrolysis of 1 µmole TAME/ minute.
nd = not determined
* % of live weight.
** /*** diets were of the same batches
\# The N content in the ileal chyme of the upper and the lower half of the small intestine was significantly higher in the raw soy protein fed piglets than in the toasted soy protein fed ones. This indicates that the protein digestibility was decreased.
Trypsin inhibitor activity was high in soy flour and low in soy concentrate.
a,b means in the same column of each study with different superscript differ significantly ($P < 0.05$).

inhibits pancreatic secretion by inactivating an intraluminally-secreted peptide, possibly a CCK-releasing factor. Evidence for a CCK-releasing factor is recently reported by Fushiki and Iwai (1989) showing that trypsin and chymotrypsin do not directly interact with the luminal surface of the small intestine but by a trypsin-sensitive, cholecystokinin-releasing peptide. This also implies that when this peptide is absent or inactivated the negative feedback mechanism cannot work. The fact that the negative feedback mechanism did not work in the animals mentioned in Table 3 could possibly be related to the absence or inactivation of this peptide. It would be interesting to study whether this peptide is absent or indeed inactivated in piglets, veal calves and guinea pigs and which role it plays in the negative feedback mechanism.

b. Relation of pancreas activity and protein digestibility
The results in Table 3 show that with a lower pancreatic enzyme secretion the protein digestibility was also lower. It is a valid explanation that with a lower secretion of pancreatic enzymes the protein digestibility is also lower. But it could also be argued that pancreas activity is lower due to lower protein digestibility. The experiments presented in Table 3 show that the trypsin activity in the intestinal chyme was reduced when ANFs are present in the feed. Due to this reduction, the protein digestion may be incomplete. As a result there are less amino acids available for the pancreas to produce enzymes. Evidence for this statement is shown by Green et al. (1986). They re-interpretated the results reported by Booth et al. (1960), shown in Table 4.

Table 4 Pancreatic hypertrophy in rats fed soybean meal

Dietary protein	Final body weight (g)	Pancreas weight	
		% of body weight	Absolute (g)
Casein	1470	0.56	0.83
Raw soybean meal	89	0.85	0.76
Raw soybean meal + amino acids	1420	0.93	1.32
Heated soybean meal	148	0.50	0.74

The results show that the absolute weight of the pancreas of the rats fed raw soybean meal enriched with amino acids, was distinctly higher compared to raw soybean meal alone or to heated soybean meal. This indicates that with raw soybean meal alone the pancreas was not activated because of an insufficient supply of amino acids. Gorrill and Thomas (1967) suggest also that the hyposecretion of the pancreactic enzymes could possibly be related to a deficiency in essential amino acids for protein synthesis. Solomon (1987) discussed that the availability of tryptophan is essential for pancreatic secretion. In our study with raw peas a low ileal digestibility of tryptophan, cystine and threonine was found (Paragraph 3.1). The low digestibility of these amino acids could possibly be related to the lower pancreatic activity. It would be interesting to study further whether pancreatic enzyme secretion, when feeding raw peas or raw soy protein, can be stimulated by the addition of amino acids.

c. pancreas activity and other factors
In the studies described by Gorrill and Thomas (1967) and Huisman and van Weerden (unpublished results) distinct differences in pH and buffer capacity in the abomasum and duodenal chyme were found in veal calves when soy protein based diets were fed instead of all milk protein diets. Gorrill and Thomas (1967) suggest that the greater total trypsin and chymotrypsin activities of intestinal contents from calves fed whole milk compared with those from calves fed the soy diets could partly be explained by the lower pH of abomasal and upper intestinal contents. It should be investigated as to whether the reduced pancreas activity in the veal calves in the studies described by Gorrill et al. (1967), Gorrill and Thomas (1967) and Khorasani et al. (1989) can be related to differences in buffer capacity and pH in the duodenum. These possible effects may also have played a role in the studies with piglets and guinea pigs (Table 3).

Conclusions

The results of our research into animal species differences clearly showed that the piglet is much more sensitive than rats or chickens to factors present in peas and common beans. It was concluded that the latter species of animals could be used as valuable models in order to study pathogenic aspects of ANFs. For nutritional evaluations, however, it is essential to study ANF effects in the target animal. In relation to human nutrition, one relevant question is which species could be recommended as a model for man. Graham and Aman (1987) stated that " of all domesticated animal species, the pig is in gastro-intestinal physiology, diet and size most similar to man". It was demonstrated that the low apparent ileal protein digestibility in raw peas is partly associated with ANFs. A part of the low apparent protein digestibility could possibly be related to antigenicity of the pea protein or other factors causing increased secretion of endogenous protein. Pea carbohydrates increased the intestinal chyme flow, but did not affect the apparent ileal protein digestibility. Using ^{15}N studies, it was demonstrated that the true digestibility of protein in raw peas was high. This means that the lower apparent protein digestibility must be attributed to an increased secretion of endogenous protein. Referring to the observation that pancreatic activity seems to be suppressed by raw peas the endogenous protein must originate from elsewhere, probably the intestinal wall. Information about true protein digestibility is very important for (bio)technologists. Our results demonstrate that treatments aimed at increasing protein digestibility of peas should focus on reducing the factors causing an increased secretion of endogenous protein. Using toasted common beans it was demonstrated that there was considerable secretion of endogenous protein, but the true digestibility of protein of these beans was also relatively low. Thus, with common beans (bio)technologist need to pay attention to eliminating the factors causing increased secretion of endogenous protein and to the structure of the protein. Our experiments with common beans and peas showed that the trypsin activity in pancreatic tissue and pancreatic juice decreased together with apparent protein digestibility. The same tendency has been observed in guinea pigs and veal calves fed different soy products. Various points have been discussed which may be associated with these observations.

References

Agricultural Research Council (ARC) (1981). The nutrient requirement of pigs. Commonwealth Agricultural Bureaux, England.

Bardocz, S. Grant, G., Brown, D.S. and Pusztai, A. (1989). Stimulation of polyamine synthesis and growth of the small intestine by dietary kidney bean lectin. In: J. Huisman, A.F.B. van der Poel and I.E. Liener (editors). Recent adcances of research in antinutritional factors in legume seeds. Pudoc , Wageningen, The Netherlands, 39-42.

Birk, Y. (1989). Protein protease inhibitors of plant origin and their significance in nutrition. In: J. Huisman, A.F.B. van der Poel and I.E. Liener (editors). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 239-250.

Booth, A.N., Robbins, D.J., ribelin, W.E. and DeEds, F. (1960). Effect of raw soybean meal and amino acids on pancreatic hypertrophy in rats. Proceedings Society Experimental Biological Medicine, 104, 681-683.

Combs, G.E., Connes, R.G., Berry, T.H. and Wallace, H.D. (1967). Effect of raw and heated soyabean meal on gain, nutrient digestibility, plasma amino acids and other blood constituents of growing swine. Journal of Animal Science, 26, 1067-1071.

De Lange, C.F.M. (1989). Endogenous protein in digestibility studies in pigs. Ph.D. Thesis University of Alberta, Edmonton, Canada.

De Lange, C.F.M., Souffrant, W.B. and Sauer, W.C. (1990). Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the ^{15}N-dilution technique. Journal of Animal Science, 68, 409-418.

Fauconneau, G. and Michel, M.C. (1970). The role of the gastrointestinal trace in the regulation of protein metabolism. In: H. Munro (editor). Mammalian Protein Metabolism. Academic Press, New York, US.

Fushiki, T. and Iwai, K. (1989). Two hypotheses on the feedback regulation of pancreatic enzyme secretion. The FASEB Journal, 3, 121-126.

Gallaher, D. and Schneeman, B.O. (1986). Nutritional and metabolic response to plant inhibitors of digestive enzymes. In: M. Friedman (editor). Nutritional and toxicological significance of enzyme inhibitors in foods. Plenum Press, New York, US, 167-185.

Gorrill, A.D.L., Thomas, J.W., Stewart, W.E. and Morrill, J.L. (1967). Exocrine pancreatic secretion by calves fed soybean and milk protein diets. Journal of Nutrition, 92, 86-92.

Gorrill, A.D.L. and Thomas, J.W. (1967). Body weight changes, pancreas size and enzyme activity, and proteolytic enzyme activity and protein digestion in intestinal contents from calves fed soybean and milk protein diets. Journal of Nutrition, 92, 215-223.

Graham, H. and Aman P. (1987). The pig as a model in dietary fibre digestion studies. Scandinavian Journal of Gastroenterology 22 (Supplement 129), 55-61.

Grant, G., Greer, F. McKenzie, N.H. and Pusztai, A. (1985). Nutritional response of mature rats to kidney bean (Phaseolus vulgaris) lectins. Journal of Science of Food and Agriculture, 36, 409-414.

Grant, G., Greer, F., McKenzie, N.H. and Pusztai, A. (1987). Changes in the small intestine and hind leg muscles of rats induced by dietary soyabean (Glycine Max) proteins. Medical Science Research, 15, 1355-1356.

Green, G.M., Levan, V.H. and Liddle, R.A. (1986). Interaction of dietary protein and trypsin inhibitor on plasma cholcystokinin and pancreatic growth in rats. In: M. Friedman (editor). Nutritional and toxicological significance of enzyme inhibitors in foods. Plenum Press, New York, US, 123-132.

Hasdai, A., Nitsan, Z., Volcani., R. and Birk, Y. (1989). Growth, digestibility and enzyme activities in the pancreas and intestines of guinea pigs fed on raw and heated soya-bean flour. British Journal of Nutrition, 62, 529-537.

Khorasani, G.R., Ozimek, L., Sauer, W.C. and Kelley, J.J. (1989). Substitution of milk protein with isolated soy protein in calf milk replacers. Journal of Animal Science, 67, 1634-1641.

Liener, I.E. and Kakade, M.L. (1980). Protease inhibitors. In: I.E. Liener (editor). Toxic constituents of plant foodstuffs. Academic Press, New York, US., 7-71.

Liener, I.E., Nitsan, Z., Srisangnam, C. Rackis, J.J., and Gumbmann, M.R. (1985). The USDA trypsin inhibitor study. II. Time related biochemical changes in the pancreas of rats. Qualitas Plantarum. Plant Foods Human Nutrition, 35, 243-257.

Miyasaka, K. and Green, G.M. (1983). Effect of rapid washout of proximal small intestine on pancreatic secretion in conscious rat (Abstract). Gastroenterology, 84, 1251.

Müller, H.L., Kirchgessner, M. and Roth, F.X. (1989). Energy utilization of intra caecally infused carbohydrates and casein in sows. In: Energy metabolism of farm animals. Proceedings of the 11th symposium. (Y. van der Honing and W.H. Close, compilers). Pudoc, Wageningen, The Netherlands, 123 - 126.

Oliveira de, J.T.A., Pusztai, A. and Grant, G. (1988). Changes in organs and tissues induced by feeding of purified kidney bean (Phaseolus vulgaris) lectins. Nutrition Research, 8, 943-947.

Palmer, R.M., Pusztai, A., Bain. P. and Grant, G. (1987). Changes in rates of tissue protein synthesis in rats induced in vivo by consumption of kidney bean (Phaseolus vulgaris) lectins. Comparative Biochemistry and Physiology, 88C, 179- 183.

Pusztai, A. (1989). Biological effects of dietary lectins. In: J. Huisman, A.F.B. van der Poel and I.E. Liener (editors). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 17-29.

Santoro, L.G., Grant, G. and Pusztai, A. (1989). Degradation of glycoprotein II (phaseolin), the major storage protein of Phaseolus vulgaris seeds. In: J. Huisman, A.F.B. van der Poel and I.E. Liener (editors). Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, The Netherlands, 363-367.

Sauer, W.C. and Ozimek, L. (1986). Digestibility of amino acids in swine: results and their practical applications. A review. Livestock Production Science, 15, 367-388.

Solomon, T.E. (1987). Control of exocrine pancreatic secretion. In: L.R. Johnson (editor). Physiology of the gastrointestinal tract, second edition. Raven Express, New York, 1173-1207.

Souffrant, W.B., Darcy-Vrillon, B., Corring, T., Laplace, J.P., Köhler, R., Gebhardt, G. and Rerat, A. (1986). Recycling of endogenous nitrogen in the pig. Archiv für Tierernährung, 36, 269- 274.

Toullec, R. and Guilloteau, P. (1989). Research into the digestive physiology of the milk-fed calf. In: E.J. van Weerden and J. Huisman (editors). Nutrition and digestive physiology in monogastric farm animals. Pudoc, Wageningen, The Netherlands, 37-55.

Van der Poel, A.F.B. and Huisman, J. (1988). Effect of steam treatment of dry bean (Phaseolus vulgaris) with extreme high lectin content on ileal digestibility in pigs. Proceedings of the 4th International Seminar on Digestive Physiology in the Pig. Jablonna, Poland, 297-301.

Van der Poel, A.F.B., Mollee, P.W., Huisman, J. and Liener, I.E. (1990). Variations among species of animals in response to the feeding of heat processed beans (Phaseolus vulgaris). 1. Bean processing and effects on growth, digestibility and organ weights in piglets. Livestock Production Science, 25, 121-135.

Van Weerden, E.J., Huisman, J. van Leeuwen, P. and Slump, P. (1985). The sensitivity of the ileal digestibility method as compared to the faecal digestibility method. Proceedings of the 3rd International Seminar on Digestive Physiology in the Pig. Copenhagen, Denmark, 392-395.

Visitpanich, T., Batterham, E.S. and Norton, B.W. (1985). Nutritional value of chickpea (Cicer arietinum) and pigeon pea (Cajanus cajan) meals for growing pigs and rats. I. Energy content and protein quality. Australian Journal of Agricultural Research, 36, 327-335.

Yen, J.T., Jensen, A.H. and Simon, J. (1977). Effect of dietary raw soybean and soybean trypsin inhibitor on trypsin and chymotrypsin activities in the pancreas and in the small intestinal juice of growing swine. Journal of Nutrition, 107, 156-165.

There is a growing interest in Europe to be self-supporting with regard to the protein supply for animal diets. Peas and beans growing well under European climatic conditions could provide alternatives to soya. However, these legume seeds contain the same classes of antinutritional factors (ANFs) as those found in raw soybeans. The use of such seeds in the raw state, therefore, is seriously hampered due to the presence of these ANFs. The role of ANFs in animal nutrition may become more important in the future. This is related to the expectation among zootechnicians that in the future farm animals will grow faster and deposit more body protein because of advances in animal breeding, health care and housing. It has been shown that the feed intake capacity has not increased in these fast growing animals, so they will therefore require relatively more highly digestible protein in the future. As a result, feedstuffs with a high protein content will become more important. However, most plant protein-rich seeds contain ANFs. The ANFs in peas, beans and soybeans have negative effects on digestibility and performance. In this respect it is necessary to find economically feasible inactivation processes which eliminate ANF activity. To achieve this, it is essential to know more about the way ANFs affect the digestion and absorption processes in animals. In this thesis, firstly a literature review (Chapter 1) was prepared on the occurrence and role of ANFs in peas (Pisum sativum), common beans (Phaseolus vulgaris) and soybeans (Glycine max). The main aspects to be considered were the state of the art concerning the action of ANFs in monogastric animals, the effect of ANFs on nutritional value, and the analytical methods for determining these ANFs. Also, recommendations for future research are given. The literature review (Chapter 1) shows that there are many unclear points related to the mode of action of ANFs in the animal. Major points being:

- Most research into nutritional effects of ANFs in animals is carried out using small laboratory animals such rats, mice and chickens. An important question is whether results obtained in these animals are applicable to pigs.
- Peas and beans always contain more than one ANF. In most studies whole ANF-containing seeds were fed to the animal. Information obtained in these studies gave no insight into the specific effects of separate ANFs. Only a limited amount of research has been done using isolated ANFs, and even then it was only carried out on small laboratory animals. To understand the relevance and the way ANFs act in the target animal, it is necessary to use isolated and purified ANFs in the investigations.
- When a low apparent protein digestibility is measured it is not clear whether this is related exclusively to ANFs or whether the native protein itself may also be resistent to the hydrolysis by digestive enzymes.
- Many analytical methods are not adequate. This has hampered the real identification of ANFs.
- In the literature, lectin research is mainly focussed on the lectins present in Phaseolus vulgaris. Information about the mode of action of lectins in other seeds is limited.
- There is insufficient information about the possibilities of eliminating ANF-activity.
- There is insufficient information about the threshold levels, being the dietary levels of ANFs which can be tolerated without causing negative effects.

In this thesis, aspects of the first three points were studied. The other points are being studied in related programmes.

Animal species differences between piglets, rats and chickens were studied in three experiments. The results are described in Chapter 2. With common beans in the diet, performance was much more depressed in piglets than in rats or chickens. The piglets even lost weight. Weight loss in the piglets was also evident when extra protein was included in the diet. This indicates that a toxic factor must be associated with the reduced performance and not an insufficient amino acid supply. Protein digestibility was also markedly more depressed in piglets than in rats. The pancreas weight increased in the rats and chickens but not in the piglets. Increase in pancreas weight in rats and chickens may be related to the trypsin inhibitors present in the beans. Weights of the spleen and thymus were reduced in piglets but not in rats or chickens. With peas a reduction in weight gain was observed in piglets, but not in rats or chickens. Pancreas weight increased in the rats and chickens but not in the piglets. In all the animal species the weights of spleen and thymus were hardly affected by peas. Kidney and liver weights were not affected by either peas or beans. The results show that piglets are much more sensitive than rats or chickens to factors present in peas and beans. Some effects in piglets were the complete opposite of those found in rats and chickens. It is concluded, therefore, that ANF-research should be carried out using the target animals.

In order to study which factor in peas caused the negative effects on protein digestibility, different fractions from peas were prepared: a pea protein isolate from which ANFs and carbohydrates were removed, a protein fraction with very high concentrations of ANFs and a fraction consisting of a mix of soluble and insoluble carbohydrates and free of protein and ANFs. Two pea varieties were involved, a summer variety with low trypsin inhibitor levels and a winter variety with relatively high levels of trypsin inhibitors. The fractions prepared from both varieties were applied in apparent ileal and faecal digestibility experiments with piglets. The results of these studies are described in Chapter 3. The apparent ileal protein digestibility of raw peas was with both varieties 14 units lower than in the pea protein isolate. Strikingly, the apparent ileal digestibility of some essential amino acids (S-containing amino acids, tryptophan and threonine) was very low at ileal level. The addition of pea carbohydrates to diets did not alter the apparent ileal protein digestibility. Small intestinal chyme flow increased due to pea carbohydrates. This effect could be related to a release of osmotic active components from the pea carbohydrates into the ileal chyme during the digestion process. The addition of pea-ANFs to a diet with pea protein isolate (low in ANFs) as the sole protein source, reduced the apparent ileal protein digestibility by about seven units. Weight gain of the piglets fed the diet enriched with ANFs was about 17% less compared with the control piglets. This demonstrates that ANFs are an important factor in explaining the reduced weight gain when more than 15-20% peas are included in the diets of piglets. The difference in apparent ileal protein digestibility between raw peas and pea protein isolate was 14 units. The other seven units which could not be attributed to ANFs could possibly be related to other factors such as antigenicity of the pea protein. True ileal and faecal protein digestibility of peas and common beans were measured using the ^{15}N dilution technique. The results of this study are presented in Chapter 4. The apparent ileal protein digestibility of the raw summer and winter pea varieties were 79% and 74%

respectively, the true protein digestibilities were between 93% and 95%. The apparent faecal protein digestibility was 85% for both varieties, the true faecal protein digestibility of both pea varieties was between 96% and 98%, respectively. These results indicate that native raw pea protein is highly digestible, and that digestion is nearly completed in the small intestine. The low apparent protein digestibility must be almost completely related to the secretion of endogenous protein. Common beans were studied in toasted form because the piglets refused the diets when raw Phaseolus beans were included. These beans were tested only for ileal and not for faecal digestibilities. Apparent ileal protein digestibility of the toasted beans was about zero. The true protein digestibility was about 66%. The very low apparent ileal protein digestibility must therefore, be related to a very high secretion of endogenous protein. It was concluded that measurements of true protein digestibility are important for (bio)technologists. In order to improve protein digestibility it is necessary to know whether the treatments need to be focussed on the inactivation of ANFs and elimination of e.g antigenicity or to changes in protein structure. Our results show that with peas it is relevant to pay attention to factors causing an increased secretion of endogenous protein and not to the protein structure. With common beans, treatments should be directed to both: to factors causing an increased secretion of endogenous protein and to the protein itself. It was demonstrated (see GENERAL DISCUSSION), that when raw pea and soya protein are fed to piglets, guinea pigs and veal calves, trypsin activity in the small intestinal chyme and pancreatic activity was reduced and also protein digestibility was decreased. The lower pancreatic activity indicates that the low levels of trypsin inhibitors did not activate the negative feedback mechanism which in turn caused a hypersecretion of pancreatic enzymes. This negative feedback mechanism seems to be not present in pigs, veal calves or guinea pigs. This is in contrast to what is stated for rats. To elucidate which factor primarily is responsible for this observation, ANFs or possibly protein quality further research is required.

Er is in de EG in toenemende mate interesse om meer eiwitrijke zaden te verbouwen. Deze interesse heeft onder andere te maken met factoren als:
- er is een graanoverschot in de EG. Door stimulering van de teelt van eiwitrijke zaden kan het areaal granen verkleind worden.
- er is een behoefte om meer zelfvoorzienend te zijn ten aanzien van eiwitrijke veevoedergrondstoffen, teneinde minder afhankelijk te zijn van de sojabonen producerende landen.

Een groep van eiwitrijke zaden die onder Europese klimaatsomstandigheden goed groeien, zijn onder andere peulvruchten, zoals erwten en voerbonen. Echter deze zaden bevatten antinutritionele factoren (ANFs) die de toepassing in de veevoeding in rauwe vorm ernstig kunnen belemmeren. In het kader van dit proefschrift werd nader onderzoek gedaan naar de effecten van ANFs op de nutritionele waarde van erwten en voerbonen. Voor het onderzoek werden ANFs als volgt gedefinieerd: ANFs zijn natuurlijke niet vezel-stoffen die bij opname met het voer negatieve effecten veroorzaken op groei of gezondheid van het dier. In deze definitie zijn vezels uitgesloten omdat ze in de humane voeding als positief gekarakteriseerd worden. In deze definitie zijn ook stoffen uitgesloten die ontstaan door chemische of andere behandelingen. In een literatuurstudie is nagegaan welke ANFs in erwten en voerbonen vóórkomen, welke functie ze in de plant en de zaden hebben en op welke wijze ze negatieve effecten in het dier veroorzaken. Tevens is nagegaan of de huidige analysemethoden voor ANFs adequaat zijn. Het literatuuronderzoek werd gericht op erwten (Pisum sativum) en voerbonen (Phaseolus vulgaris). Het bleek dat in de erwten en rauwe voerbonen dezelfde ANFs voorkomen als in soja (sojabonen en sojameel). Veel onderzoek ten aanzien van het effect van ANFs op de stofwissseling is uitgevoerd met soja. Besloten werd daarom ook de gegevens van soja in het literatuuronderzoek te betrekken. Uit het literatuuronderzoek blijkt onder andere dat de ANFs een beschermende functie in de planten en het zaad hebben. Ze worden daarom wel biopesticiden genoemd. Er zijn sterke aanwijzingen dat de beschermende functie van ANFs in het zaad verband houdt met verstoringen van "stofwisselingsprocessen" bij de "aanvallers" zoals insecten, bacteriën, schimmels, enz. Onder andere is aangetoond dat verteringsenzymen in de darm van insecten worden geïnactiveerd door enzyminhibitoren aanwezig in de plant. Lectinen remmen de activiteit van bacteriën, schimmels en virussen. Ook is aangetoond dat tengevolge van lectinen de larven van kevers gedood kunnen worden. Omdat er overeenkomsten zijn met de stofwisselingsprocessen van mens en dier, mag ervan uitgegaan worden dat de stofwisselingsprocessen in mens en dier op dezelfde wijze verstoord zullen worden.

Uit het literatuuronderzoek werd duidelijk dat in erwten, voerbonen en sojabonen de lectinen en trypsine inhibitoren de belangrijkste ANFs zijn. Trypsine inhibitoren zijn peptiden die zich kunnen binden aan de verteringsenzymen trypsine en chymotrypsine. Door de binding wordt de activiteit van het verteringsenzym geremd. De belangrijkste groepen inhibitoren in erwten, voerbonen en soja zijn de "Kunitz soybean trypsin inhibitor groep" en de "Bowman-Birk trypsin inhibitor groep". De Kunitz inhibitor komt voornamelijk voor in soja, de Bowman-Birk inhibitor ook in

erwten en voerbonen. De Kunitz inhibitor remt voornamelijk trypsine en de Bowman-Birk zowel trypsine als chymotrypsine. Door de inactivering van het trypsine tengevolge van de binding met de inhibitor kunnen er twee effecten ontstaan:

a. door de inactivering van het trypsine in de dunne darm kan het eiwit minder goed worden afgebroken.
b. als reactie op de verlaagde trypsine activiteit in de dunne darmchymus worden endocrine cellen in de mucosa gestimuleerd tot het produceren van het hormoon cholecystokinine-pancreozymine (CCK-PZ). Door een stijging van het gehalte aan dit hormoon in het bloed wordt de pancreas gestimuleerd tot een verhoogde secretie van de enzymen trypsine en chymotrypsine. Op deze wijze tracht de pancreas de inactivering van genoemde enzymen te compenseren. Door de gestimuleerde pancreas secretie komt er meer endogeen eiwit in de vorm van enzym-eiwit in de dunne darm. De produktie van deze enzymen gaat ten koste van aminozuren die voor de eiwitaanzet in het dier benut zouden kunnen worden. De pancreasenzymen zijn met name rijk aan S-houdende aminozuren. Door een overmatige produktie aan trypsine en chymotrypsine kan er een tekort aan deze aminozuren ontstaan.

De verlaagde eiwitvertering tengevolge van de remming van verteringsenzymen en de verhoogde secretie van pancreas enzymen resulteert er in dat de schijnbare eiwitvertering verlaagd wordt. Bij kleine dieren, zoals ratten, muizen en kuikens, is vastgesteld dat er tengevolge van de extra stimulering van de pancreas een hypertrophy (vergroting) en hyperplasia (vermeerdering van cellen) kan optreden. Lectinen zijn peptiden die worden gekenmerkt door de eigenschap zich te kunnen binden aan suikers. Lectinen kunnen verschillen in affiniteit voor suikers. Een bekend effect van lectinen is dat zij zich kunnen binden aan suikers in glycoconjugaten van de rode bloedcellen waardoor er haemagglutinatie ontstaat. Het oppervlak van darmepitheel bevat eveneens glycoconjugaten. Afhankelijk van de suikersamenstelling van deze glycoconjugaten kunnen lectinen zich hier aan binden. Tengevolge van deze binding kunnen er morphologische en functionele veranderingen in de darmmucosa ontstaan, waardoor de vertering en absorptie van nutriënten verstoord wordt. Ook kan de permeabiliteit van de darmwand vergroot worden tengevolge waarvan peptiden vanuit het darmlumen de bloedbaan kunnen bereiken waardoor er immunologische effecten ontstaan. Tengevolge van de verstoorde vertering en absorptie ontstaat er een rijker substraat voor bacteriën waardoor de bacteriële ecologie in zowel de dunne als de dikke darm verstoord kan worden. Niet alle lectinen zijn in de zelfde mate schadelijk. Lectinen van voerbonen zijn zeer schadelijk, lectinen van erwten zijn aanzienlijk minder schadelijk, terwijl lectinen van tomaten onschadelijk zijn. In erwten, voerbonen en soja kunnen nog andere ANFs voorkomen, zoals tanninen, alpha-amylase inhibitors, flatulentie factoren en fytine zuur. Tanninen komen in bontbloeiende variëtieiten voor. Bij erwten en voerbonen is deze factor meestal van ondergeschikt belang. Andere factoren komen meestal in zulke lage doseringen voor dat ze als weinig relevant beschouwd kunnen worden.

Uit het literatuur onderzoek bleek dat er nog veel onduidelijkheden omtrent ANFs zijn.

- Veel ANF-onderzoek is uitgevoerd met kleine dieren, zoals ratten, muizen en kuikens. Een belangrijke vraag is of de resultaten verkregen met deze diersoorten geëxtrapoleerd kunnen worden naar het varken. Besloten werd dit onderwerp als onderdeel van het proefschrift te bestuderen (Hoofdstuk 2).

- Veel onderzoek is uitgevoerd met voedering van het complete zaad. Deze zaden echter bevatten altijd meer dan één ANF, bovendien zijn er andere fracties aanwezig zoals het eiwit en de koolhydraten. De negatieve effecten die in deze proeven verkregen werden, kunnen dus het gevolg zijn van meerdere factoren. Hierdoor is er geen goed inzicht in welke mate de verschillende factoren een bijdrage leveren aan het negatieve effect. Dit aspect werd met erwten als model, bestudeerd (Hoofdstuk 3).
- Het is niet bekend of een lage schijnbare eiwitvertering uitsluitend dient te worden toegeschreven aan de ANFs of dat het eiwit zelf ook minder goed verteerbaar is. Dit aspect werd onderzocht in het onderzoek beschreven in hoofdstuk 4.

Het onderzoek naar de ANF-effecten in verschillende diersoorten (Hoofdstuk 2), toonde duidelijk aan, dat jonge biggen gevoeliger en ten opzichte van bepaalde criteria anders reageren op ANFs in erwten en voerbonen dan ratten en kuikens. Bij voedering van voerbonen was de groei en de vertering bij jonge biggen aanzienlijk meer verlaagd dan bij ratten en kuikens. Het pancreasgewicht was bij ratten en kuikens verhoogd, maar bij de biggen verlaagd of niet beïnvloed. Het gewicht van de milt en de thymus was bij de biggen afgenomen, terwijl bij de ratten en kuikens geen veranderingen in gewichten werden waargenomen. Bij voedering van erwten was er een groeivertraging bij de biggen, maar niet bij kuikens en ratten. Het pancreasgewicht was tengevolge van de erwtenvoedering toegenomen bij ratten en de kuikens, maar niet bij biggen. De gewichten van de milt en de thymus waren bij geen van de drie diersoorten beinvloed door de voedering van de erwten. De toename van het pancreasgewicht bij de ratten en de kuikens tengevolge van de voedering van erwten en voerbonen kan worden toegeschreven aan trypsine inhibitoren. Dat er bij biggen geen vergroting van de pancreas optreedt is in overeenstemmming met de literatuur. De verschijnselen van atrofie ten aanzien van de milt en de thymus waargenomen bij voedering van voerbonen, wijzen er op dat het immuunsysteem beïnvloed kan zijn. De resultaten toonden aan dat ratten en kuikens geen goede modellen zijn voor het bestuderen van ANF-effecten in biggen. ANF-onderzoek dient dus met het doeldier uitgevoerd te worden. Voor onderzoek bij de mens wordt in veel gevallen de rat als model gebruikt. Het is de vraag of ratten wel het meest geschikte diermodel voor de mens zijn. In de literatuur is beschreven dat het varken van alle gedomesticeerde dieren het meest met de mens overeenkomt ten aanzien van de fysiologie van het maag-darm kanaal, de voeding en de grootte. Mede gezien onze resultaten is het aan te bevelen het varken als model voor de mens te gebruiken.

Ten behoeve van het onderzoek naar de factoren welke in de erwten verantwoordelijk zijn voor de negatieve effecten op de groei (hoofdstuk 3), werden de volgende fracties uit rauwe erwten bereid: een eiwitisolaat waaruit de ANFs en koolhydraten waren verwijderd, een eiwit-isolaat met zeer hoge ANF-activiteit (ANF-concentraat) en een fractie bestaande uit een mengsel van oplosbare en onoplosbare koolhydraten, vrij van ANFs. Uit het onderzoek uitgevoerd met deze fracties bleek, dat de schijnbare ileale eiwitverteerbaarheid van rauwe erwten 14 eenheden lager was dan van het eiwitisolaat waaruit de ANFs en koolhydraten verwijderd waren. In vervolgonderzoek bleek dat de koolhydraten van erwten toegevoegd aan rantsoenen, geen negatief effect op de schijnbare ileale eiwitvertering veroorzaken. Door toevoeging van koolhydraten van erwten aan voeders was de passage van ileumchymus wel aanzienlijk verhoogd. Dit effect moet

waarschijnlijk geassocieerd worden met een toename van osmotisch active componenten in de darminhoud. Werd het ANF-concentraat aan het voeder toegevoegd dan daalde de ileale eiwitvertering met 7 eenheden. Hieruit kan geconcludeerd worden dat ongeveer de helft van het verschil in ileale vertering tussen rauwe erwten en erwten eiwit-isolaat verklaard kan worden uit de ANFs. Het andere deel van het verschil in de schijnbare ileale eiwitvertering moet mogelijk toegeschreven worden aan veranderingen in de structuur van het eiwit in het eiwit-isolaat. Het produceren van het eiwitisolaat gebeurt namelijk door manipulatie van de pH. Het moet niet uitgesloten worden geacht dat hierdoor de eiwitstructuur veranderd is.

Het onderzoek naar de ware eiwitverteerbaarheid werd met erwten en bonen uitgevoerd. In een klassieke eiwitverteringsproef wordt gemeten hoeveel van een bepaald eiwit wordt opgenomen en hoeveel met de mest (faecale verteerbaarheid) of aan het einde van het ileum (ileale verteerbaarheid) wordt uitgescheiden. Het totaal uitgescheiden eiwit bestaat uit enerzijds onverteerd voereiwit en anderzijds uit endogeen eiwit, afkomstig van verteringsenzymen, mucinen, bacteriën, enz. De eiwitverteerbaarheid berekend op basis van uitscheiding van totaal eiwit, wordt de schijnbare verteerbaarheid genoemd omdat ze geen exacte maat is voor de ware vertering van het eiwit. De ware verteerbaarheid kan worden berekend door de schijnbare eiwitverteerbaarheid te corrigeren voor het aandeel van het endogeen eiwit. In ons onderzoek werd de hoeveelheid uitgescheiden endogeen eiwit bepaald met de ^{15}N verdunningstechniek. Dit wordt gedaan door de biggen tijdens de proefperiode ^{15}N-L-Leucine continu in het bloed te infuseren. Door deze infusie worden alle eiwitten in het dier, dus ook het endogeen eiwit, gelabeld met ^{15}N. Aangenomen wordt dat de verhouding van ^{14}N:^{15}N in de weefsels en het endogeen eiwit overeenkomt met die in de aminozurenpool in het bloed. Door analyse van de verhouding ^{14}N:^{15}N in de faeces of ileuminhoud kan nu berekend worden welk deel van de totale N afkomstig is van het endogeen eiwit. Met dit gegeven kan de schijnbare eiwitverteringscoëfficient gecorrigeerd worden voor het aandeel van het endogeen eiwit. Het onderzoek werd uitgevoerd met twee erwten rassen: de zomererwt FINALE met lage gehalten aan trypsine inhibitoren en de wintererwt FRIJAUNE met hoge gehalten aan trypsine inhibitoren. De schijnbare ileale eiwitverteerbaarheid voor de twee variëiteiten was 79 en 74% en de schijnbare faecale eiwitverteerbaarheid 85% voor beide variëteiten. De ware ileale eiwitverteerbaarheid was 95% en 93%, en de ware faecale verteerbaarheid was 96 en 97%, respectievelijk. Deze resultaten tonen aan dat de ware verteerbaarheid van het natieve rauwe erwteneiwit zeer hoog is. Ook bleek dat het eiwit van de erwt vrijwel volledig in de dunne darm wordt verteerd. De lage schijnbare verteerbaarheid van het rauwe erwteneiwit dient voor het grootste deel te worden toegeschreven aan een overmatige uitscheiding van het endogeen eiwit. Nader onderzoek zal moeten aantonen of de overmatige uitscheiding aan endogeen eiwit geheel kan worden toegeschreven aan ANFs of dat andere factoren mede een rol gespeeld hebben. Het onderzoek met de voerbonen werd uitgevoerd met stoom behandelde (getoaste) bonen. De opzet was het onderzoek met rauwe bonen uit te voeren. De voeropname was echter zeer slecht met de rauwe bonen. Besloten werd de bonen te toasten gedurende 40 minuten bij 102°C. Door de toasting was de voeropname verbeterd. Van deze getoaste boon werd alleen de ileale verteerbaarheid vastgesteld. De schijnbare ileale eiwitverteerbaarheid was ca. -4% terwijl de ware verteerbaarheid ca 66% bedroeg. Berekend kon worden dat per 100 gram eiwit opname ca. 34 gram

onverteerd voereiwit en ca. 70 gram endogeen eiwit het einde van het ileum passeerde. Ondanks de toasting was er dus een zeer hoge uitscheiding aan endogeen eiwit. Met ongetoaste bonen kan een nog grotere endogene uitscheiding verwacht worden. Deze resultaten verklaren waarom in de proeven beschreven in hoofdstuk 3 de biggen in gewicht afvielen wanneer rauwe bonen verstrekt werden.

In de "GENERAL DISCUSSION" is nader ingegaan op de resultaten die in ons onderzoek ten aanzien van de pancreas werden verkregen. In één van de verteringsproeven met erwten werd gevonden dat de trypsine activiteit in het pancreasweefsel bij voedering van rauwe erwten lager was dan bij voedering van het erwteneiwitisolaat. Bij voedering van dezelfde rantsoenen aan biggen voorzien van een pancreas cannule, werd met de rauwe erwten een lagere secretie van trypsine gevonden dan bij voedering van het erwteneiwitisolaat. In de literatuur is zowel met biggen als cavia's onderzoek beschreven waarin in een vergelijking van rauw sojaeiwit met getoast sojaeiwit een overeenkomstig effect te zien is, namelijk met rauw sojaeiwit een lagere pancreasactiviteit dan met getoast sojaeiwit. Bij mestkalveren werd een overeenkomstig effect waargenomen, bij voedering van een eiwitisolaat bereid uit soja was de pancreasactiviteit lager dan bij voedering van melkeiwit. In elk van deze proeven werd parallel aan de lagere pancreasactiviteit een verlaging van de eiwitverteerbaarheid geconstateerd. In een andere proef met mestkalveren, waarin geen verteringsmetingen werden uitgevoerd, werd eveneens een lagere pancreas activiteit gemeten wanneer melkeiwit gedeeltelijk werd vervangen door sojabloem. In een viertal proeven met jonge biggen, waarin rauwe soja in het voeder was opgenomen, en in een proef met mestkalveren, waarin sojabloem werd verstrekt, werd ook de trypsine activiteit in de dunnedarminhoud gemeten. In beide proeven was met de voedering van sojaprodukten de activiteit van trypsine in de dunnedarminhoud verlaagd. De verlaagde activiteit van trypsine in de dunnedarminhoud kan worden toegeschreven aan de remmingsactiviteit van trypsine inhibitoren. In de literatuur wordt beschreven dat er bij ratten en andere kleine proefdieren tengevolge van een verlaagde activiteit van trypsine in de dunne darminhoud een negatieve terugkoppeling optreedt waardoor de secretie van pancreas enzymen gestimuleerd wordt. De resultaten van dit proefschrift wijzen uit dat dit effect niet optreedt bij de biggen, cavia's en mestkalveren. De waargenomen verlaagde eiwitverteerbaarheid kan verklaard worden uit de verlaagde proteolytische activiteit in de dunne darm en de verlaagde secetie van pancreasenzymen. In de "GENERAL DISCUSSION" zijn verschillende factoren genoemd die mogelijk verband houden met het feit dat het negatieve terugkoppelings mechanisme niet functioneerde bij de biggen, cavia's en mestkalveren. Recentelijk is gevonden dat de trypsine en chymotrypsine niet rechtstreeks het terugkoppelings mechanisme activeren maar dat een speciaal eiwitmolecuul hierin als medium functioneert. Het kan zijn dat dit eiwitmolecuul niet geactiveerd wordt in genoemde diersoorten, waardoor de pancreas niet gestimuleerd zou kunnen worden. Ook kan niet geheel uitgesloten worden geacht dat door de verlaagde trypsine activiteit in de dunne darm het eiwit onvolledig wordt verteerd. Hierdoor zouden er onvoldoende aminozuren ter beschikking kunnen zijn om de pancreas optimaal te laten functioneren. Nader onderzoek zal moeten uitwijzen welke factoren voor genoemde waarneming primair verantwoordelijk zijn.

Johannes Huisman werd geboren op 8 augustus 1938 te Garderen. Na de lagere en middelbare landbouwschool volgde hij in de avonduren de MULO. Vervolgens werd aangevangen met een HBS-studie die door omstandigheden niet voltooid kon worden. In 1974 slaagde hij cum laude voor het diploma Register Ingenieur. In 1985 werd aan hem volledige vrijstelling verleend voor het propaedeutisch examen in de studierichting T20 aan de Landbouwuniversiteit. In 1987 werd aan hem voor de zelfde studierichting vrijstelling van het doctoraal examen verleend. Van 1 juli 1960 tot 1 mei 1984 was hij verbonden aan het ILOB (Instituut voor Landbouwkundig Onderzoek van Biochemische produkten). Sinds 1 mei 1984 is het ILOB opgenomen in de TNO-organisatie onder de naam TNO-Instituut voor Diervoeding en Fysiologie (ILOB-TNO). De huidige functie is wetenschappelijk medewerker en hoofd van de sectie voedingsfysiologie.
In zijn vrije tijd is hij amateur archeoloog.