CRAMBE MEAL: EVALUATION, IMPROVEMENT AND COMPARISON WITH RAPESEED MEAL

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buitengewoon hoogleraar op het vakgebied van de veevoeding in het bijzonder de voeding van herkauwers.

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CRAMBE MEAL: EVALUATION, IMPROVEMENT AND COMPARISON WITH RAPESEED MEAL

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Proefschrift

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Crambe abyssinica has gradually been introduced in agriculture as a new oil-bearing crop. Its oil contains 55 to 60% erucic acid (C22:1, Δ13), desirable as lubricants, plastic additives or as a raw material for chemical synthesis. The defatted meal has high protein content which provides potential as an animal feed. However, crambe seeds contain glucosinolates as a negative factor in nutrition. The aim of this study was to obtain a clear view of the possibilities and constraints of crambe by-products as feedstuff. The investigation showed the decorticated and defatted crmabe meal contains nearly 50% protein with an amino acid profile similar to rape. Crambe meal has low contents of cell wall constituents and high energy digestibility in both rats and pigs. The seed pericarp is fibrous and therefore poorly digested in cows. The level of glucosinolates in crambe seed is higher than in traditional rapeseeds, with an epi-progoitrin domination. Several approaches were investigated to remove crambe glucosinolates, which revealed possibilities and inclusion levels in animal feeding. Furthermore, two studies were carried out on rapeseed meal concerning detoxification treatments and their effect on the nutritive value. The final chapter discussed crambe meal's perspectives and future research areas.
PhD thesis, Department of Animal Nutrition, Wageningen Agricultural University (WAU), Haagsteeg 4, 6708 PM Wageningen, The Netherlands.

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Stellingen

1. Investigation of crambe is not only justified by its high content of erucic acid, its oil-free residue with a protein content of 50% is an equally good reason. *This thesis.*

2. The higher digestibility for non-ruminants of crambe meal as compared to rapeseed meal is caused by its low proportion of seedcoat. *This thesis.*

3. The finding that heating makes crambe or rapeseed meal more palatable to livestock can only partly be explained by the destruction of glucosinolates and sinapine. In: *Chapter 6 and Can. J. Anim. Sci. 1993, 73:679-697.*

4. Glucosinolates in animal feeds can be decomposed and subsequently evaporated by steaming. *This thesis.*

5. Detoxification by extraction makes crambe or rapeseed meal more palatable for animals, part of their nutritional value is also lost. *This thesis.*

6. Increased heating not only decreases the contents of lysine and cystine in crambe and rapeseed meal, but also lowers their digestibility. *This thesis.*

7. Because the glucosinolates in Crucifare plants act as protectants against insects, their potential as natural pesticides should be investigated.

8. Nutritionists can give an important contribution to setting long-term breeding goals by plant breeders.

9. Because the country produces the largest quantity of rapeseed in the world and conventional rapeseed species are still predominating, rapeseed research is particularly relevant for China.

10. In China, a shift from conventional rapeseed to double low varieties, together with improved seed processing, will not only improve human health, it will also increase the amount of protein available for livestock by 20-40%.

11. When social welfare, security and organization are taken as criteria, socialism is more easily recognized in countries like Denmark and the Netherlands than in those socialist labeled countries.

12. The complicated, bureaucratic and costly visa granting procedure is a good example that the Netherlands is not a highly developed country in every aspect.

13. The population of China benefits more from persuading them to eat fibrous bread and unpolished rice in order to improve human health and food supply, than placing emphasis on observing human rights.

PhD thesis: *Crambe meal: evaluation, improvement and comparison with rapeseed meal.*
By Yonggang Liu, 1994-9-12

Preface

This thesis is the result of several years of research which was intensified over the last three years. Its successful completion is not only a scientific accomplishment, but also an achievement of co-operative effort between institutions in three countries: People's Republic of China, The Netherlands and Denmark. It can be viewed as an illustration of an ancient Chinese proverb which states "More logs make a bigger fire." Among the many people and organizations who have contributed to this project, I am particularly indebted to the following ones:

I am most grateful to Professor Dr Ir S. Tamminga, my Dutch promoter. At the beginning of 1992, while working at the Research Institute for Livestock Feeding and Nutrition (IVVO-DLO) in the Netherlands, I wrote to him and inquired about the possibility of studying for a Ph.D. at Wageningen Agricultural University. He was quick and enthusiastic in his response and promptly arranged my study. My gratitude to him stems not only from the opportunity he offered me, but also from his constructive contribution to the thesis, his invaluable encouragement and general concern. Whenever there were problems, he gave a positive impulse when it was needed most. I can not imagine that the thesis could be completed without his consistent prodding. I also owe very much to Professor Dr B.O. Eggum, my Danish promoter. He warmly accepted me and organized a period of study at the National Institute of Animal Science (NIAS). His support and scientific contribution ensured the finalization of this thesis. Moreover, his working spirit, efficiency, open mind and friendly attitude all together made himself outstanding and unforgettable.

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I was extremely fortunate to meet retired Dutch Professor Dr Ir A. J. H. van Es. I stayed in his home on several occasions. He is not only highly knowledgeable in animal and human nutrition, in which he guided me in great detail, but also he and his wife have a special interest in China. We had countless daily discussions on numerous topics that altogether widened my horizons enormously. Their continuous support and encouragement

always strengthened my self-confidence during difficult periods. They deserve all my heartfelt respect and provided me with an example of human kindness I shall never forget. I am indebted to them forever.

In the Netherlands I was assisted by many colleagues at the IVVO-DLO. I especially thank Mr V.A. Hindle, he not only provided valuable co-operation throughout this project, but also critically reviewed each manuscript with the advantage of being a native English speaker. Dr Ir S. F. Spoelstra introduced me to the institute and provided both spiritual and financial support. Ir G.C.M. Bakker kindly accommodated me in Lelystad with Dutch hospitality and friendliness. Mr L.B.J. Sebek spent much time kindly introducing me to the essential techniques at the institute. I will never forget other friendly colleagues at the institute: Dr Ir Y. van der Honing, Dr Ir A. W. Jongbloed, Dr Ir Y. S. Rijpkema, Dr Ir A. M. van Vuuren, Mr B. Smits, Drs H. Everts, Ir N. P. Lenis and Dr Z. Mroz. They accepted me in a friendly atmosphere which was inductive to many useful discussions. I owe a sincere gratitude to Mr R. Terluin, Mr L. H. de Jonge and their co-workers in the metabolism unit and laboratory for their assistance. At the NIAS in Denmark I am particularly grateful to Dr S.K. Jensen and Dr A. Just, for their invaluable collaboration and friendliness.

My acknowledgement should further extend to the Chinese National Education Commission and my native institute: RIAVS in Chengdu, for providing me with the opportunity and financial support to take part in the overseas training, which resulted in my first stay in the Netherlands that initiated the Ph.D. project. Gratitude is also due to the Wageningen Agricultural University as well as the National Institute of Animal Science, for sponsoring my international trips and residence in the Netherlands and Denmark, respectively.

Last, but by no means least, my sincere thanks will go to my dear wife and son for all their love during the painfully long separation; to my parents, parents-in-law, brothers and sisters, for all the inspiration and indirect support. Without their assistance, this study would not have been realized.

Contents

Chapter 1

General Introduction

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General Introduction

In recent years a number of potentially new crops have been considered for introduction in agricultural practice. This is expected to lead to a diversification in traditional agriculture, to a reduction of surpluses and to a broadening of the existing crop rotation schemes. It has been discovered that "novel" oil-bearing plants produce certain fatty acids with unique chemical structures and special physical properties, desirable for industrial applications or as alternative sources of certain important raw materials. The oleochemical industry has expressed great interest in utilization of these renewable vegetable oils with specific fatty acids as ingredients in the production of a range of specialty products (Rexen, 1992; Van Soest and Mulder, 1993).

Over the past years, some of the novel oil-plants have drawn much attention. For example, *Crambe abyssinica* oil contains 55 to 60% erucic acid (C22:1, Δ13), which possesses high stability with high smoke and flash points. It lubricates and remains fluid at relatively low temperatures. These properties make the oil desirable as lubricant, plastic additive or as a raw material for the synthesis of nylon and plastic film, and has potential in other applications (USDA, 1990). *Euphorbia lagascae* yields vernolic acid (Δ12, 13-epoxy, 9c-octadecenoic acid), which can be used in paints and coatings and also as stabilizers in polymers. *Limnanthes alba* contains large amounts of long chain fatty acids (C20 and C22), with a high stability against oxidation. The oil has application in cosmetics, lubricants and plastics. *Dimorphotheca pluvialis* contains conjugated double-bonded hydroxy fatty acid, dimorphecolic acid (Δ9-hydroxy, 10t,12t-octadecadienoic acid), useful for the manufacturing of lubricants, coatings, plasticizers and foam plastics. *Calendula officinalis* yields a high level of calendic acid (8t,10t,12c-octadecatrienoic acid), which displays a high viscosity and strong tendency to air-drying. The oil has been suggested as an alternative source to a special water-proof paint and coating oil (Muuse et al., 1992; Van Soest and Mulder, 1993) that is traditionally produced in China from the tung tree (*Aleurites fordii*, New Encyclopædia Britannica).

Processing these oil seeds results in large quantities of residues, either as cake or meal. Such by-products could have potential in livestock feeding adding a possible source of income; if not, the cost of disposal could be expensive. Therefore, profitable use of the by-products would improve the economics of growing those novel crops.

1. Description of crambe

Crambe abyssinica Hochst. Ex. R. E. Fries is a member of the Cruciferae family, native to the Mediterranean region. The species designation "*abyssinica*" might imply that the centre of origin of this member of the genus *Crambe* was *Ethiopia Abyssinia*, but Vavilov (1946) did not include it among plants considered for the Central Ethiopia Abyssinia. The genus Crambe comprises approximately 20 species (Lessman and Anderson, 1992).

Crambe *abyssinica* is an erect annual herb with numerous branches, growing to a height of 60 to 90 cm depending on the season and the population density. Flowers are white and very small. The spherical siliques are usually one-seeded and indehiscent. Seed weight ranges between 7.0 to 7.5 mg, which is about twice the weight of rape seeds (Whitely and Rinn, 1963). In many aspects crambe resembles rapeseed. It is also a cool-season crop; medium-light to heavy soils that are fertile and well drained are suitable for crambe production. The crop is planted in spring and matures in about 100 days. It has fewer pests than rapeseed (Erickson and Bassin, 1992). Seed output varies depending on many factors. In the USA the production averages 1,700 kg ha^{-1} with a maximum of 2,800 kg ha^{-1} (Dyne et al., 1990). In the Netherlands the seed yields have been registered at between 1,700 and 2,500 kg ha^{-1} on a semi-commercial scale, but the best performance in field trials was 3,500 kg ha^{-1} (Van Soest and Mulder, 1993). The equipment used in rapeseed or soybean harvesting and processing can be applied to crambe and it seems unnecessary to develop special equipment for this crop (Erickson and Bassin, 1992; Carlson et al., 1985).

Generally speaking, the amount of pericarp in whole crambe seeds ranges between 25 to 40%. After decortication, the seeds contain 36 to 54% oil with most samples between 40 and 50% (Earle et al., 1966), of which 55 to 60% is erucic acid, this is the highest among the oil-bearing plants under examination (Erickson and Bassin, 1992).

The cultivation of crambe started as late as 1940's when the first seeds were introduced into the Connecticut Agricultural Experimental Station in the United States (White and Higgins, 1966). The earliest evaluations of a number of strains of crambe began in 1958 and in 1962 in Texas and Indiana, respectively. Later, crambe was evaluated in several other states in the USA (Lessman and Anderson, 1982). Its potential value as an industrial oil crop and favourable agronomic adaptability has led in recent decades to extensive research and cultivation of the crop in many countries in the world (Mazzani, 1954; Brunn and Matchett, 1963; Lessman and Anderson, 1982). More recently, crambe has been cultivated on semi-commercial or even commercial scale on thousands hectares in both Europe and North America for the production of erucic acid (Abbe, 1993, Van Soest and Mulder, 1993).

2. Nutrition and toxicity of crambe by-products

Information concerning the chemical composition of crambe meal dates from the 1960s, thereafter more data became available from initial analyses on the meal's chemical components. Because the crop seeds are usually harvested as pod-intact, decortication is required. The crambe pericarps are rich in fibre and contain little protein. Nearly half of the decorticated and defatted seed material is crude protein with 5.5 g lysine per 16 g N (Carlson and Tookey, 1983). The meal contains 8-9% ash, 6-7% crude fibre (Baker at al., 1977b). Carbohydrates remain largely unclear. There is evidence to suggest that crambe by-products may be useful dietary ingredients for beef (Perry et al., 1979; Carlson and Tookey, 1983 and Caton et al., 1993) and dairy cattle (Liu et al., 1993). For monogastrics, crambe meal was primarily evaluated in rats, the results showed that the protein efficiency ratio (PER) was even higher than that of casein (Pereira et al., 1981).

As a cruciferae plant, Crambe seeds contain high levels of glucosinolates, ranging

between 55 and 70 μmol g^{-1} seeds grown in the Netherlands (Steg et al., 1994). The content in the decorticated and defatted meal varies between 50 and 160 μmol g^{-1} (Anderson 1993 and Liu et al., 1994a), this is much higher than in conventional rapeseed meals. Only traces of glucosinolates were found in the pericarps (Earle et al., 1966; Liu et al., 1994b). The high glucosinolate level creates a problem to the introduction of crambe by-products for livestock feeding. Hesketh et al. (1963) showed that the inclusion of crambe meal in chicken diets caused a depression in feed intake and growth rate, and Van Etten et al. (1969) reported the toxins in crambe meal to be lethal to rats. In addition, there are numerous other evidences showing the toxicity of the glucosinolates present in rapeseed (Tookey et al., 1980). For instance, trials with rats showed that the purified epi-progoitrin significantly decreased protein biological value (Bjerg et al., 1989). Several authors conducted experiments to decompose or remove the glucosinolates from crambe meal, including methods as: toasting with ammonia (Kirk et al., 1966a), with sodium carbonate (Mustakas et al., 1968) or with other chemicals like ferrous sulphate (Kirk et al., 1971); microwave treatment (Medeiros et al., 1978; Lessman and Kirleis, 1979), irradiation (Lessman and McCaslin, 1987) and aqueous extraction (Kirk et al., 1966b; Mustakas et al., 1976; Baker et al., 1977a and Kirleis and Brown, 1980) were also considered. These treatments in general reduced the toxicity of the meal with variable effect, but this was accompanied by nutrient losses or high costs.

3. Aims and motivation of present study

Domestication and development of conventional oilseed crops like rapeseed or soybean took place over thousands of years before the properties of these crops were generally understood. In contrast, crambe was not exploited until very recently, neither its oil nor its by-products. Many aspects of crambe remain unknown. Since investigations have mainly focussed on crambe oil and its agronomy, evaluation on the by-product utilization in livestock feeding has attracted only limited attention, and current knowledge of crambe by-products is neither complete nor conclusive. Since the increasing demand for a new source of erucic acid could make crambe an important crop during the next decades, more research on its by-products is therefore considered necessary in order to obtain a clear view of their possibilities and constraints as livestock feeds. Available information requires a critical review; more data concerning nutritive composition and toxicity are essential; detoxification methods should be carefully examined and a selection is needed to provide effective options in processing and utilization of the final products. Moreover, the digestibility and availability of nutrients to the livestock should be studied in more detail.

4. The outline of the thesis

In accordance with the objectives of this study, the first chapter gives a general introduction followed by a literature review as the second chapter. Chapter 3 contains a description of the chemical composition and a laboratory evaluation of the by-products as feedstuffs; Chapter 4 elucidates rumen degradation and intestinal digestion of the by-products in dairy cows; information on crambe meal digestion in monogastrics is presented in Chapter

5; Chapter 6 illustrates the removal of the glucosinolates in crambe meal by various treatments. Although comparisons between crambe, rapeseed and other oilseed meals were already made in these chapters, two special studies were conducted into detoxification treatments and their effect on the nutritive value of rapeseed meal. Descriptions of these studies are given in Chapters 7 and 8. The final chapter, Chapter 9, contains a general discussion of the results.

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Chapter 2

Crambe Meal: A Review of Nutrition, Toxicity and Effect of Treatment

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Crambe Meal: A Review of Nutrition, Toxicity and Effect of Treatment

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Abstract

Processing of *Crambe abyssinica* for oil production results in a considerable quantity of residues which could be utilized as animal feed. Dehulled crambe meal contains approximately 46% protein with a well-balanced amino acid profile. Experiments with rats and mice indicate that the protein quality of crambe protein is comparable to casein. Unfortunately, crambe meal contains 8-10% glucosinolates and an endogenous hydrolyzing enzyme (thioglucosidase, TGSase) which limits its feeding potential. Native levels of such toxic substances have been shown to be lethal to mice, rats and chickens when fed at increased dietary levels. Heat-toasting, chemical and micro-wave treatments are able to decrease the content of glucosinolates considerably, but meal treated as such is still considered unsuitable for monogastric animals because some toxic products from hydrolysis of glucosinolates are left in the meal. Irradiation treatment could provide an alternative to decrease glucosinolates content, but more research is needed in this field. Water-washing after TGSase inactivation has proved effective in the removal of most glucosinolates as well as their hydrolysis products. However, other soluble fractions in the meal may also be partly washed away during the washing procedure. This treatment is costly and may create problems for the environment. Information on nutritional evaluation of crambe meal and effect of treatments are reviewed in this paper.

1. Introduction

Crambe abyssinica Hochst ex R.E. Fries is an annual herb of the family Cruciferae, native to the Mediterranean region. It contains high levels of erucic acid in oil, which can be used in many purposes: rubber additives, plastics, coatings, lubricants, etc. When broken-down to lower molecular-weight chemicals it can be used in the production of polymers, such as plastic film (Wolff, 1966).

Approximately 46% of the dehulled crambe seed weight is oil, of which the erucic acid content is as high as 55-60%. Before oil extraction, the dehulled seeds contain almost 30% protein, which has been shown to have a good amino acid profile and promising nutritional quality (Carlson and Tookey, 1983). However, like other Cruciferae crops, crambe seed contains enzyme-responsive glucosinolates, which preclude using the seeds or meals in animal feeding.

Recently, for various reasons, interest in growing crambe as a commercial crop has

increased in several countries, including the United States, Canada, Germany and the Netherlands. Attractive aspects of growing crambe may be: (a) a domestic source of high-erucic oil; (b) an alternative feed protein source resulting from the by-products; and (c) an alternative crop for farmers. Obviously, the economic viability of growing crambe depends on the possibilities and constraints of using these by-products as animal feedstuffs. Research on this subject is in progress within the Dutch Agricultural Research Programme. In this paper, we discuss some aspects of crambe meal, such as chemical composition, anti-nutritional factors and detoxification methods to improve its feeding value, based on data in the literature.

2. Chemical composition and protein quality

2.1 Chemical analysis of crambe

An indication of the composition of whole and dehulled crambe seed is shown in Table 1. Nearly half of the dehulled seed weight is oil. The oil content depends on the dehulling procedure: from well-dehulled seeds we extracted over 500 g oil per kilogram crambe seed harvested in the Netherlands. The amount of crude protein in crambe ranges from 20% in the whole seeds to almost 50% in the dehulled and defatted seed meals (Kirk et al., 1966a; Mustakas et al., 1968; 1976; Baker et al., 1977a; IVVO, unpublished data, 1992).

The protein from crambe has been isolated and named as "crambin" (Van Etten et al., 1965). According to Teeter et al. (1981), crambin consists of a single chain of 46 amino acids with a calculated molecular weight of 4720. Van Etten et al. (1965) reported that amino acids accounted for 85-87% of the meal nitrogen (N) and this figure can be increased to 95-97% if the meal was first subjected to acetone/water (98:2) extraction. Furthermore, the nitrogen in crambe was more soluble than soybean nitrogen at the pH of minimum solubility (pH 3.5-4.5, 40% vs 10%), but a higher pH was required for maximum solubilization of crambe nitrogen. If classified according to solubility in different aqueous solvents, about 12 % of the crambe N is non-protein, and a large portion of the crambe proteins seem to be globulins. In Table 2 the amino acid profile of crambe is compared with protein from rapeseed meal. Data from our own laboratory are also included in this table. The amino acid profile of rapeseed meal is derived from Dutch Feedstuff Table (CVB, 1991). Clearly most of the essential amino acid contents are comparable or even higher in crambe meal. This means that crambe meal could supply more amino acids to animal rations than rapeseed meal because the total protein content is higher. Comparing with soybean meal, the content of sulphur-containing amino acids (Met+Cys) in crambe meal is approximately 4.2 - 4.7 g/16 g N, whereas soybean meal contains only about 2.9 g/16g N.

Information concerning starch, cell-wall ingredients and mineral contents in crambe meal is still limited. Carlson et al. (1985) reported that crambe meal contained 96 g sucrose, 29 g dextrose and 277 g other carbohydrates per kilogram sample. According to our analyses, a sample of dehulled and defatted crambe meal contained 107 g neutral detergent fibre (NDF) and 74 g acid detergent fibre (ADF) per kilogram dry matter. Crambe hulls contribute largely to the fibre content of the whole seed meal. In a sample of partly dehulled crambe

cake we found that 261 g NDF and 212 g ADF per kilogram dry matter. For extracted meal, 10 g kg^{-1} phosphorus and 22 g kg^{-1} sulphur were reported (Van Etten et al., 1965).

Table 1 Average composition of crambe seed and meal (g kg^{-1} in DM)

Constituents	Whole seed	Dehulled seed
Lipids	350	465
Fat-free basis:		
Protein (N×6.25)	310	490
Crude fibre	220	65
Acid detergent fibre	–	75
Ash	75	85
N-free extract	400	355
Glucosinolates	45-70	80-100

Source: Mustakas et al., 1968, 1976; Kirk et al., 1966a; Baker et al., 1977b.

A recently analysed sample of crambe hulls contained about 85 g protein, 417 g crude fibre, and 565 g NDF and 500 g ADF per kilogram dry matter. Their in vitro digestibility of organic matter was found to be only 45% (IVVO, unpublished data, 1992). Approximately 20 g calcium and 10 g phosphorus per kilogram hulls were reported (Lambert et al., 1970). However, the seeds can be easily dehulled using conventional oil extraction facilities to produce a high-protein, low-fibre meal (Carlson and Tookey, 1983; Carlson et al., 1985).

Table 2 Comparison of amino acid composition of crambe meal (CM) and rapeseed meal (RSM).

Amino acid	Range (g/16 g N)		Amino acid	Range (g/16 g N)	
	CM	RSM		CM	RSM
Arginine	5.7-7.3	5.8	Alanine	3.8-4.2	4.5
Histidine	2.2-2.7	2.5	Aspartic acid	6.0-7.6	7.3
Isoleucine	3.7-4.1	4.2	Cystine	2.6-2.8	2.5
Leucine	5.9-6.8	6.8	Glutamic acid	14.2-17.0	18.0
Lysine	4.9-5.7	5.0	Glycine	4.7-5.3	5.2
Methionine	1.6-1.9	2.1	Phenylalanine	3.4-4.0	4.0
Proline	5.5-6.2	5.9	Threonine	3.1-4.6	4.3
Serine	3.5-4.1	4.6	Valine	4.5-5.6	5.5
Tyrosine	2.5-3.0	2.9	Tryptophan	1.0-2.0	1.2

Source: Earle et al., 1966. Baker et al., 1977a and Pereira et al., 1981; CVB, 1991.

2.2 Evaluation of crambe protein

An experiment conducted by Pereira et al. (1981) showed that crambe meal may be an

excellent protein source for rats. Diets containing 18% detoxified crambe meal and 10% protein, supplemented an extra 0.25% L-lysine and/or 0.10 DL-methionine to diets for rats did not improve growth in rats or the ratio of feed to live weight gain, indicating that neither lysine nor methionine was the first limiting factor in the diets. During the beginning of 1960s some authors judged the proportions and availability of amino acids in crambe meal to be adequate for the growth of rats and pigs (Carlson and Tookey, 1983). Owing to the high glucosinolate content of crambe meal, its value as a protein source may largely depend on the extent of detoxification. Several research groups have evaluated crambe protein quality after glucosinolates were removed by extraction with aqueous acetone or with water. Van Etten et al. (1969a) determined protein efficiency ratios (PER) in rats fed crambe meals which had been extracted once or twice with acetone/water (89: 11). Their results are shown in Table 3. The PER of unheated crambe meals were as good as or even better than the casein control. Baker et al. (1977b) also found that heated and water-washed crambe meals had a PER comparable to or higher than the casein control. From this evidence we can conclude that a significant level of high-quality, well-balanced and digestible protein is present in crambe meal.

Table 3
Protein efficiency ratios (PER) for aqueous acetone and water extracted crambe meals (CM) for rats

Diet constituent	Protein in diet	PER[1] (g gain/g protein consumed)
Casein control	11.8	2.50
CM, aq acetone 1[2]	20.1	2.55
CM, aq acetone 2[2]	20.1	2.75
Casein control	11.4	2.50
CM, water wash 1[3]	19.7	2.53
CM, water wash 2[3]	20.8	2.75

1. Adjusted for casein 2.50.
2. Meal autolyzed but not heated; washed once (1) or twice (2) with acetone/water (89:11). Van Etten et al., 1969b.
3. Meal moistened and crisped after defatting; washed on a continuous filter at two meal to water ratios. Baker et al., 1977b.

3. Glucosinolates and aglucon products

3.1 Glucosinolates and their metabolism

Dehulled, defatted crambe meal contains 80 - 100 g kg^{-1} glucosinolates (Table 1), higher than traditional rapeseed meal (30 - 70 g kg^{-1}). More than 90% of the glucosinolates will be transformed naturally into epigoitrin (epi-PG). Its structure and hydrolysis have been detailed by several authors (Tookey et al., 1980; Carlson and Tookey, 1983). In the seed, epi-PG is biologically separated from the glucosinolate hydrolyzing-enzyme system called thio-

glucosidase (TGSase). A reaction between epigoitrin and this enzyme may occur if the seed is crushed, if it germinates, or when the plant tissues are softened (Tookey et al., 1980). Enzymes of this type have been identified as glucoproteins with sulphydryl groups essential to their activity (Tookey et al., 1973). Heat destroys the enzyme. However, Oginsky et al. (1965) and Tani et al. (1974) mentioned that a certain kind of intestinal bacteria (e.g. *Enterobacter cloacae*) was able to exhibit TGSase activity. Thus, ingested epi-PG may still be hydrolysed to aglucon products in the digestive tracts of livestock consuming crambe seed or meal.

Table 4
Performance of rats receiving crambe meal (CM), epigoitrin (epi-PG) or aglucon products

Diet constituent	Percent added	Body wt % of control	Relative organ wt (g/100g body wt)		
			Liver	Kidney	Thyroid
Controls	–	100	2.7-3.5	0.61-0.65	4.4-8.5
CM aq acetone-Extraction	10	105	2.7	0.74	8.0
CM aq acetone-Extraction	30	97	3.4	0.68	8.4
CM, autolyzed to 1.3% vinyl-OZT	10	85*	3.7	0.68	20.8*
Vinyl-OZT	0.23	85*	4.0	0.62	14.7*
Epi-PG	0.85	85*	4.7	0.81*	9.0
CM, no TGSase, 7.6% epi-PG	10	77*	4.56	0.86	12.9*
CM, TGSase activity, 7.6% epi-PG	10[1]	41*	9.3*	1.54*	13.4*
CM autolyzed to 0.8% nitrile mix	10	All animals died within 21 days			
Nitrile mix	0.2	All animals died within 14 days			
Nitrile mix	0.1[2]	17*	5.6*	1.5**	6.1

Source: Van Etten et al., 1969b. * $P<0.01$.
1. Four of 5 rats died within 35 days.
2. Two of 5 rats died within 84 days.

The formation of toxic aglucon products from glucosinolates has been thoroughly studied in crambe seed meal. Products of the epi-PG/TGSase reaction may be any of the following four derivatives: (R)-5-vinyl-oxazolidine-2-thione (vinyl-OZT); 1-cyano-2(S)-hydroxy-3-butene (cyanobutene); erythro- and threo-1-cyano-2(S)-hydroxy-3(R)(S), and 4-epithiobutanes (epithiobutanes), (Daxenbichler et al., 1965; 1967; 1968; Van Etten et al., 1969a). According to Tookey et al. (1980), glucose and an acid sulphate ion are always released as products from such reactions. The organic aglucon portion may undergo an intramolecular re-arrangement following the hydrolysis to give an isothiocyanate. Without such a rearrangement, the aglucon forms a nitrile, often with the loss of sulphur. Alternatively, a re-arrangement to produce an organic thiocyanate may occur. It appears that only one enzyme is necessary to hydrolyze epi-PG, but a number of factors are involved in determining which of the several aglucon products will predominate. The feeding value of crambe meal will depend on the

relative toxicity of intact epi-PG and on the levels of aglucon products present. These products are toxic and have a bitter taste that makes the meal unpalatable.

3.2 Determination of toxicity in raw crambe meal (CM)

Hesketh et al. (1963) fed broilers with raw, dehulled and defatted crambe meal as a protein source. Diets containing 5-42% crambe meal produced growth depressions proportional to the amount of crambe consumed. Similarly, Van Etten et al. (1965) found that rats fed crambe meal at levels of 15-25% of their diets displayed a considerable loss in weight and even died within 90 days. Such toxicity is always observed in rats and chickens fed raw crambe meal which contains both intact glucosinolates and active TGSase (Kirk et al., 1966b; Mustakas et al., 1968, 1976; Van Etten et al., 1969b; Baker et al., 1977b; Pereira et al., 1981). Van Etten et al. (1969b) conducted an experiment to define the toxicity and feeding value of crambe meal. Their experimental design and results are condensed in Table 4. Data in Table 4 clearly show the toxicity of individual aglucon products derived from hydrolysis of glucosinolates in crambe meal. From the above experiments we may explain the toxicity of glucosinolates and their hydrolyzed products as follows: 1) vinyl-OZT seems non-lethal to animals but enlarges the thyroid and impairs iodine-intake; 2) nitriles are the most toxic and even lethal to animals; 3) epi-PG has no direct toxicity to animals but it can be easily hydrolyzed to vinyl-OZT, isothiocyanates or nitriles by the native plant TGSase or intestinal bacteria. Therefore, animals consuming diets containing epi-PG tend to show pathological indications of these three kinds of toxic substances.

4. Detoxification of crambe meal

It is obvious that aglucon products, especially the nitriles, are undesirable constituents of crambe meal. At least thermal inactivation of TGSase without hydrolyzing or decomposing the glucosinolates is necessary. Several methods of detoxification were tested: conventional toasting; processing with chemical additives; irradiation; and water-extraction after inactivation of TGSase.

4.1 Conventional processing (toasting)

In the conventional processing of crambe, moist heat or micro-wave treatment can be used to provide the energy for enzyme inactivation (Medeiros et al., 1978; Lessman et al., 1979; Kirleis and Brown, 1980; Pereira et al., 1981). Such treatment seems to be able to reduce the content of glucosinolates as shown in Table 5. However, steam heating obviously decreased the content of available lysine and increased nitrile level. Meal treated in this way is generally considered unsuitable for monogastric animals at significant dietary levels, unless glucosinolates and aglucon products have been removed through an additional treatment.

4.2 Processing with chemical additives

Kirk et al. (1966b) treated crambe meal during conventional processing steps with ammonia; Mustakas et al. (1968) with soda ash (sodium carbonate). Glucosinolates were reported to be absent from the ammoniated meal; the presence of nitriles was not

investigated. Soda ash treatment left 1.7-3.0% epi-PG and 0.2-0.7% nitriles in the meal (Table 5). Meanwhile, there was a 28% reduction in total lysine (Mustakas et al., 1976). In the ferrous sulphate-treated meals 0.5-0.6% epi-PG and 0.8-0.9% cyanobutene were left. Originally the purpose of these chemical treatments was to destroy glucosinolates and prevent the formation of aglucon products, but substantial destruction of glucosinolates was often counterbalanced by formation of a certain amount of cyanobutane or other aglucon products in the meals (Carlson and Tookey, 1983).

An intensive study of the chemical treatment of rapeseed meal was reported by Fenwick et al. (1986). They indicated that for rapeseed meal, the most effective combination of 5% alkali plus 1% ferrous sulphate, reduced the total glucosinolate content by 80%, but simultaneously very high (>30 μmol g^{-1} defatted meal) levels of nitriles were produced. These results confirm the suggestion that the analysis of nitriles in addition to viny-OZT and isothiocyanates should be used to monitor the effectiveness of processing techniques. For crambe, chemically treated meal had a better feeding value for rats and chicks than untreated meals. However, at a dietary level of 20-30%, the meals still limited growth to 70-80% of the casein fed control animals. Also the thyroids, livers and kidneys of crambe fed animals were often enlarged compared with the control animals (Baker et al., 1977b). In general these percentages (20 to 30% in diet) seem to be higher than those used in practical feeding.

Table 5 Analysis of crambe meal after various treatments

Meal treatment	Nutrients (DM basis), %				Toxins, %		Lysine
	CP	CF	Ash	NFE	epi-PG	Nitriles	g/16gN
None	48.1	4.9	8.5	38.1	9.5	0.10	6.2
Steam cooking	49.4	4.9	8.6	36.7	6.7	0.40	5.4
Soda ash+steam cooking	48.6	4.9	12.8	33.3	1.7	0.70	3.3
Steam cooking plus water-extraction	54.2	6.8	9.6	29.0	2.9	0.16	5.4
Soda+steam cooking plus water-extraction	56.0	6.7	11.0	25.9	0.0	0.20	3.9
Soda plus steam cooking, water extraction twice	59.1	9.1	11.0	20.4	0.0	0.10	3.9

Source: Mustakas et al., 1976.

4.3 Irradiation

A procedure involving irradiation of crambe seed with cesium 137 and cobalt 60 at levels up to 75 and 76 Mrad has been tested by Lessman and McCaslin (1987). A glucose test showed that endogenous TGSase was no longer reactive with glucosinolates at levels of irradiation as low as 50.4 Mrad. Furthermore, the content of erucic acid in the oil showed a slight increase after treatment and other fatty acids were left relatively unchanged, according to the authors. Data about feeding value of irradiated crambe meal will be needed to assess this procedure further.

4.4 Water extraction after inactivation of TGSase

A practical method to detoxify crambe meal may be water extraction. This process consists of conventional or chemical inactivation of TGSase, followed by washing of the meal with water to extract nearly all of the glucosinolates (Mustakas et al., 1976). However, according to Baker et al. (1977a), 20-25% solid losses should be accepted with this procedure. Although the growth rates of chicks and rats fed the single water-washed steam-cooked meal at a 20-30 % ration inclusion rate were not significantly different from the controls, the meal still was not completely devoid of both epi-PG and aglucon products. Growth and organ pathology correlated with the levels of these constituents. A continuous filter for repeated washing of crambe meals gave meals of 50% protein with good amino acid balance and a PER equivalent to casein (Baker et al., 1977b). Only traces of aglucon products and 0.6% or less of epi-PG were detected in the washed meals. Chicks and rats consuming 20-30% of the washed meals in their rations had gains, feed consumption and feed efficiencies ranging from 85 to 100% of the controls. Performance correlated with residual glucosinolate contents in the meals. The procedure of repeated washing with water is shown in Fig. 1.

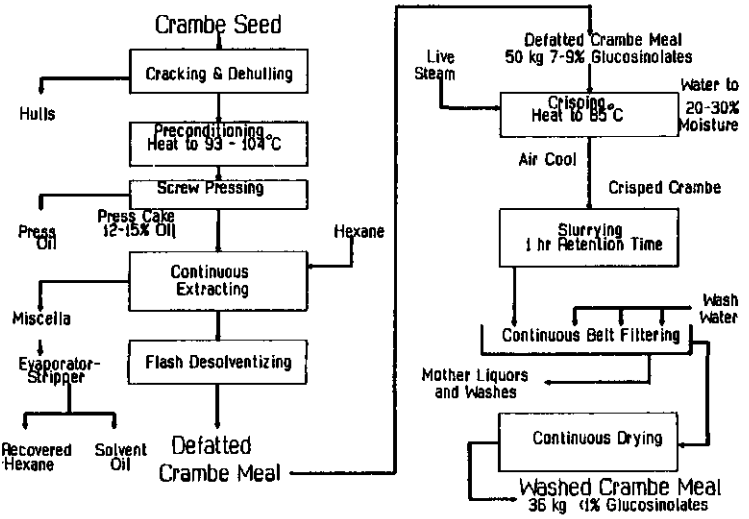

Fig. 1. Flow sheet: continuous water extraction of crambe glucosinolates. Adapted from Baker et al., 1977a.

Another acceptable method to inactivate TGSase is to immerse the whole crambe seeds into hot water prior to washing to extract the toxic substances (Kirleis and Brown, 1980). The meals immersed for 3 min at 100°C to inactivate TGSase followed by washing with water showed nitrogen solubilities and functional properties comparable with microwave-treated products. Compared to soybean meal, both washed meals gave superior gains and feed efficiencies in rats receiving diets containing 10% crude protein and equivalent gains at 15.0 and 17.5% protein. Increasing the levels of microwave and hot water-treated crambe meals in the diets to provide up to 17.5% crude protein had no adverse effect on animal performance, indicating that detoxification of the meals by water washing was effective.

According to the authors, the poorer performance of rats fed on the microwave treated-crambe meals may be related to a significantly lower available lysine level (4.4%) compared to the hot water-treated crambe meal (5.3%), due to some overheating of the seeds during the microwave procedure.

From the results of these studies it is concluded that a practical method of detoxification may be: hot water or microwave treatment to inactivate TGSase in whole seeds, followed by oil extraction and washing the meal in water. Some authors are convinced that epi-PG and/or aglucon products recovered from aqueous washes could find use as pesticides (Tookey et al., 1980) or as special chemicals (Carlson and Tookey, 1983). It should be considered that although the water-washing procedure is effective in the removal of nearly all of the glucosinolates in crambe meal, a considerable part of other soluble fractions in the meal would be washed away simultaneously, making up a total of 20 - 25% of dry matter in the meal (Baker et al., 1977a). This leads to the loss of some potential feeding value on one hand, and poses the problem of recovery of these soluble fractions to prevent environmental pollution. Finally, the high costs involved in buying the extra equipment for detoxification (Baker et al., 1977b) might limit the application of this technique.

For rapeseed processing, an interesting alternative detoxifying approach has been demonstrated by Naczk et al. (1986). They used methanol-ammonia and hexane as a two-phase solvent system with a limited addition of water to extract both toxic substances and rapeseed oil, resulting in a satisfactory reduction in the total content of glucosinolates with less loss of potentially digestible components. Patents on this subject have been drawn up (Schlingmann et al., 1980, 1982). Trials are needed to verify whether the techniques described in rapeseed meal are applicable in the defatting and detoxification of crambe.

5. Crambe meal for livestock

5.1 Monogasitric animals

The effect of crambe meal incorporated into poultry rations has been reported in a number of studies. Baker et al. (1977b) observed that chicks fed 20% conventionally washed crambe meal showed lower weight gains (14-16%) than those fed soybean meal. Feed efficiency ranged from 91 to 95% of that of control animals. When crambe was washed, usually no thyroid problems were reported. If not washed, serious problems due to epi-PG or aglucon products were observed (Baker et al., 1977; Kramer et al., 1983; Sim et al., 1985). Kramer et al. (1983) reported that the presence of sinapine in crambe meal taints brown-shelled eggs with a fishy taste. Therefore, the authors recommended that the level of crambe meal in rations for layers should not exceed 5%.

Incorporating crambe meal without detoxification into diets for pigs may have detrimental effects on voluntary intake because of their very sensitive taste. A much better response can be expected from pigs fed a ration containing detoxified crambe meal. Unfortunately, no report concerning pig feeding value of crambe meal has been found so far.

5.2 Ruminants

In situ and in vitro ruminant digestibility data from our own research revealed that dehulled crambe meal may be suitable for ruminants, because its in vitro digestion of organic matter was over 85% (compared to 76% for rapeseed meal, double zero. IVVO, 1992,

unpublished data). Crambe protein was found to be rapidly degradable in the rumen (IVVO, recent experiments).

The depressing effect on voluntary intake of rations containing raw crambe meal also appears to be important in beef cattle, though some publications stressed this particularly in monogastric animals (Appelqvist and Ohlson, 1972; Baker et al., 1977b and Perry et al., 1979). Lambert et al. (1970) compared the performance of steers fed rations containing different amounts of crambe meal for 196 days, their results are listed in Table 6. Cattle receiving crambe meal as the only source of supplemental protein did not perform as well as cattle receiving soybean meal, owing to reduced intake. However, cattle receiving half of their supplemental protein from heat-carbonate-treated crambe meal performed as well as those fed only soybean meal as supplemental protein. A palatability effect still existed in the trial as shown by decreasing daily intake as the amount of crambe meal was increased. The fact that differences in feed efficiency were not significant implies that increasing the proportion of crambe meal in the ration did not reduce its nutritional quality. Animals decreased their daily feed consumption by selection, but it was partly overcome by pelleting rations or blending crambe meal and soybean meal.

Between 1972 and 1977, several long-term (152-182 days) feeding trials were conducted to obtain approval of crambe as a supplemental protein source in beef cattle rations as required by the Food and Drug Administration (FDA) of the USA (Perry et al., 1979). In the first experiment, crambe meal replaced 1/3, 2/3 or all of the soybean meal in a high energy diet. Rate of gain and daily feed intake decreased with increasing levels of crambe meal. These differences were not significant, even when crambe meal supplied all the protein in the diet. In this experiment, the crambe meal (with hulls) was prepared by toasting and contained 25% protein, 21% crude fibre, 1.1% epi-PG and 1.5% nitriles; no TGSase activity was detected. In the second experiment, crambe meal was progressively increased in three rations, with slight increases in total crude protein content. Differences in daily live weight gain were significant ($P < 0.05$), and feed consumption and feed efficiencies tended to decline as the level of crambe meal was increased. The crambe meal used contained 28% crude protein, 24% crude fibre, 1.9% epi-PG , no nitriles and no TGSase activity were detected.

Table 6 Performance of steers fed rations containing crambe meal (196 days)

	Supplementary protein from crambe (%)			
	0	33	67	100
Ration ingredients (%)				
Corn	62.1	61.8	61.9	61.9
Corn cobs	20.0	20.0	20.0	20.0
Soybean meal	9.1	6.0	3.0	–
Crambe meal	–	3.4	6.6	9.8
Protein	11.1	12.0	11.0	11.7
*Daily gain, kg**	1.18	1.12	0.98	0.86
percent of control	–	95	83	73
*Daily feed intake, kg**	9.0	9.0	8.3	6.0
Intake per kg of gain	7.9	8.1	8.1	6.9

Source: Lambert et al., 1970. Ration containing premix of vitamins, minerals, etc. (8.3-8.8%).

* Difference between 0 and 100, $P < 0.01$.

The appearance of epigoitrin or aglucon products in fat, muscle, liver or kidney tissues was studied by Van Etten et al. (1977). Cattle were fed on rations containing 10% crambe meal for up to 30 days. None of these detrimental compounds were detected in body tissues by methods sensitive to 1 ppm. In their unpublished results, no epi-PG or aglucon products were found in rumen fluid from a number of these animals, which seems to suggest that they are quickly destroyed or converted to unknown products on ingestion by cattle.

From above trials we may consider that inclusion of crambe meal in ruminants ration is still hampered by a negative influence on intake. Although relatively high dietary levels of raw or toasted crambe meal could be accepted by beef cattle, it is recommended not to supply more than a half to two thirds of the supplemental protein by crambe meal in beef diet. Application of this meal in ruminant feeding will benefit from a considerable reduction in glucosinolates as a result of proper treatment. The FDA has approved use of solvent-extracted crambe meal in beef finishing rations at a level up to 4.2% of total ration weight. Relative descriptions and restrictions about crambe meal from FDA were mentioned by Carlson and Tookey (1983). Experiments concerning dairy cattle feeding crambe meal are scarce at this stage.

6. Conclusion

Information about protein content, amino acid composition, and numerous animal feeding experiments indicate that crambe meal contains protein of good nutritional quality. The nutrients availability of the meal will increase by proper removal of seed hulls. However, crambe meal contains up to 8-10% glucosinolates and an endogenous hydrolysing enzyme (TGSase) which limits the feeding value of the meal by reducing intake and performance. High levels of inclusion of unprocessed crambe meal in diets for mice, rats and chicks may lead to high mortality.

Traditional heat-toasting and/or chemical treatments considerably decrease glucosinolates as well as TGSase, but the process may also reduce the content of available lysine. Moreover, part of glucosinolates might be converted into nitriles or other aglucon products which are still toxic to animals. Generally, meals treated by these methods may be used as a protein supplement for beef cattle, but are not suitable for monogastric diets at significant dietary levels. Removal of glucosinolates by a washing procedure may result in meals with excellent nutritional quality but some of the nutrients will also be washed away. Feeding experiments suggest that these meals could be used in chicken and rat diets at a practical dietary level. However, studies confirming the value of crambe meal in pig feeding are lacking.

Further processing research should concentrate on the development of dehulling, defatting and detoxification methods that result in a low-fibre and low-glucosinolate meal without jeopardizing the environment.

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Chapter 3

By-products of Some Novel Oil-seeds for Feeding: Laboratory Evaluation

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By-products of Some Novel Oil-seeds for Feeding: Laboratory Evaluation

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Abstract

Thirty-six samples of intact seeds or by-products from expelling or solvent extraction of the novel oil seeds *Crambe abyssinica, Dimorphotheca pluvialis, Euphorbia lagascae, Limnanthes alba* and *Calendula officinalis* were collected and tested for proximate analysis and composition of the mineral, protein and cell wall fractions. Apparent digestibility of organic components in ruminants and pigs was determined in-vitro with rumen fluid and enzyme cocktails. Crambe and limnanthes were also tested for contents of glucosinolates and their derivatives.

Decortication of crambe seeds prior to defatting improved nutritive value as expressed by decreased fibre content (from 402 to 108 g cell walls, determined as neutral detergent fibre (NDF) per kg fat-free dry matter (ffDM)), increased protein (CP) fraction (277 vs. 490 g CP kg^{-1} ffDM) and increased in-vitro digestibility (% of organic matter digested by rumen fluid (dOt): 57.3% vs. 85.0%). As glucosinolate content increases, adequate detoxification is essential to safeguard the animals consuming the feed. Decortication of euphorbia seeds resulted in improvement of feeding characteristics of its by-products (NDF 381 vs. 202 g kg^{-1} ffDM; CP 417 vs. 608 g kg^{-1} ffDM; dOt 55.3 vs. 74.9%). Detoxification of such by-products requires further study. By-products of dimorphotheca showed considerable variation in composition (84-263 g ash, 203-266 g CP and 419-538 g NDF kg^{-1} ffDM) and unsatisfactorily low digestibility (dOt 51.4±5.8%). Limnanthes meal showed a favourable amino acid profile but was less well digested (dOt 67.4%) than expected from the level and composition of the fibre fraction. Calendula meal was poorly digested (dOt 34.9%) due to its extremely high fibre content (731 g NDF kg^{-1} ffDM).

Adequate processing is essential for the profitable inclusion of some of the by-products of novel oil seeds in livestock diets.

1. Introduction

In Europe structural surplus-production of traditional arable crops intended essentially for food production has prompted the search for alternative land usage (Rexen, 1992). One of the options under consideration is growing novel oil seed crops. The oleochemical industry has expressed interest in the utilization of vegetable oils with specific fatty acids as an ingredient in the production of a range of specialty products, such as paints and coatings, detergents, cosmetics, lubricants, flavours, biodegradable polymers, etc. (Van Soest and Mulder, 1993; Muuse et al. 1992; Derksen et al., 1993). A current research programme of the Dutch Ministry of Agriculture, Nature Management and Fisheries (1990-1994) focuses

on the potential of the crops *Crambe abyssinica, Dimorphotheca pluvialis, Euphorbia lagascae, Limnanthes alba* and *Calendula officinalis*.

Given the limited lipid content of the seeds concerned, oil extraction processes will result in considerable volumes of residues accounting for at least 50% and sometimes over 80% of the original seed weight. Adequate utilization of such by-products may contribute substantially to an economically successful introduction and acceptance of the new crop. Moreover, it is obligatory that the residues do not turn into 'waste' and hence increase the burden on the environment.

Currently, in the EC about 30 x 10^6 tonnes of by-products from traditional oilseeds are annually used as feed ingredients for livestock (Eurostat, 1989). This may also provide an attractive market for by-products of novel oil seeds. However, for a successful introduction substantial evidence will be needed concerning their nutrient characteristics, effect on animal performance and quality of animal produce. Literature information on Crambe by-products for feeding has recently been reviewed by Liu et al.(1993). They also summarized the limited literature information on limnanthes (meadowfoam) (Liu et al., 1992). Data on dimorphotheca, euphorbia and calendula is scarce.

This paper reports on the chemical characteristics and in-vitro digestibility of by-products from novel oil seeds studied within the framework of the Dutch National Oil Programme.

2. Materials and Methods

2.1 Samples

Samples of whole or dehulled seeds, press cake after mechanical expelling or meal from solvent extraction were obtained from several sources. Intact seeds were provided by the Centre for Plant Breeding and Reproduction Research (CPRO-DLO) in Wageningen. These seeds were defatted by solvent extraction (s.e.) with petroleum ether (PE 40-60) at the IVVO-laboratory. The Agrotechnical Research Institute (ATO-DLO) in Wageningen supplied cakes after screw expulsion, sometimes followed by PE-extraction. TNO-Nutrition and Food Research in Zeist provided residues from different treatments, including solvent extraction (W.Vernooij, 1992, pers.comm.). In addition, a limited number of samples were received from commercial enterprises. Total sample numbers were 11, 12, 8, 2 and 3 for crambe, dimorphotheca, euphorbia, limnanthes and calendula respectively.

2.2 Laboratory analyses

Where necessary, samples were defatted by flushing with petroleum ether prior to further analyses. After freeze-drying, samples were ground in a Peppink hammer mill to pass a 1 mm sieve. Contents of dry matter (DM), crude ash (ASH), crude protein (CP), lipids (FAT), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), starch (ST) and sugars (SUG) were determined according to IVVO-standard procedures (Steg et al., 1990, Van Vuuren et al., 1991). Calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K) contents were determined spectrophotometrically after ashing at 550° C (ISO

standard procedures). Amino acid analyses were performed as described by Van Vuuren et al.(1992).

Apparent digestibility in ruminants of the feed samples was determined in vitro both with rumen fluid followed by pepsin-HCl (dOt; method of Tilley and Terry, IVVO modification) and with cellulase digestion (dOc; Steg et al., 1994). An estimation of apparent organic matter digestibility in pigs (dOp) was obtained by incubation with commercial enzymes according to Cone et al.(1993). The in vitro determinations were calibrated against the results of in vivo (adult sheep or growing pigs) tested compound feed ingredients.

The total glucosinolates content was analyzed in crambe, limnanthes and rapeseed samples by DLO-State Institute for Quality Control of Agricultural Products (RIKILT-DLO), using high performance liquid chromatography (HPLC), following ISO 9167-1. The contents of vinyl-oxazolidone-thione (OZT) and isothiocyanate (ITC) were determined according to ISO 5504.

The analyses mentioned were selectively applied to the samples under examination.

3. Results and Discussion

Information on proximate composition and mineral content of the samples analyzed is summarized in Table 1. To avoid confusion due to differences in the degree of defatting, data are displayed in fat-free DM. Hemicellulose (HC) contents were calculated as the difference between NDF and ADF; Cellulose (Cell) as the difference between ADF and ADL. For reasons of comparison information concerning rapeseed meal is also given. Table 2 contains information concerning the amino acid composition of the protein. Average and standard deviation of in-vitro digestibility is presented in Table 3.

3.1 Crambe

Contents of ash, lipids and protein in whole seeds compared favourably with literature (Carlson, 1992; Liu et al., 1993). Cell wall characteristics showed considerable variation. This may be due to a naturally occurring variation in hull proportion and seed fill as well as partial loss of hulls during storage and processing. Cell walls in crambe were highly lignified: 361 ± 22 g kg^{-1} NDF was determined as ADL and 501 ± 20 g kg^{-1} was cellulose. The hemicellulose content of 137 ± 17 g kg^{-1} NDF was relatively low. The cell-wall content, determined as NDF ranged from c. 400 g kg^{-1} fat-free DM for whole seeds to 100 g kg^{-1} ffDM for dehulled material. Figure 1 relates contents of cell walls and protein in crambe samples. A strong inverse relationship was observed between CP and NDF. Fat-free hulls contained less than 80 g protein kg^{-1}, dehulled meal almost 500 g kg^{-1}. As in rapeseed, crambe contained low levels of starch and up to 150 g kg^{-1} sugars in fat-free meal. More than 90 g kg^{-1} fat-free DM was recovered as either ash, protein, starch and sugars or polysaccharides, determined as the cell-wall fraction. The mineral content measured in crambe was similar to that suggested by Carlson (1992). Amino acid composition of the protein in whole seeds and expelled cake were found to be very similar. However, prolonged heating of the product accompanied by solvent extraction and/or detoxification may reduce

the biological availability of (in particular) lysine. Compared to literature (Liu et al., 1993; Carlson, 1992), we found low levels of cystine.

A large variation was observed in the in-vitro OM digestibilities, with both rumen fluid and a cocktail of enzymes. Hulls and undecorticated seeds and cakes (sample numbers 1, 5 and 2 respectively) were poorly digested, whereas figures for dehulled, defatted meal (3 samples) were particularly high, even exceeding those of rapeseed meal. However, Bourdon and Aumaitre (1990) observed that dehulling of rapeseed meal increased its digestibility in pigs considerably. Figure 2 relates dOt and dOc of samples with their NDF content. The variation in both digestion parameters was largely explained by the NDF-content of the samples, thus mainly related to the hull fraction. In this study, dOc was systematically higher than dOt. There is no obvious explanation for this, as both methods were calibrated in a similar fashion. However, as sieve-fractionation showed a considerable fraction of crambe disperses in water (Liu et al., 1994b), therefore these differences may be due to the different filtration methods employed. As expected, predicted OM-digestibility of undecorticated products was lower (6.4 ± 4.3 percentage units) for pigs (dOp) than for ruminants.

Limited variation was observed in the total glucosinolate content of whole seeds at 62 ± 5 μmol g^{-1} (5 samples). Dehulled seed contained 89 μmol g^{-1} and dehulled, defatted meal 190 μmol g^{-1}. The glucosinolate content of hulls was low at 0-9 μmol g^{-1}. Retention time and UV-spectrum (diode-array detection) revealed that 95-97% of the glucosinolates in crambe are harmful epi-progoitrin and 3-5% 4-OH-glucobrassicin. The amount of the glucosinolate derivative OZT was measured in samples of decorticated crambe meal, in hulls and in rapeseed meal at 20900; <750 and 800 mg kg^{-1}, respectively. Data for crambe correspond with literature (Liu et al., 1993). Recent Dutch data for rapeseed meal (Borggreve, 1992, pers.comm.) vary from less than 500 mg kg^{-1} (detection limit) to - by exception - 1500 mg kg^{-1}. Data given by Bourdon and Aumaitre (1990) show large variation, even for by-products from 00 rapeseed. Considerable reduction of the glucosinolate level in crambe meal is required in order to justify its safe utilization as feedstuff. Literature (e.g. review of Liu et al., 1993) and current research results provide evidence of appropriate treatments based on either washing procedures or moist heating with additives (Liu et al., 1994b).

3.2 Dimorphotheca

The first 5 samples collected were pressed-cakes. As expelling alone allowed for insufficient removal of the lipids (all cakes contained more than 10% lipids), additional subsequent solvent extraction was performed (7 samples). Prior to processing, separation of the two prevailing seed types: cones and wings, was executed in three experiments. The chemical characteristics of dimorphotheca samples varied greatly (Table 1). Some of the high ash contents observed (up to 263 g kg^{-1}) may be due to contamination with soil particles. Variations in the level and composition of the cell-wall fraction may be a reflection of processing: seed coats were perhaps partly separated from the endosperm during harvesting, drying or further processing. In addition, by-products from wings showed lower and less lignified cell-wall contents than by-products from cones: while for cones the average contribution of ADL to NDF was 220 g kg^{-1}, this was 170 g kg^{-1} for wings. Cell walls were

Table 1
Proximate composition and mineral content (g/kg fat-free DM) in by-products from novel oil seeds. Averages (Avg.) and standard deviation (St.D.)

		Ash	CP[1]	HC	Cell	ADL	St	Sugar	Ca	Mg	P	K
Crambe												
Undecort.	Avg.	77	277	55	200	147	7	83	14.1	2.3	8.5	13.5
	St.D.	5	4	9	2	12	3	9	1.0	.1	.3	1.5
Decort.	Avg.	99	495	46	52	23	12	122	8.3	5.1	17.4	
	St.D.	6	11	17			8	45				
Dimorphotheca												
	Avg.	145	232	93	279	93	6	32	13.2	4.7	7.4	15.6
	St.D.	92	20	12	21	12	2	8	3.0	.3	.9	2.7
Euphorbia												
Undecort.	Avg.	125	417	69	181	131	21	30	25.6	5.8	12.8	10.9
	St.D.	7	17	3	5	6	2	4	3.5	0.2	0.4	0.4
Decort.		112	608	34	103	65	10	48	11.2	8.5	19.7	
Limnanthes												
Meal	Avg.	155	319	52	137	51	9	77	15.0	2.7	8.6	19.6
Calendula												
Meal	Avg.	53	136	132	419	180	3	17	5.8	2.6	3.7	12.0
	St.D.	7	13	25	50	12						
Rapeseed												
Meal	Avg.	81	396	50	119	80	62[3]	105[3]	8.3[3]	4.3[3]	12.7[3]	14.7[3]
	St.D.	1	20	9								

[1] CP = Crude Protein; HC = Hemicellulose; Cell = Cellulose; ADL = Acid Detergent Lignin; St = Starch.
[2] ADL not determined.
[3] Tabulated data (CVB, 1992).

Table 2
Amino acid composition (g per 16 g N) of by-products from novel oil seeds

		LYS[1]	MET	CYS	THR	ILE	ARG	PHE	HIS	LEU	TYR	VAL	ALA	ASP	GLU	GLY	PRO	SER	ΣAZ
Crambe																			
Seed	Avg.	5.3	1.2	1.7	4.4	3.8	6.5	3.6	2.4	6.2	2.7	4.8	4.1	6.2	15.0	5.0	5.4	4.4	83.0
	St.D.	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0	0.1	0.1	0.1	0.0	0.3	0.3	0.1	0.1	0.1	1.2
Press cake	Avg.	5.2	1.5	1.9	4.6	3.9	6.4	3.6	2.5	6.1	2.8	4.8	4.1	6.7	15.4	5.2	5.8	4.6	85.0
Dimorphotheca																			
Cones	Avg.	3.9	1.4	1.3	4.0	4.1	6.7	3.8	2.5	6.4	2.7	4.9	4.4	9.0	15.9	5.4	3.7	5.4	85.6
Wings	Avg.	3.5	1.3	1.2	3.7	4.0	6.1	3.6	1.9	6.2	2.5	4.8	4.2	8.5	14.4	5.0	3.6	4.2	78.8
Euphorbia																			
Seed	Avg.	3.2	2.3	1.0	3.1	4.3	13.0	4.5	2.5	6.0	2.6	5.4	4.2	10.1	14.6	4.2	3.8	5.4	89.4
	St.D.	0.3	0.1	0.0	0.1	0.1	0.8	0.1	0.2	0.1	0.3	0.2	0.1	0.2	0.6	0.3	0.3	0.2	2.1
Limnanthes																			
Meal	Avg.	4.8	1.2	1.7	3.6	3.5	7.7	3.3	2.1	5.9	2.4	4.2	3.9	6.7	12.6	4.9	4.0	3.9	77.0
Calendula																			
Meal	Avg.	1.7	1.0	0.6	3.3	3.9	4.4	3.7	2.3	5.5	2.0	4.7	4.0	7.9	14.3	4.8	1.7	4.1	70.0
Rapeseed[2]																			
Meal		5.0	2.1	2.5	3.3	4.2	5.8		2.5	6.8	2.9	5.5	4.5	7.3	18.0	5.2	5.9	3.6	85.0

[1] LYS, lysine; MET, methionine; CYS, cystine; THR, threonine; ILE, isoleucine; ARG, arginine; PHE, phenyalanine; HIS, histidine; LEU, leucine; TYR, tyrosine; VAL, valine; ALA, alanine; ASP, aspartic acid; GLU, glutamic acid; GLY, glycine; PRO, proline; SER, serine; ΣAZ, sum of amino acids, % of CP.
[2] Tabulated data (CVB, 1992).

composed mainly of cellulose (60±4%). Low starch and sugar contents were observed in wings, cones or mixtures of both. More than 900 g kg^{-1} fat-free DM was recovered as either ash, protein, cell walls or starch and sugars. Contents of Ca, P, Mg and K were not clearly related to the ash content and no significant differences in mineral composition were observed between cones and wings. The protein fraction of the fat-free DM averaged 232 g kg^{-1} with a maximum of 266 g kg^{-1}. These values are considerably lower than data presented by Knowles et al.(1965), presumably for samples containing lower levels of seed coats. Amino acids represented 856 and 788 g kg^{-1} crude protein in cones and wings, respectively (Table 2). The ratios between amino acids differed only marginally. However, the contents of lysine, methionine and cystine were rather low in comparison to rapeseed meal, indicating only a moderate nutritive protein quality to non-ruminants in dimorphotheca by-products.

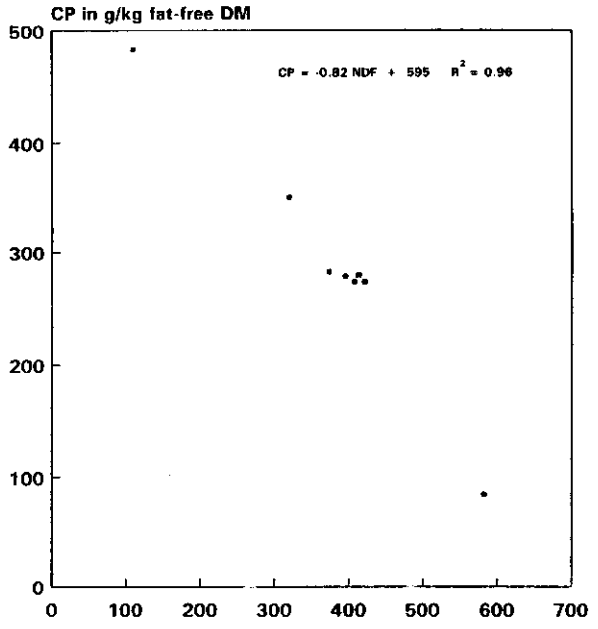

Fig. 1 Crambe: relationship between protein and fibre

The in-vitro OM digestibility showed considerable variation, ranging from 42.7 and 47.2% (cones) to 64.2 and 65.4% (wings) for dOt and dOc respectively. Figure 3 shows the relationship of dOt and dOc with lignin content in the samples. Although correlation of the digestibility parameters was higher with ADL than with other cell-wall characteristics, the variation explained by linear regression was lower than for crambe samples. Average predicted digestibility of the samples in ruminants was rather low (Table 3). However, considerably lower predictions of OM-digestibility were observed for pigs. The difference between dOc and dOp was 15±3 percentage units (6 samples), the highest dOp was determined at 42.2%. The low apparent digestibility of the samples as received presents a clear constraint on the nutritive quality of dimorphotheca by-products, in particular for non-ruminants. However, the data presented by Knowles et al.(1965) suggest that careful removal of the seed coat may result in improved feed quality of the residues. Further study on this aspect is essential to clarify the true potential for nutritive improvement of dimorphotheca derivatives.

Table 3
In-vitro OM-digestibility (%) of by-products from novel oil seeds

		dOt[1]	dOc	dOp
Crambe				
Undecort.	Avg	57.3	60.4	52.9
	St.D.	6.5	5.7	4.0
Decort.	Avg.	85.0	88.2	
Hulls	Avg.	44.5	49.0	
Dimorphotheca				
	Avg.	51.4	53.4	36.9
	St.D.	5.8	5.5	5.7
Euphorbia				
Undecort.	Avg.	55.3	59.3	50.5
	St.D.	1.2	1.2	3.4
Decort.	Avg.	74.9	75.0	79.2
Limnanthes				
Meal	Avg.	66.7	74.3	61.2
Calendula				
Meal	Avg.	34.9	35.7	18.7
	St.D.	3.1	9.6	2.1
Rapeseed				
Meal	Avg.	76.1	78.2	68.0[2]

[1] dOt= incubation with rumen fluid; dOc= enzyme incubation for ruminant feeds; dOp= enzyme incubation for pig feeds.
[2] tabulated data (CVB, 1992).

3.3 Euphorbia

Samples studied included whole seeds (5 samples), press cake, hulls and decorticated and solvent extracted meal. The lipid content of whole seeds averaged 500 g kg^{-1} DM and compared favourably with data of Muuse et al.(1992). By-products from undecorticated seed showed little variation in chemical characteristics (Table 1). The rather high ash content was reflected in high levels of Ca, Mg and P. Dehulling seemed to reduce in particular the Ca content. The undecorticated product contained a substantial level of protein in excess of 400 g kg^{-1}. The cell walls were highly lignified (361 $\pm$ 22 g kg^{-1} NDF being ADL). Dehulling the seeds preceding treatment resulted in a sharp increase in protein content up to 600 g kg^{-1} fat-free matter and an obvious reduction in cell walls. Hulls as such were mainly composed of cell walls (574 g NDF kg^{-1}) yet also contained 200 g protein kg^{-1}. They represented almost 500 g kg^{-1} fat-free residue after lipid removal. Starch and sugar content in Euphorbia accounted for almost all the residual fraction not contained in ash, protein or fibre. The amino acid composition of the 8 samples studied (whole seeds, press-cake, solvent extracted meal and hulls) showed limited variation (Table 2). However, the lysine fraction of the protein in hulls was slightly higher. The protein quality of euphorbia by-products for feeding should be graded as moderate since lysine and cystine fractions are rather low. Liu et

al.(1994a) indicated that the protein is highly soluble and degradable in ruminants.

The predicted ruminant digestibility of OM in undecorticated products was rather low (Table 3) and showed little variation between samples. The low digestibility is due to the low rumen degradability of the cell walls (Liu et al., 1994a). Dehulling increased OM-digestibility considerably. In-vitro digestibility (dOt and dOc) of hulls was 41.7 and 42.7% respectively. Confirming again the relationship between cell-wall content and digestibility. The predicted OM-digestibility of the undecorticated product for pigs was lower than for ruminants, the difference between dOc and dOp was 7.5 ± 1.7 percentage units. However, a high dOp value (79.2%) was observed for decorticated, solvent extracted meal.

Euphorbia plants are known to contain irritant diterpene esters, which may cause inflammation of the skin and when taken internally may cause severe gastro-enteritis (Frohne and Pfänder, 1983). These irritating and toxic substances prevail in the latex as well as in the oil. No information as yet is available on its presence in seed by-products after oil removal or concerning effective methods of detoxification. Before safe feeding can be considered, adequate information concerning these matters is essential.

3.4 Limnanthes

Both samples of pressed-cake and meal were studied. Their fat-free composition was similar. The protein content of limnanthes meal was slightly higher than that of undecorticated crambe meal. Lysine and methionine contents of the protein (Table 2) compared favourably with information provided by Throckmorton et al.(1981). The cell-wall fraction of limnanthes meal was clearly lower and less lignified than in crambe meal (Table 1). Considering this, the in-vitro OM-digestibility for ruminants was expected to be higher than observed, in particular for dOt (Table 3). This level of digestion may be due to a slow degradation of limnanthes meal in rumen fluid, as was observed by Liu et al. (1994b) during an in-situ experiment in the rumen of dairy cows. They also showed that the potentially rumen-degradable OM of limnanthes compared favourably with rapeseed meal.

Although meadowfoam belongs to the family of Limnanthaceae rather than Cruciferae, its seed meal contains significant levels of glucosinolates. The level of OZT in limnanthes meal was 1500 mg kg^{-1}, ITC remained below the detection limit (200 mg kg^{-1}). Total glucosinolates were determined at 90 μmol g^{-1} (benzyl-glucosinolate). These data compare favourably with Daxenbichler and Van Etten(1974). From a literature review (Liu et al., 1992) it was concluded that limnanthes meal could provide a satisfactory protein supplement for ruminants (sheep). Good performance should be obtainable with steam-cooked meal in diets for poultry and rabbits, but only when incorporated at moderate dietary levels.

3.5 Calendula

One sample of pressed-cake and two samples of solvent extracted meal were involved in this study. The chemical composition of calendula meal was dominated by cell wall material as 731 g kg^{-1} fat-free matter was determined as fibrous components (Table 1). The

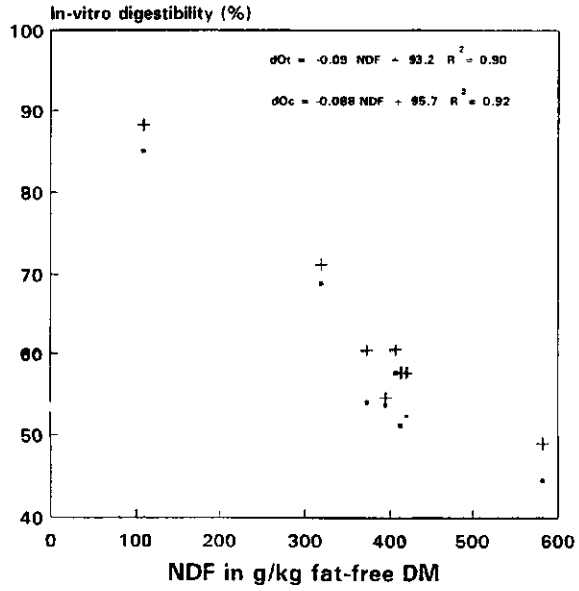

Fig. 2 Crambe: relationship between digestibility and cell wall fraction. ▪ rumen fluid (dOt) and + enzyme (dOc).

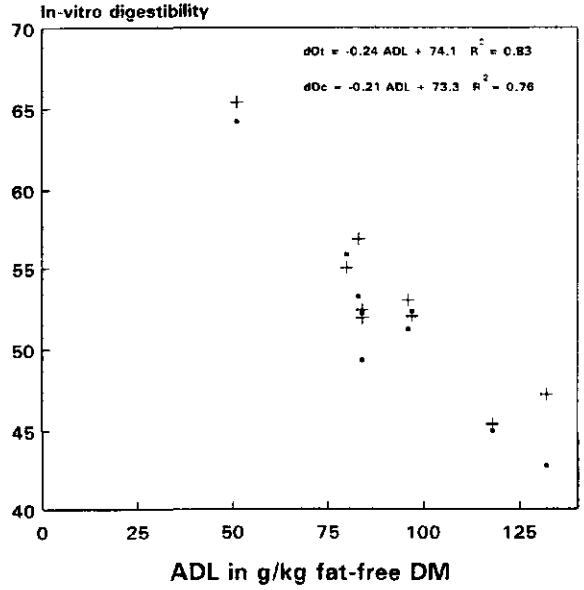

Fig. 3 Dimorphotheca: relationship between digestibility and lignin content. ▪ rumen fluid (dOt) and + enzyme (dOc).

protein content was low and, moreover, of poor quality owing particularly to a very low level of lysine (Table 2). As a result of the fibrous nature of this by-product the predicted digestibility was poor, both for pigs as well as for ruminants (Table 3). Unless removal of a large part of the cell walls becomes possible through technological treatment or breeding, feeding of calendula meal to livestock will remain unattractive.

4. Conclusions

By-products from crambe, dimorphotheca and euphorbia varied considerably in chemical characteristics, in-vitro digestibility and hence potential nutritive value. Decortication prior to lipid removal proved very effective in reducing fibre content and improving digestibility of crambe and euphorbia by-products.

Proximate characteristics of decorticated crambe meal (low fibre, high protein with a satisfactory amino acid profile) make it a potential feed ingredient for monogastrics. However, a considerable reduction in glucosinolate content will be needed to justify its safe introduction as a feedstuff.

Chemical composition and digestibility of dimorphotheca by-products did not favour their inclusion in livestock diets. It may be possible to increase its value by alternative processing.

Euphorbia meal contains a significant level of protein, which can be increased by dehulling prior to defatting. Feeding perspectives are hampered by the less than optimal amino acid profile, low fibre digestibility and lack of knowledge concerning anti-nutritional factors.

Limnanthes meal has an attractive composition but is slowly digested and may need detoxification of glucosinolates prior to inclusion in livestock diets.

Owing to its fibrous nature and low digestibility Calendula meal seems to have little potential as a feedstuff.

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Chapter 4

Rumen Degradation and Intestinal Digestion of Crambe and Other Oilseed By-products in Dairy Cows

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Rumen Degradation and Intestinal Digestion of Crambe and Other Oilseed By-products in Dairy Cows

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Abstract

The by-products of several special oilseeds (*Crambe abyssinica, Euphorbia lagascae, Dimorphotheca pluvialis* and *Limnanthes alba*) were analyzed for their chemical composition, and studied for in vitro digestibility, rumen degradation and post-rumen digestion in dairy cows. In situ nylon bag and mobile bag techniques were employed for rumen and intestine incubations. Rapeseed and soybean meals were used as reference feeds. The results indicated that some of these special oilseed by-products were potentially useful feed ingredients. Properly dehulled and defatted crambe meal showed the highest potential degradability of the samples examined. Potential rumen degradabilities (100 minus the undegradable fraction) of organic matter for crambe meal, crambe cake, crambe hulls, euphorbia meal, dimorphotheca meal, limnanthes cake, rapeseed and soybean meals were 96%, 73%, 43%, 62%, 67%, 84%, 85% and 95%, respectively. The intestinal digestibility of rumen-undegraded nutrients was largely dependent on their rumen residence time, but the fraction of undigestible protein was not. The total tract digestibilities of sample protein (100 minus undigestible fraction) were determined as 98%, 95%, 97%, 88%, 79% and 92%, for crambe meal, crambe cake, euphorbia meal, dimorphotheca meal, limnanthes cake and rapeseed meal, respectively.

1. Introduction

The overproduction of traditional food crops in European Community countries has prompted a search for new forms of agricultural land usage. In addition to the options using surplus land for recreation, nature preservation and forestry, one of the new directions under consideration is the production of raw materials for industrial use, of which new oilseeds form an important group among the potential industrial crops (Rexen, 1992). In the Netherlands, an extensive programme concerning new crops has been developed in recent years (Van Soest and Mulder, 1993; Muuse et al., 1992).

Crambe abyssinica, a member of Cruciferae family, has been successfully introduced in many countries. Its oil contains 55 to 60% erucic acid (C22:1,Δ13), useful for lubricants, plastic additives, nylon synthesis and many other potential applications. Euphorbia lagascae yields a high level of vernolic acid (Δ12,13-epoxy, 9c-octadecenoic acid), which can be used in the paints and coatings industry and also as stabilizers in polymers. *Limnanthes alba* (Meadowfoam) produces special long chain fatty acids (C20 and C22), having application in

cosmetics, lubricants and plastics industry. *Dimorphotheca pluvialis* contains a conjugated double bonded hydroxy fatty acid, dimorphecolic acid (Δ9-hydroxy, 10t,12t-octadecadienoic acid), useful for the manufacture of lubricants, coatings, plasticizer and foam plastics (Van Soest and Mulder, 1993; Muuse et al., 1992).

Oil production from the seed results in considerable amounts of by-products. In most cases more than 50 percent of the total weight remains as residues, in the form of cakes or solvent extracted meal. Obviously, the economic viability of growing these oilseeds would be largely affected by the potential application of the by-products in livestock feeding. Studies concerning chemical composition and feeding value of crambe meal were initiated in the 1970s, and the meal has been regarded as a promising feedstuff because of its high protein content and well-balanced amino acid profile (Carlson and Tookey, 1983; Liu et al., 1993). Limnanthes meal was successfully fed to rabbits, lambs and cattle in small-scale animal trials (Throckmorton et al., 1981, 1982). However, the characteristics of degradation and digestion of these by-products remain unknown. For other oilseed by-products, no information has yet been produced.

The objectives of this study were to examine the chemical composition of these oilseed by-products, to determine their degradation and digestion characteristics using nylon bag techniques in cannulated dairy cows and to compare these by-products with rapeseed and soya-bean meals with respect to nutrient content and digestion.

2. Materials and Methods

2.1 Samples and preparation

A short description of the feed samples studied is given in table 1. Most samples were provided by DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO). Crambe seeds were grown in 1990. Whole seeds were sampled for this experiment. The seeds were dehulled by an experimental crusher and hulls were collected. The dehulled seeds were ground and defatted in our laboratory using petroleum ether (PE40:60). The extraction was repeated because the residual fat content was considered to still be too high (200 g kg^{-1}) after the first extraction. Twice extractions yielded 505 g oil kg^{-1} seeds, and the level of residual lipids was 18 g kg^{-1} meal. The meal was white, fine and uniform.

Euphorbia was also grown in 1990. The seeds were dehulled before storage and further treated as for crambe. Almost the same amount of oil output was obtained (500 g oil kg^{-1} seeds) by PE40:60 extraction. The meal appeared similar to crambe except slightly more seed coat residues remained.

Both crambe and limnanthes cakes were industrially processed. After dehulling, seeds were cold-pressed.

The dimorphotheca sample contained meal from both types of seeds (cones and wings). Seeds were defatted by solvent extraction following pressing by expelling. The rape (double zero) and soya-bean meals used in this study were received from commercial enterprises.

After collection, all the samples were ground through a 3 mm sieve, thoroughly mixed

and stored at 3°C.

2.2 Animal and experimental design

Four lactating cows (Friesian cross Holstein) were used for this study, cannulated both in the rumen (cannula 10 cm in diameter) and the duodenum, and housed in the metabolism unit of IVVO. Each cow received 15 kg dry matter (DM) daily of a ration consisting (DM basis) of half grass silage and artificially dried grass, and half of commercial concentrates.

Table 1 Sample description

Sample	Variety	Description
Crambe meal	*Crambe abyssinica*	Dehulled, solvent-extracted
Crambe cake	*Crambe abyssinica*	Partly dehulled and pressed
Crambe hulls	*Crambe abyssinica*	Separated from whole seeds
Euphorbia meal	*Euphorbia lagascoe*	Dehulled, solvent-extracted
Dimorphotheca	*Dimorphotheca pluvialis*	Screw-press+solvent-extr.
Limnanthes	*Limnanthes alba*	Screw-pressed
Rapeseed meal	*Canola*	Commercial source
Soybean meal		Commercial source

2.2.1 Rumen incubation

About 5 g (DM) feed samples were weighed into coded nylon bags (made of polyamide, pore size 41 μm, porosity 30%), with an inner size of 9x18 cm. The bags were tied with a plastic strip and stored in a freezer (-20°C) until required for incubation. During weighing-in the bags, duplicate samples were taken for laboratory analyses and determination of in vitro digestibility. Blanks (0 hr incubation) were treated as the other bags but without being placed into the rumen.

After incubation the bags were removed from the rumen, rinsed with cold water and immediately placed in ice to stop further microbial activity. Then the bags were washed in a washing machine for 50 min at room temperature. The washed bags were dried at 70 °C for 24 hr, weighed straight from the oven (warm) and after acclimatisation to room conditions the residues were ground through a 1 mm sieve. Ground samples were then pooled according to feed and incubation time for each cow. The pooled residues were analysed for dry matter, ash, crude protein and neutral detergent fibre contents.

2.2.2 Intestinal digestion

For the determination of intestinal digestibilities some extra bags were incubated in the rumen to provide sufficient material for the intestinal studies. These bags were allocated to three rumen incubation times (6, 12 and 24 hr), in order to examine the effect of rumen residence time on intestinal digestibility of organic matter and protein. After rumen incubation the bags were removed and handled as described previously. However, the residues were freeze-dried, pooled according to feed, animal and incubation time. Approximately 0.5 g

(DM) of this material was then weighed into small (3x7 cm; 41 μm) coded nylon bags, 6 bags per cow per feed. Prior to incubation in the small intestine the bags were incubated in a solution of 2.5 g (2000 FIP U g^{-1}) pepsin 0.1 M HCl at 39°C for 1 h. Every 20 min, 4 mobile bags were taken at random and inserted into the intestine through the duodenal cannula. The bags were retrieved from the faeces. Checks were made every 2 h after bag introduction, and the collection time was recorded for each bag. The retrieved bags were stored at -20°C until all the bags had been recovered. These bags were washed in a washing machine for 2 h at 40°C with occasional spinning at 700 rev min^{-1} (Hvelplund and Weisbjerg, 1991). The residues are pooled according to feed and rumen incubation time and analyszed for nitrogen and ash.

In order to obtain an indication of crambe meal potential in monogastric feeding, the meal was incubated in the pepsin solution and inserted in the duodenum without prior incubation in the rumen, and a comparison was made with rapeseed meal.

2.3 Laboratory analyses

Chemical analyses were performed according to IVVO's standard procedures (Steg et al., 1990). Laboratory samples of the feeds were prepared by grinding to pass a 1 mm mesh after freeze-drying. Feeds as well as incubation residues were analyzed for DM, ash (ASH), crude protein (CP) and neutral detergent fibre (NDF). In addition, feed samples were analysed for acid detergent fibre (ADF). Organic matter digestibility of feed samples was determined in vitro both with rumen fluid followed by pepsin-HCl (dOt) (method of Tilley & Terry, IVVO modification) and with cellulase digestion (dOc) (Steg et al., 1993).

Contents of total glucosinolates were analysed in crambe, limnanthes and rapeseed samples, by DLO-State Institute for Quality Control of Agricultural Products (RIKILT-DLO), using high performance liquid chromatography (HPLC), following the ISO method of 9167-1. The content of vinyl-oxazolidine-thione (OZT) was determined according to ISO 5504.

2.4 Calculation model and statistical analyses

Feeds were assumed to consist of 3 fractions: the instantly disappearing fraction from the nylon bags during washing without rumen incubation (W, zero incubation time); the rumen-undegradable fraction after 336 h incubation (U, Robinson et al., 1986) and the slowly rumen-degradable fraction ($D = 100 - W - U$). The degradation rate of the D fraction (k_d, % h^{-1}) was calculated according to a first-order degradation model, including a test for a lag phase preceding the onset of rumen degradation (Van Vuuren et al., 1991). Organic matter and crude protein digestibilities in the small intestines were calculated on the basis of rumen residues (Hvelplund et al., 1992).

Data were analysed using analysis of variance. Factors in the model for rumen degradation were feeds (F) and animals (A). The effect of rumen residence time was introduced as a factor in the investigation of intestinal digestion.

3. Results

3.1 Chemical composition of samples and in vitro digestibility

Chemical components of the samples are given in Table 2. The protein content of dehulled crambe meal was almost 50%, 10 percentage units higher than that of rapeseed. The crude fibre content of crambe meal was comparable with soya-bean meal and its in vitro digestibilities (dOt and dOc) were slightly lower than those of soybean but considerably higher than those in rapeseed meal. Contrary to this, crambe hulls, as an extreme sample of these by-products, contained about 50% fibre and a low level of crude protein, its in vitro organic matter digestibility was the lowest among the samples involved. The fibre content and in vitro digestibility of crambe cake fell in between those of meal and hulls. The high lipid contents of both crambe and limnanthes cakes indicate that they were not satisfactorily defatted. Upon comparison with rapeseed meal, euphorbia meal had a relatively high protein content, but its fibre constituents were also high.

The contents of total glucosinolates in crambe meal, hulls, limnanthes cake and rapeseed meal were 190 μmol g^{-1}, 9 μmol g^{-1}, 90 μmol g^{-1} and 12 μmol g^{-1}, respectively; the contents of the main hydrolysis derivative, OZT, were 21 mg g^{-1}, 0 mg g^{-1}, 2 mg g^{-1} and 1 mg g^{-1}, respectively.

3.2 Rumen degradation

Statistical analysis revealed that there was no significant difference in rate or extent of rumen degradation between animals in this experiment. Inclusion of a lag phase preceding degradation did not improve data fit. Therefore, the rates of degradation presented are derived without considering a lag phase. The degradation characteristics of the different feeds are shown in Table 3

3.3 Digestion in the intestines

Results are listed in Table 4. The bag residence time in the intestines averaged 11 h but varied, especially for dimorphotheca and limnanthes. Increasing the rumen residence time caused an increase in disappearance of OM and CP. By far the largest rumen degradation was observed during the first 6 h, after which it slowed. A linear decrease in intestinal digestibility of the rumen-undegraded nutrients was observed for all samples studied. Crambe and rapeseed meals without prior rumen incubation showed very high intestinal digestibility, for organic matter and crude protein. Crambe meal was more digestible than rapeseed meal.

The undigestible protein fractions after both rumen and intestinal incubations were calculated and listed in Table 4. They varied considerably between feeds but remained fairly constant for different rumen incubation times of each feed. The highest fraction was found in limnanthes (21%), followed by dimorphotheca (12%), rapeseed meal (8%), crambe cake (4.5%), euphorbia (3.3%) and the lowest in crambe meal (2.5%).

Table 2
Chemical composition and in vitro digestibility (in g kg^{-1} DM unless stated otherwise)[1]

	DM	ASH	CP	CL	CF	NDF	ADF	dOt(%)	dOc(%)
Crambe									
meal	968	104	494	18	52	107	74	85.0	88.2
cake	930	73	292	169	175	261	212	64.3	65.9
hulls	892	88	82	29	417	565	500	44.9	49.0
Euphor. m.	954	123	436	3	218	389	327	60.0	60.2
Dimorph.m.	949	182	192	9	224	383	348	62.3	59.3
Limnan.c	937	141	264	162	71	217	168	60.3	65.2
Rapeseed m.	880	79	383	32	133	246	196	76.1	78.2
Soybean m.	879	72	538	25	62	80	59	89.7	90.4

[1]Samples analyzed in duplicate. DM, dry matter; CP, crude protein; CL, lipids; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; dOt, in vitro rumen fluid digestibility of organic matter; dOc, in vitro enzyme digestibility of organic matter.

4. Discussion

4.1 Chemical composition and in vitro digestibility

Reference data for the special oilseed by-products studied here are limited, especially for dimorphotheca and euphorbia. For dehulled and defatted crambe meal, a crude protein content of nearly 50% has been reported, as well as about 7% crude fibre (Earle et al., 1966; Carlson and Tookey, 1983). In this trial the crude fibre content of crambe meal was found to be 5%, indicating an effective dehulling. The crambe hulls contained 41.7% crude fibre, 56.5% NDF and 50% ADF, suggesting a high content of cell wall constituents and low level of semi-cellulose. There was 8.2% crude protein, at the upper limit ofthe 4-8% crude protein reported by Lambert et al. (1970). Nutrient contents in press cake are variable, depending largely on the extent of dehulling and defatting. Information concerning NDF, ADF contents and in vitro digestibility of crambe meal has not been found elsewhere.

Some information concerning the chemical composition of dimorphotheca was given by Knowles et al. (1965). The seed weight was reported to represent 37% of the total weight of whole seeds, indicating a high proportion of hulls. Dehulled seeds contained 41% oil. In oil-free meal, 50% crude protein was found. Our sample contained much lower crude protein (19%), suggesting there is a considerable scope for quality improvement of the meal by paying more attention to proper dehulling. Throckmorton et al. (1982) reported contents of 22-26% crude protein, 7% crude fibre and 29% ADF in limnanthes cake, which were comparable with our findings when the difference in residual lipids content was taken into account.

As expected, in vitro digestibilities using both rumen fluid and commercial enzymes

were similar. Crambe meal had the highest in vitro organic matter digestibility among the oilseed by-products with the exception of soya-bean meal. Crambe hulls had the lowest in vitro digestibility of both dOt and dOc methods, presumably due to their high fibre content. Euphorbia meal, dimorphotheca meal and limnanthes cakes showed similar in vitro digestibilities (59 to 65%) with, however, much lower fibre content in limnanthes cake.

Table 3
Fraction (expressed as percentage) of instantly washable (*W*), rumen slowly degradable (*D*), rumen-undegradable (*U*) and rate of degradation (k_d, % per hr) of organic matter, crude protein and neutral detergent fibre[1]

	W	D	U	k_d
Organic matter				
Crambe meal	57^{A}	39^{D}	4^{F}	13.1^{a}
cake	52^{B}	21^{F}	27^{D}	8.7^{c}
hulls	17^{G}	26^{E}	57^{A}	13.4^{a}
Euphorbia meal	42^{D}	20^{F}	38^{B}	8.5^{c}
Dimorph. meal	24^{F}	43^{C}	33^{C}	11.6^{b}
Limnanth. cake	46^{C}	38^{D}	16^{E}	2.9^{e}
Rapeseed meal	25^{F}	60^{B}	15^{E}	9.3^{c}
Soybean meal[2]	28^{E}	67^{A}	5^{F}	6.4^{d}
SEM[3]	0.12	0.11	0.11	0.08
Crude protein				
Crambe meal	56^{B}	42^{D}	2^{G}	18.4^{a}
cake	78^{A}	13^{G}	9^{D}	11.4^{d}
hulls	29^{D}	37^{E}	34^{A}	13.8^{c}
Euphorbia meal	80^{A}	16^{G}	4^{F}	19.2^{a}
Dimorph. meal	37^{C}	46^{C}	17^{B}	15.8^{b}
Limnanth. cake	57^{B}	28^{F}	15^{C}	4.7^{f}
Rapeseed meal	18^{E}	75^{B}	7^{E}	9.9^{d}
Soybean meal[2]	8^{F}	86^{A}	6^{E}	6.1^{e}
SEM[3]	0.26	0.16	0.05	0.12
Neutral detergent fibre				
Crambe meal	11^{A}	65^{B}	24^{F}	9.5^{a}
cake	10^{A}	24^{D}	66^{B}	9.9^{a}
hulls	0^{D}	19^{E}	81^{A}	7.5^{bc}
Euphorbia meal	2^{C}	18^{E}	80^{A}	6.5^{c}
Dimoph. meal	0^{D}	49^{C}	51^{C}	8.5^{ab}
Limnanth. cake	7^{B}	51^{C}	42^{D}	2.3^{d}
Rapeseed meal	0^{D}	68^{B}	32^{E}	5.7^{c}
Soybean meal[2]	0^{D}	90^{A}	10^{G}	3.5^{d}
SEM[3]	0.25	0.10	0.13	0.18

1. Data are compared within the same column and same fraction. Different capital letters indicate $P<0.01$, and different small letters mean $P<0.05$.
2. For soybean meal, U fractions are derived from 48 hr rumen incubation instead of 336 hr.
3. Standard error of means.

Our data on glucosinolate contents in crambe are in agreement with previous literatures (180 to 250 μmol g^{-1} on oil-free basis, Van Etten et al., 1965; Earle et al., 1966; Liu et al.,

1993). Such a content in crambe meal is clearly higher than that in traditional, unimproved rapeseed meal. Over 90% of the glucosinolates in crambe were characterised as the

Table 4
Relationship between rumen degradation and intestinal digestion of sample with different rumen residence time

	Rumen incubation time (h)				
	0	6	12	24	SEM[1]
OM disappearance in rumen, %					
Crambe meal	–[2]	75	91	93	0.17
Crambe cake	–	58	67	69	0.51
Euphorb.meal	–	52	56	57	0.29
Dimorph. meal	–	45	53	56	1.45
Limnanth. cake	–	66	73	77	0.24
Rapeseed meal	–	48	64	75	1.07
Residual OM digestibility in intestines, %					
Crambe meal	93	68	48	34	1.42
Crambe cake	–	40	35	32	0.50
Euphorb.meal	–	23	17	14	0.49
Dimorph. meal	–	31	24	13	0.55
Limnanth. cake	–	49	43	33	1.30
Rapeseed meal	79	56	48	37	0.58
CP disappearance in rumen, %					
Crambe meal	–	82	95	96	0.13
Crambe cake	–	84	89	90	0.23
Euphorb.meal	–	90	95	95	0.19
Dimorph. meal	–	66	76	79	0.67
Limnanth. cake	–	54	63	67	0.82
Rapeseed meal	–	49	68	82	1.79
Residual CP digestibility in intestines, %					
Crambe meal	97	83	60	49	0.79
Crambe cake	–	70	61	56	0.58
Euphorb.meal	–	65	38	31	0.91
Dimorph. meal	–	63	55	40	0.40
Limnanth. cake	–	51	49	35	1.10
Rapeseed meal	92	82	75	65	0.29
CP not digested in intestines, % of original					
Crambe meal	3.0	3.1	2.0	2.0	0.20
Crambe cake	–	4.8	4.3	4.4	0.13
Euphorb.meal	–	3.5	3.1	3.4	0.10
Dimorph. meal	–	12.6	10.8	12.6	0.52
Limnanth. cake	–	22.5	18.9	21.4	0.92
Rapeseed meal	8.0	9.2	8.0	6.3	0.34

1. Standard error of means.
2. Not measured.

potentially harmful epi-progoitrin, making additional treatment necessary for including the meal in livestock diets (Carlson and Tookey, 1983; Liu et al., 1993). Only trace glucosinolates were found in the crambe hulls, agreeing with the finding of Earle et al. (1966). A level of 90 μmol g^{-1} glucosinolates was determined in our limnanthes cake, which is in accordance with the result of Throckmorton et al. (1982).

4.2 Rumen degradability

4.2.1 Reference feeds

The *D* fraction of CP in rapeseed meal was similar to that found by Van Straalen and Tamminga (1990) differing by two percentage units (75% vs. 73%). The *U* fraction was also comparable (7% vs. 6%), but the degradation rate (k_d) of our sample was lower (9.9% h^{-1} vs. 13.8% h^{-1}). Our sample contained slightly more protein (38.3% vs. 36.9% in DM), and the soluble fraction in our sample was lower (18% vs. 21%). The difference observed may be due to a more intensive heating during processing of our rapeseed sample, because heating decreases solubility and rumen degradability, of protein in particular (Moshtaghi Nia and Ingalls, 1992). For soya-bean meal, fraction *W*, *U*, and degradation rate k_d of protein were reported as 6.2%, 0.1%, and 8.3% hr^{-1}, respectively, by Van Straalen et al. (1990); and the slow rumen-degradability was calculated as 93.7%, higher than our results. However, as indicated in Table 3, the *U* fractions of soya-bean protein in our study were derived from a 48 h incubation, and the longest rumen incubation of the other samples were 336 h.

4.2.2 Crambe

The rumen degradabilities of OM and CP were higher in crambe meal than rapeseed meal. Crambe meal was more soluble, and the rate of degradation of the slowly degradable fraction (k_d) was higher than of rapeseed meal. This trend was even more obvious when comparing crambe meal with soybean meal. However, the data from this trial may not be appropriate to enable an accurate prediction of the rumen degradation characteristics of industrially processed crambe meal, due to differences in heating intensity. Van Straalen et al. (1990) compared the degradation of raw soybeans with toasted soybeans, indicated that industrial processing may alter protein fractions (*W*, *U*, k_d) considerably. Recently, Moshtaghi Nia and Ingalls (1992) showed that heating significantly reduced dry matter and nitrogen disappearance of canola meal in the rumen, with a simultaneous increase of its digestibility in the intestines. A high nitrogen solubility of crambe protein was previously reported by Van Etten et al. (40% minimum solubility at pH 3.5 to 4.5). This may vary considerably, depending on the extent of heating (Liu et al., 1994).

As expected, crambe hulls showed a very large part of NDF to be undegradable, but their potential organic matter degradability was still up to 43%, conforming a possible acceptance by cattle (Lambert et al., 1970). Low degradability was observed in crambe cake. The degradation rates of OM and CP were lower in crambe cake than in either meal or hulls, as shown in Table 3. This is probably related to the high lipid content which may influence activity and/or population of rumen micro-organisms.

4.2.3 Euphorbia

Euphorbia meal displayed a high protein degradability (96%), which seems mainly due to its high washable fraction of protein (80%). However, its OM degradability was low (62%). Its large fraction of cell wall constituents being rumen-undegradable will be largely responsible for this.

4.2.4 Dimorphotheca

One-third of the OM and half of the NDF in dimorphotheca was undegradable. The sample had a high level of ADF (350 g kg^{-1}), yet degradation rates of OM, CP and NDF were relatively high. These data suggest that further dehulling may improve nutrient availability.

4.2.5 Limnanthes

Potential OM degradation of limnanthes cake was equivalent to rapeseed meal, but its degradabilities of CP and NDF were not as high as those in rapeseed meal. Degradation rates of OM, CP and NDF were considerably lower in the limnanthes cake than in the other feeds (Table 3). However, its CP, OM and NDF fractions were highly soluble comparing with the reference feeds. An in vivo digestion trial using lambs showed that the nutrients in limnanthes cake were less digestible than in cottonseed meal, but lamb growth performance in the trials was desirable (Throckmorton et al., 1982).

4.2.6. Relationship between in situ and in vitro digestibility

In this study, potential in situ degradabilities (100-*U*) of OM are generally higher than both dOt and dOc digestibilities, but there is a close correlation between the results from these two methods. The correlation coefficient (r) between in situ and dOt is 0.91, and in situ and dOc is 0.93, both are statistically significant ($P < 0.01$).

4.2.7. Rumen-undegraded protein fraction

This fraction was calculated using the equation of Ørskov et al. (1979), assuming a rumen passage rate of 6% h^{-1}. When using 0.1% as a U fraction of soybean protein (Van Straalen and Tamminga, 1990), the rapeseed and soya-bean meals had respectively 35 and 42% rumen-undegraded protein, similar to those summarized by Van Straalen and Tamminga (1990). For the other feeds percentages were: crambe meal 12, crambe cake 13, crambe hulls 45, euphorbia meal 8, dimorphotheca meal 30, limnanthes cake 31. Euphorbia meal, crambe meal and cake showed low rumen-undegraded protein fractions.

4.3 Intestinal digestibility

For crambe and rapeseed samples not subjected to prior rumen incubation, intestinal disappearance of CP was 97.1 and 92.3%, respectively. As shown in Table 4, nutrient degradation was very rapid during the first 6 hr of rumen incubation. Rumen residence time influenced intestinal digestibilities for all the feeds studied.

The protein fractions that remained undigested after both ruminal and intestinal incuba-

tions varied considerably between feeds but were not systematically affected by the durations of rumen incubation. Most of these data fit with the rumen undegradable protein fractions (U). These results are in good agreement with the observations of Hvelplund et al. (1992), who suggested that there is a protein fraction which is both undegradable in rumen and undigestible in intestines.

5. Conclusion

Proximate composition, in vitro digestibility, rumen degradation and intestinal digestion show that some of the oilseed by-products under study are potentially useful feed ingredients, such as crambe and limnanthes. Their nutrient contents and availabilities may vary, largely owing to the origin of the by-products and method of processing; especially dehulling, defatting and extent of heating. In general, dehulled and defatted oilseed meals have high protein contents that are easily and rapidly degradable in the rumen. The quantity and digestibility of rumen escape protein is clearly affected by rumen residence time. Large fractions of cell-wall ingredients are undegradable in the rumen and dehulling has been demonstrated to be a potential means of reducing cell-wall content and increasing overall degradability and digestibility, as shown in crambe.

Additional study is required for euphorbia and dimorphotheca, and also for more information concerning processing effects on the nutritive value of these by-products, the effects of glucosinolates and other anti-nutritional factors on digestibility and animal performance, and the levels at which these by-products can be included in practical diets for livestock.

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Chapter 5

Crambe Meal: Digestibility in Pigs and Rats in Comparison with Rapeseed Meal

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Crambe Meal: Digestibility in Pigs and Rats in Comparison with Rapeseed Meal

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Abstract

Seed meals from *Crambe abyssinica* (CM) and modern variety rape (RSM) were comparably studied for composition and digestibility. The whole seeds' structural elements (pericarp, seedcoat and endosperm) were measured through hand-separation. In the digestion trial the decorticated and defatted meals were fed to four pigs (liveweight 35 kg) fitted with ileo-caecal valve cannulas. Digestibility data were obtained from a change-over experiment in which both meals were included at 300 g kg^{-1} in the diet. Then the energy digestibility and nitrogen utilisation of the meals were determined in a balance trial with rats.

The whole crambe seeds contained 300 g kg^{-1} pericarp. After decortication, the seeds possessed 89 g kg^{-1} seedcoat, significantly lower than in rapeseed (160 g kg^{-1}). Compared with RSM, CM contained considerably higher level of protein (504 vs. 367 g kg^{-1}) and lower level of fibre constituents (e.g. 65 vs. 139 g kg^{-1} for crude fibre). Apparent faecal digestibilities of gross energy for CM and RSM in pigs were 78.3 and 73.2% ($P<0.05$); of organic matter 78.7 and 73.2% ($P<0.05$); and of protein 76.8 and 79.1%, respectively. However, the apparent ileal digestibility of protein in CM was lower than in RSM (63.0 vs. 71.7%, $P<0.01$); of lysine and cystine was also lower (61.1 and 62.5% for CM vs. 75.3 and 71.2% for RSM, $P<0.01$). The difference in protein digestibility was presumably due to the overheating on CM during the detoxification treatment. Phosphorus faecal digestibility was 43.4 and 22.5% for CM and RSM ($P<0.01$). In the balance trial, rats received diets in which all nitrogen was originated from either CM or RSM. The animals showed that CM had clearly higher apparent energy digestibility than RSM (93.4 vs. 79.6%, $P<0.01$). Apparent protein digestibility was similar (78.4 vs. 78.8%). However, the biological value (BV) and net protein utilisation (NPU) of CM were low, i.e. 46.8 and 41.7%, compared with 87.1 and 77.6% for RSM ($P<0.01$), which is presumably due to the high level of glucosinolates in CM. It is therefore concluded that CM is highly digestible, and that there is large scope for enhancement in protein utilisation by optimizing the detoxification treatment.

1. Introduction

Crambe abyssinica, a member of the Cruciferae family, has been increasingly investigated and cultivated in many countries as a potential oilseed crop, due to its high content of erucic acid which has many industrial applications, and the production of protein-rich by-products. It has been recognized that the decorticated and defatted crambe meal may become an excellent protein source for livestock, owing to it has high protein content and

well balanced amino acids, as were reviewed by Carlson and Tookey (1983), and Liu et al. (1993). For non-ruminants, observations showed that the unprocessed crambe meal was of undesirable palatability to piglets (Liu et al., 1994b) and even toxic to rodents due to the presence of high level of glucosinolates (Van Etten et al., 1969). However, certain treatments can minimize the toxicity and improve its feeding characteristics, as shown by a number of investigations (Kirk et al., 1971; Baker et al., 1977; Kirleis and Cline, 1981). A short-term experiment using pigs showed that detoxified crambe meal has potential in pig feeding (Liu et al., 1994b).

So far information concerning crambe nutritional aspects is very limited. No data have been found on literature concerning nutrients' digestibility in non-ruminants. Therefore, the present study was undertaken to investigate ileal and total-tract digestibility of crambe meal in pigs, and overall digestibility as well as protein utilisation in rats. Since in many aspects crambe is comparable to rapeseed in terms of botanic characteristics, seed processing, nutritional and anti-nutritional components, batches of rapeseed by-products were employed for comparison in this investigation.

2. Materials and Methods

2.1 Experiment with pigs

Test feeds

Crambe seed was grown in the Netherlands in 1992 and harvested as intact pod (with pericarp). The whole seed was air-dried, and decortication was performed mechanically, which enabled a removal of the pericarp. After this processing the clean seed was obtained which was considered comparable with the commonly used rapeseed. The rapeseed sample used in this study was from double low variety of low erucic acid and low glucosinolates. Both crambe and rape seeds were defatted by screw-pressing and the defatted materials were used for chemical analyses and animal trials.

In order to reduce the level of glucosinolates and their derivatives from crambe meal, the defatted material was ground and mixed with 20 g kg^{-1} sodium carbonate powder and toasted for 1 h at 110^{0}C (Liu et al., 1994b). After heating, the meal was dried at room temperature. The glucosinolate level in the rapeseed meal was considered unharmful, no extra heat treatment was thus performed on rapeseed meal. For the experiment, both crambe and rapeseed meals were ground through a 3 mm sieve and stored at 4^{0}C. The maize used as a basal feed in this study was of standard quality as used by feed manufacturers. Premixes of minerals and vitamins were added to the maize to supply all necessary vitamins and minerals in accordance with the animal's requirements (CVB, 1994).

Animals and cannulation

Four barrows (Duroc × Finnish Landrace × Dutch Landrace) averaging 35 kg live weight were surgically fitted with so-called steered ileo-caecal valve cannulas (SICV), followed by a 14-day recovery period. When pulling a thread connected to an inner ring

installed inside the ileum, the ileo-caecal valve can be steered into a simple T-cannula fitted on the caecal-colon, the ileal digesta can thus be quantitatively collected during a fixed period. The detailed cannulation procedure was described by Mroz et al. (1991). The animals were individually housed in metabolism pens at the Research Institute for Livestock Feeding and Nutrition (IVVO-DLO, Lelystad). In addition to weekly weighing, the pigs were weighed immediately prior to and after termination of the experimental periods.

Table 1
Chemical composition of crambe and rapeseed meals used in pig digestion trials, in g kg^{-1} on DM basis

	Test feeds		Diets[a]	
	Crambe	Rapeseed	Crambe	Rapeseed
Dry matter, g kg^{-1}	842	951	855	892
Organic matter	916	929	960	956
Ash	84	71	40	44
Crude protein	442	325	204	174
Crude fat	123	115	74	74
Crude fibre	57	123	32	56
N-free extract	294	366	650	652
Starch	47	67	476	448
Sugar	90	122	38	51
Hemicellulose	51	58	16	19
Cellulose	45	105	14	36
Pectin	28	53	9	18
NDF[b]	116	239	36	81
ADL[c]	20	76	6	26
Phosphorus	15.3	11.5	6.9	6.0
Phytin-P	9.6	9.6	-	-
Calcium	7.3	6.7	2.4	5.0
Gross energy, MJ kg^{-1}	21.54	21.10	19.74	19.54

a, Diets consisted of 300 g kg^{-1} test feeds and 700 g kg^{-1} maize fortified with minerals and vitamins;
b, Neutral detergent fibre;
c, Acid detergent lignin.

Experimental

For diet formulation, a preliminary estimation of net energy (NE) content was made for the test feeds according to the Dutch Feeding System (CVB, 1994), based on the feeds' proximate composition. This enabled a feeding level design of 2.3 × M (maintenance = 293 kJ NE/liveweight$^{0.75}$). During the adaptation period, crambe or rapeseed meal were gradually increased from 0 to 300 g kg^{-1}. Water was supplied at approximately 3 litres per kg feed dry matter. The animals were fed twice daily at 5:50 and 16:50 h.

The digestion study was performed in a 2×2 change-over experiment. The interval between both periods was six days. Both digestibility trials consisted of four consecutive periods: an adaptation period, a preliminary period and two collection periods. In the adaptation period, the pigs were allowed seven days to accustom themselves to the diet. This was followed by a preliminary period during which the same diet was fed as in the collection

periods. During the first 10-day collection period the faeces from each animal were quantitatively collected twice daily, and stored at -20°C. This period was followed by a 6-day collection period during which ileal digesta were collected through the SICV cannulas for 12 h (6:00h to 18:00h) on the first and the sixth day. This interval was chosen to ensure a normal digestion in the gastro-intestinal tract. The ileal digesta were collected every 20 min and temporarily stored in ice. Hourly batches of the digesta were weighed and stored at -20°C until required for analysis.

Chromium was used as an indigestible marker, added at an amount of 0.4 g chromic oxide (Cr_2O_3) per kg basal feed. Prior to addition to the basal feed, chromic oxide was thoroughly mixed with maize starch as carrier in the proportion (w/w) 1:3 and ground through a 0.5 mm sieve, to ensure a homogeneous distribution in the feed. It was assumed that the marker was entirely indigestible and completely recovered via the faeces (Bakker and Jongbloed, 1994). Since the apparent digestibility of phosphorus (P) was to be measured in the test feeds, no extra P was added to the diets. Calcium (Ca) was standardized at 3×digestible P content.

Digestibility data of the basal feed (maize) were obtained from a previous experiment executed according to the same procedure, by feeding a diet containing maize plus premix of vitamins and minerals. Apparent digestibilities of the components of the test feeds were calculated by difference. The digestible energy (DE) was calculated by multiplying gross energy with its digestibility coefficient, and the NE in MJ kg^{-1} of the test feeds was according to the formula (Dutch Feeding System CVB, 1994).

NE (MJ kg^{-1}) = $(10.8*X_1+36.1*X_2+6.3*X_3+12.7*X_4-0.63*Sui)/1000$

Where X_1 ... X_4 were digestible components respectively for crude protein, crude fat, crude fibre and N-free extract, in g kg^{-1}. Sui: sugar, in g kg^{-1}.

2.2 Experiment with rats

Feeds

The crambe seed was from the same batch and decorticated in the same manner as used for the pig trial. The rapeseed was from a double low variety. Owing to small quantities required for the rat trial, both seeds were defatted by solvent extraction on lab scale. In order to inactivate the myrosinase and partially decompose the glucosinolates, the rapeseed and crambe meals were autoclaved at 110°C for 10 and 40 min, respectively. The processed meals were air-dried, ground through a 3 mm sieve, and stored at 4°C until required for analysis and diet formulation.

Animals and experimental procedure

The experiment was executed at the National Institute of Animal Science in Denmark. Two groups of male Wistar rats were employed with an initial liveweight of 65±0.5 g. Each group consisted of five animals that were housed individually in plastic box cages. The cage had a wire-base allowing excreta to drop through. Each cage was equipped with a funnel, net and bottle to enable separate and quantitative collection of urine and faeces. The urine

was collected in 100 ml bottles and preserved with 50 ml 5% H_2SO_4, faeces were collected daily and stored at -20°C until required for analyses.

The animals were provided with 10 g dry matter (DM) containing 150 mg N/rat/day, i.e. the diet contained 94 g crude protein per kg DM. The diets were formulated in a way that all dietary N originated from test feeds, the remainder was autoclaved N-free maize starch. Minerals and vitamins were provided in accordance with the animal's requirements (Eggum, 1973). The rats were fed once daily at the same time during the whole experiment, with free access to water supply. The animals were housed under controlled conditions of temperature 22-23°C and relative humidity 60-70%. Four days were allowed for adaptation and the following five days for collection of urine and faeces. During the collection period feed consumption was recorded and feed refusals were taken into account. Further details of the experimental procedure were previously described by Eggum (1973).

At the end of the balance trial the crambe diet was diluted: half of the protein supply was shifted from crambe to casein in an attempt to improve feed intake. The rapeseed meal diet remained unchanged. All rats were kept an extra 15 days and fed ad libitum, then the animals were euthanized using CO_2 and several of their organs (thyroid, liver and kidney) were examined and weighed.

The true protein digestibility (TD), biological value (BV) and net protein utilisation (NPU) and digestible energy (DE) were calculated according to Eggum (1973). In this procedure, the corrections for metabolic N and endogenous N were derived from an experiment with eggs, which were assumed to be 100% digestible.

2.3 Analytical methods

Since crambe was normally harvested as intact seed pods (with pericarp), a fractional analysis was performed to obtain data on relative ratio of their structural elements. After drying duplicate samples of 2 g seed pods were gently broken, and further separated into three fractions: pericarp, seedcoat and endosperm. This was performed by hand-picking. Each fraction was weighed. Rapeseed (without pods) was measured for the seedcoat and endosperm in the same way.

Chemical analyses were carried out according to standard procedures based upon prescriptions given by the International Organization of Standardization (ISO, Geneva) or the Dutch Standardization Institute (NNI) and specified by document number (NEN). Laboratory samples were prepared following ISO 6497. Feed, ileal and faecal samples were analysed for contents of moisture (DM, ISO 6496); ash (A, ISO 5984); crude protein (CP) as Kjeldahl N (ISO 5983), crude lipids (Cfat, ISO 6492), crude fibre (CF, NEN 3327), gross energy (GE, ISO 1928). For the analysis of starch, an enzymatic method was used according to the description of Van Vuuren et al. (1993). Neutral detergent fibre (NDF), cellulose, hemicellulose and acid detergent lignin (ADL) were determined according to Van Soest (1967). Pectin was determined following the method of Dekker and Richards (1972). The concentration of calcium was determined by atomic absorption, and that of phosphorus by spectrophotometry. Urine of rats was analysed for N and gross energy. Amino acids were determined by high performance liquid chromatography (HPLC) according to ISO TC 34/SC.

Content of intact glucosinolates was measured by HPLC according to Sørensen (1990). Oxazolidine-thione (OZT) and isothiocyanates (ITC) were analysed after hydrolysis at pH 7 with myrosinase (Sigma Chemicals Co.), according to Wetter and Young (1976). Chromium content was measured based on the method of Williams et al. (1962) and further described by Bakker and Jongbloed (1994). Analyses on feeds and diets were performed on air-dry materials, and on faeces and ileal digesta were conducted in freeze-dried materials. N in faeces, digesta and urine was analysed on fresh samples.

Data were reported as means and standard deviations. Student's T-test was performed where relevant.

3. Results and Discussion

3.1 Seed structural elements and chemical composition

The whole crambe seeds used in this study contained 698 g kg^{-1} pure seed after removal of pericarp material. The result of 30% pericarp is in agreement with the 25-40% observed by Earle et al. (1966). The pericarp was reported to be fibrous and low protein content (82 g kg^{-1}) and also low in digestibility (Liu et al., 1994a). The decorticated crambe seed was found to contain 89 g kg^{-1} seedcoat in this study, comparable with the early result of 80 g kg^{-1} (Earle et al., 1966). Compared with rape, crambe seedcoat was thin and light-coloured, with higher protein content (230 g kg^{-1} for crambe vs. 160 g kg^{-1} for rape). The seedcoat content of rapeseed averaged on 160 g kg^{-1}, in good agreement with the data reviewed by Bell (1993). This meant that the seedcoat in oil-free material will be about 160 g kg^{-1} for crambe meal and 300 g kg^{-1} for rapeseed meal. Similar to rape, the relative lysine content in crambe seedcoat protein was higher than in endosperm (7.4 vs. 5.5 g per 16 g N, Earle et al., 1966). Due to the high level of cell wall constituents in the rapeseed coat, its energy digestibility was found to be around 20% in pigs (Bell, 1993). This results imply that defatted crambe meal is very likely to be more digestible than rapeseed meal for non-ruminants.

Table 1 shows the proximate composition of the rapeseed and crambe meals as well as their diets. Both test feeds contained similar levels of residual fat. Crambe meal studied contained a very high level of crude protein, up to 504 g kg^{-1} when calculated on oil-free DM basis, whereas rapeseed sample had 367 g kg^{-1} on the same basis. Considerable differences were found in the carbohydrate fractions: crambe contained 65 g kg^{-1} crude fibre, but the rapeseed had 139 g kg^{-1} (oil-free DM basis). The hemicellulose content in crambe meal was slightly lower than in rapeseed sample, whereas cellulose in crambe meal was less than half (42%) of that presented in rapeseed meal. Levels of NDF and pectin in crambe sample were also only half of those in rapeseed sample, and the level of ADL was 26% of that in rapeseed sample. These results are in good agreement with our previous findings reported by Steg et al. (1994) and Liu et al. (1994b). Differences in fibre contents between crambe and rapeseed meals are largely attributable to their seedcoat proportions as discussed previously. The crambe sample in this study was found to contain less sugar than rapeseed.

Steg et al. (1994) reported that solvent extracted but unheated crambe meal contained 152 g kg^{-1} sugar whereas rapeseed meal had 105 g kg^{-1}. Similar differences can also be found in the experimental diets as shown in Table 1.

Table 2
Crambe and rapeseed meals: apparent faecal and ileal digestibility (%) in pigs[a]

	Crambe	Rapeseed	Difference
Faecal (overall)			
Dry matter	76.4 (4.6)	70.1 (1.4)	*
Organic matter	78.7 (4.2)	73.2 (1.2)	*
Ash	50.8 (9.5)	29.9 (5.0)	**
Crude protein	76.8 (3.6)	79.1 (1.9)	ns
Crude fat	86.7 (4.7)	78.6 (2.4)	*
Crude fibre	60.1 (10.0)	38.3 (1.2)	**
N-free extract	81.9 (5.0)	78.1 (1.2)	ns
Phosphorus	43.4 (14.8)	22.5 (6.2)	**
Gross energy	78.3 (3.6)	73.2 (1.5)	*
Ileal			
Dry matter	51.1 (7.3)	48.2 (5.2)	ns
Organic matter	55.3 (7.0)	51.1 (5.1)	ns
Ash	5.4 (12.0)	10.1 (11.3)	ns
Crude protein	63.0 (3.1)	71.7 (1.9)	**

a, mean and standard deviation; ns, not significant; * $P<0.05$; ** $P<0.01$.

The amino acid profiles of both test feeds are presented in Table 3. The results for rapeseed meal used in this study are similar to the literature data for samples without subjecting to high temperature treatment, as indicated by 6 g lysine per 16 g N (Jensen et al., 1994). The data of crambe amino acids are in line with literature results (Steg et al., 1994 and Liu et al., 1993) except for lysine, which was only 3.9 g per 16 g N in this study, agreeing with our previous findings, where lysine content fell from 5.5 to 3.9 g after moist-heating with sodium carbonate (Liu et al., 1994b). There is evidence that processing rapeseed meal at high temperature and long duration decreases lysine content as well as its availability in poultry (Anderson-Halfermann et al., 1993) and in rats (Jensen et al., 1994), due to the Maillard reaction. During rapeseed meal processing the heat treatment releases glucose from glucosinolates, and this together with reducing saccharides will actively react with ε-NH_2 of lysine, resulting in a significant decrease in both lysine content and its biological value (Jensen et al., 1994). On the other hand, a toasting treatment was considered essential for crambe acceptance by the pigs (Liu et al., 1994b). The total content of glucosinolates in crambe meal decreased from over 120 μmol g^{-1} to 1 μmol g^{-1} after treatment, and the content of OZT+ITC declined from over 20 mg g^{-1} to 8 mg g^{-1}. Rapeseed meal was analyzed to contain 4 μmol g^{-1} intact glucosinolates and 6 mg g^{-1} OZT+ITC.

As presented in Table 1, in this study crambe meal contained 17 g kg^{-1} P in oil-free DM, about 62% of it existing as phytin-P, whereas in rapeseed meal the corresponding values were 13 g kg^{-1} and 83%.

3.2 Apparent total-tract digestibility in pigs

The levels of glucosinolates in the test feeds did not cause any palatability problem in spite of 300 g kg^{-1} dietary inclusion levels. Apparent digestibility data are presented in Table 2. For rapeseed meal, the overall digestibility of GE was found to be 73.2% in this investigation, which is comparable with previous reports: 75% by Keith and Bell (1991); 74.3% by Bourdon and Aumaitre (1983). Our study resulted in 79% apparent digestibility coefficient for crude protein. According to Rundgren (1983), low glucosinolate rapeseed meals average a digestibility of 79% for protein, and for GE between 64 - 80%, depending on the extent of seeds' dehulling. The digestibility coefficients for GE precisely followed the data for organic matter.

The organic matter apparent digestibility for crambe meal was 5.5 percentage units higher than that of rapeseed meal ($P<0.05$). Similar trends were evident for the other components such as crude fat, N-free extract. Crude fibre digestibility in crambe meal was remarkably higher (21.8 percentage units) than in rapeseed meal ($P<0.01$). This may be explained by lower levels of cellulose and lignin, as discussed earlier. The lower fibre content also inversely affected digestibility of other components, except for crude protein of which the digestibility was slightly lower for crambe meal than for rapeseed meal. This is mainly related to the crambe treatment as will be discussed later. Interestingly, the phosphorus digestibility in crambe meal was twice of that in rapeseed meal ($P<0.01$), the fact that a higher proportion of rapeseed phosphorus was bound in phytin gives a most probable explanation. Bell (1984) reviewed that about two thirds of phosphorus in rapeseed meal was bound in phytin, and Larsen and Sandstrom (1993) reported that phosphorus retention in pigs from rapeseed meal was between 20 and 30%. However, no previous references were found concerning the digestibility of crambe phosphorus.

Based on the information in Tables 1 and 2 and the energy evaluation system (CVB, 1994), the available energy contents of the test feeds can be calculated as: crambe meal, 16.86 MJ DE and 10.61 MJ NE per kg DM; rapeseed meal, 15.45 and 9.86, respectively.

3.3 Apparent ileal digestibility in pigs

At the ileal level there was also a tendency towards a higher digestibility of both DM and OM for crambe than for rapeseed, although the difference was not significant. Table 3 shows the apparent ileal digestibility of nitrogen and amino acids. The ileal digestibilities of amino acids for rapeseed meal in this study were generally in agreement with data reported by Sauer et al. (1982) and Yin et al. (1994). Apparent protein digestibility at the ileal level was considerably lower than that at the faecal level for both samples, and crambe had a significantly lower protein digestibility than rapeseed ($P<0.01$). Differences for lysine, cystine, asparagine and glycine, were 14.2, 8.7, 9.5 and 10.5 percentage units, respectively ($P<0.01$). The digestibility of other amino acids was slightly lower in crambe than in rapeseed sample.

Table 3
Amino acid content of crambe and rapeseed meals and their apparent ileal digestibility in pigs[a]

	Content, g per 16 g N		Apparent digestibility, mean (Sd), %		
	Crambe	Rapeseed	Crambe	Rapeseed	Difference
Nitrogen, g kg^{-1}	70.7	51.9	63.0 (3.1)	71.7 (1.9)	**
Alanine	4.3	4.6	68.8 (4.7)	73.7 (2.0)	*
Arginine	5.8	6.2	84.5 (1.8)	84.6 (1.3)	ns
Aspartic acid	6.7	7.9	63.5 (3.0)	73.0 (1.6)	**
Cystine	2.8	2.5	62.5 (4.0)	71.2 (4.0)	**
Glutamic acid	15.8	15.9	76.7 (2.7)	82.1 (1.5)	*
Glycine	5.5	5.3	65.8 (2.7)	76.3 (2.2)	**
Histidine	2.5	2.8	77.1 (2.4)	79.1 (2.2)	ns
Isoleucine	4.2	4.3	71.2 (4.5)	74.7 (1.7)	ns
Leucine	6.5	7.0	74.5 (5.2)	77.6 (2.6)	ns
Lysine	3.9	6.0	61.1 (2.5)	75.3 (1.7)	**
Methionine	1.8	2.0	81.9 (3.4)	84.3 (1.1)	ns
Phenylalanine	4.0	4.1	76.7 (5.4)	77.7 (2.5)	ns
Proline	6.3	6.2	77.4 (2.2)	78.6 (2.5)	ns
Serine	4.1	4.6	69.7 (3.1)	73.4 (2.7)	ns
Threonine	4.8	5.0	64.6 (2.2)	69.2 (2.9)	*
Tryptophan	1.2	1.3	70.5 (1.7)	76.0 (3.0)	*
Tyrosine	3.1	3.4	74.7 (6.2)	75.0 (2.4)	ns
Valine	5.1	5.7	69.0 (4.8)	71.7 (1.7)	ns

a, * $P<0.05$; ** $P<0.01$; ns, not significant; Sd, standard deviation.

From this investigation it can be assumed that not only lysine content, but also the ileal digestibility of lysine and crude protein in crambe meal was impaired by the severe detoxification treatment. An in-vitro study using pepsin incubation showed that the digestibility of crambe protein fell from 90 to 72% after a severe toasting with sodium carbonate, whereas toasting without an alkali resulted in only 3 percentage units of decrease (Liu et al., 1993, unpublished observation). The negative effect of overheating on lysine content and availability in mono-gastric animals is generally observed (Van der Poel, 1990; Anderson-Halfermann et al., 1993; Jensen et al., 1994). Other probable reasons for the low ileal protein digestibility of CM could be due to the effect of anti-nutritional factor (epi-progoitrin) which may cause more endogenous N excretion as shown in the rat trial. However, this would not only hamper the digestibility of lysine and cystine. Bjerg et al. (1989) concluded that epi-progoitrin has a strong negative effect on feed intake but little on true digestibility. Therefore, there is still considerable scope for detailed research on this aspect. Future study should be focussed on developing a more appropriate detoxification treatment which does not reduce protein utilisation.

3.4 Post-ileal digestion

Both ileal and faecal digestibility coefficients enabled an estimation of hind-gut digestion. Obviously all post-ileal digestibility coefficients are positive and some of them are rather high

in percentage: for crambe 25.3 for DM, 23.4 for OM, 12.6 for protein; for rapeseed the respective figures are 21.9; 22.1 and 7.4. Our coefficients for protein in rapeseed meal are similar to the data reported by Yin (1994), but figures for DM are considerably higher. The post-ileal digestion of protein differred obviously between crambe and rapeseed: the higher post-ileal digestibility of rape protein is probably due to its lower anterior-caecum digestibility, since it has been recognized that diets with higher fibre or components with lower ileal digestibility will facilitate a higher post-ileal fermentation, due to a higher level of available substrates (Schroeder et al., 1989). The energy released in the hind-gut is partially utilisable by pigs, whereas the N digested from large intestines is of no nutritional value for pigs because it is absorbed as urea and thereafter excreted in urine (Zebrowska et al., 1977).

3.5 Digestibility and N utilisation in rats

Results from the trial with rats are shown in Table 4. The crambe meal used was toasted at 110^{0}C for 40 min, which was less severe than that applied for the pig trial. About 55 μmol intact glucosinolates remained in the toasted crambe meal, corresponding to 11 μmol in the diet. The treatment was insufficient to remove the palatability problem: the rats showed feed refusals during the adaptation period. Intake improved during the collection period and the animals completed the trial successfully and all grew during the experiment.

As shown in Table 4, the GE digestibility coefficient for rapeseed meal was comparable to the previous results (Jensen et al., 1994). The figure for crambe meal was significantly higher than for rapeseed meal ($P<0.01$), which may be largely due to the low fibre content. However, the coefficient for crambe meal observed in this trial was extremely high and can not be easily explained. For crude protein both apparent and true digestibility coefficients were similar between the two samples. However, the BV and NPU values for crambe were significantly lower than those of rapeseed meal ($P<0.01$) due to a larger amount of N excreted in urine. It is most likely that the high level of epi-progoitrin in the crambe diet decreased the N utilisation as Bjerg et al. (1989) observed that epi-progoitrin has little influence on protein digestibility, but decreased the BV significantly, from as low as 0.5 μmol in the diet of rats. The mode of action has not been fully understood, presumably glucosinolates or catabolic products interfere with proteins, enzymes or cofactors in the animal enzymes.

Despite the fact that crambe meal was the only source of dietary protein for the rats, the animals showed no visible problems in health during the balance trial. During the subsequent period of fifteen days, the dilution of crambe meal by casein resulted in an obvious improvement in feed intake. Average growth of rats on crambe was 2.3 g per day compared to 2.5 g for the control animals during this period. Examination of the internal organs revealed enlargements as presented in Table 4, particularly the thyroid. Presumably, the negative influence was already developed during the balance period due to the high glucosinolate content in the diet. 3.5 Comparison of the results from pigs and rats

Table 4
Digestibility, N utilisation and size of some internal organs of rats fed crambe or rapeseed meals as sole protein source[a]

	Crambe Mean (Sd)	Rapeseed Mean (Sd)	Difference[b]
Balance trial			
GE digestibility, %	91.4 (0.8)	79.6 (2.6)	**
CP, apparent dig., %	78.4 (1.5)	78.8 (0.9)	ns
CP, true dig., %	88.4 (1.5)	89.0 (0.9)	ns
Biological value, %	46.8 (4.1)	87.1 (1.6)	**
Net protein utilisation, %	41.7 (4.0)	77.6 (0.8)	**
Internal organs			
Liver, g	4.4 (0.3)	5.3 (0.2)	
g per 100 g body wt	4.6 (0.4)	4.1 (0.1)	*
Thyroid, mg	10.5 (1.5)	7.8 (1.3)	
mg per 100 g body wt	11.0 (1.8)	6.1 (1.0)	**
Kidney, g	1.0 (0.1)	1.1 (0.1)	
mg per g body wt	10.2 (0.3)	8.5 (0.6)	**

a, For the balance trial, rats were provided with 10 g dry matter and 150 mg N per rat per day. All dietary N originated from either crambe or rapeseed meal as test feed. Other ingredients were starch plus vitamins and minerals.
b, $P<0.05$; ** $P<0.01$; and ns, not significant.

The apparent digestibility coefficients of crude protein found in rats are in good agreement with those from pigs: for crambe protein the coefficients were 78.4% for rats and 76.8% for pigs; for rapeseed 78.8% for rats and 79.1% for pigs. If apparent ileal digestibility is used as an indication of protein and lysine utilisation in pigs, the data show that crambe meal has clearly lower protein values than rapeseed meal. As discussed earlier, the lower ileal value of crambe protein in pigs is assumed to be due to the over-toasting that lowered the protein availability, whilst the low value of BV and NPU in rats could be due to the possible interference from the residual epi-progoitrin.

Both rats and pigs showed that energy digestibility of crambe meal is significantly higher than of rapeseed meal, despite a considerable difference in extent of digestion between the two types of animals, i.e. 15 percentage units for crambe and 6 percentage units for rapeseed. This difference might be partially due to differences in sample preparations.

3.6 Conclusion

In conclusion, this study revealed that decorticated and defatted crambe meal contained a significantly higher level of protein and lower level of fibre than rapeseed meal. Subsequently, its apparent digestibilities of gross energy, organic matter, lipids and fibre were significantly higher in both pigs and rats. On the other hand, the investigation also indicates the protein in crambe meal as processed in this study to be of lower nutritive value than the double low variety of rapeseeds, which is presumably due to over-toasting and/or

the residual anti-nutritional factor present in the crambe sample. It is expected that both ileal digestibility and utilisation of crambe protein can be enhanced by improvement of the detoxification treatment.

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Chapter 6

Crambe Meal: Removal of Glucosinolates by Heating with Additives and Water Extraction

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Crambe Meal: Removal of Glucosinolates by Heating with Additives and Water Extraction

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Abstract

In order to remove glucosinolates from dehulled and defatted crambe meal, various treatments involving heating with or without chemical additives and aqueous extraction were investigated on laboratory scale. Results showed that the levels of vinyl-oxazolidine-thione (OZT) and isothiocyanates (ITC) in crambe cake were reduced by 60% after heating at 100-110°C for 60 - 80 min and reduced by 95% upon addition of 2-3% sodium carbonate (Na_2CO_3) or 1% ferrous sulphate ($FeSO_4 \cdot 7H_2O$). Simultaneously, up to 2.5% dry matter of crambe cake was lost during heating, which is mainly attributed to the evaporation of the glucosinolates as well as their derivatives. More glucosinolates were decomposed in the presence of a high moisture content. A 10% reduction of lysine content was observed during heating and a further 10% by addition of the chemicals. Water extraction removed nearly all the glucosinolates from the meal, but recovery of dry matter and protein was largely dependent on sample pre-treatment and filter porosity. Prior heating increased dry matter recovery from around 50% to 80%, and crude protein from 45% to about 90%.

The palatability of crambe meals was tested in piglets (from 20 to 38 kg liveweight) during a 4-week period, in which the proportion of crambe meal in the diet was increased by 1% daily. A minimum dietary inclusion (3%) of untreated crambe meal made the diet unpalatable and led to poor growth of the piglets (44 g day^{-1}). However, the intake of diets containing up to 20% toasted crambe meals was equal to the control, resulting in the same growth rate (570 g day^{-1}) as on a commercial diet. The results suggest that adequate treatment of crambe meal can result in a product with good property as an ingredient in pig diets.

1. Introduction

Over the past few years, *Crambe abyssinica* Hochst ex R.E. Fries, a member of the Cruciferae family, has been introduced in many countries, as a new source of industrial oil having high erucic acid content (Liu et al., 1993). The refined triglyceride oil is well suited as a mould lubricant in the continuous casting of steel, and the isolated erucic acid is highly desired by the plastics industry (Muuse et al., 1992). The by-products resulting from crambe defatting may be used as a feed ingredient for livestock (Pereira et al., 1981; Carlson and Tookey, 1983; Liu et al., 1993).

Previous work in our laboratory showed that the nutrients in dehulled and defatted crambe were easily degradable in the rumen and highly digestible in the intestines of dairy cows (Liu et al., 1994). However, the main constraint of feeding crambe by-products to livestock is

their high level of glucosinolates, which render them toxic to non-ruminants (Hesketh et al., 1963; Van Etten et al., 1969b) and lower their palatability to ruminants (Lambert et al., 1970 and Perry et al., 1979). Epi-progoitrin has been identified as the main source of toxicity (Tookey et al., 1980).

The nutritional acceptance of crambe meal can be enhanced by either decomposing the glucosinolates or removing them by extraction. According to previous reports, the glucosinolates may be diminished by sodium carbonate (Mustakas et al., 1968), ammonia-heating (Kirk et al., 1966a), and ferrous sulphate (Kirk et al., 1971 and Fenwick et al., 1986). Enzymatic hydrolysis or decomposition of epi-progoitrin results in isothiocyanates (ITC), which are largely volatile or may further cyclise to vinyl-oxazolidine-thione (OZT), both are found to be goitrogenic to animals (Van Etten and Tookey, 1980). If such hydrolyses occur in acidic medium, as in the natural pH of crambe meal (5.0 - 5.3), nitriles will be produced (Uda et al., 1986). Nitriles are detrimental to animals (Tookey et al., 1980) but their formation can be prevented by increasing pH from 5 to 6.5 - 7.5, leading to a shift from nitriles to ITC (Uda et al., 1986).

Toasting has often been used to decompose the glucosinolates in rapeseed and crambe by-products. However, the influences of temperature, duration of heating, moisture and additives have not yet been thoroughly investigated. After treatments, the amount of decomposed glucosinolates was often found to be much less than theoretically expected, such differences remain unaccounted for (Austin et al., 1968; Kirk et al., 1971).

Because the glucosinolates and most of their derivatives are water soluble, detoxification through water extraction was considered to be desirable (Kirk et al., 1966b; Mustakas et al., 1976; Baker et al., 1977), yielding an apparently non-toxic meal with improved nutritional quality (Pereira et al., 1981). However, high percentage dry matter loss during the extraction has remained a problem, owing to both nutrient loss and waste water handling.

The objectives of this study were to systematically investigate the influence of heat treatments, level of chemical additives and water extraction in relation to the efficiency of detoxification and recovery of nutrients. Finally, a palatability trial was conducted in piglets to obtain an indication of the effect of such treatments on the feeding potential of the meal for pigs.

2. Materials and Methods

2.1. Crambe

The seeds were harvested in the Netherlands in 1992, industrially dehulled and defatted by cold-pressing and the residue was pelleted as crambe cake without being subjected to high temperature. Further solvent extraction was performed in our laboratory using petroleum ether (PE40:60). Both cake and solvent extracted meal were used in this investigation. Their chemical composition is listed in Table 1.

2.2. Heating

A laboratory autoclave (Presto Cooker-canner, 15 l, Rotterdam, Netherlands) was used, with adjustable temperature and pressure. The toasting procedure in our trials followed that of Kirk et al. (1971). Approximately 20 g crambe cake or meal was weighed into nylon bags (9 × 18 cm, pore size 40μm) in triplicate, to determine the effects of temperature, duration, chemical additives and moisture. For use in the palatability trial, batches of 20 kg dehulled and defatted crambe meal were toasted at 110^{0}C for 60 min. After 15 min of preheating, the sample was moistened by spraying with a 15% quantity of water at room temperature and stirred to obtain a uniformly warm and moist penetration. The extra moisture was evaporated during and after steaming.

Previous experiments conducted by Kirk et al. (1971), Fenwick et al. (1986) and Uda et al. (1986) suggested that sodium carbonate (Na_2CO_3) and ferrous sulphate ($FeSO_4 \cdot 7H_2O$) are most effective in decomposing the glucosinolates and these were also used as additives in our trials. The testing levels were 0, 1, 2 and 3% for sodium carbonate and 0, 0.5, 1.0 and 1.5% for ferrous sulphate. Sodium hydroxide (NaOH) was additionally examined in order to decrease minerals left in crambe by-products. These chemicals were added as powder except for sodium hydroxide which was added as a solution in water. The chemicals were thoroughly mixed with crambe samples before toasting. Preheating was allowed for 15 min as preconditioning in each run.

Table 1
Composition and glucosinolate content of dehulled and defatted crambe (DM basis)

Press cake	Extracted meal	
Fat, g kg^{-1}	247	20
Crude protein, g kg^{-1}	374	494
Crude fibre, g kg^{-1}	44	52
Ash, g kg^{-1}	72	104
Nitrogen-free extracts, g kg^{-1}	263	330
Glucosinolates, μmol g^{-1}	116	168
OZT[a], mg g^{-1}	16±0.8	22±2.1
ITC[a], mg g^{-1}	8±1.7	13±1.3

a. OZT and ITC, vinyl-oxazolidine-thione and isothiocyanates, respectively, measured after enzymatic (myrosinase) hydrolysis at pH 7.0.

2.3. Water extraction

A series of sieves (screen porosity 0.500, 0.250, 0.125, 0.063 mm) were mounted on a sieve shaker (Fritsch Laborgerätebau, Germany; type 03.502). The shaker was equipped with a top cover allowing water spraying through the top during shaking; extracts were collected at the bottom of the column. For each washing run, the crambe sample was slurried with water and aloowed to settle for 30 min, then the slurry was transferred to the top sieve, and shaken for 20 min. Fresh water was sprayed from the top and shaking was repeated. Residues were collected from each of the sieves, dried at 70^{0}C, weighed and ground through

a 1 mm sieve and analysed for nitrogen content. The extracts were then filtered through Whatman filter paper, and then centrifuged at 1000 × g for 15 min. The supernatant was removed and precipitates were treated as described previously.

2.4. Palatability trial

Dehulled and defatted crambe meal was used in this trial. Twenty weaned piglets (Dutch Landrace, average liveweight 20 kg) were allocated at random to five treatments, each containing four animals (two castrates and two females) penned as a group. The animals were allowed a few days for adaptation and fed individually. The treatments were: (A) commercial starter diet (control); (B) crambe meal without additional treatment; (C) crambe meal toasting only; (D) crambe meal toasting, plus 3% sodium carbonate; (E) crambe meal toasting, plus 1% ferrous sulphate.

The main ingredients of the commercial diet (%) were: barley 55.9, cassava 15.0, soya bean meal 7.5; fish meal 5.4; meat meal 2.0; maize gluten feed 10.0; linseed meal 2.5. The calculated values (kg^{-1} dry matter) were: net energy 9.5 MJ (CVB, 1992), crude protein 175 g, lysine 11.2 g, calcium 8 g and phosphorus 5.9 g. This diet was to formulated to provide basic amino acids, minerals and vitamins. Control piglets were fed at a level of 2.8 times maintenance (293 kJ $kg^{-0.75}$liveweight), in accordance with the requirement for their growth. In the other treatments, the commercial diet was substituted gradually for crambe meals at a daily substitution rate of 1% up to a maximum of 20% after 20 days, providing the same feeding quantity as for the control animals. Observations at the maximum inclusion level were made for 1 week. When feed refusals occurred, for the animal concerned no further increase in crambe was applied for 1 week. The animals were weekly weighed. Feed refusals were collected, weighed and analysed. Feed intake, growth rate and health status of the animals were monitored.

2.5. Analyses

Proximate analyses were used to determine the chemical constituents of the crambe samples following routine procedures. Amino acid profiles before and after various heat treatments were determined using high performance liquid chromatography (HPLC) following the LKB Biochrom (1986) method, but methionine and tryptophan were excluded in this analysis. Total glucosinolates were measured by DLO-State Institute for Quality Control of Agricultural Products (RIKILT-DLO), using HPLC. The contents of OZT and ITC were determined separately using the ultra-violet absorption after enzymatic hydrolysis at pH 7 according to the method of Wetter and Youngs (1976). The myrosinase (thioglucosidase EC 3.2.3.1) used was a commercial product from Sigma Co. Amsterdam. pH was determined in aqueous dispersion (1 g sample in 10 ml distilled water) after 30 min.

3. Results and Discussion

3.1. Decomposition of glucosinolates by heating

3.1.1. Initial trial

This trial was conducted to investigate the efficacy of the glucosinolate decomposition by heating crambe cake with or without various additives. Table 2 shows the results after heating for 30 min. The data in the table show that the treatments reduced OZT and ITC contents, and this decomposition was accelerated by addition of alkalies and ferrous salt. The results agreed favourably with the literature from Mustakas et al. (1968); Kirk et al.(1971) for crambe meal and Fenwick et al.(1986) for rapeseed meal. Therefore, further trials were conducted to verify the influence of various reaction conditions.

3.1.2. Heating time and temperature

Fig. 1 shows the results of variation in heating temperature and duration. About two-thirds of OZT+ITC were lost during heating at 100°C for 60 - 80 min in the absence of additional moisture. This was further confirmed by the HPLC analysis, showing that only one-third of total glucosinolates (33 μmol g^{-1}) remained intact after 80 min at 100°C. Similar results were repeatedly observed during the successive trials using solvent-extracted meal. As heating temperature and time increased, content of both derivatives and intact glucosinolates decreased. After heating for 1 h at 115°C, no intact glucosinolates and only traces of OZT and ITC were detected, indicating a complete destruction of the glucosinolates.

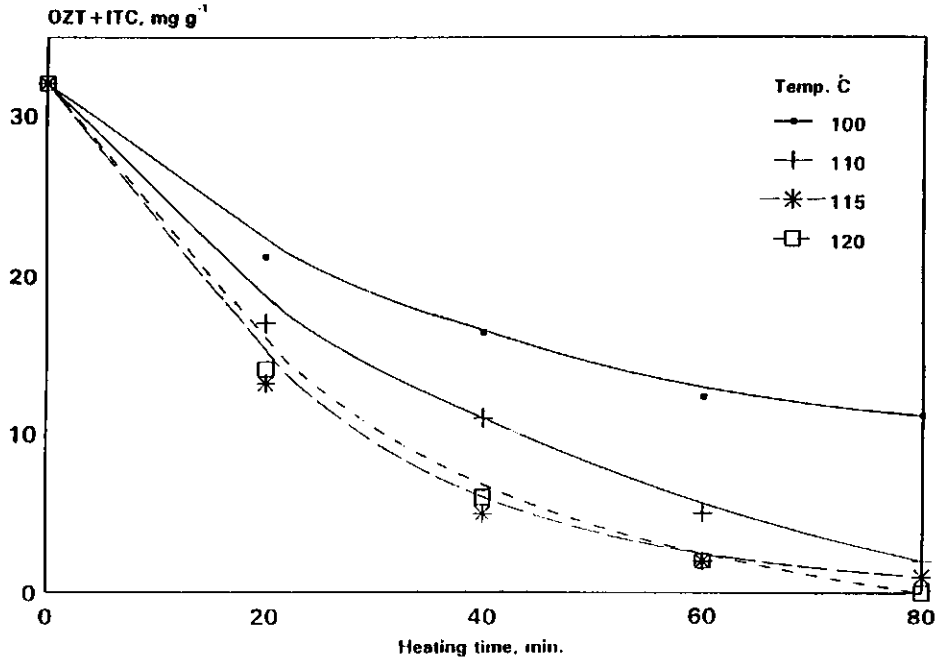

Fig.1 Glucosinolate decomposition (OZT & ITC) in crambe cake after heating at certain temperature for various periods.

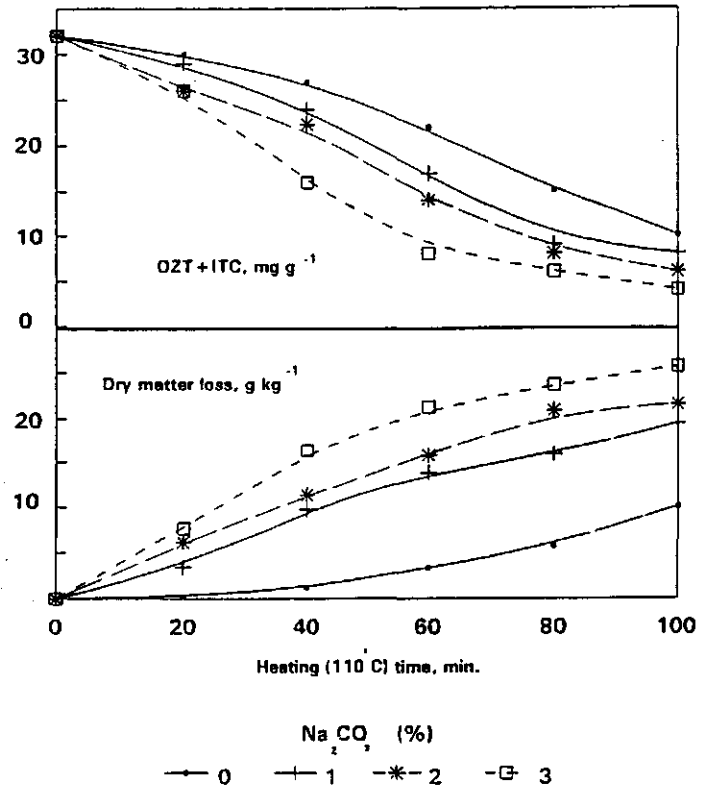

Fig.2 Glucosinolate decomposition and dry matter loss in crambe cake after heating with sodium carbonate.

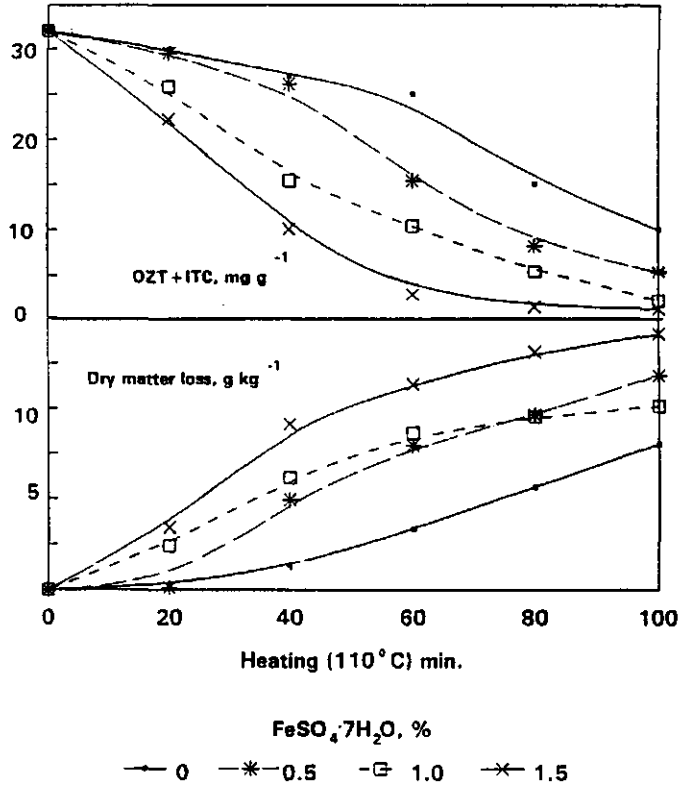

Fig.3 Glucosinolate decomposition and dry matter loss in crambe cake after heating with ferrous sulphate.

Table 2
Crambe cake: decomposition of glucosinolates by heating with chemical additives at 110°C for 30 min, (mg g^{-1} on fat and moisture free basis)

	OZT[a]	ITC[a]
Crambe cake	20.6	11.0
Heating only	14.6	8.5
Heating with:		
A. 1% NaOH[b]	12.1	6.6
B. 3% Na_2CO_3	12.9	9.9
C. 3% $FeSO_4 \cdot 7H_2O$	5.8	2.2
D. B+C	4.5	1.2

a. Abbreviation as in Table 1.
b. Added as solution.

3.1.3. Chemical additives

In this trial, various levels of sodium carbonate and ferrous sulphate were added as chemical additives. Samples were subjected to different heating time. Results are illustrated in Fig. 2 and 3. The disappearance of glucosinolates was obviously accelerated by the addition of increasing levels of both chemical additives. These results are in accordance with those obtained by Kirk et al. (1971).

Along with the destruction of glucosinolates, dry matter losses were observed during heating (Fig.2 and 3). Moreover, such a dry matter loss was proportionally correlated with the rate of disappearance of glucosinolates. This observation was further confirmed when using solvent-extracted crambe meal instead of cake. The dry matter loss was always accompanied by an unpleasant smell evaporating from crambe samples, in accordance with the previous finding that some volatile flavours are derived from glucosinolates of Cruciferae plants (Van Etten and Tookey, 1980). Recently, Kim and Rhee (1993) reported that a decrease of pungency in radish was accompanied by a decrease in 4-methylthio-3-butenyl isothiocyanate derived from its parent glucosinolate. Chemically, epi-progoitrin (2-hydroxy-3-butenyl-glucosinolate) consists of three fractions: glucose, sulphate (both non-volatile), together accounting for about 65% of the total molecular weight, and a radical CH_2=CH-CHOH-CH_2-CNS, which may be converted into volatile ITC and/or non-volatile nitriles, depending upon the pH of the medium. The formation of nitriles will only be quantitatively meaningful at pH < 6.5; at pH > 7.0 volatile ITC is formed (Uda et al., 1986). Therefore, addition of sodium carbonate will result in more dry matter evaporation in comparison with ferrous sulphate. Based on the molecular weight of epi-progoitrin (411), the crambe cake under study was calculated to contain 47.6 mg glucosinolates g^{-1} dry matter (DM). Some of the components in glucosinolates are non-volatile and non-toxic (glucose and sulphate). Figs. 2 and 3 show that 25 mg g^{-1} DM was lost during heating, which provides a reasonable explanation for the gap in quantity of glucosinolates detected before and after Na_2CO_3-treatment. The lower dry matter loss upon addition of ferrous salt is likely to be due to the formation of non-volatile nitriles at pH < 5, as observed by Kirk et al. (1971).

Table 3
Influence of moisture on decomposition of glucosinolates in crambe (measured OZT+ITC, mg g^{-1} on fat-free basis) at various heating times (minute at 110°C)

Heating time (min)	Moisture, %[a]		
	10	30	Difference[b]
Press cake			
0	29		
20	26	18	**
30	20	14	**
40	16	12	**
SEM[c]	0.3	0.2	
Extracted meal			
0	30		
40	15	13	*
60	12	9	**
80	9	4	**
SEM[c]	0.1	0.1	

a. Adjusted by spraying water, triplicate samples.
b. *, $P<0.05$ and **, $P<0.01$.
c. Standard error of means.

3.1.4. Effect of moisture

Table 3 shows the effect of different moisture levels during heating on the decomposition of glucosinolates. More glucosinolate had disappeared at increased moisture levels in both cake and solvent extracted meal. Our results agreed with the observations of Van Etten et al. (1969a). The moisture effect may be attributable to the requirement of at least 1 mole H_2O to hydrolyse 1 mole of glucosinolate in the early stage (Tookey et al., 1980).

3.1.5. pH change

A pH change was clearly observed during heating crambe material as illustrated in Fig. 4. The natural pH of crambe meal (and cake) was determined to be slightly above 5.0. As expected, pH increased after addition of sodium carbonate and decreased with ferrous sulphate. However, the medium pH increased for all treatments after heating, then dropped as heating continued. Kim and Rhee (1993) found that the decomposition of glucosinolates resulted in a decrease in pH. These changes in pH are possibly due to decomposition of the glucosinolates, initially to isothiocyanates and further to alkaline thiocyanate ion (SCN^-) and alcohols (Tookey et al., 1980), which are likely to be evaporated in the latter stage.

3.1.6. Protein and amino acids

No obvious decline in crude protein content was found after heat treatments. However, lysine content decreased by heat treatment, in particular when chemicals were added (Fig. 5). Carlson et al. (1985) reported similar observations during crambe processing. Lysine damage was attributed largely to Malliard reactions. In our study, decreases in lysine occurred in both sodium carbonate and ferrous sulphate, whereas Kirk et al. (1971) found such reduction only in the case of alkaline additives, like sodium carbonate. The levels of other amino acids under examination remained unchanged.

From these investigation it can be concluded that the glucosinolates in crambe meal can be decomposed by steam treatment. Addition of sodium carbonate, ferrous sulphate or extra moisture accelerates the detoxification as measured by OZT and ITC. Some of the toxic components are assumed to be evaporated as a pungent vapour during heating. In practice, heating for 60 - 80 min at 110°C in the presence of both sodium carbonate and moisture may result in an adequate detoxification. When using ferrous sulphate as additive, further investigation of nitriles is required.

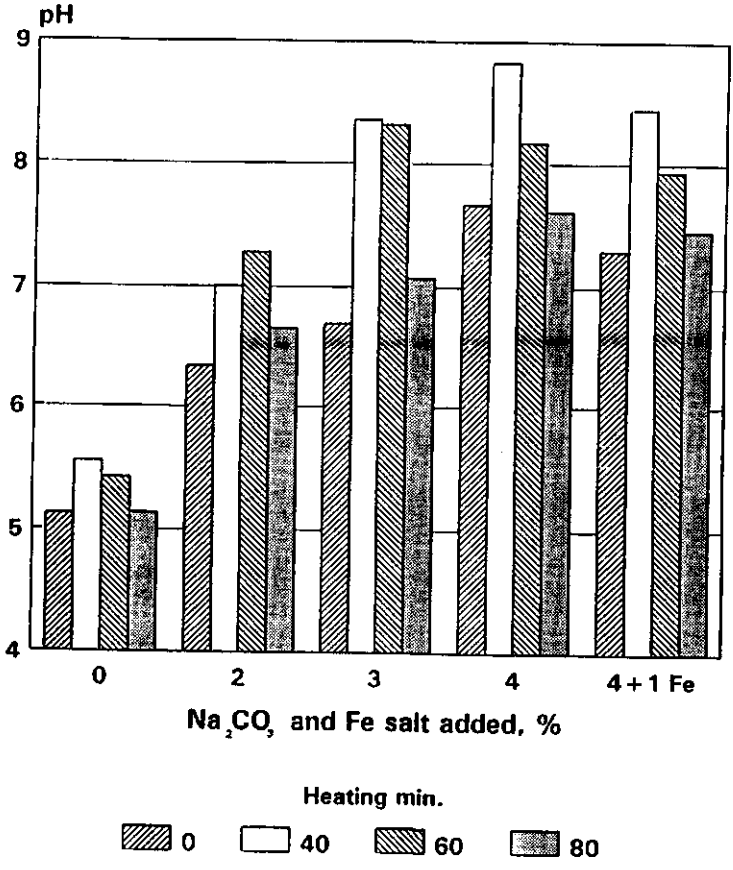

Fig. 4. Change in pH upon heating crambe meal (at 110°C) with or without additives.

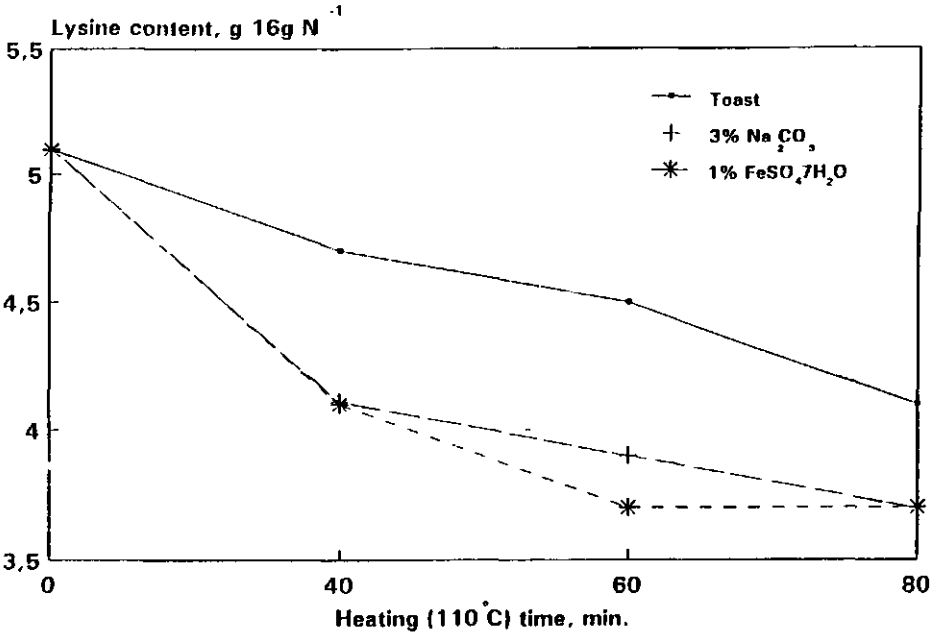

Fig. 5. Reduction in lysine content of crambe meal after various heat treatments.

3.2. Water extraction

3.2.1. Efficiency of glucosinolate removal

The results are shown in Fig. 6. The OZT and ITC in recovered fractions were negligible in the analysis method sensitive to 0.15 mg g^{-1}, or at very low levels (Treatments D, E and F), indicating the effective removal of the glucosinolates from the meals. The influence of different water to sample ratios on the detoxification efficiency was clear but relatively limited in this study. No systematic differences in levels of residual toxins were found amongst the various sieve fractions. These results agreed with the observations of Baker et al. (1977). Eventually, the solids presented in the abstracts can account for almost all the glucosinolates which had disappeared during the extraction, according to Kirleis and Brown (1980).

Comparing Treatments D, E and F with the other treatments, prior heating slightly reduced the solubility of the glucosinolates. This may be due to denaturing of protein and other constituents leading to binding or encapsulation of the glucosinolates or their derivatives. Mustakas et al. (1976) also found 29 mg g^{-1} epi-progoitrin in steamed and water-extracted meal, but they did not investigate a crambe sample without pre-heating.

3.2.2. Nutrient recovery after extraction

Fig. 6 illustrates the information concerning nutrient recovery after extraction. Extracting

unheated crambe meal always yielded deep yellow extracts unless a high water to sample ratio (50:1, Treatments A and B) was used. This ratio resulted in large dry matter loss through a 0.063 mm filter, for both cake and meal. Further filtration through Whatman 41 filter paper to recover more dispersed material was ineffective. After centrifugation at 1000 × g for 15 min, 15 - 21% of the original dry matter and 16 to 26% of the original crude protein were additionally recovered for Treatments A and B. The sediments of centrifugation of A and B contained higher crude protein (391 g kg^{-1} and 589 g kg^{-1}, respectively, for A and B) than the others, indicating that a considerable amount of protein was solubilized in the extracts. Dry matter recovery was improved by using less water. A ratio of 8:1 and a single filter 0.063 mm (Treatment G) resulted in an adequate toxin removal and 70% dry matter recovery. However, further reduction of water:sample ratio for an unheated sample resulted in a paste that did not pass through the filter.

Prior heating slightly decreased the removal of glucosinolates, but clearly improved the recovery of both dry matter and crude protein (C vs. D, G vs. F). In this study, the highest nutrient recovery (DM 79.1% and CP 90.2% in Treatment F) was obtained by using a

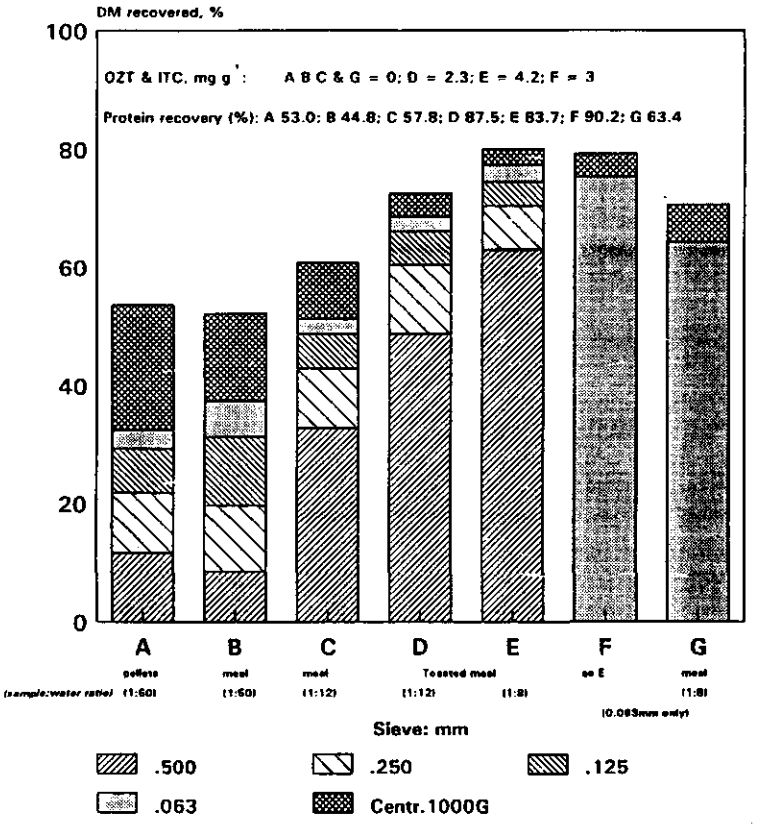

Fig. 6. Water extraction of glucosinolates from crambe meal.

water:sample ratio of 8:1 and a single filter 0.063 mm for toasted sample. It is known that crambe protein has a high solubility (Liu et al., 1994). Total protein recovery from unheated samples was rather low (44 - 63%) even including the portion from centrifugation. Prior heating increased this to 84 - 90%, showing that toasting is particularly effective to decrease protein solubility in crambe. It appears almost impossible to exceed 80% DM recovery when extracting a crambe sample by normal filtration. In semi-pilot scale studies, Mustakas et al. (1976) using toasted crambe meal and Liu et al. (1987) using rapeseed meal, both obtained approximately 75% recovery. The dry matter loss is in part attributable to the glucosinolates and substantially to soluble carbohydrates, e.g. sucrose and dextrose (Baker et al., 1977; Kirleis and Brown, 1980).

The results have demonstrated that water extraction can efficiently remove the toxins, but nutrient recovery will largely depend on sample pre-treatment and filter porosity. Prior heating significantly increases the recovery of both protein and dry matter. About 80% dry matter recovery can be expected when the meal is pre-heated. Research on waste water handling is necessary if the method is to be applied in industry.

3.3. Palatability for piglets

Growth performance of the piglets is illustrated in Fig. 7. The contents of OZT+ITC in crambe meals were 34 mg, 10 mg, 6 mg and 3 mg g^{-1}, respectively, for untreated, toasted, sodium carbonate and ferrous sulphate treated crambe meals. Piglets fed untreated crambe meal had feed refusals from the 3% dietary inclusion level, which did not improve during the following 2 weeks at the same inclusion level and some animals even refused to eat. Consequently, the animals showed a slight loss in weight (-28 g day^{-1}) during the first week, with limited liveweight gain during the second week (76 g day^{-1}). After this the crambe meal in this group was withdrawn and the animals were fed with commercial diet as for the control animals. This resulted in a rapid recovery in growth rate (Fig.7). In the contrast, animals receiving treated crambe meal consumed as much feed as those on the commercial diet, and reached the target dietary inclusion level, i.e. 20% crambe meal in their diets in 20 days, without feed refusals. The animals also displayed an equal growth rate of 570 g average daily gain. No disorders were observed from feeding treated crambe at 20% inclusion level in this initial experiment.

The experiment indicates that the untreated crambe meal is unpalatable to pigs even at minimum dietary inclusion level, but this can be overcome by toasting. In a short term trial piglets receiving treated crambe meal at 20% dietary inclusion level performed equally well as those fed a commercial diet, suggesting that the meal has a potential feeding value to pigs. Additional information is required before a recommendation can be made for including crambe meal in pig diets.

4. Acknowledgements

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concerning the experimental design of the animal trial and the assistance of V. A. Hindle in reviewing the text are gratefully appreciated.

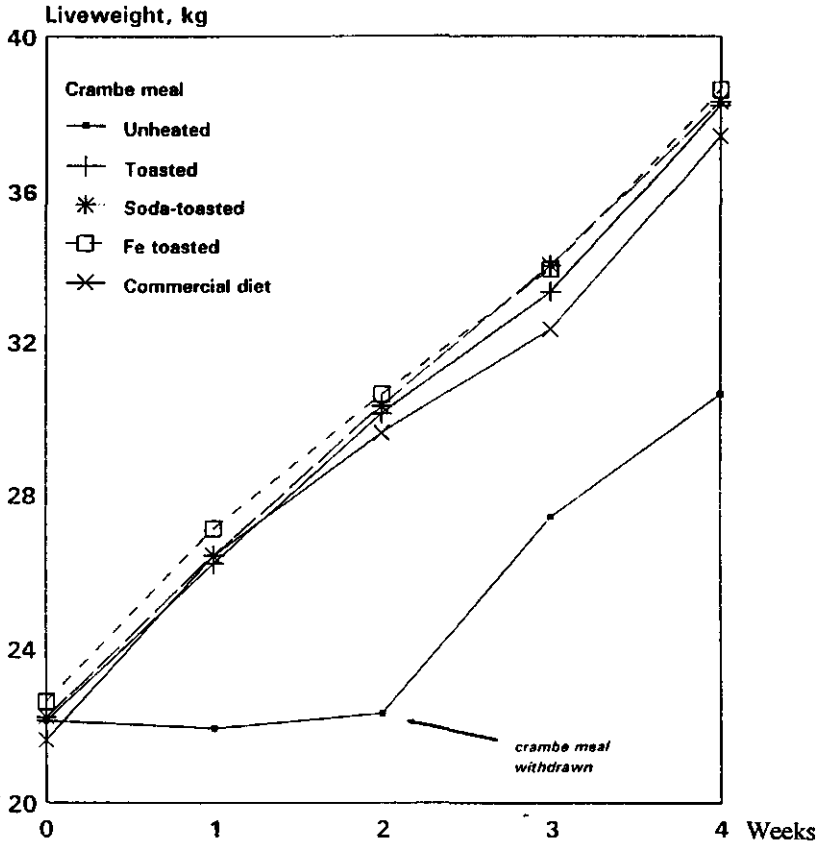

Fig.7 Average growth performance of piglets fed crambe meal at increasing levels (1% daily up to 20%). Allowance according to growth requirements.

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Chapter 7

The Effect of Heat Treatment on Glucosinolates and Nutritional Value of Rapeseed Meal in Rats

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The Effect of Heat Treatment on Glucosinolates and Nutritional Value of Rapeseed Meal in Rats

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Abstract

Samples of rapeseed meal were studied for the influence of heat-treatment on glucosinolates and nutritional value. After toasting at 100°C for 15, 30, 60 and 120 min., the total content of glucosinolates decreased 24, 46, 70 and 95%, respectively. 4-Hydroxy-glucobrassicin was found to be more heat-sensitive than the aliphatic glucosinolates progoitrin and gluconapin. Protein solubility decreased from 85% before heating, to 81, 61, 52 and 40% after their respective toasting. Total lysine content dropped from 5.93 to 5.72 and 4.91 g per 16 g N after 30 and 120 min. toasting respectively. Other amino acids remained relatively unchanged except cystine level dropped 12% after two hours toasting. The content of some mono-, di- and oligosaccharides decreased after toasting, particularly sucrose. True digestibilities (TD) of rape protein in rats were 77.0, 73.9, 72.1, 72.9 and 71.2%, respectively after 0, 15, 30, 60 and 120 min. toasting ($P<0.05$). Biological value (BV) and net protein utilization (NPU) also decreased significantly when toasting time was longer than 60 min. ($P<0.05$). Four commercial rapeseed meal samples, i.e. Danish extracted meal, Danish pressed cake, German extracted meal and Chinese extracted meal, were also investigated for their glucosinolates and nutritional quality following the same procedures. These samples displayed the same pattern as after various extent of heat-treatment. This experiment suggests that up to 30 minutes toasting can be accepted in practice without significantly jeopardizing nutritional quality of protein. Furthermore, besides determination of glucosinolates, protein solubility may be measured as an indication of protein quality after heat-treatment.

1. Introduction

Meal of double low rapeseed varieties is an excellent protein source with a well balanced amino acid profile (Bell, 1984; Bille et al, 1983a). However, the content of glucosinolates and their degradation products restrict the amount of rapeseed meal in the diet to monogastric animals. Hydrolyzed products of the glucosinolates are often more harmful than the intact glucosinolates (Bille et al., 1983b, Bjerg et al., 1989). Heat treatment prior to pressing and/or extraction is a common practice for reducing the toxicity by inactivating myrosinase, lipase and improving crushing capacity and oil yield of the seeds (Bandholm, 1991). If the crushing involves extraction with hexane an additional heat treatment with steam is performed in order to reduce the content of residual hexane in the meal (Grant et al., 1987).

Prolonged heat treatment of rapeseed meal will result in degradation of intact glucosinolates. Reported values in commercially processed rapeseed meals are 40 to 60% degradation of aliphatic glucosinolates (Daun, 1986) and up to 100% degradation of the

indole glucosinolates (Campbell and Cansfield, 1983). Overheating will also decrease lysine availability. It is thus of great importance to adjust the heat treatments during processing to keep the nutritional value of the rapeseed meal, and avoid unnecessary degradation of glucosinolates and loss of available lysine.

The purpose of this investigation was to measure the effect of toasting low-temperature extracted meal on the contents of glucosinolate and lysine, protein solubility and nutritional value in balance trials with rats. In addition, four commercial rapeseed meal samples of different origins were examined in the same way in order to reveal whether the differences in processing conditions can be justified by these techniques.

2. Materials and Methods

2.1. Sample and heat treatment

Seeds of double low rapeseed varieties were supplied from the production line of Scanola A/S DK-8100 Aarhus in Denmark. After flaking and heating to 95°C for 5 minutes the flaked seeds were defatted by extraction with hexane without further subjecting to high temperature. The meal was collected and toasted in a laboratory-scale toastor. Heat was provided by steam and toasting temperature was kept at 100°C. During toasting moisture content was increased from 5.5% to 8.6-10.8%. Toasting duration was 0, 15, 30, 60 and 120 minutes, respectively. After toasting the samples were dried, analyzed for protein solubility, amino acids and nitrogen utilization in rats.

Four samples of commercial rapeseed meals were collected. They were: Danish pressed cake from Scanola A/S, Danish extracted meal from Aarhus Oil A/S; German extracted meal and Chinese extracted meal. No additional treatment was performed for these samples.

2.2. Chemical analyses

Total content and individual glucosinolates in rapeseed meals were determined by high performance liquid chromatography (HPLC) of intact glucosinolates according to Sørensen (1990). Nitrogen and other proximate compositions were measured following the method of AOAC (1984). N-solubility was examined by measuring Kjeldahl N solubilized in the solution of 0.2 M KOH. Amino acid was analyzed by the procedure of Mason et al. (1980). Carbohydrates were determined by HPLC according to the method of Bach Knudsen and Li (1991).

2.3. Rat trial

Five Wistar male rats were used for each sample for the N-balance trial. The live weight of the animals were about 65 ± 0.5 g before the experiment. They were kept individually in metabolism cages, four days for adaptation and five days for collection of excrements. Each animal received 10 gram dry matter and 150 mg nitrogen per day, free access to water supply. To determine the influence of heat treatment, 50% of the dietary nitrogen (N) was

originated from the toasted rapeseed meal and the other 50% from wheat gluten supplied with 1 g DL-methionine per 100 g. Thereby it was ensured that lysine was the first limiting amino acid in the diet. Due to the low glucosinolate content in the diets (about 2 μmol g^{-1} diet), it was assumed that any reduction in biological value (BV) was caused by the declining of available lysine. However, due to different glucosinolate level in the four commercial samples the same methodology could not be applied for those samples. This meant that all dietary nitrogen was supplied by test rapeseed meal and the remaining parts were N-free maize starch. Vitamins and minerals were provided in the diets in accordance with their requirement. Any feed refusals were taken into account. The equipments allowed that the faeces and urine were collected separately, which enabled a calculation of digestibility and utilization of nitrogen. The details of the procedures and facilities were previously described by Eggum (1973).

At the end of the balance period the rats fed the four commercial rapeseed samples were allowed to feed ad libitum on their diets for another two weeks. The rats were then killed by CO_2 and their thyroid, liver and kidney were removed and weighed.

2.4. Data processing

The differences between treatments were calculated by variance analyses. Other statistics comparisons were further applied when necessary.

3. Results and Discussion

The proximate composition and carbohydrates of rapeseed meal sample before and after toasting as well as commercial samples are shown in Table 1.

3.1. Effect of heat treatment on glucosinolates

The effect of heat treatment on the content and profile of glucosinolates are illustrated in Figure 1. The total content of glucosinolates in unheated defatted rapeseed meal was around 16 μmol g^{-1}. As shown in the figure, there was a linear decline in the content of total glucosinolates after toasting. The first 15 min. toasting degraded 24% of the glucosinolates, while 46% was degraded after 30 min., 70% after 60 min. and 95% after 120 min. A correlation coefficient between toasting time and content of total glucosinolates can thus be calculated as -0.955 ($P < 0.01$), in good agreement with an observation by Liu et al. (1994a). They reported a similar reduction pattern of glucosinolates by measuring the residual level of oxazolidine-thione and isothiocyanates after toasting *crambe abyssinica* seed meal, and concluded that the decomposed glucosinolates were virtually vaporized during toasting. However, Bille et al. (1983a) found that only 13% of total glucosinolates were removed during toasting at 100^{0}C for 30 min.

Three main glucosinolates occurred in the rapeseed meal samples: progoitrin, gluconapin and 4-hydroxy-glucobrassicin. 4-Hydroxy-glucobrassicin was the most sensitive glucosinolate to heat treatment. The reduction of this glucosinolate was 36, 70, 93 and 97% respectively

after 15, 30, 60 and 120 min. toasting. This is in agreement with the observations from Campbell and Cansfield (1983) and Daun (1986). They found that almost 100% of the indole-glucosinolates were decomposed during processing on the normal oil extraction line, whereas only 40-60% of the aliphatic glucosinolates were decomposed.

Table 1
Effect of heat treatment on the contents of crude protin, HCl-fat and various mono-, di- and oligosaccharides in rapeseed meals, percentage in dry matter.

	Toasting, min.			Commercial sample[1]			
	0	30	120	A	B	C	D
Dry matter	95.4	91.9	91.7	90.2	93.7	92.2	90.7
Protein	38.2	39.1	39.6	37.6	34.4	39.4	42.4
HCl-fat	5.7	5.7	5.9	5.6	15.6	3.5	4.1
Stachyose	2.09	2.07	1.92	2.75	2.01	2.19	1.44
Raffinose	0.43	0.43	0.42	0.57	0.37	0.40	0.42
Sucrose	8.48	8.17	7.33	9.69	7.45	8.14	5.28
Glucose	0.23	0.22	0.34	0.28	0.19	0.28	0.36
Fructose	0.21	0.21	0.50	0.25	0.17	0.27	0.54
Total CHO[2]	11.5	11.1	10.5	13.5	10.2	11.3	8.0

1. A, Danish extracted meal; B, Danish press cake; C, German extracted meal and D, Chinese extracted meal.
2. Content of carbohydrates measured by HPLC.

The glucosinolate contents for the commercial samples varied: the German one had the lowest content of 6 μmol g^{-1} and the Chinese one showed the highest of 28 μmol g^{-1}. The high content of glucosinolates in Chinese rapeseed meal was previously reported by Liu et al. (1994b) that the conventional rape varieties are still overwhelmingly planted in China, producing seeds with high content of glucosinolates and erucic acid. According to the level of glucosinolates, particularly 4-hydroxy-glucobrassicin determined in this trial, samples of both Scanola and Aarhusolie had not or only slightly been heated; but the German and especially Chinese samples may have had rather severe heat-treatment.

3.2. Effect of heat on protein, amino acids and carbohydrates

3.2.1. Protein solubility.

Figure 2 shows the influence of heat treatment on nitrogen solubility. The nitrogen solubility of rapeseed meal prior to heating was 85%. However, it was linearly decreased to 81, 61, 52 and 40%, respectively after 15, 30, 60 and 120 min. toasting, giving a negative coefficient -0.934 ($P<0.05$). Carlson et al. (1985) reported a negative correlation of -0.88 between nitrogen solubility and processing temperature. This decline in protein solubility was found to be associated with a decrease of lysine content as shown in the same figure. The commercial samples displayed N-solubility values variable presumably in accordance with

the heat treatment to which they had been previously subjected.

3.2.2. Amino acids

The contents of amino acids after various heating times and the four commercial samples were presented in Table 2. The lysine content in unheated rapeseed meal sample was 5.9 g per 16 g N, and this was decreased to 5.7 and 4.9 g, respectively after 30 and 120 min. toasting, corresponding to the disappearance of 3.4 and 17.2% of the total lysine. The reduction in lysine content followed the same pattern as protein solubility although the number of measurements was less than protein solubility. For the commercial samples, the two Danish samples had rather high nitrogen solubility and also lysine content was close to 6 g per 16 g N. The German extracted meal showed lower values. On the contrast, the Chinese meal displayed rather low nitrogen solubility and also lysine content was rather low, reflecting an intensive heat-treatment during processing. As shown in Table 2, the heat treatment had little influence on the other amino acids except the level of cystine dropped 12% after 120 min. toasting. There was also a tendency showing that the longer toasting time slightly reduced the content of nitrogen as amino acid (Table 2).

Table 2
Amino acid content of treated, commercial rapeseed meals and wheat gluten, in g per 16 g N

	Toasting, min.				Commercial sample[1]			
	0	30	120	Gluten	A	B	C	D
Alanine	4.41	4.45	4.44	2.60	4.63	4.49	4.49	4.37
Arginine	6.12	6.22	5.79	3.50	6.19	6.13	6.21	5.70
Asparat acid	7.54	7.58	7.40	3.20	7.81	7.42	7.51	6.55
Cystine	2.49	2.41	2.19	2.16	2.50	2.49	2.37	2.54
Glutamic acid	17.86	16.30	16.37	33.50	17.27	17.05	17.56	18.33
Glycine	5.24	5.27	5.25	3.27	5.34	5.18	5.24	4.93
Histidine	2.74	2.80	2.71	2.18	2.88	2.80	2.79	2.86
Isoleucine	4.30	4.40	4.34	3.85	4.28	4.21	4.27	4.05
Leucine	6.95	7.04	7.09	6.86	7.18	6.96	7.12	6.81
Lysine	5.93	5.72	4.91	1.62	5.98	5.86	5.59	4.39
Methionine	2.02	1.99	2.03	1.68	2.05	1.17	2.02	2.01
Phenylalanine	4.12	4.16	4.17	5.26	6.49	4.01	4.15	3.92
Proline	6.36	6.53	6.65	12.45	4.60	6.39	6.64	6.93
Serine	4.60	4.65	4.63	5.05	4.60	4.53	4.57	4.30
Threonine	4.49	4.51	4.46	2.41	4.51	4.42	4.43	4.06
Tryptophan	1.32	1.34	1.31	0.91	1.34	1.30	1.31	1.27
Tyrosine	3.27	3.38	3.39	3.64	3.37	3.30	3.31	3.02
Valine	5.51	5.66	5.43	4.18	5.61	5.51	5.57	5.36
N as protein, %	81.87	81.07	79.48	83.83	82.66	80.81	81.75	78.54

1. A, Danish extracted meal; B, Danish press cake; C, German extracted meal and D, Chinese extracted meal.

Due to the close correlations between the decreases in glucosinolates, lysine and protein solubility after heat treatment, this experiment suggests that, in practice, a protein solubility

is a useful measurement as an indication of protein quality after heat treatment. Determinations of amino acids as well as animal's digestibility are complicated, time-consuming and also rather expensive.

3.2.3. Carbohydrates

In order to reveal the likely reaction between lysine and reducing sugars, the content of several carbohydrates in the rapeseed samples was measured as shown in Table 1. The total content of carbohydrates was decreased with increasing heat-treatments. On the other hand, the decrease of sucrose was not followed by a corresponding increase of glucose and the reducing fructose. Taking into account the data of the commercial samples, it appeared that the more intensive heating resulted in a greater reduction of the carbohydrate content. It is known that decomposing one molecule of glucosinolates releases one molecular free glucose (Van Etten et al., 1969). The disappearance of carbohydrates, especially the reducing ones, are believed to actively react with the ϵ-NH_2 of lysine (Maillard reaction). When calculate the lysine reduction on a molecular basis, 120 min. toasting resulted in the disappearance of 26 μmol lysine from one gram rapeseed meal. This decrease is almost equal to the decrease in glucosinolate and carbohydrate content expressed as mono saccharide equivalents (15.2 + 5.4 $\approx$ 21 μmol per g rapeseed meal). It is thus obvious to assume that the decrease in lysine content is caused by reactions between the nucleophilic ϵ-amino group on lysine and some of the glucosinolate break-down products and/or reducing carbohydrates.

3.3. Balance trial with rats

Table 3
True digestibility (TD), biological value (BV) and net protein utilization (NPU) of rapeseed proteins after different toasting periods in rats.

Toasting time, min.	TD_{diet}, %[1]	TD_{rape}, %	BV, %	NPU, %
0	88.5	77.0[a]	71.2[a]	62.9[a]
15	87.0	73.9[b]	71.1[a]	61.8[a]
30	86.0	72.1[bc]	71.3[a]	61.4[a]
60	86.9	72.8[bc]	68.3[ab]	58.6[ab]
120	85.6	71.2[c]	65.8[b]	56.3[b]
SEM[2]	1.96	1.84	2.20	1.96

1, Half of dietary N was provided by wheat gluten. Figures without same superscript letter indicate $P<0.05$.
2, SEM, mean of standard deviation.

The digestibility and utilization of rapeseed meal after various toasting are shown in Table 4. To reveal the influence of the heat treatment on the availability of lysine, half of dietery protein was originated from wheat gluten because of very low level of lysine (1.62 g per 16 g N). Two hours' toasting resulted in about 3 percentage units of dietery protein TD decreases ($P>0.05$). The real difference was probably insulated by the inclusion of wheat gluten, because a significant difference appeared when calculated as pure rape protein by difference as shown in Table 4 ($P<0.05$). The decreases in protein quality after toasting

were further revealed by BV and NPU measurements, reflecting a decrease of lysine availability. More than 30 min. toasting resulted in a considerable decline in both BV and NPU and two hours' toasting decreased 5.4 percentage units of BV and 6.6 of NPU ($P<0.05$). Moreover, there is again a close correlation between protein solubility and BV (0.859, $P<0.05$).

The results from the commercial samples are presented in Table 4. The values of TD, BV, NPU and digestibility of energy (ED) precisely followed the same manner as that of protein solubility and lysine content. German extracted meal showed lower values than those of Danish samples and the Chinese extracted meal displayed the lowest digestibility and protein availability ($P<0.05$).

The weight of thyroid, liver and kidney from the rats fed the Chinese extracted meal showed a negative effect from the glucosinolates ($P<0.05$, Table 4). The rats receiving Danish extracted meal as only protein source had a significant higher thyroid weight ($P<0.05$) than the animals fed German extracted meal which had the lowest level of glucosinolates.

Table 4
True digestibility (TD), biological value (BV), net protein utilization (NPU), energy digestibility (ED), and the size of internal organs of rats fed four commercial rapeseed meals[1]

Sample[2]	TD %	BV %	NPU %	ED %	Thyroid[3] mg/100g	Liver[3] g/100g	Kidney[3] g/100g
A	81.8^{a}	92.0^{ab}	75.3^{ab}	62.7^{ab}	9.25^{b}	4.98^{ab}	0.90^{a}
B	83.5^{a}	95.3^{a}	79.6^{a}	64.9^{a}	8.62^{ab}	4.39^{a}	0.90^{a}
C	81.2^{a}	89.3^{b}	72.5^{b}	56.2^{ab}	7.10^{a}	4.38^{a}	0.88^{a}
D	75.4^{b}	74.6^{c}	56.3^{c}	53.7^{b}	10.23^{b}	5.35^{b}	1.04^{b}
SEM	1.20	2.10	2.20	4.45	0.78	0.32	0.05

1. For statistics, figures with different superscript letters indicate $P<0.05$. SEM, mean of standard deviation.
2. A, Danish extracted meal; B, Danish press cake; C, German extracted meal and D, Chinese extracted meal.
3. The relative size of organs is expressed as per 100 g rats.

4. Conclusion and implication

For rapeseed meal a short-term toasting is essential to inactivate myrosinase and lipase. More intensive heating can break down glucosinolates as shown in this experiment. On the other hand, overheating the rapeseed meal resulted in a significant impairment in protein quality, and very likely also a negative influence on energy digestibility as determined in the commercial samples. This investigation suggests that up to 30 min. toasting at 100°C can be accepted without seriously lowering nutritional value. Furthermore, several criterions were compared for monitoring heat treatment. Besides a determination of total amount and the relative proportion of the glucosinolates, protein solubility can be used as an indication of rapeseed protein quality for monogastric animals after heat treatment. On the other hand, this criterion can also be used to reflect the heat-treatment to which the sample in question had been previously subjected. It is simple, quick and inexpensive.

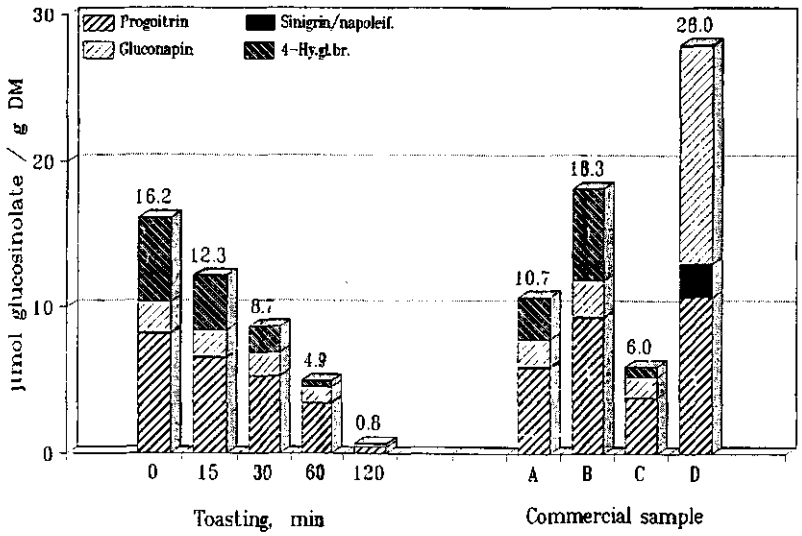

Figure 1. Influence of heat-treatment on the individual glucosinolates of toasted and commercial rapeseed meal samples. Toasting temperature: 100°C. Commercial sample: A, Danish extracted meal; B, Danish press cake; C, German extracted meal and D, Chinese extracted meal.

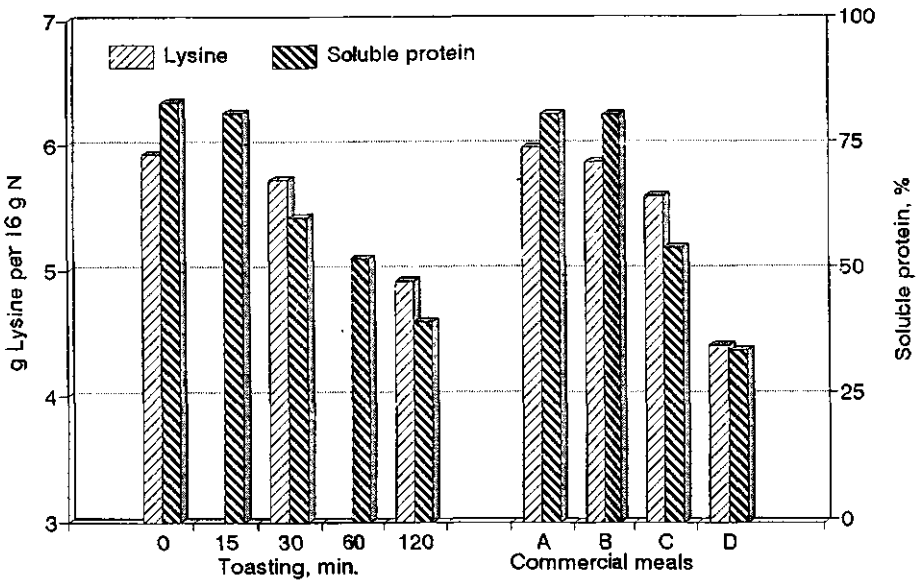

Figure 2. Influence of heat-treatment on the protein solubility and lysine content of toasted and commercial rapeseed meal samples. Toasting temperature: 100°C. Commercial sample: A, Danish extracted meal; B, Danish press cake; C, German extracted meal and D, Chinese extracted meal.

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Chapter 8

A Suvey of Nutrients and Toxic Factors in Commercial Rapeseed Meal in China, and Evaluation of Detoxification by Water Extraction

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A Suvey of Nutrients and Toxic Factors in Commercial Rapeseed Meal in China, and Evaluation of Detoxification by Water Extraction

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Abstract

Information is presented concerning the utilization of Chinese rapeseed by-products as animal feed. Results were obtained from more than 200 samples from various oil mills. For screw-pressed cake and prepress-solvent extracted meal, respectively, crude protein averaged 389 and 432 g kg^{-1} on a dry matter basis; residual lipids 106 and 19 g kg^{-1}; crude fibre 132 and 138 g kg^{-1}; ash 87 and 99 g kg^{-1}; calcium 8 and 9 g kg^{-1}; phosphorus 11 and 12 g kg^{-1}; lysine 3.7 and 3.4 g per 16 g N. The meal was found to contain 1.5 ± 1.1 mg g^{-1} oxazolidine-thione and 1.4 ± 1.0 mg g^{-1} isothiocyanates; whereas the cake contained 2.3 ± 2.2 and 2.7 ± 1.8 mg g^{-1} respectively. Approximately 5-6 g kg^{-1} tannins and 20 g kg^{-1} phytates were determined. Detoxification involving water extraction alongside seed processing was examined on a pilot scale, and the meal was evaluated in feeding trials with pigs, broilers and rabbits. Water extraction removed nearly all glucosinolates and significantly increased crude protein content. Remarkable improvement in animal growth performance was achieved when feeding detoxified meal compared with untreated feed. The treatment rendered the meal suitable for use as a main protein supplement as proved by feeding trials.

1. Introduction

Since 1985 the output of oilseed rape in China has exceeded 5×10^6 t, making it the world's largest rapeseed producer. Its total production reached 7.65×10^6 t in 1992 and is increasing steadily, according to FAO statistics (1992). Compared with other parts of the world where most rape varieties have been replaced by "double zero" strains with low glucosinolates and low erucic acid, conventional high-glucosinolate rape species (B. *Nupus*, B. *Campestris* and B. *Juncea* etc.) still dominate, about 415,000 ha, 7.6% of the total rape area has been converted to the new varieties (Bao, C.Y., person. commun. 1992). This is probably caused by a number of reasons: lower seed output from "double zero" ones, inferior disease-resistance and less awareness of the side-effects from consuming high erucic acid oil.

Traditionally, the huge amount of residue from the rapeseed crushing industry was used as fertilizer because of the high glucosinolate content caused low palatability and toxicity to animals. However, this has gradually been changing along with the rapid advances in animal husbandry throughout the country. The advantages of availability and low price have allowed rapeseed by-products to become an important protein supplement in livestock feeding, particularly in Southern China, where other protein sources are scarce. However, nutrient

content, toxic factors or anti-nutritional factors (ANFs) and the feeding value of the by-products under local processing conditions have not been systematically investigated. The available data are out of date because of new developments in processing and analysis.

In view of the overwhelming interests in the conventional rapeseed, proper detoxification of the by-products has long been considered to be of great importance in the country. In addition to traditional processing, a number of approaches have been studied for removal of glucosinolates and hydrolyzed aglucons to improve the quality of rapeseed by-products. These include chemical and physical treatments (Fenwick et al., 1986); ammoniation (Keith and Bell, 1982); aqueous ethanol extraction (Van Megen, 1983); methanol ammonia-water extraction (Naczk et al., 1986); heat treatment followed by water extraction (Rauchberger et al., 1979); preparation of protein concentrates (Jones, 1979); and aqueous enzymatic processing (Jensen et al., 1990). Detoxification was often achieved but economical feasibility remains questionable under many circumanstances. Some of the glucosinolate-free by-products would therefore appear only to be appropriate as ingredients in human or pet foods due to the high costs of processing. However, heat treatment followed by water extraction has its attraction for the Chinese rural areas because of the cheap water supply and high efficiency of detoxification without damaging nutrients in the meal, if the extraction adapted correctly. An improved procedure would provide a better combination of detoxification and oil recovery to produce desirable protein supplements, which would benefit both livestock feeding and oil processing.

The objectives of the current study were: (1) to investigate nutrient content and the level of ANFs in industrially processed rapeseed by-products; (2) to examine the detoxification procedure combining oil recovery with glucosinolate removal by heat treatment plus water extraction; and (3) to evaluate the nutritional value of rapeseed meal produced by the current procedure in comparison with conventional rapeseed meal in feeding trials with pigs, broilers and rabbits.

2. Materials and Methods

2.1. Sample collection

At present two oil recovery procedures are mainly used in China: screw pressing and prepress plus solvent extraction. The screw pressing includes: seed cleaning, preconditioning (preheating the seeds to 60^{0}C), flaking and screw pressing. In practice this is done by various expellers. Through this procedure 80-90% of the oil can be extracted out from the seeds, providing pressed cake as a by-product. Better equipped oil mills can extract the cake further with hexane or other solvents to recover the residual oil, then the meal is toasted above 100^{0}C to remove and recycle the solvents. Thus, the extracted meal remains as a by-product. In this investigation, 150 cake and 50 meal samples were collected from various oil mills throughout the main rapeseed producing areas.

2.2. Water extraction

A water extraction system was designed and tested in co-operation with the Guanghan oil-mill. The principle of the procedure was to combine oil recovery and detoxification in a single system using water extraction. First, the seeds were toasted at 100-110°C for 30-40 min to inactivate the enzyme thioglucosidase (myrosinase). Then the seeds were ground and extracted with water. In this procedure a micro-grinder was used to ensure adequate grinding (10 to 20 μm diameter particles) essential for efficient oil recovery. Rape seed and water were continuously introduced into the system at room temperature; the ratio of seed to water was 1:8 (weight to weight). The extraction was conducted in six containers similar in size and connected by tubes, each container was equipped with an agitator inside. Oil was separated from the mixture in a descending filtration tower. Waste water was removed by centrifugal filtration and wet meal was repeatedly extracted by adding water to remove residual oil and toxic substances, the filtration was then repeated. The meal was press-filtrated and heat dried. The waste water was pooled and fermented to minimize the risk of pollution. The procedure is illustrated in Fig 1. After treatment, proximate chemical composition, amino acids, total glucosinolates, oxazolidine-thione (OZT), isothiocyanates (ITC) and phytates were determined.

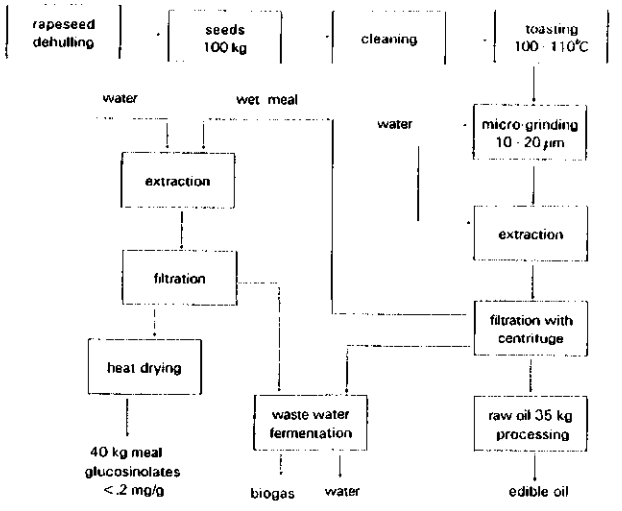

Fig. 1. Flow sheet of water extraction for removal of both oil and anti-nutritional factors during seed processing.

2.3. Feeding trials

2.3.1. Pigs

Forty-eight weanling pigs (Danish Landrace male × Chenghua female) were castrated and randomized into six treatments, each containing eight animals, four replicates of two animals each, males and females were allocated equally to each treatment. Pigs were housed in an

experimental building where the temperature averaged 22°C (range 18-24°C) and relative humidity was about 85%. Diets 1 - 4 were formulated as isoenergetic and isonitrogenous. Basic diets consisted of maize, wheat bran, silkworm, peanut meal and blood meal. Minerals were supplemented on all diets. The first four diets (see Table 4) were designed to determine whether and to what extent the detoxified rapeseed protein could replace dietary silkworm protein, a by-product of silk processing and locally an important animal protein for piglets and poultry feeding. The silkworm used in this trial contained 580 g crude protein and 80 g lipids per kg. Diets WM_{155} (washed meal 155 g per kg diet) and UM_{155} (untreated meal 155 g per kg diet) were designed to compare the performance of pigs receiving either treated or untreated rapeseed meal as sole protein supplement at 15.5% dietary inclusion level. The animals were allowed 8 days for adaptation to their diets and surroundings. They were fed twice daily with wet feed (3:1 water:feed), each feeding time last approximately for 1 h. Water was supplied when most pigs had finished eating. The animals were kept from 16 kg to 55 kg. Body weight and feed consumption were measured at the end of every 20 days. Feed intake, feed conversion, and growth rate were criteria of comparison.

2.3.2. Broilers

Five hundred one-day-old chicks were used, randomly allocated to five treatments, each containing 100 birds. Groups of 20 were housed in a metal cage giving five replicates. Temperature was kept at 32°C during the first week, followed by a weekly decrease of 2°C, until room temperature (15 to 18°C) at the age of 4-5 weeks. Air humidity was increased to 90% during the first 2 weeks by placing water trays in the room. Diets contained 9.1 MJ ME kg^{-1} and 20% crude protein for the first 4 weeks, and 9.4 and 18% for the subsquent 4 weeks. All treatments were kept isoenergetic and isonitrogenous by adjusting the dietary level of soybean meal (for protein) and animal fat (for energy). Premixes of vitamins and minerals were supplemented to all treatments. Diets consisted of maize, wheat bran, fish meal, silkworm and peanut meal. Different dietary levels of detoxified rapeseed meal were included in the diets (0, 5, 9, 18 and 30%). Feed and water were ad libitum. Feed intake, growth rate and feed conversion were determined and the carcasses were examined. The slaughtered birds were additionally subjected to histopathological examination, in order to detect possible negative effects from the glucosinolates in the diets.

2.3.3. Rabbits

One hundred and twenty Sichuan White rabbits were allocated to four treatments, each containing 30 animals. Groups of six were kept in bamboo cages as replicates, creating five replicates for each treatment. The experimental concentrates consisted of maize, wheat bran, grass hay meal, soy flour, peanut meal and silkworm as annotated (see Table 6). The intention was to use the treated rapeseed meal to replace silkworm and soy flour at 0, 3.5, 7 and 14% levels in concentrates. The feeds were pelleted and supplied twice daily, fresh grass was provided after the concentrates had been consumed. Water was provided ad libitum. The animals were fed for 60 days, then five from each treatment (one from each group) were slaughtered for determination of carcass quality and possible influence of glucosinolates.

2.4. Chemical analyses

The chemical composition of all samples was determined (i.e. moisture, crude protein, ether extracts, crude fibre, ash, calcium and phosphorus) according to routine procedures (AOAC, 1984). Amino acids were analyzed using reflux hydrolysis under nitrogen in 6 M HCl (at 110^{0}C for 24 h) and separation of the amino acids was performed with high pressure liquid chromatography (Hughes and Wilson, 1982). Tryptophan was measured separately after alkaline hydrolysis (Miller, 1967). Contents of glucosinolates, OZT and ITC were measured using the method of Wetter and Young (1976). Tannins, phytates and nitriles were analyzed by the Analytical Centre of the Sichuan Agricultural Academic Institution at Chengdu.

2.5. Data processing

Data were calculated and compared using analysis of variance, and the least significant difference (LSD) was used for comparison of the means where relevant.

3. Results and Discussion

3.1. Nutrient and toxic factor content in commercial rapeseed cake and meal

3.1.1. Nutrients

The content of proximate constituents and amino acids is shown in Table 1. Variation appeared to be limited. As expected, the difference in nutrient contents between the two processing procedures (screw pressing and prepress extraction) was significant ($P < 0.05$) owing to a large extent to different levels of residual lipids left in the by-products. The same trend can also be found in amino acid content. The level of residual lipids displayed considerable variations indicating that the efficiency of oil recovery varies between oil-mills.

The level of crude protein (N $\times$ 6.25) found in our study averaged 440 g kg^{-1} on oil-free dry matter (DM) basis, which is considerably higher than the average found in 115 Canadian commercial rapeseed meal samples (373 g kg^{-1} on the same basis, Bell and Jeffers, 1976), and similar to canola meal (Keith and Bell, 1991). The average residual lipid content was 212 and 39 g in Canadian rapeseed cake and meal, respectively, as presented in the same report (Keith and Bell, 1991). We found 106 and 19 g kg^{-1}, respectively. This indicates that defatting in Chinese oil mills was performed more completely than in Canada. Levels of other ingredients (crude fibre, ash, etc.) found in this investigation were similar to those found by Bell and Jeffers (1976).

The lysine content in meal was not only lower than in cake (3.4 vs 3.7 g/16g N), but also the contents in our samples were much lower than from other findings (5.5 g by Clandinin et al., 1981, Bell and Keith, 1991; 5.0 g by Liu et al., 1993a). Methionine level (1.7 g/16g N) was also slightly lower than in the figure of Liu et al. (1993a, 2.1 g). Levels of other essential amino acids, such as threonine and tryptophan, were similar. The low lysine content in our samples may be explained partly by severe processing conditions. Recently it was

found that total lysine was reduced by up to 20% after moist-heating (Liu et al., 1993b). In many rural oil mills heating time and temperature can not be easily regulated, and the main objective of processors is to recover as much oil as possible. High oil recovery was achieved as shown in Table 1, but lysine in the by-products was partly destroyed. Probably, ileal digestibility of some amino acids was also impaired. Yang S. et al. found 62 and 71% ileal digestibilities, respectively, for lysine and threonine in Chinese rapeseed meal (pers. commun., 1989); whereas 77 and 85% was reported in Canadian rapeseed meal by Sauer et al. (1982).

Table 1
Contents of proximate components and amino acids in commercial Chinese rapeseed by-products (g kg^{-1} on DM basis)

	Pressed cake, n = 150	Extracted meal, n = 50
Crude protein	389 ± 35[a]	432 ± 25
Ether extract	106 ± 44	19 ± 9
Crude fibre	132 ± 27	138 ± 19
N-free extract	292 ± 64	312 ± 28
Ash	87 ± 16	99 ± 21
Calcium	8 ± 2	9 ± 2
Phosphorus	11 ± 2	12 ± 2
Amino acids[b]		
Arginine	19.9	20.8
Cystine	9.0	9.9
Histidine	9.1	9.8
Isoleucine	13.5	14.6
Leucine	24.9	26.6
Lysine	14.5	14.8
Methionine	6.6	7.2
Phenylalanine	14.8	16.5
Threonine	15.3	16.8
Tryptophan	4.5	4.9
Tyrosine	10.0	11.0
Valine	17.7	19.8

1. Standard deviation.
2. Data of amino acids from 15 cake and 23 meal samples.

3.1.2. Toxic factors

Analyses of toxic factors were performed in some of the collected samples. Results are shown in Table 2. In rape seeds, the level of total glucosinolate expressed as OZT+ITC was determined as 12 mg g^{-1}, which will double when calculated on oil-free basis. The contents of ITC and OZT in by-products were low and varied considerably. Moreover, the content of these toxic factors in meals was clearly lower than in cakes, glucosinolates and their hydrolysed products in particular ($P<0.01$). The content of nitriles also varied.

Table 2
Content of anti-nutritional factors (ANFs) in rapeseed by-products (mg g^{-1})

	Pressed cake	Extracted meal
Oxazolidinethione	2.3 ± 2.2 (n=138)	1.5 ± 1.1 (n=44)
Isothiocyanates	2.7 ± 1.8 (n=144)	1.4 ± 1.0 (n=43)
Nitriles	1.3 ± 1.1 (n=8)	0.8 ± 0.6 (n=6)
Tannins	5.4 ± 1.3 (n=20)	5.2 ± 1.7 (n=40)
Phytates	21.0 ± 3.6 (n=36)	19.6 ± 5.3 (n=20)

As shown in Table 2, the total level of OZT + ITC in pressed cake was 5 mg g^{-1} and in extracted meal was 3 mg g^{-1}, respectively. Bell and Jeffers (1976) reported that 7 mg g^{-1} was found as an average of 115 samples of commercial rapeseed meal. Nearly half of the OZT and ITC were decomposed after solvent-extraction. Such a decomposition is attributable to the high temperature (90 - 110^{0}C) and moisture during desolventizing-toasting, which reduces glucosinolates markedly as reported by several authors (Carlson et al., 1985; Liu et al., 1993b). A similar observation was reported in canola processing: only a minor reduction of glucosinolate occurred after screw-pressing but a pronounced decrease was found after prepress and solvent extraction, i.e. 38.4, 35.8 and 21.6 μmol g^{-1}, respectively, for seeds, pressed cake and extracted meal (Keith and Bell, 1991). Severe toasting condition (e.g. high temp. and long duration) will, to a larger extent, decompose the glucosinolates. Part of the decomposed glucosinolates like glucose, sulphates and nitriles would remain in the meal and others are assumed to evaporate (Liu et al., 1993b). This may be the reason for low levels of OZT+ITC in Chinese rapeseed by-products. Practically, the farmers have been feeding locally available rapeseed by-products as a routine protein supplement without encountering a serious problem of toxicity, provided dietary inclusion levels are not too high.

Nitriles in rapeseed meal were measured in this study. The data varied probably due to rape varieties, processing conditions, and also the analytical method employed. Nevertheless, the average of our data is similar to that of Campbell et al. (1980) in Canadian rapeseed meals. Nitriles are generally considered to be very toxic among the aglucon products resulting from hydrolysis of glucosinolates. Several treatments may largely reduce the contents of the glucosinolates but often tend to increase the amount of nitriles. Therefore, measurement of nitriles was suggested as an additional monitor for such detoxification treatments (Fenwick et al., 1986).

In this investigation the tannin contents were 5 - 6 mg g^{-1}, it is believed that most of tannins come from pericarp and hulls. Bell (1984) reported 15 mg g^{-1} in rapeseed hulls. Tannin is generally classified as an ANF owing to its property of binding protein molecules to make them resistant to enzymatic hydrolysis in the digestive tract of non-ruminants. The content of phytates in the cake and meal was constant and compared favourably with the results of Bell (1984).

3.2. Detoxification by water extraction

The water extraction was capable of processing a few tonnes of rape seeds per working day. Oil recovery averaged 88%, equivalent to about 35% of total seed weight. The meal represented approximately 75% of the weight of the oil-free material. In the initial trial, a batch of high glucosinolate rapeseeds was processed and about 800 kg detoxified meal was produced for the feeding trials. Unlike those routinly produced, commercial rapeseed cake and meal with a deep-brown colour and some hard lumps, the water extracted meal displayed a light colour, indicating no "browning reaction" had occurred and probably less seed pericarp remained in the meal after the water extraction process. A comparison of the composition of commercial rapeseed meal and water extracted meal is given in Table 3. This pilot extraction removed 95% of ITC and OZT.

After the extraction, the crude protein content increased from 403 to 568 g kg^{-1}, at the expense of disappearance of some washable or soluble constituents, such as carbohydrates. Part of the seed pericarps were removed during micro-grinding and extraction, resulting in a reduction of crude fibre, as can be seen in Table 3. Our results agreed with those of Baker et al. (1977). However, this treatment involves a loss of 20-25% of the oil-free dry matter. The organic matter together with solubilized ANFs in the waste water could become a potential source of pollution. Fermentation appeared to be effective to minimize this, but further research is required before the procedure is used on a large scale.

Table 3
Comparison of chemical composition of treated rapeseed meal with conventional meal (DM basis, g kg^{-1})

	Conventional meal	Detoxified meal
Crude protein	403	568
Crude fibre	129	79
Ether extract	20	18
Ash	84	94
N-free extraction	299	240
Calcium	7	12
Phosphorus	12	15
Lysine	12	23
Methionine	15	19
Tryptophan	2	2
Oxazolidine-thione, mg g^{-1}	3	0.2
Isothiocyanates, mg g^{-1}	2.7	nd[1]
Phytates	21	4

1. nd, not detectable.

3.3. Feeding trials

3.3.1. Pigs

The pigs were kept to a final liveweight of 55 kg except the groups 5 and 6, which grew very slowly. Results are shown in Table 4. The daily gain of the animals in this trial averaged 480 g. There was no significant difference in growth rate among the first three treatments, indicating that detoxified rapeseed meal is nutritively capable of substituting for

silkworm to a moderate extent. On the other hand, an improvement in feed intake and growth rate was obtained in pigs receiving the diet with 6.3% detoxified rapeseed meal supplemented with 1 g lysine per kg diet ($P<0.05$, WM_{63L}), suggesting there is a certain amino acid unbalance in the diet.

Table 4
Pig performance at different dietary levels of washed rapeseed meal (WM) in comparison with untreated meal[1]

	WM_0	WM_{32}	WM_{63}	WM_{63L}	WM_{155}	UM_{155}
Diet design and nutrients, g kg^{-1}						
Silk worm	60	30	–	–	–	–
Washed meal	–	32	63	63	155	–
Untreated meal	–	–	–	–	–	155
L-lysine·HCL	–	–	–	1	–	–
Digestible energy ($MJ\ kg^{-1}$)	10.1	10.1	10.1	10.1	10.1	10.0
Crude protein	161	160	158	158	160	135
Lysine	7	7	6	7	6	4
No of pigs	8	8	8	8	8	8
Initial wt (kg)	16.1±1.5	16.2±2.1	16.2±2.1	16.1±1.2	16.3±1.6	16.1±1.8
Ending wt (kg)	54.4±4.4	55.1±4.7	54.8±5.5	58.4±4.9	48.4±5.1	29.2±4.6
Daily gain (g)	479[b]	487[b]	483[b]	528[a]	401[c]	164[d]
Feed intake ($kg\ day^{-1}$)	1.51[b]	1.48[b]	1.47[b]	1.60[a]	1.23[c]	0.80[d]
Feed conversion	3.15[b]	3.05[b]	3.04[b]	3.03[b]	3.10[b]	4.86[a]

1, Other dietary ingredients include maize, wheat bran, peanut meal and blood meal. The diets WM_{155} and UM_{155} consisted of maize and wheat bran as basic ingredients and no other protein supplements. Different superscript letters indicate $P<0.05$.
2, Digestible energy.

Table 5
Performance of growing broilers receiving different dietary levels of detoxified RSM[1]

	Dietary levels of detoxified rapeseed meal, g kg^{-1}				
	0	50	90	180	300
No. birds	100	100	100	100	100
Mortality (%)	3	0	1	2	5
Live wt. at 6 wk (g)	1197[b]	1227[a]	1211[ab]	1183[b]	897[c]
Live wt. at 8 wk (g)	1770[b]	1847[a]	1760[b]	1790[b]	1467[c]
% carcass of live wt.	75.7	75.1	74.4	75.0	75.9
Free fat (g bird)	9.0	21.8	12.2	4.8	2.8
Feed intake (kg per 8 wk)	4.2	4.2	4.2	4.3	3.6
Feed/Gain (8 wk)	2.4	2.3	2.4	2.4	2.5

1. Different superscripts indicate $P<0.05$.

Compared with the other treatments, pigs receiving untreated rapeseed meal as sole protein supplement (UM_{155}) had both low feed intake and low growth rate, and the animals on this diet had a poor appearance throughout the whole experimental period. Their growth rate was even lower than half of that of the control group (164 vs. 401 g day^{-1}). Presumably this is due to low palatability of their diet and side-effects from the glucosinolates. Nevertheless, no clinical signs were evident. On the contrary, pigs fed detoxified meal as the sole protein supplement (WM_{155}) showed much higher feed consumption and growth rate ($P<0.05$), indicating the effectiveness of detoxification.

From these results it can be concluded that inclusion of 15.5% untreated rapeseed meal as the sole protein supplement in diets of weanling pigs is not to be recommended. Inclusion of the water-extracted meal at the same level gave better results although feed intake and weight gain were somewhat below expectations, but feed conversion was reasonable.

3.3.2. Broilers

The growth rate, feed conversion and carcass traits of the broilers are shown in Table 5. There were no significant differences among the groups with 0%, 9% and 18% treated meal in their diets, but the birds fed with 5% treated meal in their diet appeared to display slightly better feed conversion and growth rate than the others. The animals fed 18% treated meal displayed a reasonable growth. However, the animals fed 30% treated rapeseed meal grew very slowly ($P<0.05$), with some mortality. Carcass examination did not reveal visible abnormalities. However, including such a high percentage of detoxified rapeseed meal in broiler diets should not be recommended.

Table 6
Performance of rabbits receiving different dietary levels of water extracted rapeseed meal (WM)

Treatment	WM_0	WM_1	WM_2	WM_3
Basal diet[a]	860	860	860	860
Silk-worm (g kg^{-1})	70	35	–	–
Soy flour (g kg^{-1})	70	35	70	–
Water Extr. RSM (g kg^{-1})	–	35	70	140
No. of animals	30	30	30	30
Initial wt (kg)	0.45	0.45	0.45	0.45
Ave. daily gain (g)	22.0	22.9	21.7	21.8
No. of death	5	5	4	4
Feed consumption per kg gain				
concentrate (kg)	2.9	2.9	2.8	2.8
grass (kg)	8.0	7.9	8.1	8.0
Carcass (% of live weight)	55.1	54.9	53.4	55.4

a. Basal diet contained (/kg) maize 330 g, wheat bran 330 g, artificially dried grass meal 115 g, extracted peanut meal 80 g, calcium carbonate 10 g and mineral premix 5 g.

3.3.3. Rabbits

A few animals (18) died of diarrhoea caused by intestinal disease and their data were thus excluded. The feed conversion, growth rate and carcass percentage of the rabbits are listed in Table 6. There were no significant differences in performance found between the WM-fed rabbits and those receiving the control diet containing soy flour and silk-worm. This indicates that replacing silkworm and soy flour by detoxified rapeseed meal will not impair nutritive value of the diets, even at rather high dietary inclusion levels.

4. Conclusions

This study has indicated that the Chinese rapeseed by-products contain relatively high crude protein and low residual lipids. Lysine is particularly low because of the inadequate defatting processing. Residual glucosinolates are also low. By-products with a higher lysine level can be obtained by paying more attention to the processing conditions in Chinese oil mills. More research is required to obtain further information concerning nutrient availability and viability of the detoxification procedure in local practice.

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Chapter 9

General Discussion

1. Introduction

Theoretically, processing of whole crambe seeds results in about 70% of the seed material as residue, including pericarp and defatted material. The importance of the residues stems not only from the economic acceptability of the crop, but also from environmental concern by their eventual disposal. There are indications from previous investigations with crambe by-products showing their potential in livestock feeding, however, the feed industry is often reluctant to accept new ingredients without clear information encompassing many characteristics. This is due to a decreasing market and to restrictions set by governments in attempts to guarantee safe feeding, animal welfare and to reduce consumer's doubts. Therefore, the task of this project was to carry out a systematic assessment on the feeding potential of crambe by-products and to obtain a clear view of their possibilities and constraints in livestock feeding.

In evaluating an unknown feedstuff, it is logical that the first and fundamental step is a detailed description of its chemical and nutritional properties. Secondly, it is essential to obtain more knowledge about acceptability and utilization by livestock, by determination of palatability, degradation and digestion by non-ruminants and ruminants. A third aspect is the investigation of the anti-nutritional factors, especially relevant because crambe belongs to the Cruciferae family. Finally, introduction of crambe as an animal feed requires data concerning safe inclusion levels in the diet and possible secondary effects on animal performance.

2. Proximate composition of crambe feeds

2.1 Structural elements of crambe seeds

Usually, crambe is harvested as intact pods, i.e., seed plus pericarp. After removal of pericarp, which can be called decortication, the seed contains both endosperm and seedcoat. In this study it was determined that the whole seeds contained 30% pericarp. Earle et al. (1966) reported a considerable variation in pericarp proportion from 25 to 40%. The variation may be due to the cultivation of crambe under different geographic and agronomic conditions (Carlson and Tookey, 1983). The decorticated seed contained about 9% seedcoat and 91% endosperm (Chapter 5). The amount of oil extracted from decorticated seeds ranged between 35 and 50% (Earle et al., 1966; Daxenbichler et al., 1970; Carlson and Tookey, 1983). Our analyses (Chapter 3) revealed that the meal from decorticated and defatted seed did not show large variation in proximate characteristics.

2.2 Crambe pericarp

Crambe pericarp showed high fibre contents: about 42% crude fibre, 56% neutral detergent fibre (NDF) and 50% acid detergent fibre (ADF) were reported (Chapter 4). This

indicates that crambe pericarp is highly lignified: about 30% acid detergent lignin (ADL) in NDF, and 48-50% cellulose (Chapter 3). The hemicellulose content of 12-17% was relatively low. Its protein content was also low (8%). The implication of these results is that crambe pericarp are likely to show a low digestibility. A proper procedure in the seed processing is required to remove the pericarp as completely as possible.

Table 1
Protein, essential amino acids, carbohydrates and other constituents in crambe seed meal in comparison with rapeseed, g kg^{-1} unless otherwise stated, on fat-free DM basis.

	Crambe	Rapeseed
Dry matter	900	900
Organic matter	916	929
Crude protein	495	396
Essential amino acids, g per 16 g N		
Lysine	5.3	5.5
Methionine	1.8	2.1
Cystine	2.8	2.5
Threonine	4.6	3.3
Tryptophan	1.2	1.3
Arginine	6.5	5.8
Leucine	6.2	6.8
Isoleucine	4.2	4.2
Carbohydrates		
Crude fibre	65	139
Sugar	122	105
Starch	54	76
Hemicellulose	55	65
Cellulose	52	118
Neutral detergent fibre	132	270
Acid detergent lignin	23	86
Pectin	32	60
Soluble non-starch polysaccharide	138	133
Insoluble non-starch polysaccharide	168	303
Dietary fibre	328	549
Phosphorus	17.4	13.0
Phytin-P	10.9	10.8
Calcium	8.3	7.6
Gross energy, MJ/kg cake (12% fat)	21.54	21.10

Source: Chapters 3-6; Liu and Jensen, recent analysis.

2.3 Crambe meal

The proximate composition of crambe by-products is dependent on the extent of decortication and oil extraction. In our experiments, seeds with seedcoat but without pericarp were investigated, defatted crambe material contained nearly 50% crude protein on a dry matter basis; 85% of nitrogen in amino acids. Amino acid composition in general was more similar to that of rapeseed protein than to soya, with a lysine content of 5.2-5.8 g per 16 g N. Crambe protein had a higher content of sulphur-containing amino acids (Met+Cys) than soya protein (4.6 g vs. 2.9 g per 16 g N). Crambe protein was also found highly soluble (90%) (Van Etten et al., 1965), which implies easy of digestion by animals but resulting in increased nitrogen loss when detoxifying the meal by aqueous extraction, as shown in our study (Chapter 6).

As shown in Table 1, about 40% of decorticated and defatted crambe material consisted of carbohydrates, of which starch and sugar accounted for 40%. In general, crambe meal contained significantly lower levels of indigestible fibre fractions than rapeseed meal, for instance, as percentage of those in rapeseed meal, crude fibre was 47%, cellulose 44%, NDF 49%, ADF 27% and insoluble non-starch polysaccharides 55%. This is of great importance in non-ruminant feeding.

Phosphorous and calcium contents in crambe meal were 1.7 and 0.8%, respectively. As percentage, crambe had less phosphorus bound in phytins than rapeseed meal, which implies a higher P-digestibility in monogastrics as was demonstrated in Chapter 5. Crambe meal contained 1 ppm selenium that is similar to rapeseed meal. Contents of other trace minerals in crambe meal are reported in Chapter 3.

3. Glucosinolates and effect of treatment

3.1 Content and characteristics of glucosinolates in crambe

3.1.1 Content

It is well-known that crambe contains high levels of glucosinolates (Earle et al., 1966; Van Etten at al., 1965; 1969a and Tookey et al., 1980). In early literature the results reported were obtained by the methods available for glucosinolate analysis at that time, for instance, sulphate ion determination, sulphur balance, silver complexing or water extraction. With these methods crambe meal was often found to contain 8-11% glucosinolates (McGhee et al., 1965 and Earle et al., 1966). This content was much higher than in conventional rapeseed species (3-7%, Bell, 1984). Developments in analytical methodology, particularly the high performance liquid chromatography (HPLC), has improved analytical accuracy and specificity. According to our determinations, the whole crambe seeds grown under Dutch circumstances contained 62 ± 5 μmol glucosinolates per gram dry matter (Steg et al., 1994), which was approximately four times higher than in modern rape varieties (Chapter 7). A typical profile of crambe glucosinolates is shown in Table 2 which was performed on HPLC

after desulphation according to Sørenson (1990). In the hulls only traces of glucosinolates were detected at levels around 2 μmol g^{-1} (Chapter 4, Daxenbichler et al., 1965).

The amount of glucosinolates remained intact after oil extraction is dependent on processing procedure. Extraction without high temperature results in a meal containing 120 - 170 μmol g^{-1}, equivalent to 5-7% in the meal when calculated on the basis of 411 dalton for epi-progoitrin. In practice, however, a large proportion of intact glucosinolates will decompose as will be discussed later.

Table 2
Total and individual glucosinolates in crambe seeds in comparison with rapeseed, μmol g^{-1}

	Crambe	Rapeseed
Epi-progoitrin	66.9	0.0
Progoitrin	0.0	10.7
Gluconapin	0.4	1.7
Glucoraphanin	0.0	0.1
4-Hydroxy-glucobrasssicin	0.6	1.2
Glucobrasssicin	0.0	0.1
Total	67.9	13.8
as aliphatic, %	99.1	90.6

Source: Liu and Jensen, recent analyses.

3.1.2 Profile

The profile of crambe glucosinolates differs from that of rape: epi-progoitrin, i.e. 2(S)-hydroxy-3-butenyl-glucosinolate, one of the aliphatic glucosinolates, accounts for nearly all of total glucosinolates, and only very small quantities of indole-glucosinolates were found; whereas the modern rape seeds contained not only significant proportions of the aliphatics such as progoitrin and gluconapin, but also indole side-chain glucosinolates like 4-hydroxy-glucobrassicin (Chapter 7, Tookey et al., 1980; Sørensen, 1990). The significance of branched chains is more vulnerable to hydrolyze into different derivatives (Tookey et al., 1980). Moreover, their heat sensitivity is also affected as presented in Chapter 7.

As will be discussed later, the toxicity problems associated with glucosinolates mainly come from their hydrolysed products namely oxazolidine-thoine (OZT), isothiocyanates (ITC) and nitriles. During our study we measured both OZT and ITC in crambe meal, showing figures between 30,000 and 35,000 ppm after hydrolysis by myrosinase.

3.2 Anti-nutritional influence

3.2.1 Palatability

It is known that intact glucosinolates in rapeseed meal impair palatability due to their bitterness (Bjerg et al., 1989; Bourdon and Aumaitre, 1990; Bell, 1993). Our experiment with crambe showed a similar influence very clearly. The diet containing unheated crambe

meal was extremely unpalatable for young pigs, even at a minimum inclusion level of 3% (Chapter 6), corresponding to about 5 μmol epi-progoitrin per gram diet. When fed at this level for a fortnight the pigs showed a loss in body weight because of eating little or nothing. However, after withdrawing the crambe meal from their diet the animals showed a rapid recovery in growth. The palatability problem of crambe meal was also found in rats even though the meal had been autoclaved at 110^{0}C for 40 min. When all the dietary nitrogen originated from crambe meal, corresponding to 11 μmol per gram diet, feed intake of the rats was very low and their body weight decreased (Chapter 5). Hesketh et al. (1963) observed that including crambe meal into chicken diet depressed intake and consequently retarded growth.

Low palatability of rapeseed meal is one of the main constraints on feeding the meal to non-ruminants. However, when feeding the modern variety rapeseed meal to pigs, intake is often not a problem unless at very high dietary inclusion levels (e.g. 15 - 20%) (Bourdon and Aumitre, 1990). This is owing to that epi-progoitrin in crambe or progoitrin in rapeseed, together with sinapine, are the main responsible compounds that depress animal's appetite. On the other hand, it is generally believed that intact glucosinolates appear to be non-toxic, the problems arise with their hydrolysis products (Chapter 2 and Tookey et al., 1980).

3.2.2 Toxicity of the hydrolysed products

Crambe seeds contain both glucosinolates as well as the myrosinase that can hydrolyse them, but in separate compartments. As soon as the seeds are broken and softened, the glucosinolates are set free and hydrolysed by the enzyme. A glucose is released first followed by a sulphate ion from the glucosinolate molecules. Further reaction depends on the side chain structure of the glucosinolate in question and medium condition (Uda et al., 1986). Aglucon products are ITC or OZT (epi-goitrin). The mechanism of these hydrolyses is rather complicated and not yet entirely understood. Although myrosinases are the naturally occurring hydrolysis enzyme, there is evidence to suggest that certain bacteria present in the intestines can also hydrolyse the glucosinolates and thus subsequently contribute to the toxicity to animals (Tani et al., 1974; Tookey et al., 1980).

Hydrolysed aglucons are physiologically harmful to both animals and humans. The thyroid is the most commonly affected organ due to the aglucons inhibiting iodine uptake (Tookey et al., 1980). Our experiment with rats revealed enlargements of thyroid, kidney and liver, yet the animals still showed reasonable growth while consuming diets containing autoclaved crambe meal (Chapters 5 and 7). A more specific study on crambe meal was reported by Van Etten et al. (1969b). On a diet containing 0.13% OZT, rats showed a growth of only 85% of the control and their internal organs were enlarged. The same was found when the diet contained glucosinolates but without active myrosinase. Where the diet contained both glucosinolates and active myrosinase, rat growth was only 41% and thyroid, liver and kidney were enlarged. However, on diets containing 10 to 30% aqueous extracted crambe meal, the rats showed normal growth comparable to the control animals, and no affect was found on thyroid, kidney or liver size.

In chickens, liver haemorrhages often occurred after consumption of rapeseed meal. In a pig trial, after long-term feeding on a diet containing rapeseed meal in which the myrosi-

nase had been inactivated, the animals displayed thyroid and liver enlargement proportionally correlated to the quantity of glucosinolates consumed (Bourdon and Aumaitre, 1990). This was probably because some bacterial strains in the intestines were able to hydrolyse the glucosinolates as found by Tani et al. (1974). Thus, the potential toxicity of crambe or rapeseed meals still existed, even though the myrosinase had been inactivated.

Therefore, it can be concluded that a proper detoxification treatment of crambe meal is required before feeding it to animals; more attention should be paid to the possible influence on animal health and the quality of the animal products.

3.3 Detoxification

Similar to rapeseed processing, the objective of crambe treatment is 1) to remove or minimize the toxicity of glucosinolates, and 2) to improve or at least maintain the nutritional quality of the meal. Since numerous research has been carried out concerning the characteristics of glucosinolates in rapeseed, and the knowledge and principles acquired can generally be applied in crambe treatment. These are: 1) both myrosinase and glucosinolates are heat-sensitive; 2) glucosinolates and their hydrolysed products are soluble either in water or certain organic solvents. On the other hand, differences between crambe and rapeseed are evident, such as the level and profile of the glucosinolates as discussed earlier.

In accordance with the properties of glucosinolates, the effects of heat treatment and solvent extraction were investigated.

3.3.1 Heat treatment

The first step of detoxification was to inactive the myrosinase, in order to prevent enzymatic hydrolysis of the glucosinolates, which has been the practice in rapeseed processing. For crambe, it was reported in literature that the moisture content in seed was of great importance. Heating at 68^{0}C for 30 min, at seed moisture levels higher than 10% the enzyme inactivation was far more efficient than at levels below 8% (Carlson et al., 1985). Since there was no evidence to show differences in myrosinase between crambe and rape, it can be considered that the inactivation for crambe can be achieved in practice by preconditioning: heating up to 100^{0}C for about 10 min with steam to provide extra moisture. Probably, inactivation of ground or flaked samples may be easier than in whole seeds (Van Etten et al., 1969a).

Prolonged heating was needed to decompose the glucosinolates, which was clearly demonstrated by the results with rapeseed meal. A significant linear relationship was found between extent of heating and glucosinolate disappearance (Chapter 7). Among the three main glucosinolates (two aliphatic and one with indole side chain) in rapeseed, the indole glucosinolate 4-hydroxy-glucobrassicin was found to be most heat-sensitive. This is in good agreement with Campbell and Slominski (1990), who reported that almost 100% of indole-glucosinolates were removed during desolventation-toasting processing in normal oil plants, whilst only 40 - 60% of aliphatic-glucosinolates disappeared. Results from the investigation described in Chapter 7 suggest that toasting at 100^{0}C for 30 min is appropriate for double zero varieties of rapeseed in practice, because the total level of intact glucosinolates was

reduced from 16 to 8 μmol g^{-1} and the protein quality was not significantly jeopardized.

Similarly, toasting crambe meal also resulted in significant decomposition of the glucosinolates as illustrated in Chapter 6. However, after toasting at 110°C for 40 min the content of intact glucosinolates decreased from 150 to 55 μmol g^{-1}. Palatability was largely improved but the meal remains toxic owing to the high content of glucosinolates (Chapter 5). Further reduction in glucosinolate content was achieved by prolonging the heating time to 80 min, which decomposed almost all intact glucosinolates in our study (Chapter 6). However, as discussed later, such a toasting damaged protein quality.

An investigation was performed on the processing of crambe seeds on a commercial scale. Most of the crambe glucosinolates remained intact after preconditioning, flaking and screw-pressing. A significant decomposition occurred during desolventation-toasting, which resulted in a meal containing less than 30 μmol glucosinolates per gram meal (Carlson et al., 1985). This glucosinolate level was considered, to a certain extent, acceptable for beef cattle according to Perry et al. (1979) and Anderson et al. (1993), but a further reduction is considered necessary for non-ruminants.

Addition of either sodium carbonate or ferrous sulphate prior to toasting resulted in a more complete decomposition of the glucosinolates (Chapter 6). We compared three inclusion levels for sodium carbonate (1, 2, and 3%) and ferrous sulphate (0.5, 1, and 1.5%). The results showed that decomposition of the glucosinolates was the best with the higher inclusion levels under the same toasting conditions. This was in agreement with the results found by Kirk et al. (1971) and Mustakas et al. (1968).

During toasting we found that crambe meal lost a few percentages of dry matter. As illustrated in Chapter 6, where more glucosinolates were decomposed the dry matter losses rose up to 2.5%. The content of glucosinolates existing in the crambe cake examined was 4.5%, glucose and sulphate in the molecules were theoretically non-volatile. The disappearance of the glucosinolates was accompanied by a pungent smell during the steaming. Based on the volatility of ITC and calculations considering the molecular weight of the glucosinolates, we suggest that the dry matter which disappeared consisted mainly of decomposed glucosinolates. This provides an acceptable explanation for the quantitative gap between the glucosinolates and decomposed products in the dry matter before and after processing, as reported by Austin et al. (1968) and Kirk et al. (1971).

In conclusion, removing of the bulk of the intact glucosinolates from crambe meal requires a toasting at 100 - 110°C for 60 - 80 min, where sample moisture content is above 10%. Addition of 1% ferrous sulphate or 2% sodium carbonate prior to toasting would enhance glucosinolate decomposition. The more intensive toasting which crambe meal requires arises from lower heat-sensitivity of the epi-progoitrin and the higher total content compared to rapeseed meal.

3.3.2 Aqueous extraction

Because the glucosinolates and their hydrolysed products are water soluble, aqueous extraction has been considered as an option for crambe detoxification (Mustakas et al., 1976 and Baker et al., 1977a). Most glucosinolates can be removed from crambe by water

washing, and in this way apparently non-toxic crambe meal may be obtained (Baker et al., 1977b; Kirleis and Brown, 1980). Due to the high content of glucosinolates in Chinese rapeseed meal, we conducted a pilot-scale trial to investigate the efficiency of detoxification and subsequently the nutritional quality of the extracted rapeseed meal. As described in Chapter 8, the water extraction was proved to have removed nearly all glucosinolates as well as their derivatives, which markedly improved the acceptability of the meal to pigs, broilers and rabbits. Moreover, a significant growth enhancement was achieved. However, such a procedure is accompanied by a dry matter loss that has not been systematically investigated.

Thus, a series of experiments were performed on crambe meal to investigate the influence of sample pre-treatment, water to sample ratio, filter porosity, and recovery of nutrients (Chapter 6). A high water to sample ratio resulted in a complete removal of the toxins at the expense of nearly half of the dry matter, due to the high solubility of both crambe protein and carbohydrates. Screen porosity between 0.25 and 0.063 mm had limited influence on dry matter recovery due to disappearance of some constituents in solubilized or suspended forms. However, a toasting prior to extraction dramatically increased nutrient recovery from less than 50% to nearly 80%, a small quantity of OZT and ITC remained after extraction. This experiment suggested as advisable extraction procedure: a preliminary toasting, water to meal ratio of 6:1 and filter porosity of 0.06 mm. This procedure will provide a 90% recovery of protein and 80% of the dry matter. Meanwhile, nearly all the glucosinolates and some soluble carbohydrates will be removed.

3.4 Evaluation of the detoxification treatments

Removal of the glucosinolates by toasting is simple, efficient and inexpensive, and has been successfully used for improving rapeseed meal quality (Jensen et al., 1994). Crambe glucosinolates can also be removed by an adapted toasting procedure as mentioned previously. Palatability problems were overcome by such treatments, and piglets fed diets containing treated crambe meal grew as fast as those fed on a commercial diet, with similar liveweight gains (570 g/day). In a digestion trial the pigs accepted a diet containing 30% treated crambe meal for two months without feed refusals. Nevertheless, further study is required concerning animal health after consumption of crambe diets, although no apparent disorders were encountered during our study.

When feeding legume seeds or rapeseed meal to ruminants, a toasting treatment has been found useful in improving protein availability by reducing rumen degradation rate (Aguilera et al., 1993). For non-ruminants, Van der Poel (1990) found that toasting of ANF-containing legumes improved protein ileal digestibility in piglets. However, excessive toasting in many circumstances may destroy some amino acids and depress protein availability. An experiment with chickens showed that, as autoclaving time increased, contents of lysine and cystine in soybean meal decreased and true digestibility of several amino acids also decreased significantly (Parsons et al., 1992). In our study, Chapter 7 describes how moist-toasting decreased protein quality in rapeseed meal fed to rats. Heating at 100^{0}C for 60 to 120 min clearly decreased lysine content due to the Maillard reaction. This occurred to a greater extent in toasting crambe or rapeseed meals than other protein sources, because the reducing sugars are already present in meal, and also released from the glucosinolates. In addition or even

more likely, some glucosinolate break down products as isothiocyanates also react readily with free amino groups through formation of schiff bases. Furthermore, both true protein digestibility and biological value were decreased significantly. Processing of crambe meal resulted in a 10% loss of lysine after toasting, addition of sodium carbonate or ferrous sulphate resulted in a further 10% loss (Chapter 6). From an in vitro experiment it was observed that toasting crambe meal with 3% sodium carbonate decreased protein digestibility from 91% (unheated sample) to 76%; whereas toasting without chemicals or with 1% ferrous sulphate only led to a 5 percentage unit decrease (Liu et al., unpublished data), indicating that particularly the addition of alkaline lowers protein quality. The pig digestion trial suggested that the ileal digestibility of both protein and lysine was negatively influenced by excessive toasting with alkaline as reported in Chapter 5. A likely explanation of this protein quality impact is again the binding of free amino groups to reducing carbohydrates and other reactive compounds through formation of schiff bases, and such reaction increases with increasing pH.

Due to complexities in evaluating protein quality after heat treatment, an attempt was made to relate heat treatment to protein quality. Appreciable correlation was found between protein solubility and other criteria, such as decline in lysine content, protein true digestibility and biological value (Chapter 7). This study yielded a recommendation that nitrogen solubility could be used as an easy way to measure protein damage after toasting. Unheated rape or crambe protein should have a minimum solubility of 85%, whereas an intensively heated sample displayed a solubility of 40%.

Another disadvantage of toasting is that small quantities of ITC and OZT remain in the meal. Recommendation in Denmark is: diets for starter pigs should contain no more than 1 μmol glucosinolates and for growing-finishing pigs no more than 2 μmol (Bjerg et al., 1989). Dutch feed legislation (1988) regulates that the level of OZT and ITC should not exceed 1,000 ppm in compound feed for non-ruminants, and 2,000 ppm for ruminants. Assuming this criteria also valid for crambe meal, our investigations suggest it is possible to include the treated meal in compound feed for pigs or poultry at levels between 10-15%, because a proper toasting can remove nearly all intact glucosinolates and minimize ITC+OZT levels to below 8,000 ppm.

Water extraction has the advantage of being highly efficient in detoxification without lowering protein quality. Crambe meal extracted in this way showed no apparent toxicity as shown in a chicken trial conducted by Baker et al. (1977b). The method is especially useful for small scale sample preparation. A disadvantage is that dry matter losses often range from 20 - 30%, although nearly half originates from glucosinolates contained in the meal. However, the high solubility of both protein and glucosinolates offers an alternative use for crambe meal: to separate and isolate these two components by water extraction. Crambe protein can be used as a food or feed ingredient as with rapeseed meal (Jone, 1979), and the isolated glucosinolates may find an application as pesticide (Carlson and Tookey, 1983).

4. Feeding Potential for Non-ruminants

4.1 Apparent digestibility

A literature survey prior to this study failed to provide any information on crambe digestibility. Therefore, in this project digestion trials were carried out in both pigs and rats, and a comparison was made with rapeseed meal. A general evaluation of crambe meal as non-ruminant feed has been made based upon the data from these experiments. Additionally, these data could possibly be used as reference for poultry diet formulation, since digestibility coefficients are currently scarce in poultry.

4.1.1 Energy

The digestibility trial with rats revealed that the gross energy (GE) in solvent extracted crambe seed meal was highly digestible, with a coefficient of 93%, whereas, in the same experiment, rapeseed meal was found to be 80% digestible. For screw-pressed crambe cake, GE digestibility was found to be 78% at faecal level in pigs, and 73% for rapeseed cake. This figure for rapeseed meal was in agreement with values (75%) reported by Keith and Bell (1991); and (74%) by Bourdon and Aumaitre (1990). The apparent digestibility coefficients of all components in crambe cake were higher than those in rapeseed cake: crude fat 87% vs. 79%, crude fibre 60% vs. 38%, and N-free extracts 82% vs. 78%. The lower value for crambe protein digestibility may be due to the inadequate treatment as discussed in Chapter 5. The differences in apparent GE digestibility coefficients between rats and pigs are probably not only due to animal species but also to sample preparations. Both trials showed the same tendency towards higher digestibility in crambe by-products than in rapeseed. The reason is that crambe meal contains significantly less cell wall constituents.

According to Dutch feeding standards (CVB, 1994), these coefficients imply that crambe cake contains 16.86 MJ digestible energy and 10.61 MJ net energy per kg dry matter for pigs, whereas the respective figures for rapeseed cake were 15.45 and 9.86, differences of 9% and 8%.

4.1.2 Protein and amino acids

For crude protein, the rats showed apparent and true digestibilities of 78 and 88% for the solvent extracted crambe seed meal, which are similar to those of rapeseed meal (79 and 89%). However, the Biological Value (BV) and Net Protein Utilization (NPU) of crambe protein were at 48 and 43%, significantly lower than those from rapeseed meal (87 and 78%). Since the crambe meal was subjected to a toasting at 110^{0}C for 40 min, protein damage was not considered serious, as indicated by a true digestibility comparable to that of rapeseed meal. The lower BV and NPU were thus most probably attributable to the anti-nutritional factor (glucosinolates) present in crambe meal at a rather high level (55 μmol g^{-1}), according to Bjerg et al. (1989). In order to avoid interference from glucosinolates, Pereira at al. (1981) used aqueous acetone or water extracted crambe meal to determine crambe protein value in rats, and found the protein to be of excellent quality: its protein efficiency ratio (PER) was higher than that for casein in all cases (2.75 vs. 2.5) as discussed in Chapter 2.

In the pig trial, the apparent protein digestibilities were 77 and 64% at faecal and ileal levels, respectively; whereas these digestibilities for rapeseed meal protein were 79 and 71%,

respectively. Some of the amino acids in crambe meal displayed distinctly lower ileal digestibilities than those in rapeseed meal, lysine (61% vs. 75%) and cystine (63% vs. 72%) in particular. According to the findings of Parsons et al. (1992), we presumed that the low ileal digestibility of protein and lysine was attributable to the severe treatment to which the crambe meal had been subjected. Despite the damaged protein or amino acids were still digestible in the hind gut, this nitrogen was generally considered to be unavailable to monogastrics. These results indicate scope for improvement in nitrogen utilization through optimizing detoxification treatment.

Although, in general, interference of glucosinolates and treatment complicated the picture of crambe protein digestibility, considering the crambe amino acid profile, the low content in cell wall constituents and the true protein digestibility obtained with rats, one can predict that crambe protein could have a slightly higher apparent ileal digestibility than rapeseed meal in pigs. But, due to the counter-effect of treatment to remove glucosinolates, it is reasonable to assume that adequately treated crambe meal will have an apparent ileal protein digestibility of 70-75%, with a similar coefficient for lysine digestibility.

Our digestion trial with pigs showed that the crambe phosphorus was 43% digestible, which is significantly higher than in rapeseed (23%). This is probably owing to that the proportion of crambe phosphorus bound in phytin was less than that in rapeseed meal (63% vs. 83%).

4.2 Animal performance

Although at present there are insufficient data to confirm feeding performance of crambe meal for non-ruminants, in this project such information was obtained from both our trials and literature. In rats, unlike previous observations showing animals often dying from crambe toxicity, our experiments showed no mortality on a diet containing crambe meal as sole protein source with 11 μmol epi-progoitrin per gram diet. The animals apparently grew well when the crambe meal provided half of the dietary nitrogen, despite the fact that the glucosinolates had previously caused varying degrees of enlargement in thyroid, kidney and liver. Inclusion of soda treated or water extracted crambe meals in diets at 30% for rats and 20% for chickens, resulted in growth equivalent to those fed soybean meal for 90 and 30 days respectively (Mustakas et al., 1976 and Baker et al., 1977b).

Similar results were found with young pigs. After the crambe meal had been detoxified by toasting, alone or with chemicals, the animals displayed a reasonable gain similar to that on a commercial diet, even at a dietary inclusion level of up to 20%. Furthermore, a diet containing 30% treated crambe meal was found acceptable by pigs. Therefore, based on experience with rapeseed meal, it appears that for pigs half of the dietary protein can originate from the treated crambe meal.

Although the above observations seem inconclusive, it is realized that the highly digestible gross energy and high protein content in crambe meal are of great advantage to non-ruminant feeding, and certain treatments can make the meal acceptable to both pigs and chickens. Adequate detoxification is still a crucial point for the efficient utilization of crambe protein. More research is therefore required.

5. Feeding value for ruminants

5.1 Apparent digestibility

As with non-ruminants, literature concerning crambe digestibility for ruminants was not available. In the present study, crambe's apparent digestibility was estimated initially using in vitro procedures either with rumen fluid followed by a pepsin-HCl treatment (dOt, method of Tilley and Terry, IVVO modification) or by incubation with cellulase (dOc) (Chapter 3). Then the rumen degradability and intestinal digestibility of crambe samples were determined by in situ incubation in dairy cows. The main results were presented in Chapters 3 and 4.

The in vitro digestibility of the whole crambe seeds ranged from 50 to 60%. As expected, the crambe pericarp showed a rather low in vitro digestibility (<50%). The coefficient for press cake was between 60 to 65%. However, figures for decorticated and defatted meal were particularly high (85-88%), exceeding those of rapeseed meal (75-78%). This is in accordance with the chemical analyses showing that there was a high cell wall content in crambe pericarp. The in vitro digestibility of crambe organic matter was strongly influenced by the NDF content, this relationship was linear: the higher the NDF content, the lower the digestibility (Chapter 3).

5.2 Degradation and digestion in cows

5.2.1 Rumen degradation

The results of in situ degradation and digestion in cows are elucidated in Chapter 4. For comparison, rapeseed and soybean meals were determined simultaneously and their degradation was found to be comparable with those from other authors as summarized by Van Straalen and Tamminga (1990). For crambe pericarp, the undegradable fraction of the organic matter (U, after 336 h in rumen) was 57%, the degraded fraction (43%) included 17% that disappeared readily upon washing and 26% that could be degraded by microbes. Thereby, degradation of the crambe pericarp investigated was poor. However, the potential degradability (100-U fraction) in the defatted crambe meal was found to be very high, up to 96%, slightly higher than that of rapeseed meal (95%). The press cake showed 73% potential degradability.

The NDF in crambe pericarp was found to be extremely undegradable (U=81%), whereas the NDF in defatted seed meal had a U fraction of only 24%. This again indicates that proper decortication of crambe seeds is important for improving the nutritional quality of the by-products.

In our study it was found that crambe protein was rapidly degraded in the rumen, with a degradation rate of 18% h^{-1} (k_d) for crambe meal and 11% h^{-1} for press cake. Rapeseed and soybean proteins were degraded at rates of 10 and 6% h^{-1}, respectively. Assuming an outflow rate (k_p) of 6% h^{-1} results in an estimated 12.5% by-pass protein in crambe meal and 13% in cake, these values are considerably lower than those for rapeseed (35%) and soybean meals (42%). The rapid degradation of the crambe protein in our study was due, to a large

extent, to the fact that the crambe samples were not subjected to high temperature treatment. Crambe protein had a high solubility, therefore the washable fraction (W) was exceptionally high (56%) in comparison with rapeseed (18%) and soybean meal (8%). It has been found that steaming canola meal to different degrees significantly decreased the DM and N degradation in the rumen, subsequently increased the digestibility of DM and N in the lower gastrointestinal tract (GI) in all treatment times. After steaming at 127°C for 45 min, N disappearance from the rumen declined from 74 to 19%, and increased in the lower GI from 16 to 64% (Moshtaghi Nia and Ingalls, 1992). Toasting the legume seeds yielded similar results (Aguilera et al., 1992).

5.2.2 Intestinal digestion

The digestibility of rumen-undegraded fractions was measured using the mobile nylon bag technique. In our study samples of crambe and rapeseed meals were inserted into the duodenum without prior rumen incubation, in order to obtain a direct indication of digestibility in the ruminant intestines. The digestibility coefficients were 93% for crambe OM and 79% for rapeseed OM. For protein these were 97% vs. 92%. In this study it was found that the rumen residence time had a significant influence on intestinal digestibility: the longer the samples were retained in the rumen, the lower the percentages of the rumen residues that were digested in the intestines, this tendency was observed for all samples studied. On the other hand, the total digestive tract digestibility remained almost consistent: after 12- or 24-hour rumen incubations the GI digestibility of protein was 98% for crambe meal, and 96% for the cake (Chapter 4). This suggests that most of the crambe protein is digested either in rumen or intestines, whilst only a very small part escapes degradation or digestion.

5.3 Feeding value

5.3.1 Energy value

Chemical analyses and digestion data enabled calculation of the availability of both energy and protein in crambe by-products. When typical compositions of crambe by-products are chosen based on our analyses as given (Table 3), together with digestibility coefficients from both the in vitro and in situ investigations and applied to various constituents, an estimation of feeding values is possible as displayed in Table 3. Taking into account a fat content of 12% for partially decorticated and defatted material in crambe cake, the NE value is slightly lower than that of the solvent extracted meal. Due to the extremely high fibre content in the pericarp (hulls), and to the fact that less than half of pericarp OM are digestible, its NE value is less than half that for meal or cake. According to the estimated values, the conversion rates from gross energy (GE) to net energy for lactation (NE_l) are thus 44, 39 and 19%, respectively for crambe meal, cake and pericarp.

5.3.2 Protein value

The availability of crambe protein was evaluated according to the Dutch "intestinal digestible protein (DVE) system", which consists of three fractions: undegraded feed CP digested in and absorbed from the small intestine as amino acids (DVBE); microbial protein

digested in and absorbed from the small intestine as amino acids (DVME); and endogenous losses resulting from digestion (DVMFE) (CVB, 1994 and Tamminga et al., 1994). In Chapter 4 by-pass protein coefficients were obtained for an unheated crambe sample and adapted to represent a sample after industrial processing. In Table 3 a coefficient of 0.34 was used, which is the tabulated figure for rapeseed meal (CVB, 1994). This resulted in a protein availability of 46% (100×DVE/CP in feed) for crambe meal and 43% for cake. Pure crambe pericarps (hulls) contributed little available protein to the animals.

Table 3 Crambe by-products: chemical composition and feeding value for ruminants

	Extracted meal		Press cake		Pericarp	
	Content	Dig[a]. %	Content	Dig[a]. %	Content	Dig[a]. %
Constituents, g per kg DM						
Org. matter	900	90	905	82	900	45
Crude protein	500	92	440	85	90	65
Crude fat	22	80	120	92	33	80
Crude fibre	61	65	130	50	417	20
N-free Extract	317	92	215	88	361	65
Feeding value, in DM						
Gross energy, MJ kg^{-1}	19.54		21.38		18.24	
Metab. energy, MJ kg^{-1}	12.81		13.84		6.54	
Net $energy_{lact.}$, MJ kg^{-1}	8.61		8.35		3.50	
By-pass CP, % of CP[b]	34		34		45	
DVE, g kg^{-1}	230		190		27	

a. Digestibility coefficients derived from in vitro and in situ determinations, and attributed to various components.
b. Figures for meal and cake were from tabulated data for rapeseed meal (CVB, 1994).
c. DVE, true protein digested in and absorbed from the small intestine (CVB, 1994).

5.4 Feeding trials

As mentioned in Chapter 2, a few feeding trials were conducted in beef cattle. Crambe meal used in early experiments contained less crude protein (25 - 31%) and decortication was often incomplete. Lambert et al. (1970) fed crambe meal to replace 1/3, 2/3 or all of the soybean meal in diets for steers. This crambe meal contained 26% crude protein. Daily gain and feed intake decreased significantly as crambe meal in the diets increased. Feed efficiency was similar on all treatments. It was also observed that the cattle selectively consumed the rations containing crambe meal, leading to lower feed consumption and gain. Pelleting and blending of crambe with other protein sources like soybean meal improved acceptability, suggesting crambe could replace up to 2/3 of the soybean meal in diets for growing cattle. Several years later, Perry et al. (1979) conducted similar experiments with finishing beef cattle. After observing two trials with slight increases and two trials with slight decreases in

performance, both of which were not significant, they concluded crambe meal quality to be important to beef cattle performance. Finishing cattle showed no adverse response to crambe meal up to a 8.5% inclusion level in the diet.

A more recent study was conducted to compare crambe meal with sunflower meal, both were included in the pelleted supplement to a large group of steers (n=1474). Crambe fed animals appeared to consume slightly more feed during the first three months but this decreased during the last forty days. Over the entire feeding period, crambe meal fed steers consumed 0.23 kg per day less dry matter and had a 2.8% lower daily gain. Feed intake fluctuated widely in both treatment groups. There were no differences in quality grade or liver abscess scores as reported by Anderson (1993). The author also conducted another trial with beef cattle to investigate: 1)100% soybean meal; 2) 67% soybean meal plus 33% crambe meal; 3) 33%soybean meal plus 67%crambe meal and 4) 100% crambe meal. The protein supplements were pelleted and the animals averaged 283 kg liveweight. Other ingredients used were dry rolled maize grain, maize silage and chopped wheat straw. Throughout the trial (180 days) no differences were detected in feed intake, feed conversion or growth rate.

Research was also performed to investigate the possible accumulation of glucosinolate compounds in beef tissue. Tissue and fat samples were tested for epi-progoitrin, unsaturated nitriles, episulfide nitriles or goitrin at a sensitivity of 1 ppm. No traces were found in samples of beef fat taken near the kidney, liver and shoulder muscle tissue from steers fed defatted crambe seed meal at up to 10% of the diet (Van Etten et al., 1977). Anderson et al. (1993) observed that steers fed crambe meal had increased marbling score compared to the control animals, similar to the results found when feeding rapeseed meal (Heidker and Klopfenstein, 1989).

These studies resulted in a legislation of crambe utilization in beef cattle feeding by the FDA in USA, in which toasted crambe meal is allowed as a protein source at an amount not exceeding 4.2% of the total ration dry matter (Carlson, 1992).

6. Comparison of crambe and rapeseed by-products

Comparison of well-known and comparatively new products can often provide a better basis for understanding and acceptance. Pereira et al. (1981) compared crambe protein with casein in rats. The crambe protein used in their study was carefully prepared by aqueous extraction. They measured protein efficiency ratio (PER) and concluded that crambe protein had a higher PER value than casein. For livestock, several authors have compared crambe with soybean or sunflower meals in rations for beef cattle (Chapter 2), and found comparable feed conversions but feed intake to be sometimes lower on crambe diets. In our view a better comparison for crambe is rapeseed: they belong to the same family of Cruciferae plants; processing procedures and proximate composition are similar and both contain glucosinolates. Therefore, rapeseed meal was used as a reference throughout this study, although soybean meal and other novel oilseed meals were also used for additional comparison (Liu et al., 1993b).

6.1 Nutritional and anti-nutritional aspects

Despite the fact that proximate compositions are similar in crambe and rapeseed by-products, they differ clearly in two aspects: first, crambe meal possesses a significantly higher level of crude protein. Our analyses revealed that, after removal of pericarps, the defatted crambe seed meal contains approximately 50% crude protein on fat-free DM basis, about 5-8 percentage units higher than that in double zero rapeseed meal and over 10 percentage units higher than in conventional rapeseed meal. Both proteins have a similar amino acid profile, and contain less lysine but more sulphur-containing amino acids than soybean protein. This implies that crambe meal is a well-qualified protein supplement to livestock, especially non-ruminants. Secondly, crambe meal obviously contains less fibre: as indicated in Table 1, NDF is half, cellulose 42% and ADL 26% of that found in rapeseed meal when calculated on the same basis. This is presumably owing to that crambe seeds contains considerably less seedcoat than rapeseeds (8 vs. 15%; 16 vs 28% in fat-free meal, author's recent experiment). Therefore, a higher digestibility for crambe meal is predictable (Chapters 3-5).

The total glucosinolate content in crambe seeds averaged 62 μmol g^{-1} (Chapter 2, Steg et al., 1994). Our recent determination using HPLC showed that the seeds grown under Dutch circumstances contained 68 μmol g^{-1} and 99% of them were aliphatics as listed in Table 2. In contrast, a qualification of being modern double low rapeseed variety requires a total content of aliphatic glucosinolates below 30 μmol g^{-1} (Daun, 1986), and in some varieties the level is in fact as low as about 10-12 μmol g^{-1} (Chapter 7). Conventional rape species often contain about 50 μmol g^{-1} (Bell, 1993). It has been known the aliphatic glucosinolates to be most harmful ANF, especially progoitrin in rapeseed or epi-progoitrin in crambe (Bjerg et al., 1989). Unlike crambe, there are an appreciable proportions of indole-side chain glucosinolates present in rapeseeds. These glucosinolates are readily broken and thereafter non-detrimental in the diets. The fact that 3% crambe meal in the diet impairs the pig's appetite (Chapter 6) would probably not occur when feeding rapeseed meal, although the palatability problem exists (Chapter 8 and Rundgren, 1983). Several authors suggest that progoitrin and epi-progoitrin are especially responsible for hampering an animal's appetite (Lee and Hill, 1983 and Bjerg et al., 1989). This probably explains why crambe meal is less palatable than rapeseed meal. Moreover, hydrolysis of the different side-chain glucosinolates may result in diverse aglucon products varying in toxicity (Tookey et al., 1980).

Detoxification of rapeseed meal was found to be easier than crambe due to the fact that rapeseed contains less glucosinolates, of which some possess an indole side-chain. In this study water extraction was found to be very efficient for removal of glucosinolates from rapeseeds, and up to a half-hour toasting at 100^{0}C can be accepted for double zero variety rapeseeds (Chapters 7 and 8).

6.2 Feeding value

For ruminants, as shown in Table 3, defatted crambe seed meal as processed industrially, contains a higher NE value and more available protein than rapeseed meal, the main

reason is that crambe meal is more digestible and contains significantly more protein than rapeseed meal. In practice, however, nutritional value of the by-products will largely depend on the extent of decortication, because crambe pericarps are poorly digested. Further removal of crambe seedcoat could possibly increase digestibility of the meal as was for rapeseed (Bell, 1993), but there has been no information concerning crambe seedcoat composition and feasibility of seedcoat removal.

In both rats and pigs, crambe meal had a higher apparent energy digestibility than rapeseed meal. However, the ileal apparent digestibility of nitrogen and amino acids was obviously lower than with rapeseed meal. The same trend was found for biological value and net protein utilization in rats. This is largely related to the glucosinolate content and detoxification treatment as discussed earlier.

7. Outlook

7.1 Importance of crambe processing

During recent years the novel crops have been investigated intensively as possible new material sources in both industry and agriculture (Rexen, 1992; Van Soest and Mulder, 1993). With respect to crambe the prospects look good for gradual introduction to many countries, for both erucic acid and protein production. In the United States and Europe, crambe has already been planted on commercial or semi-commercial scales, showing its potential as a future oil-crop. Unfortunately, little attention has been paid to crambe by-products in spite of the fact that they represent over 70% of the seed weight. It should be realized that any utilization of the by-products, profitable or otherwise, is considered more acceptable than adding a further burden on the environment as a disposable waste. The study presented in this thesis has laid a basis of knowledge for a better understanding of such by-products as animal feeds.

The high glucosinolate content forms a considerable challenge to the by-product utilization. Due to the great success achieved in breeding the double zero rapeseeds, a search for genetic variation in crambe would seem relevant, with a view to breeding "low glucosinolate crambe". However, experience in breeding modern rapeseed has shown that such breeding programmes will require very many years before the zero-glucosinolate variety of crambe is available to the farmer.

7.2 Which treatment?

Toxicity problems with rapeseeds have attracted enormous attention, not only for conventional varieties but also for the double zero rape (Chapters 7 and 8; Rundgren, 1983; Bell, 1984; 1993). However, so far no standard procedures have been established in rapeseed meal detoxification. This is because the mechanism of toxicity from glucosinolates and their hydrolyzed products has not been entirely understood, therefore, requirement for feed legislation on glucosinolates is different from country to country, and at present, no precise and universally accepted regulation exists.

In practice, economical utilization of rapeseed by-products depends on various conditions. Choice of treatment appears not only depending on the level of toxicants, but also on the animal species to be fed and the feeding circumstances involved. For instance, conventional rapeseed species are still grown in great quantities in many countries, e.g. China. In many cases a prolonged desolventation-toasting can render the by-products acceptable to feed compounders, because limited dietary inclusion level (e.g. 10%) can generally meet feed regulations and obtain reasonable animal growth and production efficiency, as for pigs and poultry (Chapter 8). However, where growth rate is important and alternative protein sources are not easily available for non-ruminants, a more costly detoxification becomes necessary.

The situation is similar for the utilization of crambe by-products. If they are to be fed to ruminants, a well regulated toasting could be sufficient since the final by-products contain about 30 μmol glucosinolates (Carlson et al., 1985), limiting dietary inclusion level can meet feed regulations. Furthermore, ruminants are to some extent able to decompose glucosinolates in rumen and therefore the risk of toxicity is reduced. However, if crambe meal is intended for feeding to non-ruminants due to its high protein content and high digestibility, an adequate treatment is essential, otherwise serious problems will occur with both animal appetite and toxicity. The same principle used for rapeseed can be used for crambe detoxification. For example, toasting, addition of ferrous sulphate or water extraction (Baker et al., 1977a; Liu et al., 1994) can considerably eliminate such toxicity, resulting in an apparently non-toxic meal. Feeding strategy and treatment costs should always be taken into account when considering a detoxification procedure.

7.3 Future strategy

A. **Optimizing detoxification for non-ruminant feeding**

Defatted and detoxified crambe seed meal can serve as an excellent protein supplement for pigs, poultry and other monogastric animals. Water extraction is currently the most efficient way of obtaining high quality crambe protein. Research should focus on reducing the processing costs and possibilities of utilizing glucosinolates (e.g., as natural pesticides). Waste water handling should also be taken into account. Since most glucosinolates can be removed by toasting, and addition of certain chemicals can accelerate such reactions, further research is required into process regulation and reduction in protein damage. Since there are firm evidences showing that overheating decreases some essential amino acids and their bioavailability (Parsons et al., 1992; Chapters 7 and 5), A compromise between these two aspects would seem to be required.

B. **Processing for ruminant feeding**

It has been proved that cattle can withstand higher levels of glucosinolates than non-ruminants. Experiments showed that toasted crambe meal is acceptable for beef cattle, despite not being completely palatable. If the desolventation-toasting after oil extraction can remove up to 80% of glucosinolates as described by Carlson et al. (1985), in certain circumstances crambe by-products may end up as dietary ingredients for ruminants.

C. Isolating crambe protein

Several experiments have been conducted to obtain protein concentrates from rapeseed meal in an attempt to utilize this by-product (Jones, 1979; Thompson et al., 1982 and Zhou et al., 1990). Isolated rape protein can be used as ingredients in the processing of both human and pet food. Compared with rape, crambe meal contains a higher level of soluble protein, which theoretically could provide nearly half of the oil-free crambe matter as protein products compared with 20 - 35% from rapeseed meal as obtained by Zhou et al. (1990). Furthermore, if the isolated glucosinolates can find a market in near future, a combined procedure can be developed to extract both protein and glucosinolates simultaneously, which would considerably improve economic consequence of the processing.

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SUMMARY

The general objective of the present study was to investigate the feeding potential of crambe meal, this included 1) a review and evaluation of the available information on the subject concerned; 2) an investigation into the possibilities of detoxification or treatment to improve the acceptability of the by-products by animals; 3) a study of the digestibility and utilization of the by-products in both non-ruminants and ruminants; and 4) a comparison with rapeseed by-products. The background, objectives and scope of this research project are stated in Chapter 1.

Chapter 2 contains a literature review which presents and evaluates the available information on this subject. The study showed that the defatted crambe seed by-products have a high level of protein with a well-balanced amino acid composition; but also a high glucosinolate content. Over 95% of crambe glucosinolates are identified as epi-progoitrin, which is rather harmful to livestock. A few feeding trials were conducted in the past to evaluate crambe meal as a feed ingredient for beef cattle, but information on digestibility was lacking.

In Chapter 3 a wide range of crambe samples were obtained and analyzed for chemical and nutritional composition. Data were accumulated concerning both nutrients and anti-nutritional factors in crambe grown under Dutch circumstances. The results revealed that crambe pericarp is fibre-rich and highly lignified; whereas defatted seed material contained nearly 50% crude protein with an amino acid profile similar to rape protein. Crambe meal showed considerably lower levels in fibre constituents: both crude fibre and neutral detergent fibre contents were only half of those in rapeseed meal, cellulose was about 40% and acid detergent lignin was less than one third of those in rapeseed meal. The seeds contained on average 62 μmol g^{-1} glucosinolates, which subsequently remained in the meal after defatting, levels of around 150 μmol g^{-1} were recorded if the sample was not treated at high temperature. Some additional information on structural elements of crambe seeds can be found in Chapter 5. Whole seeds contained 30% pericarp, after decortication the seeds had 9% seedcoat, whereas decorticated rapeseed contained 16% seedcoat, this explained largely why crambe meal showed low fibre content and high digestibility in both rats and livestock.

Apparent digestibility and rumen degradability of crambe by-products were determined in dairy cows as presented in Chapter 4. It was found that, after removal of pericarp, organic matter and protein in the defatted crambe seed meal were highly digestible as shown by both in vitro and in situ incubation experiments; whereas crambe pericarp were poorly digested. For unheated crambe meal the fraction of by-pass protein was low due to its quick disappearance from the rumen. This experiment yielded basic data on degradability and digestibility of crambe by-products, and comparison was made with several other oilseed by-products.

Information on digestibility from non-ruminants was obtained from both pigs and rats as elucidated in Chapter 5. Defatted crambe seed meal was highly digestible, especially gross energy. Apparently, crambe protein was as digestible as rapeseed meal, but protein quality and utilisation were presumably hampered by over-toasting. Probably, protein utilisation was influenced by residual glucosinolates where moderate toasting was applied. Based on information from this study, the available energy for pigs was estimated and it was also

assumed that crambe protein and lysine could have a digestibility similar to that found in rapeseed meal after optimization of treatment. Moreover, crambe meal showed a considerably higher phosphorus digestibility in pigs compared to rapeseed meal. In rat trial it was found that the glucosinolates in crambe meal caused enlargement of thyroid, liver and kidney. Unheated crambe meal was almost entirely unacceptable to young pigs, as shown by loss in appetite and liveweight of pigs fed crambe at a 3% dietary inclusion level.

Since crambe contained high levels of glucosinolates and the meal was very unpalatable to pigs and rats, several detoxification approaches were investigated as illustrated in Chapter 6. These included toasting, chemical treatment and aqueous extraction. The aqueous extraction was found to be very efficient in removing glucosinolates, but the treatment caused 20 - 30% dry matter loss. Toasting was also very useful in inactivating myrosinase and further decomposing the glucosinolates. A significant removal of the glucosinolates can be achieved by toasting at 100-110^{0}C for 60 min, and the reaction can be quickened if the moisture rose above 10% and/or certain chemicals were added prior to toasting (e.g. Na_2CO_3 or $FeSO_4 \cdot 7H_2O$). Such treatments removed nearly all intact glucosinolates, and made the meal acceptable to pigs. The treatment created the possibility of including crambe meal into pig diets, even though feed legislations were taken into account. On the other hand, the prolonged toasting lowered nitrogen solubility and lysine content. A compromise is therefore needed.

Owing to a good similarity between crambe and rapeseed, investigations on crambe detoxification were also conducted on rapeseed meal. Results are reported in Chapters 7 and 8. Additional knowledge concerning properties of glucosinolates became available from these studies. The treatment target was to eliminate toxicity from glucosinolates whilst maintaining nutritional value, particularly of protein and amino acids. Results in Chapter 7 yielded a clear picture of their relationship. Glucosinolates were destroyed as toasting increased but protein quality was impaired. The investigation recommended an appropriate toasting procedure for double zero rapeseed meal in practice (100^{0}C 30 min), and nitrogen solubility as monitor of damage to protein quality. Chapter 8 dealt with conventional rapeseed meal for which a water extraction procedure was thoroughly investigated. Information was found useful for improvement in processing and utilization of high glucosinolate rapeseed meal.

In the general discussion, Chapter 9, a systematic assessment of crambe by-products is presented. This chapter contains a compilation and evaluation of all the available data, including chemical composition; anti-nutritional aspects; degradation and digestion in ruminants; digestibility in non-ruminants; estimation on energy and protein values; detoxification treatment and comparison to rapeseed by-products. Properties of crambe by-products are discussed and future research directives are suggested.

SAMENVATTING

In het kader van onderzoek naar nieuwe gewassen voor de akkerbouw is er belangstelling voor de teelt van *Crambe abyssinica*. De Nederlandse benaming van dit gewas is 'Afrikaanse zeekool' of ook 'crambe'. Het zaad van crambe bevat olie met een hoog gehalte aan erucazuur, waarvoor de oleochemische industrie hoogwaardige toepassingen kent. Bij de produktie van olie uit crambezaad komt een aanzienlijke hoeveelheid restmateriaal beschikbaar. Vanouds wordt restmateriaal na de oliewinning uit zaden vaak als veevoer gebruikt; dan is sprake van bijprodukten of 'meel'.

Het doel van deze studie was, de toepasbaarheid van crambemeel als veevoer te onderzoeken. De studie omvatte o.m.: 1) een overzicht en evaluatie van de beschikbare literatuur; 2) onderzoek naar de mogelijkheden van verwijderen of verlagen van het gehalte aan glucosinolaten om de bruikbaarheid van bijprodukten als veevoer te vergroten; 3) een studie van de vertering en benutting van de bijprodukten door zowel herkauwers als eenmagige dieren; en 4) een vergelijking met raapzaad-bijprodukten.
In hoofdstuk 1 zijn de achtergrond en probleemstelling van het onderzoek behandeld en is het doel van de studie geformuleerd.

Hoofdstuk 2 betreft een literatuurstudie van de beschikbare informatie over de bruikbaarheid van crambemeel als veevoer. De studie geeft aan dat de vetvrije droge stof van crambezaad een hoog eiwitgehalte heeft met een evenwichtige aminozuur-samenstelling, maar ook dat het gehalte aan glucosinolaten hoog is. Bovendien wordt meer dan 95% van deze glucosinolaten gevormd door het schadelijke epi-progoitrine. Er is wel enige literatuur over opname- en voederproeven met crambe-bijprodukten bij vleesvee, maar informatie over afbraak en vertering van nutriënten ontbreekt.

In hoofdstuk 3 is informatie gegeven over chemische samenstelling, anti-nutritionele factoren en nutriënt-gehalte (gemeten met in-vitro verteringsmethoden) van bijprodukten van crambe en andere (nieuwe) oliezaden. De gegevens zijn afkomstig van gewassen die in Nederland zijn geteeld. Uit de gepresenteerde resultaten komt naar voren dat crambehullen (pericarp) vezelrijk zijn en veel lignine bevatten. De vetvrije droge stof van het ontdopte zaad bevatte bijna 50% ruw eiwit met een aminozuurpatroon vergelijkbaar met raapzaadeiwit. Meel van ontdopt crambezaad bevatte aanzienlijk minder celwandmateriaal dan de standaard-kwaliteit raapzaadmeel: zowel ruwe celstof als NDF (Neutral Detergent Fibre), ADF en cellulose. Het zaad bevatte gemiddeld 62 μmol g^{-1} glucosinolaten, die door ontvetten niet worden verwijderd. Hierdoor werden in het meel gehalten tot ongeveer 150 μmol g^{-1} gevonden, wanneer geen hittebehandeling had plaatsgevonden. Hoofdstuk 5 geeft nog enige aanvullende informatie over de samenstelling van zaadfracties waaruit crambemeel is opgebouwd. Hele zaden hebben ca. 30% pericarp; het gehalte aan zaadhuid (epicarp) na ontdoppen van de zaden was slechts 9%, tegenover 16% bij raapzaad. Dit verschil vormt een verklaring voor de relatief lage celwandgehalten

in ontdopt crambemeel en de hoge verteerbaarheid.

Hoofdstuk 4 geeft de uitkomsten van een proef waarin de afbraak in de pens en vertering in de darmen van melkvee is gemeten van bijprodukten van crambe en andere oliezaden. De organische bestanddelen van ontdopt crambemeel waren zowel in-vitro als in-situ (in de pens) zeer goed verteerbaar. De doppen werden matig tot slecht verteerd. Bij onverhit crambemeel was de bestendigheid van de eiwitfraktie tegen afbraak in de pens laag; de darmverteerbaarheid van het eiwit was relatief hoog. In dit hoofdstuk is ook een vergelijking gemaakt met afbraak en vertering van sojaschroot en raapzaadschroot.

Informatie over de verteerbaarheid van crambemeel bij varkens en ratten is weergegeven in hoofdstuk 5. De schijnbare verteerbaarheid van de energie in een partij ontdopt en vochtig verhit crambezaadmeel was hoog. Hoewel de samenstelling en de schijnbare verteerbaarheid van het crambe-eiwit op mestniveau bij varkens vergelijkbaar was met die van raapzaad-eiwit, was de vertering ervan in de dunne darm lager, waarschijnlijk als gevolg van te langdurige verhitting bij de ontgiftingsbehandeling. De resultaten van het onderzoek maakten een schatting van de energiewaarde van crambemeel voor varkens mogelijk. Tevens werden indicaties verkregen dat na optimalisering van de behandeling een met raapzaadmeel vergelijkbare verteerbaarheid van het in het produkt aanwezige eiwit en lysine mogelijk is. De verteerbaarheid van fosfor (P) in crambemeel was opvallend hoger dan in raapzaadmeel. Adequate behandeling ter reductie van glucosinolaten is echter noodzakelijk. In een opnameproef met biggen (gerapporteerd in hoofdstuk 6) bleek dat onbehandeld crambemeel door biggen vrijwel volledig werd geweigerd, zodat zelfs bij slechts 3% crambemeel in het rantsoen groeistagnatie optrad. Uit de proeven met ratten werd de indicatie verkregen dat de eiwitbenutting werd belemmerd door residuglucosinolaten. In deze proeven werd ook een vergroting van schildklier, lever en nieren vastgesteld.

In hoofstuk 6 is verslag gedaan van onderzoek naar verschillende behandelingsmethoden van crambemeel om het gehalte aan glucosinolaten te verminderen. Als methoden werden onderzocht: uitwassen met water, vochtig verhitten en het toevoegen van chemicaliën tijdens vochtig verhitten. Door uitwassen met water konden de glucosinolaten vergaand worden verwijderd, maar de behandeling veroorzaakte tevens 20-30% verlies aan droge stof. Ook vochtig verhitten was effektief voor de afbraak van glucosinolaten. Een aanzienlijke reductie in glucosinolaatgehalte kan worden verkregen door 60 minuten verhitten bij 100-110°C; de reactie werd versneld door toevoeging van vocht en/of bepaalde chemicaliën (b.v. Na_2CO_3 of $FeSO_4.7H_2O$). Door deze behandelingen konden vrijwel alle intacte glucosinolaten worden omgezet en kon worden voldaan aan wettelijke kwaliteitseisen. In een opnameproef met biggen bleek aldus behandeld meel goed te worden gegeten. Overigens moet er rekening mee worden gehouden dat langdurige verhitting leidt tot omzettingen die een lager lysinegehalte en een lagere verteerbaarheid van dit aminozuur veroorzaken.

In verband met de vele overeenkomsten tussen crambe- en raapzaad werd een vergelijking van ontgiftigingsexperimenten bij crambe- en raapzaadmeel uitgevoerd. De resultaten van dit onderzoek zijn in de hoofdstukken 7 en 8 verwerkt. Door deze studie

kwam aanvullende kennis over de karakteristieken van glucosinolaten beschikbaar. Het doel van de toegepaste behandeling was ontgifting te bewerkstelligen met behoud van voederwaarde, in het bijzonder van eiwit en aminozuren. De uitkomsten in hoofdstuk 7 bevestigen het beeld dat intensivering van de verhitting leidt tot meer afbraak van glucosinolaten bij gelijktijdige vermindering van de eiwitkwaliteit. Het onderzoek leidde tot een aanbeveling voor vochtig verhitten van zgn. 00-raapzaadschroot in de praktijk (100°C, 30min.) met eiwit-oplosbaarheid als een graadmeter voor de aantasting van de eiwitkwaliteit. In hoofdstuk 8 is de aandacht gericht op de vanouds gebruikelijke type raapzaadschroot, waarbij een wasprocedure ter ontgifting werd onderzocht. Het onderzoek leverde nuttige informatie op voor het verbeteren van de behandeling van raapzaadschroot met een hoog glucosinolaatgehalte.

In de algemene discussie (hoofdstuk 9) is de bruikbaarheid van crambe-bijprodukten als veevoer systematisch besproken aan de hand van een evaluatie van alle beschikbare gegevens: chemische samenstelling; anti-nutritionele aspecten inclusief ontgiftigingsbehandeling; afbraak en verteerbaarheid bij herkauwers; verteerbaarheid bij monogastrische dieren; schatting van energie- en eiwitwaarden; en vergelijking t.o.v. bijprodukten van raapzaad. Tenslotte zijn suggesties gedaan ten aanzien van de richting van toekomstig onderzoek.

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Curriculum Vitae

Yong-Gang Liu was born on July 27, 1957 at Fengjie, Sichuan Province, the People's Republic of China. After completion of his primary, secondary and high school education in 1975, he had worked in a rural commune for "re-education" for two and half years, which ended in 1978. Restoration of academic examinations enabled him to enter university. He was accepted by the Department of Animal Science, Sichuan Agricultural University in Ya'an. There, he completed a four-year study and graduated with a Bachelor's Degree. After this he gained acceptance to the Animal Nutrition Institute at the same university for a three-year's study for Master's Degree, which was completed in the end of 1984, with a series of publications on selenium nutrition. Afterwards he worked in the Research Institute for Animal and Veterinary of Sichuan Province, as a researcher for about seven years. Then he was selected by the Chinese National Education Commission for an overseas training programme. In 1991 he joined the Research Institute for Livestock Feeding and Nutrition (IVVO-DLO) in Lelystad, in the Netherlands; and in 1993 the Animal Physiology and Biochemistry Department of the National Institute of Animal Science in Foulum, Denmark. During this period he carried out this Ph.D. project.