PHENOLIC CHOLINE ESTERS IN RAPESEED: POSSIBLE FACTORS AFFECTING NUTRITIVE VALUE AND QUALITY OF RAPESEED MEAL

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Summary

Feeding experiments have revealed that rapeseed meal contains other constituents than glucosinolates which affect the quality of rapeseed meal when used as food and/or feed. Sinapine, the choline ester of sinapic acid, is the best known of the phenolic choline esters occuring in cruciferous seeds. However, seven different aromatic choline esters have been described as plant products. Only sinapine has been the subject of metabolic studies as well as of studies of quality problems related to the use of rapeseed meal. This is most likely caused by lack of an available general method of analysis of aromatic choline esters.

Recently, new methods of analysis have been developed, and it is revealed that appreciable amounts of different phenolic choline esters occur in seeds of glucosinolate-containing plants. Feeding experiments using rapeseed meal have shown that sinapine disappears from the digestive tract of rats. The content of phenolic choline esters in rapeseed meals is variable but independent of the level of glucosinolates. The tast problems of meat and milk which still exist seem not to be closely related to glucosinolates, at least not in a simple manner, but a likely cause may be the phenolic choline esters.

The present report discuss briefly the above mentioned observations in relation to the efforts assigned to the improvements of rape as an oil and protein source.

Introduction

Antinutritional and toxic constituents of rapeseed are well known reasons for a greatly restricted utilisation of rapeseed meal as food and feed. This has pronounced economical consequences for the rapeseed production. Therefore, it is of utmost importance to identify the antinutritional and toxic constituents of rapeseed occurring in too high concentration for optimal utilisation of rapeseed protein.

Investigations of antinutritional and toxic compounds require more than consideration of the groups they belong to. The individual compounds in the groups need to be identified, and knowledge of their structure, chemical, biochemical and physiological properties as well as reliable methods of analysis are required. Futhermore, it is important to realise the great differences in nutritional problems encountered when different experimental animals (mice, rats, pigs, ruminants and poultry) are used, e.g. owing to differences in the stomach and digestive tract and the microorganisms present therein.

Traditionally, the problems discussed in connection with an optimal utilisation of rapeseed have been divided into main groups:

1. Erucic acid in the oil.
2. Glucosinolates - myrosinases - degradation products of glucosinolates.
3. Crude fiber.
4. Phytate (hexaphosphate of myo-inositol).
5. Tannins, including phenolic choline esters.

Erucic acid in the oil was a rapeseed problem which found its solution by use of serious and comprehensive plant breeding programs.[2]

Glucosinolates have been discussed in other papers[1,2], and it is revealed that double low rape with an acceptable composition and concentration of glucosinolates in the seeds is available. Rapeseed meal from these varieties can obviously be used instead of soybean meal in diets to pigs, young calves and dairy cows without consequences on feeding utilisation, weight gain and milk yield. However, some types of glucosinolates not yet investigated and still remaining problems with effects on internal organs call for attention. It is also important to point out that not all double low rape varieties have an acceptable low concentration and composition of individual glucosinolates.

Crude fiber is especially accumulated in the hull, but dehulling is probably too expensive compared to the obtainable quality improvement.

Phytate is in some cases discussed[2] as the reason for insufficient amounts of available metals (e.g. Zn^{2+}) in the diets but further experiments in this field are needed.

Tannins are defined by the methods of analysis as a rather unspecific heterogenous group of different phenolic compounds. Therefore, a more specific measure of phenolic compounds is required. Tannins in rapeseed account for some few percent of the rapeseed meal. It is claimed that they are located primarily in the hulls, contrary to phenolic choline esters which are also included in this group.

Results and Discussion

Phenolic choline esters form a structurally well defined group of benzoic acid and cinnamic acid derivatives which have been isolated only from seeds of glucosinolate-containing plants (Fig. 1).

Cinnamic acid derivatives:		Benzoic acid derivatives:	
Coumaroylcholine	R_3=R_5= H; R_4= OH	4-Hydroxybenzoylcholine	R_3=H; R_4= OH
Feruloylcholine	R_3= OCH_3; R_4= OH; R_5= H	Hesperalin	R_3=R_4=OCH_3
Isoferuloylcholine	R_3= OH; R_4= OCH_3; R_5= H		
Sinapine	R_3= OCH_3; R_4= OH; R_5= OCH_3		
Sinapinglucoside	R_3= R_5= OCH_3; R_4= glucopyranosyloxy		

Fig. 1. Phenolic choline esters known as seed constituents of glucosinolate-containing plants.

Sinapine is the only phenolic choline ester discussed in relation to rapeseed. This is, maybe, caused by lack of information about phenolic choline esters, especially as a result of methods of analysis, traditionally applied. Recently, new methods of analysis, based on group seperation of natural products in combination with HPLC analysis have been developed (Fig. 2), and a simple, fast and reliable determination of the individual phenolic choline esters is now possible.

Investigations of the total pool of choline esters in crucifer seeds using the new analytical technique have revealed that seeds of crucifers most often contain appreciable amounts of different phenolic choline esters and/or alkaloids. It appears, that seeds of double low rape varieties contain a pool of choline esters which most often is quantitatively dominated by sinapine as is also known from rapeseed with high glucosinolate content (Table 1, Fig. 2).

Table 1. Concentration of sinapine (µmole/g seed) in seeds of some double low rapeseed varieties and Gulliver (high glucosinolate content).

Tower	Regent	Line	Erglu	Tobin	Candle	Mary	Karat	Gulliver
24.1	24.1	28.9	25.7	20.9	22.5	23.5	30.5	25.7

However, great differences between different rapeseed cultivars as well as between single plants belonging to the same cultivar are found, ranging from about 0.2 - 2 % sinapine in the seeds. These differences may be under genetic control, but during maturation and germination of the seeds the amount and composition of the choline ester pool are submitted to great variation.

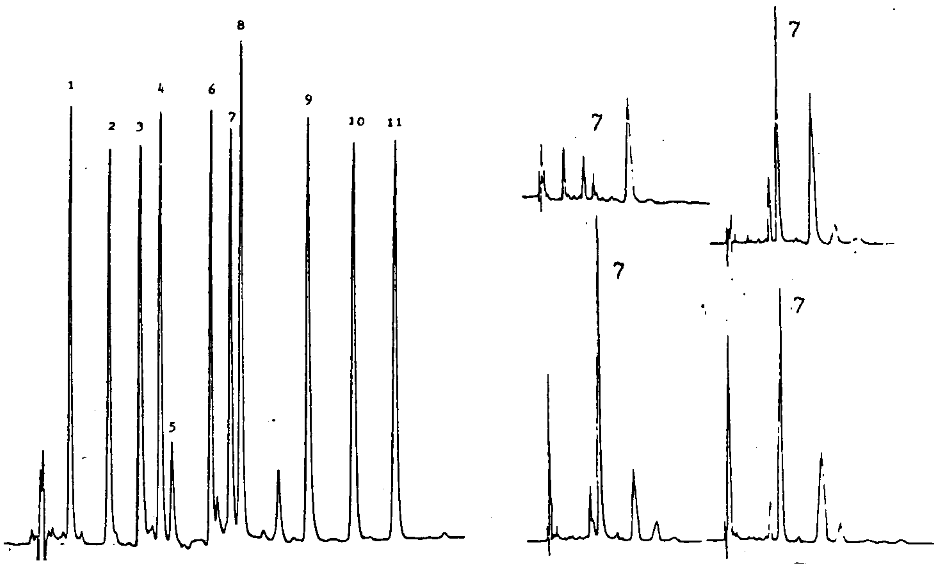

Fig.2. HPLC chromatogram of different aromatic choline esters reference compounds and of the choline ester fraction from different double low rape cultivars.

1: 3,4,5,-Trihydroxybenzoylcholine
2: 3,5-Dihydroxybenzoylcholine
3: 4-Hydroxybenzoylcholine
4: 3-Hydroxy-4-methoxybenzoylcholine
5: 2,3-Dihydroxybenzoylcholine
6: 3,4-Dimethoxybenzoylcholine
7: 3,5-Dimethoxy-4-hydroxycinnamoylcholine
8: 3-Hydroxy-4-methoxycinnamoylcholine
9: 3,4-Dimethoxycinnamoylcholine
10: 2,5-Dimethoxycinnamoylcholine
11: 2,3-Dimethoxycinnamoylcholine

Phenolic choline esters and rapeseed quality

The "fishy or crabby" taint in eggs as well as other nutritive, toxic and quality problems observed when feeding rapeseed meal to poultry, including the effect of sinapine have recently been the subject of authoritative and careful reviews[3,4]. Therefore, we will draw attention to unsolved quality questions revealed when feeding double low rapeseed meal to pigs and ruminants. Using double low rapeseed meal with a satisfactory low glucosinolate content (Line in Table 2), there are obviously no problems in obtaining high feed utilisation, weight gain and milk yield (Fig. 3). The glucosinolate and sinapine content in the applied diets are shown in Table 2 and effects on internal organs are shown in Fig. 4.

Table 2. Content of glucosinolates and sinapine in the applied Line and Erglu rapeseed meals. (μmole/g)

	total glucosinolate	Gluco-napin	Glucobras-sicanapin	Progoitrin	Napole-iferin	Glucona-sturtiin	Sinalbin	Sinapine
Line	2.96	1.06	0.31	1.45	0.03	0.11	-	17.5
Erglu	19.02	3.17	1.64	7.27	0.95	0.79	5.20	22.2

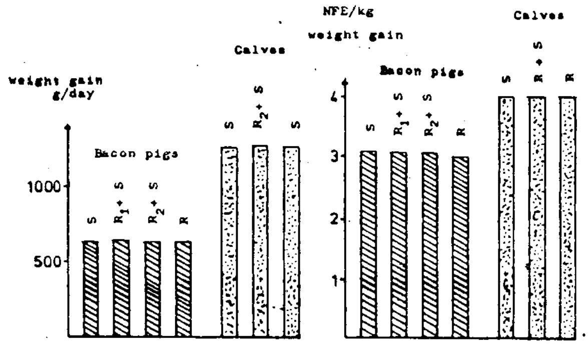

Fig. 3. Line rapeseed meal (R) fed to young calves (from 28 days to 340 kg, about 3 months)[5] and bacon pigs (20 - 90 kg)[6] compared to diets based on soybean meal (S) and mixtures of rapeseed and soybean meal. Bacon pigs: S = 18 %; R_1+ S = 6 % + 13.5 %; R_2+ S = 12%+9%; R = 24 %. Young calves: S = 16 %; R + S = 10 % + 8 %; R = 20 %.

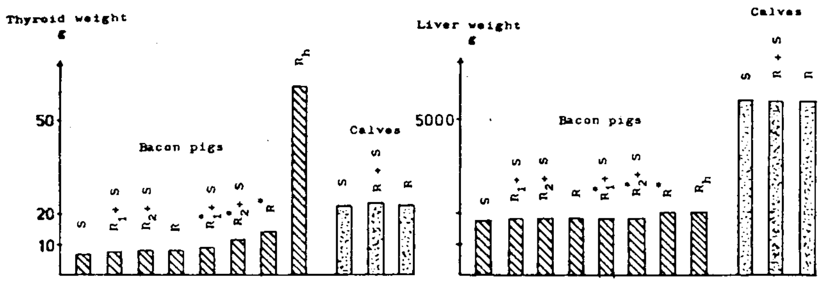

Fig. 4. Effects of rapeseed meal on weight of internal organs of animals from the experiments mentioned in Fig. 3. Results from corresponding experiments with Erglu rapeseed meal (R*) and rapeseed meal with high glucosinolates content (Rh) are also shown.

These experiments revealed, in accordance with the results discussed elsewhere[2], that a glucosinolate level as found for Line is acceptable. The effect of rapeseed glucosinolates is especially easily revealed from the thyroid weight. However, the effect often observed on the liver weight is not in a simple manner correlated to the glucosinolate level[2].

One of the remaining problems still caused by low glucosinolate--containing rapeseed meal, e.g. Line, is the appearance of a disagreeable taste of the meat of calves and of the milk of dairy cows[6].

The presence of a relative high concentration of phenolic choline esters in rapeseed meal calls for attention. Processing has an effect on the sinapine content in rapeseed meal, and it has been shown that sinapine disappears from the digestive tract of rats (Table 3). Whether it is absorbed or destroyed is not known.

It was found that the decrease in sinapine concentration as a function of toasting was followed by an increase in sinapine derivatives, maybe dimers/oligomers. It is well known that choline esters are labile in alkaline solution, but it is not advisable to treat rapeseed meal in this way before knowledge of the degradation products and their effects have been investigated.

Table 3. Concentration of sinapine (µmole/150 mg N) in dehulled Erglu rapeseed meal ($\pm$ toasting at 100°C), as well as content from the digestive tract and in faeces (µmole/g freezedried material) of rats fed a diet containing 26 % rapeseed meal.

seed	dehulled seed	dehulled and extracted Erglu rapeseed meal: untoasted	toasting 10 min	toasting 20 min	toasting 30 min	hulls
69.9	81.0	69.7	68.0	61.0	57.9	6.6

diet	stomach	small intestine	caecum	large intestine	faeces
5.9	2.2	1.6	0.4	0.3	0.1

In conclusion, the main problems which seem to restrict the utilisation of high quality rapeseed proteins as food and feed are:1. no requirement of a sufficient low level and composition of glucosinolates in double low rapeseed meal[2]; 2. no requirements of reliable methods of analysis for control of this level[1]; 3. remaining problems concerning effects on internal organs[2-4] and 4. remaining taste problems[3,4,6].

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