# NUTRITIVE VALUE OF RAPESEED MEAL: ANTINUTRITIONAL AND TOXIC EFFECTS OF INDIVIDUAL GLUCOSINOLATES (± MYROSINASES)

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## Summary

The protein quality and nutritive value of rapeseed meal from double low rape varieties have been investigated in nitrogen balance trials with growing rats. The results are compared with results from feeding experiments using other protein sources and experimental animals. Rapeseed proteins from double low rape have a well balanced amino acid pattern, and consequently, a high biological value. However, some problems concerning effects of rape constituents on various internal organs, on protein utilisation and on taste of e.g. meat and milk from the animals still remain to be solved. The effects of glucosinolates and/or their degradation products have been assigned particular attention. Different processing procedures for rapeseed meal have been investigated. It was possible to inactivate the myrosinases (thioglucoside glucohydrolase EC 3.2.3.1.) in this way but the antinutritional and toxic effects could not be eliminated completely.

Relatively great amounts of myrosinases and crystalline potassium salts of different glucosinolates have been isolated. The glucosinolates were added singly and in various amounts to a standard diet based on starch, casein + methionine, soyaoil, minerals and vitamins. In another serie of experiments active myrosinases were also added to the diets. Food conversion, protein utilization and effects on internal organs were recorded. The results revealed that intact glucosinolates (without myrosinases) could cause significant antinutritional and toxic effects. Individual glucosinolates could also act differently, although several observations were similar, and some of the effects were increased with myrosinases included in the diets.

The present contribution is a brief discussion of these results, as well as of some unsolved problems and the question concerning tolerable dietary glucosinolate concentrations.

## Introduction

Quality improvements of vegetable proteins are among the most important and most readily available means of increasing the total protein supply for both humans and animals. Especially seeds of different legumes and oilseed protein residues call for attention. Proteins from rapeseed and some other crucifers belong to this group of nutritionally superior proteins as revealed from their content of essential amino acids (Table 1). However, some rapeseed constituents reduce the value of rapeseed.

## Results and Discussion

**Table 1. Amino acid composition (g/16 g N) of some selected legumes and rapeseed protein sources.**

| | Protein sources | | | | | |
|---|---|---|---|---|---|---|
| | Legumes | | | Rapeseed | | |
| | Soy bean | Faba bean | Pea | Erglu | Tower | Candle |
| Asp | 10.85 | 11.21 | 11.10 | 7.36 | 8.65 | 8.12 |
| Thr* | 3.89 | 3.71 | 3.54 | 4.35 | 4.63 | 5.47 |
| Ser | 5.03 | 5.00 | 4.28 | 4.35 | 4.42 | 4.47 |
| Glu | 18.06 | 17.72 | 12.42 | 18.72 | 15.15 | 18.44 |
| Pro | 5.51 | 4.33 | 4.52 | 6.48 | 7.15 | 6.29 |
| Gly | 4.28 | 4.51 | 3.54 | 5.11 | 4.64 | 5.37 |
| Ala | 4.19 | 4.29 | 3.73 | 4.45 | 4.33 | 4.66 |
| Val | 5.22 | 4.81 | 3.68 | 5.15 | 4.42 | 5.43 |
| Ile | 4.58 | 4.39 | 3.08 | 3.91 | 3.24 | 4.04 |
| Leu | 7.48 | 7.53 | 6.08 | 7.10 | 6.81 | 7.20 |
| Tyr | 3.33 | 4.66 | 2.96 | 2.91 | 2.82 | 2.84 |
| Phe | 5.42 | 4.48 | 4.07 | 3.72 | 3.84 | 3.65 |
| Lys* | 6.08 | 6.26 | 6.30 | 5.90 | 5.41 | 5.98 |
| His | 2.59 | 2.66 | 2.15 | 2.75 | 2.64 | 2.59 |
| Arg | 7.23 | 10.14 | 7.77 | 6.45 | 6.62 | 6.37 |
| Met* | 1.56 | 0.87 | 0.78 | 2.06 | 1.87 | 2.16 |
| Cys | 1.49 | 1.52 | 0.90 | 2.39 | 1.56 | 2.23 |

*Essential amino acids of special nutritional interest.

Rapeseed proteins have a well balanced amino acid pattern, and consequently, a high biological value is to be expected. Unfortunately, some antinutritional, quality and toxicological problems still exist. These must be completely solved before rapeseed proteins can win general acceptance as the superior protein source it is. The main problems are frequently caused by too high concentrations of glucosinolates and the co-occurrence with thioglucoside glucohydrolase EC 3.2.3.1 (myrosinases). This can lead to serious problems caused by several of the different glucosinolates[1].

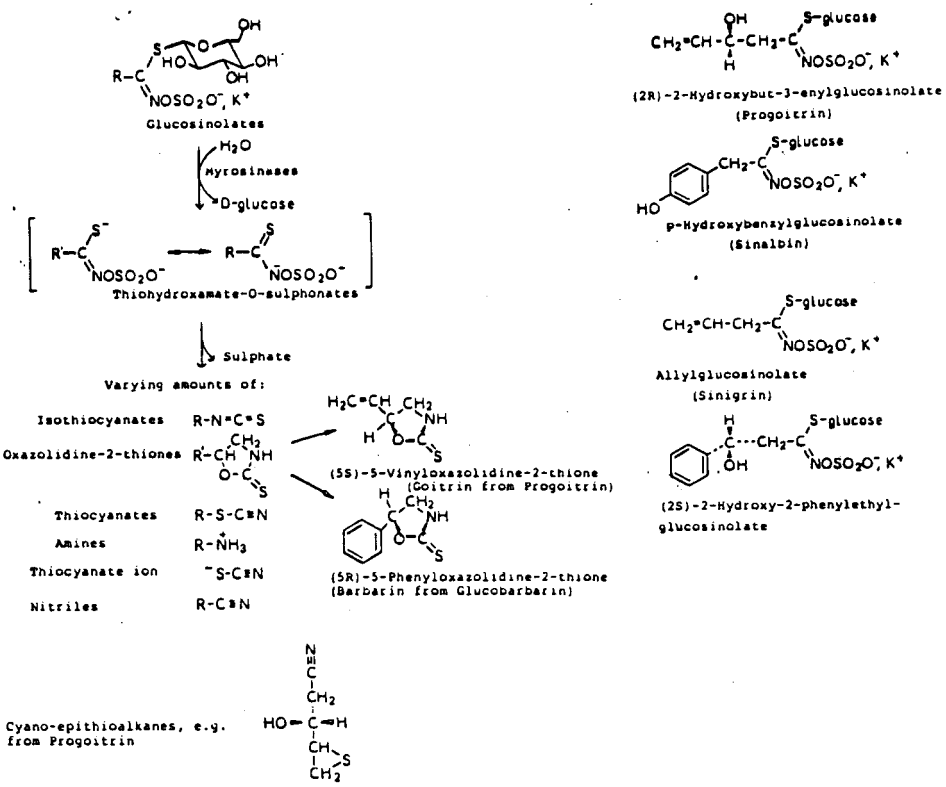


Fig. 1. Structures and names of some selected glucosinolates and known products from myrosinase catalysed degradation of glucosinolates.

Owing to the complex nature of glucosinolates and their degradation products (Fig. 1), the nutritional problems are insufficiently understood. It is realised that high levels of glucosinolates are harmful, and double low rape represents a tremendous progress. However, some but not all double low rape varieties seem to have an acceptable level and distribution of the individual glucosinolates. although problems still exist. Information of acceptable dietary glucosinolate levels and toxic effects of the individual glucosinolates and/or their degradation products are required. Possibilities of quality improvement by technological processes also require attention. Furthermore, as described elsewhere, the discussions concerning quality of double low rape suffer from problems with reliability of some of the applied analytical procedures[1].

## Protein quality and effects of technological processes.

Details of experimental procedures for chemical analyses including glucosinolate and myrosinase analyses as well as the $^{2}$N-balance trials with growing rats have been described elsewhere[2].

Table 2. Chemical composition, true protein digestibility (TD), biological value (BV), net protein utilisation (NPU) and utilisable protein (UP) of Erglu ( E.) and Candle ( C.) rapeseed exposed to different technological processes and the effect of these protein sources on growing rats.

| | E. meal extracted | E. dehulled and extracted -toast. | toast. 10 min | toast. 20 min | toast. 30 min | C. meal extracted | E. hulls | Casein+ methionin (control) |
|---|---|---|---|---|---|---|---|---|
| Protein (N x 6.25) % | 39.6 | 51.9 | 51.7 | 52.0 | 52.3 | 40.2 | 15.6 | 89.2 |
| Stoldt fat % | 4.1 | 6.8 | 7.1 | 6.4 | 5.7 | 5.2 | 14.9 | 0.3 |
| RHC % | 10.4 | 11.2 | 11.1 | 11.1 | 11.4 | 13.4 | 4.7 | 5.3 |
| Crude fibre % | 14.4 | 6.1 | 5.6 | 5.8 | 6.3 | 11.7 | 27.3 | - |
| Ash % | 7.8 | 7.1 | 7.2 | 7.2 | 7.4 | 8.8 | 5.7 | 3.1 |
| Myrosinase ( U/g protein) | 36 | 41 | ND | ND | ND | 4 | ND | - |
| µmole glucosinolate/g | | | | | | | | |
| Total | 8.83 | 19.02 | 18.70 | 18.40 | 16.54 | 12.96 | 5.11 | - |
| Gluconapin | 2.03 | 3.17 | 3.09 | 2.88 | 2.73 | 2.94 | 0.84 | - |
| Glucobrassicanapin | 1.06 | 1.64 | 1.65 | 1.48 | 1.44 | 2.01 | 0.67 | - |
| Progoitrin | 4.68 | 7.27 | 7.25 | 6.86 | 6.51 | 4.09 | 2.28 | - |
| Napoleiferin | 0.65 | 0.95 | 0.96 | 0.92 | 0.89 | 0.90 | 0.35 | - |
| Gluconasturtiin | 0.31 | 0.79 | 0.84 | 0.84 | 0.84 | 0.90 | 0.71 | - |
| Sinalbin | 0.10 | 5.20 | 4.91 | 5.42 | 4.14 | 2.13 | 0.26 | - |
| Sinapine ( µmole/150 mg N) | 83.1 | 69.7 | 68.0 | 61.0 | 57.9 | 50.6 | 6.6 | - |
| TD % | 84.9 | 91.1 | 91.0 | 90.1 | 91.1 | 85.0 | 39.1 | 100.5 |
| BV % | 86.0 | 88.8 | 87.0 | 87.5 | 84.8 | 90.3 | 90.1 | 87.6 |
| NPU % | 73.0 | 80.9 | 79.1 | 78.9 | 77.2 | 76.8 | 35.3 | 87.9 |
| UP % | 28.9 | 42.0 | 40.9 | 41.0 | 40.8 | 30.9 | 5.5 | 78.4 |
| Rat weight g | 90.3 | 91.4 | 87.4 | 86.8 | 86.8 | 84.0 | 71.2 | 95.3 |
| Liver weight g | 4.53 | 5.36 | 3.57 | 3.80 | 3.77 | 3.57 | 3.29 | 4.31 |

Each experiment comprised five male rats each weighing 70 g. They were fed in a preliminary period of four days and a balance period of five days. The results thus revealed an excellent quality of rapeseed proteins and their high biological values in accordance with the well balanced amino acid pattern (Table 1). The applied technological processes had only a slightly negative effect on BV at 30 min toasting at 100 °C. Toasting had no effect on the amino acid composition, but an effect on glucosinolates was observed and the myrosinase activity was destroyed. An effect on sinapine was also observed as discussed elsewhere[2]. True protein digestibility was in general

high. Dehulling resulted in a slightly increased TD, whereas toasting did not influence on TD. Toasting had a slight negative effect on body weight and liver weight, but when calculated as mg liver/g rat no significant effect was found.

Table 3. Effects of 26% Erglu rapeseed meal in the diet to rats.

| | Rat weight g | Liver mg/g rat | Kidneys mg/g rat | Supr.gland mg/g rat | Testicles mg/g rat | Thyroid mg/g rat |
|---|---|---|---|---|---|---|
| Rapeseed meal | 114.2 ±6.0 | 59.6 ±4.8 | 8.8 ±0.5 | 0.24 ±0.04 | 13.8 ±1.1 | 0.69 ±0.11 |
| Standard diet | 99.8 ±5.3 | 49.4 ±3.7 | 8.9 ±0.4 | 0.26 ±0.06 | 13.4 ±0.4 | 0.25 ±0.08 |

Organ weights, pathological and histological studies revealed that antinutritional compounds were present in harmful concentrations when (26%) Erglu rapeseed meal was used as the only protein source in the diets. The processing procedures applied seemed to reduce but not completely eliminate the effects of these compounds. Plant breeding seems to be a possible solution to the problems[1].

Individual glucosinolates ± myrosinases added to a standard diet

Investigations of antinutritional and toxic effects of individual intact glucosinolates in diets (± myrosinases) have been performed in experiments as mentioned above. Relatively great amounts of crystalline potassium glucosinolates and purified powdered myrosinases were isolated from different sources. The glucosinolates sinigrin, progoitrin, sinalbin and glucobarbarin (Fig.1) were used singly in different concentrations (±myrosinases) in a standard diet based on autoclaved N-free potato starch, casein, minerals and vitamins.

The results revealed (including weights, patological and histological investigations of the same internal organs as mentioned in Table 3) that great differences exist in the antinutritional and toxic effects of individual glucosinolates and degradation products thereof. Intact glucosinolates caused palatability problems and other antinutritional and toxic problems, confirming that inactivation of myrosinases by processing is not sufficient to prevent problems from glucosinolates. However, it was shown that myrosinases aggravated some of the glucosinolate problems. A glucosinolate concentration of 0.2 mg/g diet corresponds to some commercial available double low varieties. At this glucosinolate concentration rapeseed meal can be fed without having toxic or antinutritional effects from glucosinolates. Although double low rapeseed has a very high protein quality ( Table 1, 2 and 4) there are still unsolved problems when high concentrations of such rapeseed meal is fed to poultry, pigs and cattle as discussed in the following paper "Phenolic choline esters in rapeseed ...".

Table 4. Effects of intact glucosinolates (± myrosinases) added to a standard diet

| | consumed diet g/5 days | weight gain mg/g DM | TD % | BV % | NPU % |
|---|---|---|---|---|---|
| Standard diet | 52.4 | 294 | 99.8 | 90.4 | 90.2 |
| Standard diet + sinigrin ( 0.2 mg/g DM) | 52.0 | 283 | 100.0 | 89.7 | 89.6 |
| Standard diet + sinigrin ( 1.0 mg/g DM) | 51.1 | 288 | 100.0 | 87.4 | 87.7 |
| Standard diet + sinigrin ( 5.0 mg/g DM) | 44.2 | 199 | 99.2 | 75.9 | 75.3 |
| Standard diet + sinigrin ( 1.0 mg/g DM and myrosinases 0.15 U/g DM) | 51.0 | 266 | 99.7 | 81.9 | 81.7 |
| Standard diet + progoitrin ( 0.2 mg/g DM) | 52.4 | 280 | 99.9 | 89.9 | 89.7 |
| Standard diet + progoitrin ( 1.0 mg/g DM) | 50.4 | 249 | 99.5 | 88.2 | 87.7 |
| Standard diet + progoitrin ( 5.0 mg/g DM) | 40.2 | 228 | 99.6 | 77.9 | 77.6 |
| Standard diet + progoitrin ( 1.0 mg/g DM and myrosinases 0.15 U/g DM) | 49.0 | 253 | 97.5 | 83.7 | 81.6 |
| Standard diet + sinalbin ( 0.2 mg/g DM) | 52.4 | 301 | 100.0 | 88.2 | 88.2 |
| Standard diet + sinalbin ( 1.0 mg/g DM) | 52.4 | 276 | 99.6 | 84.2 | 83.8 |
| Standard diet + sinalbin ( 5.0 mg/g DM) | 50.2 | 258 | 99.0 | 81.7 | 80.9 |
| Standard diet + sinalbin ( 1.0 mg/g DM and myrosinases 0.15 U/g DM) | 51.8 | 270 | 99.5 | 84.3 | 83.8 |
| Standard diet + glucobarbarin ( 0.2 mg/g DM) | 52.2 | 297 | 99.2 | 89.9 | 89.1 |
| Standard diet + glucobarbarin ( 1.0 mg/g DM) | 52.2 | 256 | 99.1 | 90.2 | 89.4 |
| Standard diet + glucobarbarin ( 5.0 mg/g DM) | 44.6 | 298 | 99.5 | 90.0 | 89.6 |
| Standard diet + glucobarbarin ( 1.0 mg/ DM and myrosinases 0.15 U/g DM) | 47.5 | 340 | 99.1 | 90.3 | 89.5 |

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# 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.

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## 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.

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.

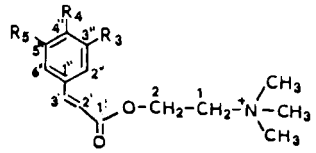
Crude fiber is especially accumulated in the hull, but dehulling is probably too expensive compared to the obtainable quality improvement.

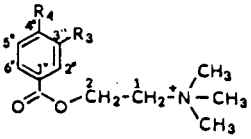
Phytate is in some cases discussed as the reason for insufficient amounts of available metals ( e.g. $Zn^{2+}$ ) in the diets but further experiments in this field are needed.

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:

| | |
|---|---|
| Coumaroylcholine | $R_3$=$R_5$= H; $R_4$= OH |
| Feruloylcholine | $R_3$= $OCH_3$; $R_4$= OH; $R_5$= H |
| 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 |

Benzoic acid derivatives:

| | |
|---|---|
| 4-Hydroxybenzoylcholine | $R_3$=H; $R_4$= OH |
| Hesperalin | $R_3$=$R_4$=$OCH_3$ |

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.

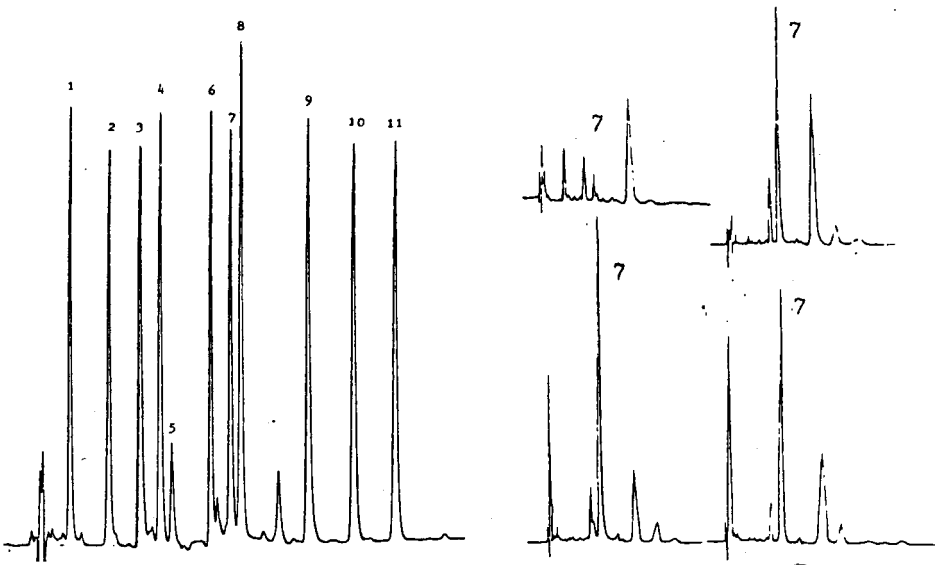



Fig.2. HPLC chromatogram of different aromatic choline esters reference compounds and of the choline ester fraction from different double low rape cultivars.

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 | Gluconas-turtiin | 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 |

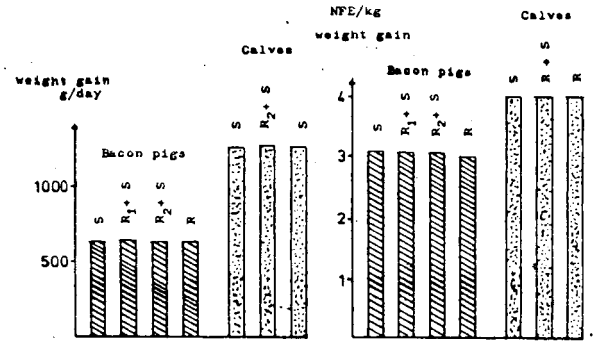



Fig. 3. Line rapeseed meal (R) fed to young calves ( from 28 days to 340 kg, about 3 months)[5] and bacon pigs ( 20 - 90 kg)[6] compared to diets based on soybean meal (S) and mixtures of rapeseed and soybean meal. Bacon pigs: S = 18 %; $R_1$ + S = 6 % + 13.5 %; $R_2$ + S = 12%+9%; R = 24 %. Young calves: 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 ( ± 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 | | | | hulls |
|---|---|---|---|---|---|---|
| | | untoasted | toasting 10 min | toasting 20 min | toasting 30 min | |
| 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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# Effect of ammoniation of canola meal on the fishy odor and trimethylamine contents of eggs produced by brown-egg layers.

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When rapeseed meal (RSM) of low glucosinolate- or high glucosinolate- type is included in the ration of brown-egg layers, some of the birds produce eggs with a fishy odor (Hobson-Frohock et al.,1973; Clandinin et al., 1974). Sinapine, was identified as the precursor of the trimethylamine (TMA), the compound responsible for the fishy odor (Hobson-Frohock et al., 1977; Clandinin et al., 1977). Goh et al., (1979a) found that laying rations containing 0.1% or more of sinapine caused the laying of eggs with a fishy odor. No fishy eggs were produced when sinapine was hydrolysed under alkaline conditions to sinapic acid and choline prior to its inclusion in the ration (Goh et al., 1979b). The effect of treating low-glucosinolate RSM (canola meal) with ammonia on its sinapine content was recently investigated by Goh et al., (1982). They found that sparging canola meal (CM) with 5% W/W ammonia and 5% W/W steam during desolventization caused hydrolysis of 65% of the sinapine in the meal. The total glucosinolate and potential 5-vinyl-2-oxazolidinethione contents of this meal were decreased 17 and 35%, respectively.

The objective of the study described, herein, was to investigate the effect of anhydrous or hydrous ammonia treatment of CM on the fishy odor and TMA contents of eggs from brown-egg layers fed rations containing the ammoniated meals.

## MATERIALS AND METHODS

Four CMs produced from *Brassica Campestris* cv Candle seed at the POS Pilot Plant Corporation, Saskatoon, Saskatchewan were used in this study. Two of the meals were prepared by sparging during desolventization with or without anhydrous ammonia (5% W/W ammonia) and two of the meals were produced by sparging with or without hydrous ammonia (5% W/W ammonia, 5% W/W steam). The proximate composition, sinapine, total glucosinolate and potential oxazolidinethione contents of the meals were reported earlier (Goh et al., 1982).

Fifty brown-egg layers (Rhode Island Red), which were found to lay eggs with a fishy odor when fed a ration containing 10% of CM were housed in floor pens (5 birds per pen) equipped with trap-nests. The birds were fed a soybean meal (SBM)-wheat based laying ration until they no longer produced eggs with a fishy odor. Two pens of the birds were continued on the same ration while two pens were fed each of the four treated-CM-containing rations (Table 1). The rations were isonitrogenous and isoenergetic. Feed and water were

supplied ad libitum. During the fourth week on ration treatment eggs were collected from each bird for fishy odor evaluation and TMA determination. Evaluation for fishy odor was performed as previously described (Goh et al., 1979a). The contents of three eggs collected from each bird were pooled, homogenized and analyzed for TMA (Goh, 1978).

Two of the layers did not produce any eggs during the collection period, hence, unequal numbers of observations per treatment resulted. The data on fishy odor scores and TMA contents were analysed statistically by least square analysis of variance (Harvey,1975). Differences among treatment means were compared by Duncan's multiple range test (Steel and Torrie, 1960) at the 0.05 level of probability.

RESULTS AND DISCUSSION

The amount of sinapine (expressed as sinapine bisulfate) in the various rations, the mean TMA contents in the homogenized eggs and the mean fishy odor scores of eggs are presented in Table 2. Statistical analysis of the TMA data showed that the TMA contents of eggs produced by layers fed the rations containing 10% of CM sparged with ammonia in the absence or presence of steam were lower than their respective CM controls (Ration 3 vs 2 and Ration 5 vs 4). The TMA contents of the eggs from the birds fed these rations paralled the decrease in sinapine contents of the rations resulting from the different sparging treatments to which the meals were subjected. Likewise the mean fishy odor scores of the eggs from the birds fed the rations containing the ammoniated meals were better than those of their respective CM controls. Only the ration containing meal ammoniated in the presence of steam (Ration 5) and the ration containing no CM (Ration 1) produced eggs that had no fishy odor. However, it should be pointed out that the average TMA content of the eggs from the ration containing CM sparged with ammonia in the presence of steam (0.76 ug/g egg) was close to the level (0.8 ug/g egg) claimed by Griffiths et al., (1979) to be detectable in eggs by organolipic evaluation. Furthermore, when one takes into account the variation in TMA content in eggs from a specific fishy egg layer or in eggs from different fishy egg layers fed the same CM-containing ration, one has to conclude, that either the ammonia-steam sparging would have to be continued for a longer period of time to bring about more complete hydrolysis of the sinapine in the meal than occurred in the meal used in this study, or, the percentage of CM used in the laying ration would have to be reduced below the level recommended for white-egg layers (10%) in order to make the ration suitable for brown-egg layers.

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Table 1. Composition of experimental rations

| | Rations[1] | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Ground wheat | 72.18 | 66.78 | 66.78 | 66.78 | 66.78 |
| Stabilised fat | 1.00 | 2.80 | 2.80 | 2.80 | 2.80 |
| Dehydrated alfalfa meal | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Soybean meal (47.5% crude protein) | 14.00 | 7.60 | 7.60 | 7.60 | 7.60 |
| CM[2] + heat | - | 10.00 | - | - | - |
| CM + heat + $NH_3$ | - | - | 10.00 | - | - |
| CM + steam | - | - | - | 10.00 | - |
| CM + steam + $NH_3$ | - | - | - | - | 10.00 |
| Ground limestone | 8.00 | 8.00 | 8.00 | 8.00 | 8.00 |
| Dicalcium phosphate | 1.40 | 1.40 | 1.40 | 1.40 | 1.40 |
| Iodised salt | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Micronutrients[3] | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 |

[1]The rations were formulated to contain 16.4% crude protein and 11.17 MJ/kg.

[2]The canola meals (CM) were produced from canola seed at the POS Pilot Plant Corporation, Saskatoon, Saskatchewan.

[3]The micronutrients supplied per kg ration: vitamin A, 6000 IU; vitamin D, 1200 ICU; vitamin E, 5 IU; riboflavin, 4 mg; calcium pantothenate, 6 mg; niacin, 15 mg; vitamin $B_{12}$, 10ug; choline chloride, 100 mg; biotin, 0.1 mg; $MnSO_4$, 0.4 g; ZnO, 0.1 g; and DL-methionine, 0.5 g.

Table 2. The trimethylamine (TMA) content and fishy odor score of eggs produced by brown-egg layers fed rations containing canola meal (CM) sparged with ammonia

| | Rations | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Treatment | control | CM + heat | CM + heat + $NH_3$ | CM + steam | CM+steam+$NH_3$ |
| Sinapine (% in ration)[1] | - | .156 | 0.083 | .146 | 0.055 |
| TMA (ug/g egg)[2] | $0.03^{a}$ | $2.42^{c}$ | $1.33^{b}$ | $2.55^{c}$ | $0.76^{ab}$ |
| ± SEM | 0.284 | 0.284 | 0.284 | 0.317 | 0.284 |
| Fishy odor score[3] | $0.00^{a}$ | $1.58^{c}$ | $0.44^{ab}$ | $0.60^{b}$ | $0.00^{a}$ |
| ± SEM | - | 0.131 | 0.131 | 0.147 | - |
| Percent of eggs scored as fishy[4] | 0 | 95 | 44 | 60 | 0 |

[1] Expressed as sinapine bisulfate.

[2] The egg samples for TMA analysis were obtained by pooling the last three eggs produced by individual birds during the fourth week on ration treatment. Mean of two replicate groups (5 birds per group).

[3] Fishy odor was scored on a scale from 0 to 4; 0 (normal), 4 (very fishy).

[4] Not analysed statistically.

# PREPARATION AND NUTRITIONAL EVALUATION OF WEANING FOOD BASED ON MUSTARD PROTEIN CONCENTRATE

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## INTRODUCTION

Mustard (B. Juncea) and Rapeseed (B.Campestris) together form a major oilseed crop of India, with an annual production of nearly 2 million tonnes. The meal from decuticled kernels contains more than 45% protein. The protein of mustard and rapeseed are well proportioned in all essential amino acids. A process has been developed at CFTRI for the removal of thyrotoxic glucosinolates in the meal and prepare a protein concentrate containing 55 to 60% protein of good nutritional quality.

In India as in many other developing countries protein malnutrition develops in children when they are weaned from mothers milk and put on solid foods. Due to economic privations these children are given only starchy foods which lack the required quantity and quality of protein and in addition lack vitamins and minerals. To overcome this problem several weaning food formulations have been developed earlier.[2,3] The present study was undertaken to prepare two weaning food formulations using edible quality mustard protein concentrate (MPC), cereals, pulses with added vitamins and minerals. While preparing weaning foods germinated legumes have sometimes been used on the basis that germination improved digestibility and nutritional quality.[4] But the beneficial effects of germination have not been observed in some instances. Hence in one weaning food formulation we incorporated germinated wheat and greengram and studied the effect of germination on nutritional quality.

## PREPARATION OF WEANING FOOD

Wheat, greengram and chick pea splits were subjected to mild roasting at approximately 80°C until a roasted aroma developed. The various ingredients shown in Table-1 were ground to pass through BSS 44 mesh and mixed in the proportion shown in Table 1.

Hot water was added in the proportion 6:1 and mixed to get a slurry which was passed through a homogeniser to make it uniform. The material was then dried in a twin drum roller drier using a steam pressure of 110-120 psig. Dried flakes scrapped from the surface of the roller were collected and passed through 44 mesh (BSS) sieve and packed in tins.

Germination was effected by soaking the seed material overnight in water and allowing to germinate for 24 hours. It was then dehusked and the material dried and mildly roasted. The germinated materials were incorporated in weaning food B. Weaning Food A is similar to B except that the ungerminated materials were used. The protein content of weaning foods ranged from 22 to 26 percent.

Mustard protein concentrate (MPC) used in the formulation of weaning food was prepared from dehusked kernels by removal of glucosinolates by aqueous extraction and removal of oil by extraction with food grade hexane. It had approximately 58% protein, less than 1 percent residual oil, 1.5% fibre, 6% ash and less than 0.15% glucosinolates.

## PHYSICAL CHARACTERISTICS OF WEANING FOOD

The physical characteristics of the weaning food are given in Table 2.

Bulk density : This was determined by filling a graduated cylinder which was being frequently tapped on a rubber sheet. The material was weighed and the bulk density was calculated as gm. per ml.

Water holding capacity : A brass cylinder of internal diameter 5 cms and height 3 cms fitted with a wiremesh bottom was used for this determination. The wire mesh was covered on the inside with a moist filter paper disc. 5 gm of the material was spread evenly on this. The cylinder was kept in a petridish containing water. The time taken for the water to wet the material inside completely was noted. The cylinder was raised to drain the excess water. The adhering water was wiped with a filter paper and the cylinder weighed. Water absorbed per gm of material calculated.

Germination caused a considerable change in the time required for water absorption. The changes taking place in the carbohydrate fractions of the cereal and pulse may be responsible for this change.

## NUTRITIONAL EVALUATION

The nutritional quantity of the product was assessed by rat bio-assay using the parameters protein efficiency ratio, net protein ratio and net protein utilization. Protein efficiency ratio (PER) was determined by the method of Osborne, Mendal and Ferry as quoted by Chick.[6] Net protein ratio (NPR) and Net Protein Utilization (NPU) was determined according to Bender and

Doell[7] and Bender and Doel[8] respectively. The results are presented in Table 4 and 5.

These parameters indicate that weaning food formulated from mustard protein concentrate is of good nutritional quality comparable with casein. Germination has shown no improvement either in PER or NPU whereas there is slight lowering of NPR. The weaning food is quite acceptable organoleptically.

After PER determination the animals were sacrificed and the total protein, haemoglobin and RBC were estimated in blood. The results are presented in Table 6.

The blood picture of the rats fed on MPC based food shows no significant difference from a casein in terms of total protein, haemoglobin and RBC contents. These results clearly indicate that MPC can be used to prepare weaning foods of good nutritional quality comparable to milk protein.

Table 1 : Ingredients Composition of Weaning Food

| | Percent |
|---|---|
| Wheat flour (roasted) | 30 |
| Rice flour | 15 |
| Mustard Protein Concentrate (MPC) | 25 |
| Chick pea flour (roasted) | 10 |
| Greengram flour (roasted) | 10 |
| Powdered sugar | 6 |
| Dicalcium phosphate | 1 |
| Sodium chloride | 2 |
| Vitamin premix* | 1 |

* *Vitamin premix contained, Vit. A 1500 IU, Vit. D 400 IU, Vit. C 30 mg, Vit. B1 0.9 mg, Vit. B2 1.5 mg and niacinamide 6.0 mg. Vit. A and D are dissolved in small quantity of hydrogenated oil and mixed with the rest of the ingredients and sugar used as filler and mixed uniformly.*

Table 2 : Physical Characteristics of MPC Based Weaning Food

| | Particle size micron | Bulk density gm/ml | Water holding capacity gms/gm product | Time for water absorption |
|---|---|---|---|---|
| Weaning Food A .... | 355 | 0.33 | 4.46 | 5 min |
| Weaning Food B .... | 355 | 0.52 | 4.23 | 20 min |

Table 3 : Composition of Diets Used for Rats Bio-assay

| | Control (Casein) | Weaning Food A | Weaning Food B |
|---|---|---|---|
| Protein source ..... | 11.7 | 38.4 | 35.3 |
| Hydrogenated fat ..... | 10.0 | 10.0 | 10.0 |
| Sugar ..... | 10.0 | 10.0 | 10.0 |
| Vit. mix ..... | 1.0 | 1.0 | 1.0 |
| Salt mixture ..... | 2.0 | 2.0 | 2.0 |
| Corn starch ..... | 65.3 | 38.6 | 41.7 |

Table 4 : Protein Efficiency Ratio of Weaning Foods (Duration of experiment 4 weeks. Mean initial weight of rats 42.8 gm)

| Diet | 10% protein level | | PER |
|---|---|---|---|
| | Wt. gain (g) | Protein intake (g) | |
| 1. Casein (control) ..... | 79.00 | 23.77 | 3.299 ±0.116 |
| 2. Weaning Food A ..... | 87.10 | 26.39 | 3.291 (28 df) |
| 3. Weaning Food B ..... | 80.40 | 24.27 | 3.304 |

@ standard error of the mean based on 28 degrees of freedom.

Results of test of significance
By Duncan's new multiple range test as given by Leon Horter in Biometrics (1960, o = 0.05, Vol. 16 PP 671-685)

| Diet | 2 | 1 | 3 |
|---|---|---|---|
| Average PER | 3.291 | 3.299 | 3.304 |

Note : a) Any two means not underscored by the same line are significantly different.
b) Any two means underscored by the same line are note significantly different.

Table 5 : Net Protein Ratio (NPR) and Net Protein Utilization (NPU) of Weaning Food

(Duration of the experiment, 10 days.
Randomised block-design-8 animals per group 10 % protein level)

| Diet | Initial wt (g) | Grain in wt (g) | Protein intake (g) | Net Protein Ratio (NPR) | Net Protein Utilization (NPU) |
|---|---|---|---|---|---|
| 1. Casein (control) ..... | 68.00 | 27.63 | 10.45 | 3.87 | 52.26 |
| 2. Weaning Food A ..... | 68.12 | 28.62 | 10.69 | 3.88 | 59.60 |
| 3. Weaning Food B ..... | 68.12 | 23.25 | 10.81 | 3.34 | 46.24 |

$S.E_m$ (28 df)

Results of test of significance (By Duncan's new multiple range test) ( P 0.05)

| Diet | 1 | 2 | 3 |
|---|---|---|---|
| Average NPR | 3.87 | 3.88 | 3.34 |
| NPU | 52.26 | 59.60 | 46.24 |

Note : (a) Any two means not underscored by the same line are significantly different
(b) Any two means underscored by the same line are not significantly different.

Table 6 : Serum Protein Haemoglobin and RBC of Rats Fed on MPC Based Weaning Food.

| Diet | Total Protein % (N x 6.25) gm/100 ml | Haemoglobin % gm/100 ml | RBC m/c mm |
|---|---|---|---|
| 1. Casein (control) ... | 7.72 | 15.75 | 8.53 |
| 2. Weaning Food A .. | 6.79 | 16.60 | 9.05 |
| 3. Weaning Food B .. | 6.68 | 15.43 | 8.37 |

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# Proposals of short nutritional evaluation test in small samples of low glucosinolate rapeseed in the early stages of plant selection

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## Introduction

It is known that rapeseed even with low glucosinolate contents have the other compounds of antinutritive character, namely phenolic derivatives, fiber or phytates, which might inhibit the feed intake and growth efficiency of animals.

The nutritional evaluation of small samples of new strains of double improved rapeseed in the early stages of selection might be important.

For that purpose the two week growth test on rats is proposed.

## Materials and methods

The animals were fed with full fat rapeseed of different strains as the only source of protein and the main source of energy. The diets were supplemented with salt and vitamin mixtures. Half kg of rapeseed sample were steam autoclaved for 15 min for myrosinase deactivation, than the corresponding amount of wheat starch was added and the mixture was ground on laboratory mill. The casein supplemented with 1% of methionine, with 20% of 0-erucic rapeseed oil diet served as the control in every set of 10 experimental groups of six rats per each sample.

The body mass gain, diet intake, PER and feed convertion coefficient served as the parameters of nutritional quality evaluation.

The thyroid weight was measured after 2 weeks of experimental period.

8 samples of rapeseed varieties of different glucosinolate content 0.22 - 7.31 mg/g of seeds/ and 42 strains of low glucosinolate /0.13 - 0.79 mg/g of seeds/ were tested.

## Results and discussion

The results are presented in Table 1 and 2. The statistical evaluation of obtained data, showed highly significant negative correlation /r = -0.86/ between all biological coefficients and the glucosinolate content in rapeseed varieties while of r = -0.18 correlation coefficient in the improved rapeseed strains.

The statistical differences were also found amoung 42 strains of double improved rapeseed in relation to body mass gain /extrema of 37 g and 63 g per 2 weeks/ and the feed convertion ratio from 246 to 348 g feed intake per 100 g of body mass gain.

Very close PER coefficients on double improved rapeseed strains to the control casein diet was shown with not significant differences among double improved rape strains, which was in agreement with a-acid composition in samples of lowest and highest PER values.

Table 1. Nutritional value of seeds of new strains of winter rapeseed compared to Start "00" and Górczański cultivars

| Protein source | Glucosinolates /mg aglycone/g seeds/ | Body weight gain /g/rat/day/ | Feed convertion /g/100g b.wt.g./ |
|---|---|---|---|
| Double improved strains | 0.13 ÷ 0.34 | 2.9 ÷ 3.9 | 265 ÷ 330 |
| cv. Start "00" | 0.63 | 3.4 | 315 |
| cv. Górczański | 6.77 | 0.3 | 1430 |

Table 2. Egzogenic amino acid content in protein /gms/16g N/ of chosen strains of rapeseed

| Amino acid | Strain No. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1115/6 | 1356/3 | 24/5 | 33/2 | 53/2 | 1370/1 | 836/5 |
| CYS | 2.09 | 2.01 | 2.28 | 2.26 | 2.20 | 2.19 | 2.26 |
| MET | 1.95 | 2.25 | 2.08 | 2.05 | 2.10 | 2.05 | 2.14 |
| THR | 4.61 | 4.59 | 4.52 | 4.64 | 4.71 | 4.44 | 4.91 |
| VAL | 5.78 | 6.03 | 5.40 | 6.22 | 5.61 | 6.23 | 5.52 |
| ILE | 4.83 | 4.49 | 4.33 | 4.41 | 4.58 | 4.22 | 4.50 |
| LEU | 7.78 | 7.67 | 7.42 | 7.44 | 7.61 | 7.31 | 7.66 |
| PHE | 4.53 | 4.31 | 4.35 | 4.44 | 4.44 | 4.31 | 4.35 |
| HIS | 2.86 | 2.80 | 2.95 | 2.87 | 2.77 | 2.82 | 2.94 |
| LYS | 6.22 | 6.26 | 6.55 | 6.53 | 6.48 | 6.33 | 7.01 |
| ARG | 6.48 | 6.55 | 6.98 | 6.59 | 6.36 | 6.00 | 5.84 |
| PER values | 3.15 | 3.25 | 4.00 | 4.08 | 3.60 | 3.48 | 3.75 |

# Influence of low glucosinolate rapeseed meal on gestation, lactation and growth of rats in long-time feeding trial

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## Introduction

Varieties of rapeseed /Brassica napus/ cultivated up to now in Poland are rich in toxic glucosinolates which severly limit utilization of rapeseed meal /RSM/ in animal feeding. During a last few years Polish plant breeders greatly reduced the level of glucosinolates in RSM with the introduction of low glucosinolate variety Start "00". For this reason it should be possible to use substantially higher amounts of RSM in animal feeding withought any depression in growth. However, it has been shown that oilseed are high in phytic acid and perhaps other binding agents which might reduce mineral availability, especialy zinc. Zinc deficiency during gestation of rats fed rapeseed protein concentrate was reported to cause a marked loss of appetite and an increase in the percentage of still-born pups and its lower weight relative to casein control animals /Jones, 1979/. In our previous feeding trials /unpublished data/ it was shown that the Zn deficiency is much more severe in rats fed water-washed RSM than untreated RSM.

Presented studies were undertaken to determine the effect of long-term feeding of rapeseed meal from the low glucosinolate variety Start "00" on growth, outcome of gestation, lactation and femur Zn and Ca content.

## Materials and methods

Seed of Start "00" variety /Brassica napus/ was treated with boiling water to destroy the enzyme myrosinase and extracted with hexane to remove the oil. The following glucosinolate composition was found /mg aglycone/ g meal/: butenyl isothiocyanate 0.50; pentenyl isothiocyanate 0.06; and oxasolidinethione 1.16. Phytate content was 3.45%.

Eight females successfully mated /mother generation/ were assigned to each of the two diets providing 20% of protein from Start meal and control - casein supplemented with methionine. Three days after parturition one pup from each litter was taken for Zn and Ca analyses in carcass. All litters were standardised up to eight pups per litter. Twenty-six days after

birth the mothers as well as offsprings, one from each litter, were killed and thyroid, liver, spleen, kidneys and femur were removed. Remaining animals were fed up to 54 days and eight rats from each group were killed and thyroid, liver, spleen and kidneys were weighted. 75 days after birth next rats were taken for measuring of organ weights and analyses of minerals in femur. Some females and males of the first generation, maintained on above diets, were mated, whereafter eight females with signs of pregnancy were put on further observations. Second gestation and lactation as well as growth of delivered rats were treated in the same way. Feed intake and weight gains were measured every 2 or 3 days.

Femur Zn and Ca contents were determined by atomic absorption spectroscopy after digestion of bone in an acid mixture as described by Brown et al. /1976/.

## Results and discussion

The details of the reproductive performance of the two generations of rats are presented in Table 1. Feeding with Start meal during gestation of the mother females caused a reduction in weight gain, feed intake and weight of the first generation of pups but did not affect the number of the pups born. Lower body weight gain of the first generation of females, maintained on Start diet from birth, had no effect on gestation and lactation but lower body weight was observed in delivered pups of second generation, 20% and 30% lower in comparison with pups of first generation and pups on control diet, respectively. Feeding Start meal reduced also weight of the young rats 21 days after birth.

The thyroids and livers of the Start-fed females were enlarged compared to the casein fed groups /Table 2/. The increased liver weight reflects, at least in part, the lower body weight of the Start-fed rats. The kidneys and spleens weight of the mother rats were not affected by consumption of the RSM.

Weight gain and feed intake data of the growing rats of the first and second generation were significantly lower than the analogous data for casein fed rats /Table 3/. This could be due in part to the presence of glucosinolates in the meal. It is well known that myrosinase inactivation does not completely inhibit the hydrolysis of glucosinolates in the gastro-intenstinal tract of rats /Appelqvist and Ohlson, 1972/. In our experiment this was proved by significantly higher thyroid weight of growing rats /Table 4/ and of females after lactation /Table 2/.

The concentration of Zn found in pups carcasses and femur of females and growing rats are shown in Table 5. No difference was observed in Zn level in pups althought concentration of this mineral in femur of mothers indicated a reduced Zn availability. The lowest levels of Zn were noted in femur of growing rats 75 days after birth. The deficiency of Zn was more deep in the second generation. No effect of tested meal on Ca femur content was observed.

It is concluded that lower weight gain of rats fed rapeseed meal is due to glucosinolate content and probably in part to some constituents involved in Zn availability. However, symptoms of toxicity were not observed during gestation of females and the number of pups born and litter size were not affected.

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Table 1. Litter size, body weight gain and feed intake of the mother rats and pups fed rapeseed meal cv. Start "00"

| | Mother generation | | First generation | |
|---|---|---|---|---|
| | Control | RSM cv.Start | Control | RSM cv.Start |
| Number of rats | 8 | 8 | 8 | 8 |
| Body weight gain during gestation/g/ | 91.6±8.2[1],a | 73.8±7.8b | 93.4±14.4a | 75.4±9.1b |
| Feed intake during gestation/g/rat/day/ | 20.2±1.6a | 15.7±1.0b | 20.9±1.5a | 16.1±1.8b |
| Number of live born litters | 8 | 8 | 8 | 8 |
| Litter size at birth | | | | |
| Live $\bar{x}$ | 10.3 | 10.0 | 9.7 | 10.6 |
| Range | 8 - 14 | 7 - 15 | 8 - 15 | 8 - 15 |
| Dead $\bar{x}$ | 0.0 | 0.0 | 0.3 | 0.0 |
| Weight of offspring | | | | |
| 3 days after birth | 8.1±0.8a | 7.2±1.0a | 8.2±1.8a | 5.8±0.4b |
| 21 days after birth | 40.3±2.1a | 33.6±2.8b | 41.2±2.4a | 29.8±2.3c |

[1] Mean ± standard deviation, a,b,c Values having different superscripts vary significantly from each other /$p<0.05$/

Table 2. Organ weight of females after lactation

| | Mother generation | | First generation | |
|---|---|---|---|---|
| | Control | RSM cv.Start | Control | RSM cv.Start |
| Thyroids, mg/100 g body wt | 7.1±1.4[1],a | 13.8±3.4b | 6.8±1.6a | 12.1±2.0b |
| Kidneys, g/100 g body wt | 0.93±0.06a | 0.92±0.05a | 0.91±0.07a | 0.94±0.09a |
| Spleen, g/100 g body wt | 0.26±0.03a | 0.25±0.02a | 0.26±0.04a | 0.27±0.02a |
| Liver, g/100 g body wt | 4.32±0.36a | 5.85±0.67b | 4.26±0.24a | 6.25±0.56b |

[1] Mean ± standard deviation, a,b Values having different superscripts vary significantly from each other /$p<0.05$/

Table 3. Feed intake and body weight gain of growing rats /21 ÷ 62 days after birth/

| | First generation | | Second generation | |
|---|---|---|---|---|
| | Control | RSM cv. Start | Control | RSM cv. Start |
| Body weight gain /g/rat/day/ | $4.8 \pm 0.2^{1,a}$ | $3.1 \pm 0.1^{b}$ | $4.9 \pm 0.2^{a}$ | $2.7 \pm 0.2^{c}$ |
| Feed intake /g/rat/day/ | $16.1 \pm 1.1^{a}$ | $10.1 \pm 0.6^{b}$ | $16.6 \pm 1.7^{a}$ | $9.4 \pm 0.7^{b}$ |

[1] Mean ± standard deviation, a,b,c Values having different superscripts vary significantly from each other /$p < 0.05$/

Table 4. Wet weight of thyroids /mg/100 g body wt/

| | Growing rats /days after birth/ | | |
|---|---|---|---|
| | 26 | 54 | 75 |
| First generation | | | |
| Control | $12.9 \pm 2.4^{1,a}$ | $6.8 \pm 2.1^{a}$ | $6.5 \pm 1.8^{a}$ |
| RSM cv.Start | $20.8 \pm 3.2^{b}$ | $14.6 \pm 2.6^{b}$ | $12.8 \pm 2.4^{b}$ |
| Second generation | | | |
| Control | $13.7 \pm 2.0^{a}$ | $7.0 \pm 1.7^{a}$ | $6.3 \pm 2.1^{a}$ |
| RSM cv.Start | $23.3 \pm 9.1^{b}$ | $15.1 \pm 1.9^{b}$ | $13.3 \pm 1.9^{b}$ |

[1] Mean ± standard deviation, a,b Values having different superscripts vary significantly from each other /$p < 0.05$/

Table 5. Influence of rapeseed meal cv. Start "00" on the level of Zn in pups carcasses and femur of females and growing rats /μg/g d.wt./

| Protein source | Pups /3 days after birth/ | Females after lactation | Growing rats | |
|---|---|---|---|---|
| | | | 26 days after birth | 75 days after birth |
| Control | 147 ± 13[1],a | 264 ± 17 a | 265 ± 18 a | 266 ± 16 a |
| RSM cv. Start | | | | |
| - mother generation | | 238 ± 13 b | | |
| - first generation | 155 ± 11 a | 223 ± 20 b | 275 ± 28 a | 194 ± 28 b |
| - second generation | 140 ± 18 a | | 210 ± 18 b | 157 ± 36 c |

[1] Mean ± standard deviation, a,b,c Values having different superscripts vary significantly from each other /$p < 0.01$/

# Influence of 1-cyano-2-hydroxy-3-butene and intact glucosinolates on nutritional value of commercial rapeseed meal

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## Introduction

The rapeseed meal /RSM/ was analysed for aglycones content in our laboratory. The results showed that commercial RSM contains compound with retention times on EGSS-X and Apiezon L GC chromatography columns similar to that of the 1-cyano-2-hydroxy-3-butene /unsaturated nitrile, CHB/. This compound was additionaly identified by GC-MS method. Earlier, Papas et al. /1978/ also found CHB in Tower RSM.

In the presented paper the effect of unsaturated nitrile and intact glucosinolates on growth of rats in two-weeks feeding trial is described.

## Materials and methods

Dietary meals were prepared as follows. Commercial RSM from Janpol variety was extracted with four portions of 80% aqueous methanol in room temperature. The extracts were combined and from one third of it methanol was removed under vacuum. The remaining water phase was than extracted four times with methylene chloride. The methylene chloride extracts were pooled and the solvent was evaporated in a rotary evaporator. When about 100 ml of methylene chloride remained, one third of methanol extracted meal was added and the solvent evaporated. The water phase was also mixed with one third of the meal and dried under vacuum. The following RSM preparations were mixed in equal portions into the test diets: $P_0$ - untreated meal, $P_1$ - detoxified meal, $P_2$ - glucosinolate containing meal, $P_3$ - nitrile containing meal.

The unsaturated nitrile and intact glucosinolate content of these preparations as determined by GLC method of Daxenbichler et al. /1970/ is given in Table 1.

Weanling male and female Wistar rats weighing 45-50 g were divided into five experimental groups of 8 rats each /40 + 4°/. The animals were housed in individual cages and were fed ad libitum. The control group was fed with diet containing 10% protein from casein supplemented with

methionine. The groups 2,3,4 and 5 were fed the test diets containing the same level of rapeseed meal /20%/. Differences in protein content of the test diets were equalized up to 10% by casein supplementation.

The body weight and feed intake were recorded every 2 days. At the end of 2 weeks the rats were killed and thyroids and kidneys were removed and weighed.

## Results and discussion

Results of the feeding test are given in Table 2. Feeding of meals $P_0$ and $P_2$ caused a very similar reduction in weight gain, feed intake and enlargement of thyroids and kidney of rats. Surprising results were obtained for CHB containing diet /$P_3$/ : weight gain and feed intake data of the growing rats were a little lower than on casein diet but the same as on detoxified meal $P_1$ and significantly higher in comparison with meals containing glucosinolates. The thyroid and kidney of rats were not significantly affected by consumption of the detoxified meal /$P_1$/ as well as nitrile-rich meal /$P_3$/.

The lack of toxicity of CHB to rats demonstrated in our experiment is in agreement with the results of Cansfield and Campbell /1980/. These authors showed that CHB was not toxic to poultry when fed as an individual compound in the level of 0.08% in the diet. For comparison in our feeding trial amount of CHB in the diet was 0.05%.

These above results are in conflict with informations published by VanEtten et al. /1969/ and Srivastava et al. /1975/. They reported that meals autolyzed to give nitriles were highly toxic to rats. However in experiment demonstrated by VanEtten and coworkers crambe seed meal autolyzed and freeze-dried was much more toxic than meal autolyzed and air-dried at 50°C, althought the amount of nitriles was not so much lowered during the air-drying /from 0.8% to 0.6%/. Weight gain of rats fed nitrile-rich meal on the level of 10% in the diet /0.06% of nitriles/ in Srivastava et al. experiment was more affected than in our. However aglycone composition in autolyzed meal is different than in meal used in our experiment. As it is known during autolysis some quantities of 5-vinylooxazolidine-2-thione are formed from the progoitrin. Additionaly, we found that in the autolyzed meal from Brassica napus 1-cyano-3,4-epithiobutene is formed from gluconapin insted of 3-butenyl-isothiocyanate. Similar enzymatic hydrolisis of gluconapin was observed by Kirk and MacDonald /1974/ in the meal from Brassica campestris. The differences in the results between the feeding trials with isolated CHB and autolyzed meal are probably caused by the content of 5-vinylooxazolidine-2-thione and split products of gluconapin and pungent tast of autolyzed meal.

It can be concluded that detrimental effect observed in feeding the commercial rapeseed meal is mainly due to its high intact glucosinolates content.

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Table 1. Glucosinolate content /mg/g/ in different preparations of commercial RSM /cv. Janpol/

| | $P_0$ | $P_1$ | $P_2$ | $P_3$ |
|---|---|---|---|---|
| 1. "As is" /without addition of myrosinase/ | | | | |
| 1-cyano-2-hydroxy-3-butene | 2.6 | 0.0 | 0.0 | 2.5 |
| 2. Hydrolysis products of intact glucosinolate /addition of myrosinase/ | | | | |
| 5-vinylooxazolidine-2-thione | 3.4 | 0.6 | 3.0 | 0.7 |
| 3-butenyl-isothiocyanate | 1.2 | 0.2 | 1.1 | 0.2 |
| 4-pentenyl-isothiocyanate | 0.2 | 0.0 | 0.2 | 0.0 |

Table 2. Influence of different preparations of rapeseed meal cv. Janpol on feed intake, body weight gain and enlargement of thyroid and kidney of rats.

| Dietary groups | Body weight gain /g/day/ | Feed intake /g/day/ | Thyroid weight /mg/100g b.wt/ | Kidney weight /g/100g b.wt/ |
|---|---|---|---|---|
| 1. Casein | 3.0 ± 0.4[1],a | 9.4 ± 0.6 a,b | 6.1 ± 1.0 a | 0.86 ± 0.04 a |
| 2. Meal $P_0$ | 1.6 ± 0.3 b | 8.5 ± 0.7 a | 9.4 ± 0.9 b | 1.03 ± 0.08 b |
| 3. Meal $P_1$ | 2.5 ± 0.4 c | 9.9 ± 0.9 b | 7.6 ± 1.7 a | 0.89 ± 0.06 a |
| 4. Meal $P_2$ | 1.7 ± 0.3 b | 9.0 ± 1.6 a,b | 9.6 ± 1.5 b | 1.03 ± 0.10 b |
| 5. Meal $P_3$ | 2.6 ± 0.3 a,c | 10.1 ± 1.6 b | 6.6 ± 1.7 a | 0.92 ± 0.06 a |

[1] Mean ± standard deviation, a,b,c Values having different superscripts vary significantly from each other /p 0.05/

# Influence of acid hydrolysis on the nutritional value of canola meal for pigs 10-25 kg liveweight

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Plant breeders have developed cultivars of rapeseed which produce meals with low levels of glucosinolates and oils with low levels of erucic acid; such cultivars are referred to as 'canola'. However, attempts to formulate diets for young pigs using canola meal as the sole protein supplement result in lower growth rates than those observed for pigs receiving other sources of protein.

In the studies to be described, the effects of pre-hydrolysis of the canola meal on digestibility and growth were examined.

Autoclaving with sulphuric acid was used for the hydrolysis. The conditions which maximized solubilization of dry matter and nitrogen with minimum acid use were: 0.6N sulphuric acid at 126$^{o}$ for 3 hours using 5 l of dilute acid per kg of canola meal. The hydrolysate was neutralized with calcium hydroxide and filtered under pressure. The filtered suspension was stored at 4$^{o}$ for evaluation. The procedure recovered 66% of the dry matter and 69% of the nitrogen from the rapeseed meal in the filtrate.

Table 1. Composition of hydrolyzate of canola meal

| Dry matter | Protein | Soluble carbohydrate | Ash |
|---|---|---|---|
| g/kg | g/kg dry matter | | |
| 16 | 340* | 140 | 270 |

*One third of the protein was 'true protein'

The high ash content of the product being due to the calcium sulphate formed by the neutralization of the sulphuric acid.

The effects of the processing in the nutritional value of the canola meal were examined by preparing diets in which 40% of the total nitrogen was provided by either the unprocessed or the processed canola. The diets contained

646 g/kg ground corn, 110 g/kg soybean meal and either 200 g/kg canola meal, or an equivalent amount of nitrogen from the hydrolyzate, with vitamins and minerals; analysis showed 187 g/kg crude protein, with the crude fiber level being lower (24 g/kg) in the hydrolyzate diet than in the unprocessed canola meal diet (46 g/kg). The unprocessed canola meal diet was mixed with water to provide the same dry matter level as in the hydrolyzate diet. The two diets were each fed to four pigs of approximately 11 kg liveweight for 21 days. There were no statistical significant differences in either food consumption (1.11 and 1.26 kg/d) or liveweight gains (0.54 and 0.52 kg/d) for the canola meal-, or its hydrolyzate-diets, respectively. All the pigs receiving the hydrolyzate diet had diarrhea. This was not due to the calcium sulphate present in the diet as a result of neutralizing the sulphuric acid with calcium hydroxide, because adding an equivalent amount of calcium sulphate to the canola meal diet did not induce diarrhea in a subsidiary experiment.

Digestibilities of the two diets were measured using chronic oxide as a digestion index and eight castrated male pigs of 15 kg liveweight. They were randomly allocated to the two diets and received 800 g/day divided between two equal meals for eight days. Feces were collected for the last four days. The apparent digestibilities of the dry matter and nitrogen were significantly lower ($P<0.01$) for the hydrolyzate diet than for the canola meal diet.

Apparent digestibilities of dry matter and nitrogen in diets containing 40% of nitrogen as either canola meal or its hydrolyzate

| | canola meal | hydrolyzate | Standard error |
|---|---|---|---|
| Dry matter digestibility | 0.83 | 0.77 | 0.04 |
| Nitrogen | 0.81 | 0.72 | 0.12 |

At the end of the collection period for the digestibility determination the pigs were given a single meal 'marked' with $^{51}Cr\ Cl_3$ and the feces monitored for gamma emissions: the times of first appearance of the marker were 26 h for the pigs receiving the canola meal diet and 12 h for the pigs receiving the canola hydrolyzate diet.

These data show that acid prehydrolysis of canola meal solubilizes two thirds of the dry matter and nitrogen but does not improve the nutritional value of the canola. The hydrolyzate passed through the digestive tract much more rapidly than the unprocessed meal resulting in a lower digestibility, and diarrhea. However, substitution of the hydrolysis product for the unprocessed canola meal did not reduce the growth rate of pigs between 10 and 20 kg liveweight.

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# Untersuchungen über die strumigene Wirkung rapshaltiger Futtermischungen in Abhängigkeit vom Glucosinolatgehalt, der Rapssorte und Fütterungszusätzen.
Versuche mit Mastküken

# Research on the thyroid enlargement dependent on different rapeseeds in feed mixtures, the content of glucosinolates and feed supplements .
Trials with chickens.

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Raps in Futtermischungen verursacht bei Mastküken eine Vergrößerung der Schilddrüsenmasse, die in Abhängigkeit von der Glucosinolataufnahme mehr als das 6-fache der Schilddrüsenmasse im Vergleich zu glucosinolatfreien Rationen betragen kann.
Bei gleichen Anteilen von 20% Rapssaat in den Versuchsmischungen wurde im Vergleich zu einer glucosinolatfreien Fütterungsgruppe der Umfang der Schilddrüsenvergrößerung durch den Glucosinolatgehalt der Rapssorte bestimmt. Darüber hinaus wurde untersucht, ob die strumigene Wirkung von Rapssaaten durch Mineralstoffzusätze vermindert werden kann. Besonders geeignet waren Zusätze von Eisen (Fe).
Die Versuchstiere wurden jeweils als Eintagsküken aufgestallt und nach einer 14-tägigen gemeinsamen Aufzuchtperiode auf die Versuchsgruppen verteilt. Während der Versuchszeit vom 15. bis 42. Lebenstag wurden die Küken in Einzelkäfigen gehalten und gefüttert. Im Vergleich zur jeweiligen Kontrollgruppe waren alle Versuchsmischungen nährstoffäquivalent. Am 42. Lebenstag wurde von allen Versuchstieren das Lebendgewicht festgestellt. Die Schilddrüsen wurden nach der Schlachtung gewogen.
Insgesamt wurden in den Mastversuchen mit männlichen Broilern (Lohmann, Cuxhaven) 12 verschiedene Rapssorten teilweise mit mehreren Wiederholungen geprüft. Neben der Wägung der Schilddrüsenmasse wurde die Wirkung der Rapssaaten auf die Futteraufnahme und die Lebendgewichtsentwicklung des einzelnen Versuchstieres gemessen.

ABKÜRZUNGEN

GSL = µmol Glucosinolate / g entfettete Rapssaat
µmol glucosinolates / g defatted rapeseed

ΣGSL = µmol Gesamtglucosinolataufnahme der Versuchsgruppen
µmol total glucosinolate- intake of groups

PROG = µmol Progoitrin / g entfettete Rapssaat
µmol progoitrin / g defatted rapeseed

PROG% = % Progoitrin am Gesamtglucosinolatgehalt
% progoitrin of the total glucosinolate content

THYR = x- fache Schilddrüsenvergrößerung ohne Eisenzulage
x- fold thyroid enlargement without iron supplement

THYR+Fe = x- fache Schilddrüsenvergrößerung mit Eisenzulage
x- fold thyroid enlargement with iron supplement

(THYR+ Fe)% = % Abnahme der Schilddrüsenvergrößerung durch Eisenzulagen
% Reduction of thyroid enlargement as influenced by the iron supplement

I: Beschreibung der Rapssaaten

No.1: Beschreibung der geprüften Rapssaaten

Description of the tested rapeseeds

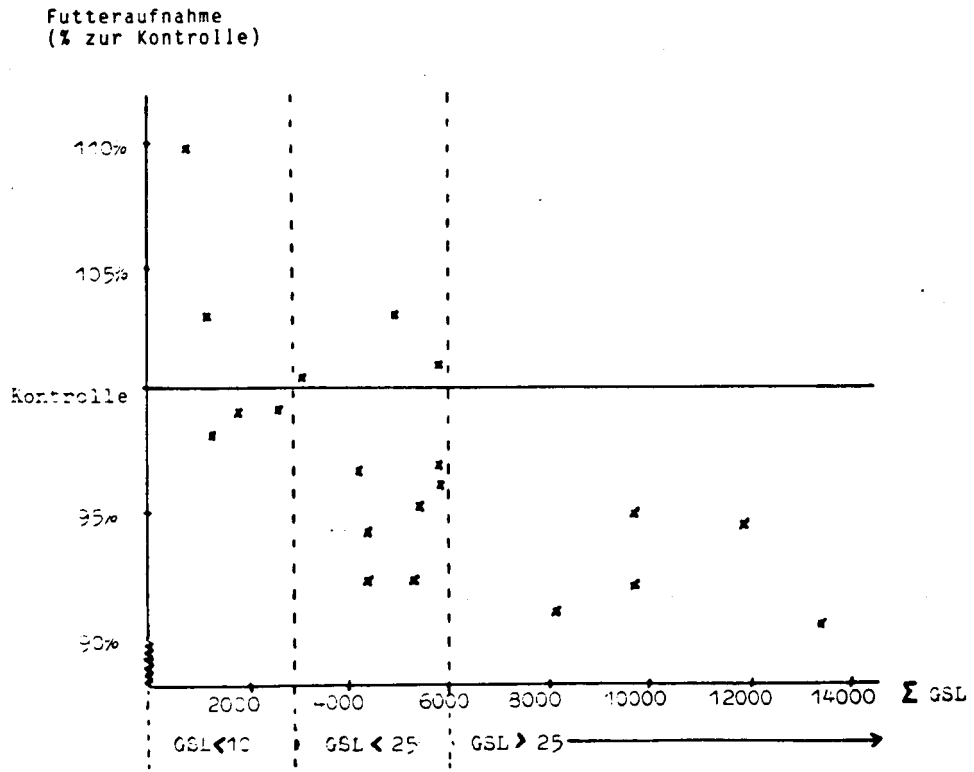
| | | GSL | PROG | (PROG%) |
|---|---|---|---|---|
| Line | aus Gulle-Kreuzung | 7,2 | 4,9 | (68) |
| Topas | aus Gulle-Kreuzung | 25,0 | 14,05 | (56) |
| Duplo | Egra x Gulle | 18,1 | 10,62 | (59) |
| Duplo | | 21,9 | 14,6 | (67) |
| Librador | | 18,9 | 12,25 | (65) |
| Ledos | [(Oro x Lenora) x Bronowski] x Rapol | 23,6 | 16,75 | (71) |
| Ledos | -"- | 45,3 | 34,5 | (76) |
| Düsse VT 5 | [(Oro x Lenora) x Bronowski] x Tantal | 9,9 | 6,3 | (64) |
| DSV SRF 13 | | 58,6 | 36,3 | (62) |
| DSV SRF 21 | [(Oro x Lenora) x Bronowski] x Petranova | 20.8 | 14,1 | (68) |
| DSV SR 56 | (Oro x Lenora) x Bronowski | 17,6 | 9,5 | (54) |
| DSV SR 57 | (Oro x Rapol) x Bronowski | 11,3 | 6,7 | (59) |
| Saphir | Ledos x Li 230/73, Li 230/73 aus Matador x Marcus | 38,9 | 24,9 | (64) |
| Tandem | eine Mehrfach-Rückkreuzung mit JetNeuf | 33,9 | 22,9 | (68) |

Die geprüften Rapssaaten weisen Glucosinolatgehalte zwischen 7 und 60 µmol/g entfettete Saat auf. Die Sorten Ledos und Librador sowie die Sorten VT 5 und SRF 13 zeigten trotz identischer Abstammung erhebliche Unterschiede im Glucosinolatgehalt. In allen Saaten betrug der prozentuale Anteil des Progoitrins etwa 60 bis 70% am Gesamtglucosinolatgehalt.

II: Mit steigendem Glucosinolatgehalt und somit steigender Glucosinolataufnahme der Tiere wurde die Futteraufnahme reduziert. In der folgenden Abbildung sind die Gruppenmittel dargestellt. (s% innerhalb der Versuchsgruppen <10)

No.2: Die Futteraufnahme (% zur Kontrolle) in Abhängigkeit von der Gesamtglucosinolataufnahme. Vergleich der Gruppenmittel.

Feed intake (% of control) as influenced by the total glucosinolate-intake. Comparison of mean value of groups.



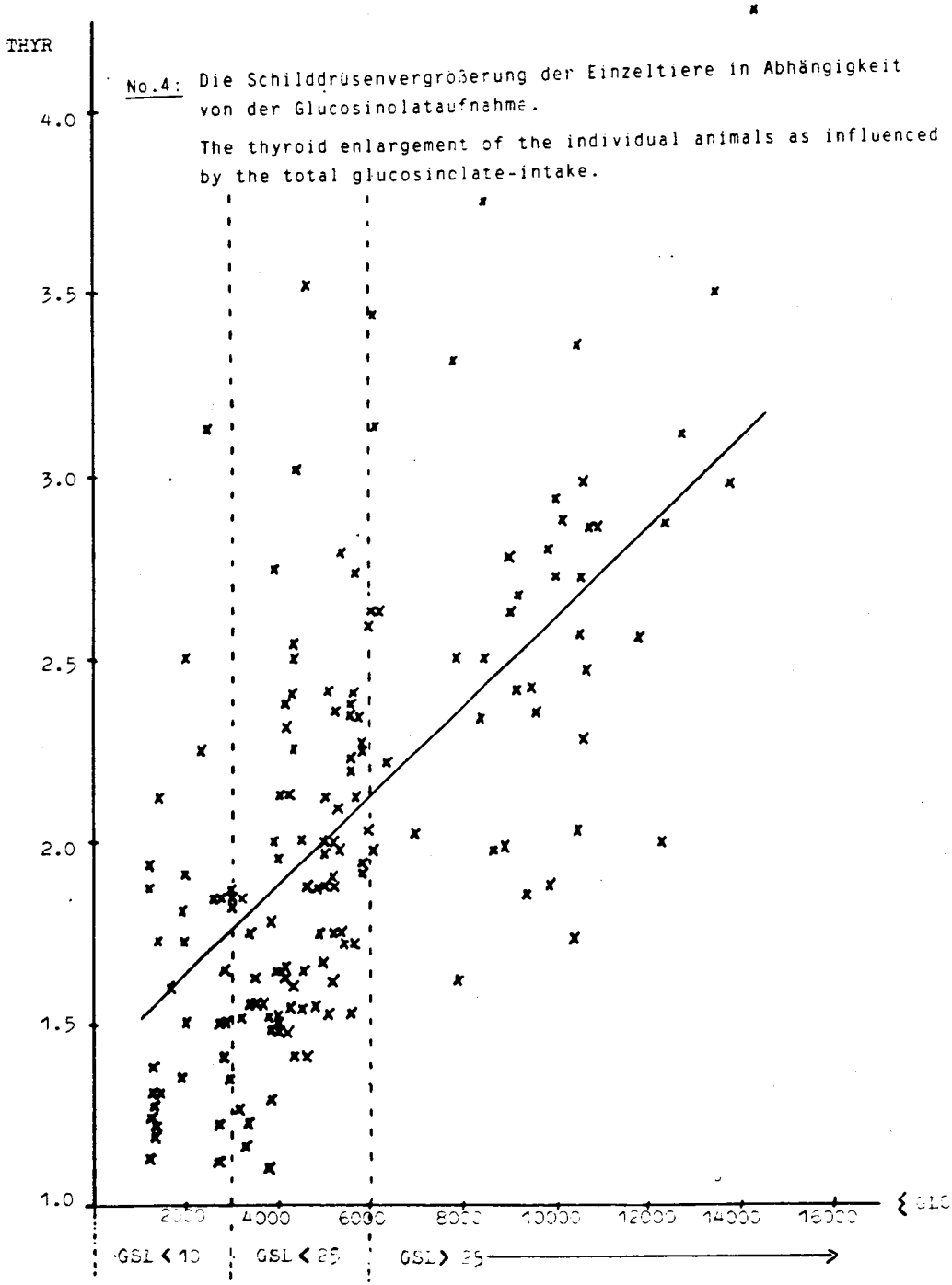
III: Mit steigender Glucosinolataufnahme nahm das Ausmaß der strumigenen Wirkung zu. Bei gleichen Anteilen in der Ration wurde der Umfang der Schilddrüsenvergrößerung durch den Glucosinolatgehalt der Rapssaat bestimmt.
In wiederholten Versuchen mit der Rapssorte Duplo blieb die strumigene Wirkung gleich.

No.3: Die Schilddrüsenvergrößerung in Abhängigkeit von der Rapssaat und der Gesamtglucosinolataufnahme. Vergleich der Gruppenmittel.

The thyroid enlargement as influenced by the rapeseed and the total glucosinolate-intake. Comparison of mean value of groups.

| Rapssorte | GSL | ΣGSL | THYR |
|---|---|---|---|
| Line | 7.2 | 1930 | 1.8 |
| VT 5 | 9.9 | 2680 | 1.6 |
| SRF 57 | 11.3 | 3140 | 1.6 |
| Duplo | 18.1 | 4230 | 1.9 |
| Duplo | 18.1 | 4230 | 1.9 |
| Duplo | 18.1 | 4420 | 2.1 |
| Librador | 18.9 | 4450 | 2.1 |
| SRF 56 | 17.8 | 4950 | 1.8 |
| Ledos | 23.6 | 5570 | 2.2 |
| SRF 21 | 20.8 | 5840 | 2.5 |
| Duplo | 21.9 | 5860 | 2.3 |
| Duplo | 21.9 | 5860 | 2.3 |
| Topas | 25.0 | 5750 | 2.2 |
| Tandem | 33.9 | 8170 | 2.5 |
| Saphir | 38.9 | 9600 | 2.3 |
| Lados | 45.3 | 9650 | 2.4 |
| Ledos | 45.3 | 10870 | 2.8 |
| SRF 13 | 58.6 | 13370 | 3.1 |

Diese Wirkung war trotz der großen Streuung der Einzeltierwerte ($s\%$ = 20 - 30) erkennbar.




No.4: Die Schilddrüsenvergrößerung der Einzeltiere in Abhängigkeit von der Glucosinolataufnahme.

The thyroid enlargement of the individual animals as influenced by the total glucosinolate-intake.

IV: Durch Eisenzulagen (200 mg Eisen als $FeSO_4$/kg Ration) konnte die strumigene Wirkung von 9 der 12 geprüften Saaten im Mittel um 50% reduziert werden.

No.5: Die Abnahme der Schilddrüsenvergrößerung durch Eisenzulagen (200 mg / kg Ration). Vergleich der Gruppenmittel.

The reduction of thyroid enlargement as influenced by the iron supplement (200 mg / kg diet). Comparison of mean value of groups.

| Rapssorte | GSL | ΣGSL | THYR | THYR + Fe | (THYR + Fe) % | |
|---|---|---|---|---|---|---|
| Line | 7.2 | 1930 | 1.8 | 1.3 | - 58 | |
| VT 5 | 9.9 | 2680 | 1.6 | 1.4 | - 35 | |
| SRF 57 | 11.3 | 3140 | 1.6 | 1.7 | | + 8 |
| Duplo | 18.1 | 4230 | 1.9 | 1.6 | - 32 | |
| Duplo | 18.1 | 4230 | 1.9 | 1.5 | - 46 | |
| Duplo | 18.1 | 4420 | 2.1 | 1.3 | - 72 | |
| Librador | 18.9 | 4450 | 2.1 | 1.6 | - 42 | |
| SRF 56 | 17.8 | 4950 | 1.8 | 1.5 | - 33 | |
| Ledos | 23.6 | 5570 | 2.2 | 1.6 | - 44 | |
| SRF 21 | 20.8 | 5840 | 2.5 | 1.8 | - 46 | |
| Duplo | 21.9 | 5860 | 2.3 | 1.7 | - 46 | |
| Duplo | 21.9 | 5860 | 2.3 | 1.6 | - 56 | |
| Topas | 25.0 | 5750 | 2.2 | 1.5 | - 67 | |
| Tandem | 33.9 | 8170 | 2.5 | 1.8 | - 44 | |
| Saphir | 38.9 | 9600 | 2.3 | 2.6 | | + 21 |
| Ledos | 45.3 | 9650 | 2.4 | 2.3 | - 10 | |
| Ledos | 45.3 | 10870 | 2.8 | 2.9 | | + 8 |
| SRF 13 | 58.6 | 13370 | 3.1 | 2.4 | - 36 | |

Wir haben Grund zu der Annahme, daß die Wirkung der Eisenzulage auf einer Beeinflussung der enzymatischen Spaltung der Glucosinolate beruht.

# Ovulation in ewe lambs given a diet containing high glucosinolate rapeseed meal

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In a preliminary experiment on the effects of a rapeseed meal (RSM) diet on reproductive efficiency in ewes (Thériez, personal communication), there was a strong trend towards reduced number of lambs born to ewes given the rapeseed meal diet. In the experiment described here, the relation between rapeseed meal and reproductive efficiency was studied with ewe lambs: Observations were made on the activity of the ovaries of lambs given a concentrate diet containing high glucosinolate rapeseed meal as the sole protein supplement.

Groups of weaned Welsh mountain ewe lambs weighing about 20kg. and Suffolk crossbred ewe lambs weighing about 37kg. were each divided into 4 pens of six lambs. Two groups of each breed or cross of lamb were given a diet containing RSM and another two groups, a diet containing soyabean meal (SBM) in place of RSM . The diets were balanced for energy and protein by the replacement of some of the barley in the SBM diet with wheat and oil in the RSM diet(Table 1) These diets were given ad libitum plus 100g of chopped hay daily for 9 weeks.

The RSM used was from an autumn sown variety grown in Britain and contained 7.90mg/g oxyzolodinethione, a hydrolysis product of the glucosinolate, progoitrin.

Table 1

The Composition of the Diets

| Ingredient % | Sbm | Rsm |
|---|---|---|
| Barley | 69.00 | 40.75 |
| Wheat | - | 20.00 |
| Soyabean meal | 23.66 | - |
| Rapeseed meal | - | 32.16 |
| Molassine meal | 5.00 | 5.00 |
| Oil | - | 1.00 |
| Salt | 0.25 | 0.25 |
| Limestone | 0.70 | 0.82 |
| Dicalcium phosphate | 1.05 | - |
| Calcined magnesite | 0.32 | - |
| Trace elements + vitamins | 0.02 | 0.02 |
| | 100.00 | 100.00 |

Feeding a diet containing rapeseed meal to the ewe lamb resulted in a significantly lower ($P<0.01$) daily live weight gain than was achieved by lambs fed the SBM diet (Table 2). The lowland Suffolk cross ewe lambs grew at a significantly ($P<0.001$) greater rate than the Welsh mountain lambs, but there was no interaction between diet and breed.

Table 2 Daily rate of liveweight gain (g/lamb) and number of Corpora lutea of lambs given diets containing soyabean meal (Sbm) or British rapeseed meal (Brsm)

| Dietary Treatment | Breed or Cross | n | Rate of gain | No. of Corpora lutea per lamb |
|---|---|---|---|---|
| Sbm | Welsh | 2 | 158.4 | 1.25 |
| | Suffolk X | 2 | 247.6 | 1.33 |
| Brsm | Welsh | 2 | 120.5 | 1.92 |
| | Suffolk X | 2 | 205.4 | 1.34 |
| Sbsm | | 4 | 203.0 | 1.29 |
| Rsm | | 4 | 163.0 | 1.63 |
| | Welsh | 4 | 139.4 | 1.59 |
| | Suffolk X | 4 | 226.5 | 1.34 |
| Statistical Analysis | | | | |
| Dietary treatment or breed | | S.E.D. | 7.79 | - |
| Dietary treatment | | P | <0.01 | NS |
| Breed | | P | <0.001 | NS |

Examination of the ovaries of the slaughtered ewe lambs showed that all the lambs had ovulated during the trial. The number of 'corpora lutea' per lamb was not significantly affected by the diet. This suggests that the feeding of high glucosinolate RSM did not markedly depress sexual development.

The inclusion of RSM in the diet produced a highly significant ($P<0.001$) increase in thyroid weight and decrease in plasma thyroxine level (Table 3). In neither case was there a significant interaction between breed and diet. These results reflect the goitrogenic property of high glucosinolate RSM, but as indicated earlier, this was not associated with delay in sexual maturity.

Table 3 Thyroid weight (mg/kg body weight) and plasma thyroxine concentration (g/100ml) of Welsh and Suffolk crossbred lambs given diets containing soyabean meal (Sbm), or British rapeseed meal (Brsm)

| | | n | Thyroid weight | Plasma Thyroxine g/100ml |
|---|---|---|---|---|
| Sbm | Welsh | 12 | 69 | 7.5 |
| | Suffolk X | 12 | 57 | 9.1 |
| Brsm | Welsh | 12 | 108 | 6.7 |
| | Suffolk X | 12 | 91 | 6.4 |
| Sbm | | | 63 | 8.3 |
| Rsm | | | 100 | 6.6 |
| Welsh | | | 88 | 7.1 |
| Suffolk | | | 73 | 7.8 |
| SE difference between means ± | | | 6.5 | 0.46 |
| Significance of difference (P) | | | | |
| Rsm v Sbm | | | <0.001 | <0.001 |
| Welsh v Suffolk | | | <0.05 | NS |

# IMPROVEMENTS IN MILK TASTE OBTAINED WITH RAPE SEED PRODUCTS

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The most frequent taste defects of milk are oxidized, rancid and unclean flavours. The acceptability of oxidized flavour is low, while rancidity, and in particular so called unclean flavours to a greater extent are identified with the flavour of milk. Rancidity is noted together with fat lipolysis in milk. Lipolytic enzyme level is high in milk, and the activity is affected by activation either through agitation or temperature changes. Screening of milk producers samples of milk shows presently 1-2% descrete rancid milk deliveries, and it is the most frequent of the defects. The oxidized flavour defect has varied, beeing at its peak in the late fifties in Norway. It was found that feeding rapeseeds and meal improved degree and frequency of this taste defect of the milk.

The effect of feeding rape seeds and meal upon oxidized flavour of milk. Rapeseeds and rape seed meal was compared with linseed and soybeans in experiments with milk cows (Meldinger fra Norges landbrukshøgskole 45, No 7, 1966). The two first experiments both had a latin square switch over design, while the third experiment was a group trial. The coarse feed was supplied as 4 kg hay and 16 kg alkali digested straw, and covered the maintenance requirement of energy. Barley was used to balance requirement of energy for production. The feeding of oil seeds and meal is given in Table 1.

Table 1. Experimental treatment in feeding milk cows rape seeds and meal

| Exp. No. | I | II | III |
|---|---|---|---|
| No of animals | 12 | 12 | 12 |
| Length of period, wks. | 2 | 3 | 6 |
| Soybeans, kg | 2.5 | 2.5 | 2.5 |
| Rapeseed + meal, kg | 1.2+1.8 | 1.0+0.0 | 1.0+1.0 |
| Rapeseed meal, kg | | 2.9 | |
| Linseed, kg | 1.0 | | 1.5 |

The rations were balanced between groups also to give equal supply of protein, carbohydrates and fat. Animals were past mid lactation yielding between 15 and 20 kg milk. Milk was judged by 4 judges, 3 students and an expert judge, on randomized samples stored 48 hrs at 4°C. A scale from 0 (without defect) till 6 was used. The results of expert judge scorings are seen in Table 2.

Table 2. Effect of feeding rape seeds and meal to milk cows upon the oxidized flavour in milk

| Exp. No. | I | II | III |
|---|---|---|---|
| Soybeans | 2.30 | 3.21 | 2.09 |
| Rape seed + meal | 1.13[a] | 1.42[a] | 1.15[a] |
| Rape meal | | 1.54[a] | |
| Linseed | 3.92[a] | | 2.82[a] |

a) Score significant different ($P < 0.01$) from control.

Rape seed or rape meal feeding, both gave less oxidized flavour in milk than when feeding either linseeds or soybeans. The mechanism by which rape seed products brought about this improvement is not quite clear. Changes in milk composition was multiple. There was a tendency toward lower polyenoic acid content and lower TBA aldehyde values in butter oil. Buttermilk contained less Cu and more membrane proteins. Milk contained more vitamin A and E and xanthine oxidase. Milk also had a higher pH. Any of these changes when feeding rape products are likely to improve milk oxidation stability. It was suggested that the resulting polyenoic acid content of the milk would be the most important single change.

The multiple changes resulting with rape seed feeding were also seen in correlation studies of milk from the University district (Meldinger fra Norges landbrukshøgskole 45, No 8, 1966). Such changes were seen from early to late lactation, from younger to older cows or in cows with and without administration of thyroxine (Meldinger fra Norges landbrukshøgskole, 45, No 22, 1966).

Thyroxine to cows had been found to increase oxidized flavour in milk (Nature 198, 192-193, 1963). It is possible that the goitrogen properties of rape seed in part was responsible for the improvement in taste, and the changes in milk composition. Other rape seed components, however, may have contributed to the regulation and brought about milk resistency to oxidized flavour.

Rancid flavour in milk when feeding rape seed products. Milks produced on good summer feeds are low in both rancid and oxidized flavour. Rancid flavour and oxidized flavour are seldom present together. Oxidized flavour occurs early in lactation and the rancid flavour often appears in late lactation. Oxidized flavour increased when protected vegetable oils were fed, while rancidity was reduced in the same milk (Milchwiss. 34, 290-291, 1979). Feeding protected low level erucic acid rape seed oil, however, was able to improve rancidity of milk, without increasing oxidized flavour (J. Dairy Res. 47, 287-294, 1979).

Improvement of rancid flavour in milk, with protected rape seed oil in the feed. Feeding protected low erucic rape seed oil to cows in negative energy balance acted against rancidity brought about by under-feeding. The 24 cows were assigned to 8 blocks, based on yields of a 4 week preliminary period. Concentrates were adjusted each week to retain energy differences. The experimental period lasted 7 weeks and all animals were in the middle of their lactation period. The 3 groups were given the treatments:

A. Control ration i.e. energy allowances according to Norw. standards.

B. Underfeeding i.e. 2 feed units for fattening (FFU) below control or standard/d.

C. Underfeeding i.e. 3.5 FFU below control level + 440 g protected rape seed oil/d.

Results are seen in Table 3.

Table 3. Effect of feeding protected rapeseed oil to underfed cows upon milk rancidity through 7 weeks

| Groups | A | B | C |
|---|---|---|---|
| mM FFA in milk | 1.0 | 1.6 | 1.1 |
| rancid flavour score | 1.4 | 2.4 | 1.9 |

Lipolysis increased from 1.0 to 1.6 mM FFA, and rancid flavour scores from 1.4 to 2.4 in underfed group (B). Lipolysis and rancid flavour scores were reduced from 1.6 to 1.1 and from 2.4 to 1.9, respectively, when protected rape seed oil was fed (C). The effect of rape seed oil may have substituted the lipoprotein lipase in compensating the low energy supply.

Rape seed and rancid flavour. A 7 week experiment with rape seed to 8 cows compared with 8 cows in control did not show any effect of rape seeds on rancidity in milk. The animals were given 20% crushed rapeseeds in the concentrate. Control group was fed soybean meal as only protein. 20% barley was given to compensate energy of fat. Results showed average content of free fatty acids to be 0.90 mM in control and 0.78 mM in rapeseed meal group. Also flavour scores came out equal. The seeds were Swedish grown with little erucic acids but normal content of glycosinolates.

Rape seed meal and rancid flavour. Normal Swedish glucosinolate containing meal and Canadian low level glucosinolate meal (Canola) was compared with soybean meal with 8 cows in the groups. The rapeseed groups got 2.6 kg meal, the control 2.0 kg soybean meal. Barley was used to cover energy requirement for production. The cows yielded 21 kg milk at start. Roughage and maintenance requirement was covered by 3 FFU grass silage and 1 FFU ammoniated barley straw. The experimental feeding lasted 6 weeks with 4 weeks for and after periods. Milk was scored and analyzed by an expert judge, as previously.

Table 4. Average differences in flavour scores, free fatty acids and fat corrected milk between experimental and control periods of the groups

| | Soybean meal | Swedish meal | Canadian meal |
|---|---|---|---|
| Unclean, score | -0.18 | 0.08 | 0.27 |
| Rancid, score | -0.64 | -0.79 | -0.69 |
| mM FFA | -0.01 | 0.03 | -0.03 |
| FCM, kg | -0.21 | -0.04 | 0.12 |

Except for the lowered rancid taste score for all groups, there were no statistical significant differences. The response of the meals were equal between the groups.

Rancidity of milk in thyroxine treatment. Twice a day 100 mg of thyroxine put on the concentrates for 5 days caused a spontaneous reduction in rancid flavour in milk. The effect was still present the week after the supplement. This effect was highly significant for rancid flavour score, content of free fatty acids and FCM.

The results are indirect indications, only, of increased rancidity when feeding goitrogenic supplements.

Table 5. The effect of thyroxine upon milk FCM, FFA and taste

| | kg FCM | mM FFA | Flavour scores | | |
|---|---|---|---|---|---|
| Week | | | Rancid | Unclean | Oxidized |
| 1 | 18.9 | 1.28 | 1.32 | 1.07 | 0 |
| 2 | 18.1 | 1.46 | 1.53 | 1.23 | 0 |
| 3 Thyroxine | 21.2 | 0.76 | 0.54 | 1.08 | 0.30 |
| 4 | 19.1 | 1.26 | 0.80 | 1.17 | 0.14 |
| 5 | 16.8 | 1.50 | 2.29 | 1.30 | 0 |
| 6 | 16.1 | 1.44 | 1.36 | 1.53 | 0 |

Unclean and unspecified tastes in milk in rapeseed feeding. The exact nature of unclean taste is not known and is usually graded together with unspecified offtaste of milk. In one of the early experiments with thyroxine (Meldinger fra Norges landbrukshøgskole, 45, No 22, 1966). Rape seed feeding (1.25 kg) when tested against linseed and linseed + thyroxine produced a small but significant increase in unclean taste.

Table 6. The effect of rape seed upon unclean taste

| | Control | Thyroxine | Rape seed |
|---|---|---|---|
| Oxidized flavour score | 1.75 | 2.58 | 1.25 |
| Unclean flavour score | 0.58 | 0.33 | 1.33 |

An unspecified off flavour was obtained by Orth and Kaufmann (Kiel Milchwirt. Forsch. Ber. 16, 245-250, 1964) when feeding mustard oil. An off flavour on milk was reported with double low (Erglu) meal by Just (NJF seminar, Göteborg 1980, Proc. 69-82).

Conclusion. Rape seed protects against oxidized flavour in milk. Protected rape seed oil acted against rancid flavour in milk.

Experiments with rape seeds and with rape seed meal did not produce significant increase in rancid flavour. Unclean taste was noted in one experiment with rape seed feeding, and not in others. Unclean taste and unspecified off flavours with rape seed feeding may occur, but is obviously not persistant, not easily detectable, and thus of less significance, particularly in situations where rape seeds is used against oxidized flavour.

# ZINC AVAILABILITY IN UNHEATED AND HEATED SEEDS OF BRASSICA JUNCEA CV RLM 198

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## INTRODUCTION

Temperature is a key-factor in the processing of Brassica oil seeds. During recovery of oil, seeds are submitted to increasing temperatures varying considerably in magnitude and duration depending on the proceedure applied (1,2,3). In a small scale village process the increase in temperature is moderate and may last for some hours. In larger processing units the temperature may approach 100°C and the duration in time may differ from minutes up to hours. The amount of water present during the temperature increase will also vary considerably in different processes.

Temperature, time and amount of water have a great impact on the quality of the seed products in terms of toxicity, availability of nutrients and acceptance in food and feed Antinutritional factors of Brassica seeds have been identified as hydrolysis products derived from glucosinolates. Furthermore phytic acid and other substances interfere with trace mineral availability. The biological activity of these seed factors is very much dependent on processing conditions and will impaire the utilization of energy and nutrients in growing and pregnant animals (4,5,6). The acceptance in foods and feeds is generally reduced by hydrolysis products from glucosinolates. However, for a particular group of Indian consumer of mustard and rapeseed oil, some of the toxic principals, so far not identified will enhence the acceptance of the oil. The significance of this toxicity for the Indian consumer is at present unknown.

Elevated temperatures in the presence of water is commonly reducing the dietary availability of zinc in Brassica napus and campestris (6,7,8,9). In any attempt to improve a process for the recovery of Brassica seed nutrients for food and feed utilization temperature, time and water are parameters of vital importance.

Recently Indian mustard Brassica juncea cv RLM 198, high in glucosinolates, has been demonstrated to possess unique properties with respect to availability of dietary zinc even at elevated temperatures (8). In order to separate different seed constituents and biologically identify those determining the availability of trace minerals, toxicity has to be controled within certain limits to permit succesful feeding trials. The present study was undertaken to find temperature, time and water conditions for minimum influence on bioavailability of seed zinc.

## MATERIAL AND METHODS

Preparation of seed meals: Seeds of Indian mustard Brassica juncea cv RLM 198 were lyophilized and grind twice in a roller mill at 20°C (Fig.1). The seed meal was deoiled in hexane at 20°C as described before (6) and stored at -20°C. Three batches of the deoiled meal were heated in deionized water at 90°C for 2, 4 and 8 minutes respectively. Meal to water ratio was 1:20. Meal and water mixture was cooled by adding ice and then frozen and lyophilized.

Chemical analysis: Zinc in meal and serum was analyzed by atomic absorption spectroscopy after wet ashing in perchloric acid and hydrogen peroxide (1:2).

Bioassay: Weanling male rats of the Sprague-Dawley strain from a commercial breedar (Anticimex, Sollentuna, Sweden) were housed in polypropylene cages with an open-grill stainless steel floor. Lighting, humidity and temperature were controled. Deionized water and food were given ad libitum for 7 days. Diets were formulated to contain 1.5, 3.0, 4.5, 6.0 and 9.0 ppm of zinc from seed meal or $ZnSO_4 \cdot 7\ H_2O$. Adequate amounts of other nutrients were incorporated in the diets as described elsewhere (10). Blood was obtained from the tail and linear correlation was calculated between serum zinc and dietary zinc. Percent available zinc was obtained from the ratio between slope of sample and slope of pure zinc sulphate.

Statistical analysis: Statistical evaluation of the slope-ratio assay was performed as described elsewhere (10).

## RESULTS AND DISCUSSION

Heating of deoiled seedmeal 2, 4 and 8 minutes did not change the zinc content significantly (Table 1). Bioavailability of seed zinc in unheated meal was high. Heating in large volymes of deionized water reduced zinc availability significantly but not to the same extent as reported for meals and protein concentrates of Brassica napus and campestris heated in tap water (7,8,9).

## ACKNOWLEDGEMENT

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Fig 1:

Preparation of seed meal for feeding trials

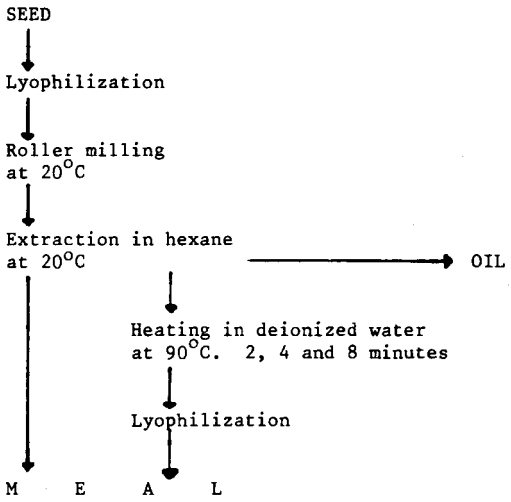


Table 1.

Influence of heat treatment on bioavailability of native seed zinc in Brassica juncea cv RLM 198

| Meal No | Heating time minutes | Zinc content ug/g | Bioavailable Zn ug/g | % |
|---|---|---|---|---|
| 1 | 0 | 91 | 67 | 74 |
| 2 | 2 | 94 | 60 | 64 |
| 3 | 4 | 92 | 39 | 42 |
| 4 | 8 | 93 | 49 | 53 |

# The Influence of Graded Levels of Protein on the Utilization of Nitrogen and Amino Acids in Canola Meal by Starter Pigs

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Previous studies for determining the protein quality and amino acid (AA) digestibility of canola meal (CM) and/or soybean meal (SBM) for swine have been primarily performed with growing and finishing pigs. Available information on the biological value (BV), net protein utilization (NPU) and AA digestibility of CM protein in starter pig diets is few and inconsistent. The objectives of this experiment were: 1) to determine the digestibility and retention of CM protein (N x 6.25) and 2) to determine the digestibilities among AA in CM when included in isoenergetic starter pig diets at graded levels.

## MATERIALS AND METHODS

Eight crossbred barrows, four from each of two litters, with an average initial weight of 9.0 kg were assigned to a 36 day nitrogen balance study. Animals were allotted to one of four dietary treatments on the basis of initial weight in a replicated 4x4 Latin square design. Protein in the diet was supplied by CM replacing cornstarch at levels of 7.8, 13.2, 17.0 and 23.8% (Table 1). The diets were balanced for digestible energy by the addition of corn oil.

Pigs were fed the diets in the form of a mash at a level of approximately 4% of body weight in three equal amounts at 0800, 1300 and 1800 h. Water was supplied ad libitum. The barrows were individually confined in 0.5 x 1.0 m metabolic crates which permitted separation and quantitative collection of feces and urine. Each of the four test periods lasted nine days. Pigs were allowed to adapt to each diet for the first five days of each period and feces and urine were collected for the next four days. Estimates of metabolic fecal nitrogen (N) and AA were determined according to the regression method (Mitchell 1924).

Data were analyzed by the least squares analysis of variance and differences between means were assessed using Student-Newman-Keuls (SNK) procedure (Steele and Torrie 1980). An additional analysis was computed in which treatment sum of squares was sub-divided into linear (L), quadratic (Q) and cubic (C) response curves (RC) and tested for significance ($P<0.05$).

## RESULTS

The composition and chemical analyses of the diets are presented in Table 1. The pigs gained an average of 168 g/day in liveweight during the 36 day trial. The apparent digestibility co-efficients (ADC) for dry matter (DM) and energy

linearly ($P<0.05$) decreased as the level of protein in the diet increased (Table 2). The ADC for N, apparent N balance and apparent NPU linearly ($P<0.05$) increased as the protein level in the diet increased. There was also a linear ($P<0.055$) increase in the apparent BV with increased levels of protein (Table 2).

The true N balance increased linearly ($P<0.05$) with increased protein level. The level of protein intake had no significant effect on true N digestion, true BV or true NPU. The co-efficients for true BV and NPU were 70.5 and 57.7%, respectively. The apparent digestibilities among all the indispensable AA linearly ($P<0.05$) and/or quadratically ($P<0.05$) increased as the dietary protein level increased (Table 3). The indispensable AA which had the lowest average apparent digestibility among the four levels of protein tested were isoleucine (73.7%), phenylalanine (74.3%), threonine (74.4%) and methionine (74.8%). No differences in the true digestibilities among the AA measured were detected (Table 3) as the level of CM protein in the diet increased.

## DISCUSSION

The observed differences among the ADC for DM and energy were most probably due to the increase in CM (high in fibre) at the expense of cornstarch which is low in fibre and high in digestible energy.

It is generally accepted that under standardized conditions, as the level of protein in the diet increases, apparent N and AA digestibility, BV and NPU increase, true BV and NPU decrease and the true N and AA digestibility remain constant (Eggum 1973). The only unexpected results in this study, therefore, were that true BV and NPU remained constant rather than decreased with increased protein levels. Partial explanation for this may be the affect of dietary fibre on microbial fermentation in the hindgut and fecal N excretion (Sauer et al. 1982). The calculated values for the true BV and NPU of CM for starter pigs were 70.5 and 57.7%, respectively.

## SUMMARY

The increased levels of CM protein in the diet of starter pigs resulted in a linear ($P<0.05$) decrease in the apparent digestibility of DM and energy and a linear increase in the apparent digestibility of N, nitrogen balance, BV and NPU. The level of crude protein had no significant effect on true N digestion (81.6%), true BV (70.5%) or true NPU (57.7%). The apparent digestibilities among the indispensable AA linearly ($P<0.05$) and/or quadratically ($P<0.05$) increased as the level of dietary protein increased. The level of protein in the diet had no significant effect on the true digestibility among the dispensable AA, with isoleucine (80.3%), threonine (80.3%), phenylalanine (81.8%) and leucine (82.0%) having the lowest average true digestibilities.

ACKNOWLEDGEMENTS

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TABLE 1. FORMULATION AND CHEMICAL COMPOSITION OF CANOLA MEAL (CM) SUPPLEMENTED DIETS CONTAINING FOUR GRADED LEVELS OF PROTEIN

| LEVEL OF CRUDE PROTEIN (%): | 7.8 | 13.2 | 17.0 | 23.8 |
|---|---|---|---|---|
| INGREDIENTS (%): | | | | |
| Canola Meal (36.5% CP) | 16.4 | 32.9 | 49.3 | 65.8 |
| Corn Starch | 69.6 | 51.1 | 32.7 | 14.2 |
| Dextrose | 10.0 | 10.0 | 10.0 | 10.0 |
| Corn Oil | -- | 2.0 | 4.0 | 6.0 |
| Premix[1] | 4.0 | 4.0 | 4.0 | 4.0 |
| CHEMICAL ANALYSIS[2] | | | | |
| Dry Matter (%) | 89.40 | 89.60 | 90.10 | 90.90 |
| Digestible Energy (MJ/kg) | 13.42 | 13.49 | 13.84 | 13.94 |
| Ether Extract (%) | 0.76 | 2.12 | 4.61 | 7.08 |
| Acid Detergent Fibre (%) | 4.84 | 7.65 | 10.34 | 14.19 |
| Neutral Detergent Fibre (%) | 7.35 | 12.50 | 15.42 | 18.96 |
| INDISPENSABLE AA'S (%) | | | | |
| Arginine | 0.46 | 0.80 | 1.04 | 1.45 |
| Histidine | 0.22 | 0.38 | 0.49 | 0.68 |
| Isoleucine | 0.32 | 0.55 | 0.70 | 0.97 |
| Leucine | 0.60 | 1.03 | 1.30 | 1.79 |
| Lysine | 0.44 | 0.75 | 0.98 | 1.34 |
| Methionine | 0.12 | 0.23 | 0.30 | 0.44 |
| Phenylalanine | 0.32 | 0.54 | 0.68 | 0.93 |
| Threonine | 0.35 | 0.62 | 0.80 | 1.08 |
| Valine | 0.41 | 0.70 | 0.91 | 1.24 |

[1] Starter premix provided the following per kg of diet: 5g iodized salt, 15g calcium phosphate, 10g ground limestone, 120.0 mg zinc, 12.0 mg manganese, 150.0 mg iron, 12.0 mg copper, 0.1 mg sodium, 0.12 mg cobalt, 0.04 mg magnesium, 0.1 mg selenium, 193.0 mg calcium, 5000 IU vitamin A, 500 IU vitamin D, 23 IU vitamin E, 4.0 mg vitamin K, 12 mg riboflavin, 45 mg niacin, 25 mg calcium pantothenate, 30 ug vitamin B, 500 mg choline chloride, 0.2 mg biotin, 1.5 mg pyridoxine, 1.0 mg folic acid, 0.15 g ethoxyquin, 275 mg ASP 250.

[2] Determined values reported on an as-fed basis.

TABLE 2. DAILY UTILIZATION OF DRY MATTER, ENERGY AND NITROGEN (N) IN CANOLA MEAL (CM) BY STARTER PIGS FED DIETS CONTAINING FOUR GRADED LEVELS OF PROTEIN[4]

| LEVEL OF CRUDE PROTEIN (%) | 7.8 | 13.2 | 17.0 | 23.8 | RC[1] |
|---|---|---|---|---|---|
| CRITERIA | | | | | |
| DIGESTIBILITIES (%): | | | | | |
| Dry Matter | 88.9 a | 85.1 b | 81.9 c | 77.5 d | L |
| Ether Extract | 72.1 a | 82.1 b | 87.4 c | 90.3 c | L,Q |
| Acid Detergent Fibre | 39.1 | 39.0 | 40.4 | 45.3 | NS |
| Neutral Detergent Fibre | 58.8 | 64.9 | 63.1 | 61.1 | Q |
| Digestible Energy | 88.4 a | 84.3 b | 81.6 c | 78.0 d | L |
| DETERMINATIONS: | | | | | |
| Apparent N digestion (%) | 69.8 a | 75.6 b | 75.2 b | 77.7 b | L |
| Apparent N Absorption (g/day) | 4.1 a | 7.4 b | 9.4 c | 13.7 d | L |
| Apparent N Retention (g/day) | 2.5 a | 4.8 b | 6.2 c | 9.1 d | L |
| Apparent BV (%)[2] | 61.0 | 65.5 | 65.7 | 66.3 | NS |
| Apparent NPU (%)[3] | 42.7 a | 49.5 b | 49.5 b | 51.6 b | L |
| True N Digestion (%) | 81.6 | 82.5 | 80.7 | 81.6 | NS |
| True N Absorption (g/day) | 4.7 a | 8.1 b | 10.1 c | 14.3 d | L |
| True N Retention (g/day) | 3.3 a | 5.7 b | 7.1 c | 9.9 d | L |
| True BV (%) | 70.2 | 70.5 | 69.7 | 69.1 | NS |
| True NPU (%) | 57.3 | 58.2 | 56.3 | 56.5 | NS |

[1]RC, significance ($P<0.05$) of the linear (L) or quadratic (Q) response curves (RC) to the dietary level of protein (NS being not significant).

[2]Biological value, N retained expressed as a percentage of the N absorbed.

[3]Net Protein Utilization, N retention expressed as a percentage of the gross N intake.

[4]a-d, means within the same row with the same or no letter are significantly different ($P<0.05$).

TABLE 3. APPARENT AND TRUE FECAL AVAILABILITIES OF AMINO ACIDS (AA) IN CANOLA MEAL (CM) SUPPLEMENTED DIETS FED AT GRADED LEVELS OF PROTEIN TO STARTER PIGS[2]

| LEVEL OF CRUDE PROTEIN (%) | 7.8 | 13.2 | 17.0 | 23.8 | RC[1] |
|---|---|---|---|---|---|
| CRITERIA | | | | | |
| APPARENT AA (%) | | | | | |
| Arginine | 81.7 a | 86.3 b | 86.4 b | 87.5 b | L,Q |
| Histidine | 82.3 a | 86.0 b | 85.6 b | 86.7 b | L,Q |
| Isoleucine | 68.9 a | 75.2 b | 74.4 b | 76.3 b | L,Q |
| Leucine | 70.1 a | 76.6 b | 76.0 b | 77.7 b | L,Q |
| Lysine | 74.4 a | 79.6 b | 79.6 b | 80.4 b | L,Q |
| Methionine | 65.5 a | 76.3 b | 77.1 b | 80.1 b | L,Q |
| Phenylalanine | 69.5 a | 75.8 b | 75.1 b | 76.8 b | L,Q |
| Threonine | 69.1 a | 76.4 b | 75.4 b | 76.7 b | L,Q |
| Valine | 71.5 a | 74.5 ab | 77.4 b | 79.0 b | L |
| TRUE AA (%) | | | | | |
| Arginine | 88.7 | 90.4 | 89.4 | 89.6 | NS |
| Histidine | 87.9 | 89.3 | 88.1 | 88.5 | NS |
| Isoleucine | 79.6 | 81.6 | 79.5 | 80.1 | NS |
| Leucine | 81.5 | 83.3 | 81.4 | 81.7 | NS |
| Lysine | 82.0 | 84.2 | 82.8 | 82.8 | NS |
| Methionine | 81.0 | 84.0 | 83.3 | 84.4 | NS |
| Phenylalanine | 81.6 | 83.2 | 81.1 | 81.3 | NS |
| Threonine | 79.0 | 82.2 | 79.9 | 80.1 | NS |
| Valine | 84.3 | 82.0 | 83.0 | 83.1 | NS |

[1]RC, significance ($P<0.05$) of the linear (L) or quadratic (Q) response curves (RC) to dietary level of protein (NS being not significant).

[2]a-b, means within the same row with the same or no letter are not significantly different ($P<0.05$).

# ESSAI D'APPRECIATION DE L'ACCEPTABILITE DU TOURTEAU DE COLZA PAR TESTS SUR CAPRINS

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La faible appétibilité du tourteau de colza représente l'un des facteurs limitants de son utilisation dans l'alimentation des ruminants. La présence d'isothiocyanates expliquerait au moins en partie ce phénomène (BERANGER et GRENET, 1969).

Pour tester l'acceptabilité d'aliments tels que le tourteau de colza, la chèvre a été utilisée comme matériel animal en raison des caractéristiques de son comportement alimentaire et en particulier du choix très élaboré des aliments ou des fractions d'aliments ingérés (MORAND-FEHR et al, 1981).

Le présent travail avait pour but :

1. de vérifier d'abord la faible appétibilité des aliments composés contenant du tourteau de colza pour les chèvres.
2. de préciser si cette appétibilité diminue lorsque la proportion de tourteau de colza augmente dans l'aliment.
3. d'étudier l'interaction entre les caractères de faible appétibilité du tourteau de colza et de forte appétibilité d'autres aliments.
4. de montrer s'il y a des différences d'appétibilité entre tourteaux de colza commercialisés en France.

## I - CHOIX DES ANIMAUX

A 70 chèvres de race Alpine âgées au moins d'un an, il a été distribué des aliments concentrés de différente composition contenant ou non de l'orge, du tourteau de soja, de la mélasse de betteraves, des pulpes de betteraves déshydratées et du tourteau de colza.

Cette expérimentation a permis d'établir la typologie relative au comportement alimentaire de ce troupeau. Trois groupes ont pu être distingués: les chèvres du premier consomment sans refus tous les aliments présentés, les chèvres du second les refusent partiellement ou totalement, les animaux du troisième en refusent certains et en acceptent d'autres. Ce dernier groupe a le meilleur pouvoir discriminant vis-à-vis de l'appétibilité des aliments. Ce sont les chèvres de ce troisième groupe qui ont participé aux expériences rapportées ici.

## II - APPETIBILITE DES ALIMENTS CONTENANT DU TOURTEAU DE COLZA

12 chèvres en lactation sont placées sur caillebotis en cage individuelle, libres de leurs mouvements. Elles reçoivent matin et soir du foin de luzerne à volonté. Une heure et demi à 2 heures après la distribution de foin elles reçoivent 300 g d'aliment concentré sous forme granulée (5 mm de diamètre), soit 600 g par jour, dans une coupelle standardisée.

Successivement, il leur est distribué pendant 3 jours un aliment standard : orge 80 %, tourteau de soja 20 %, pendant 1 jour un aliment "Mélasse" : orge 75 %, tourteau de soja 19 %, mélasse de betteraves 6 %, ensuite 3 jours l'aliment standard puis pendant 1 jour un aliment tourteau de colza non toasté : orge 72 %, tourteau de soja 18 %, tourteau de colza 10 %. Cette séquence est répétée deux fois.

Résultats :

| Aliment | Standard orge-soja | Mélasse | Tourteau de colza |
|---|---|---|---|
| Niveau de consommation g/j/chèvre moyenne $\pm$ écart-type ($\bar{12}$ chèvres) | $599{,}5 \pm 1{,}3$ | $599 \pm 2{,}1$ | $189{,}8 \pm 154{,}7$ |

La présence de 10 % de tourteau de colza dans l'aliment concentré abaisse fortement l'acceptabilité de cet aliment puisque toutes les chèvres font des refus, dont 3 refusent totalement l'aliment alors que les 12 chèvres ne font pour ainsi dire aucun refus d'aliment standard ou d'aliment mélasse.

## III - EFFET DU POURCENTAGE D'INCORPORATION DU TOURTEAU DE COLZA

Les aliments contenant 5,10,15,20 % du même tourteau de colza que précédemment ont été comparés dans un essai du même type. Comme les résultats n'ont pas été très nets, nous avons utilisé la méthode

des tests en "cafetéria" consistant à comparer deux aliments en libre choix. Chaque chèvre est mise pendant 3 minutes devant un présentoir où ont été placées quatre coupelles qui contiennent 300 g d'aliment. Le même aliment était présent dans deux coupelles.

Deux aliments à 10 et 30 % de tourteau de colza et contenant par ailleurs un mélange orge-tourteau de soja dans un rapport 4/1, ont été testés sur 10 chèvres.

Résultats :

| Aliment | Tourteau colza 10 % | Tourteau colza 30 % |
|---|---|---|
| Niveau de consommation g /chèvre moyenne + écart-type (10 chèvres) | 241,4 ± 145,4 | 65,1 ± 99,7 |

Les chèvres en présence des 2 aliments discriminent les taux d'incorporation du tourteau de colza. Ces résultats confirment des essais de comparaison indirecte où l'un des deux aliments colza est testé en présence de l'aliment standard ou de l'aliment mélasse.

Ainsi, les chèvres généralement font nettement plus de refus sur les aliments colza. Présentés seuls, les aliments colza semblent refusés dans des proportions faiblement influencées par le taux d'incorporation (au-dessus de 5 %). Présentés simultanément, les chèvres ont une préférence pour les aliments à bas taux d'incorporation.

## IV - INTERACTION ENTRE L'ACCEPTABILITE DU TOURTEAU DE COLZA ET CELLE DE LA MELASSE ET DES PULPES DE BETTERAVES.

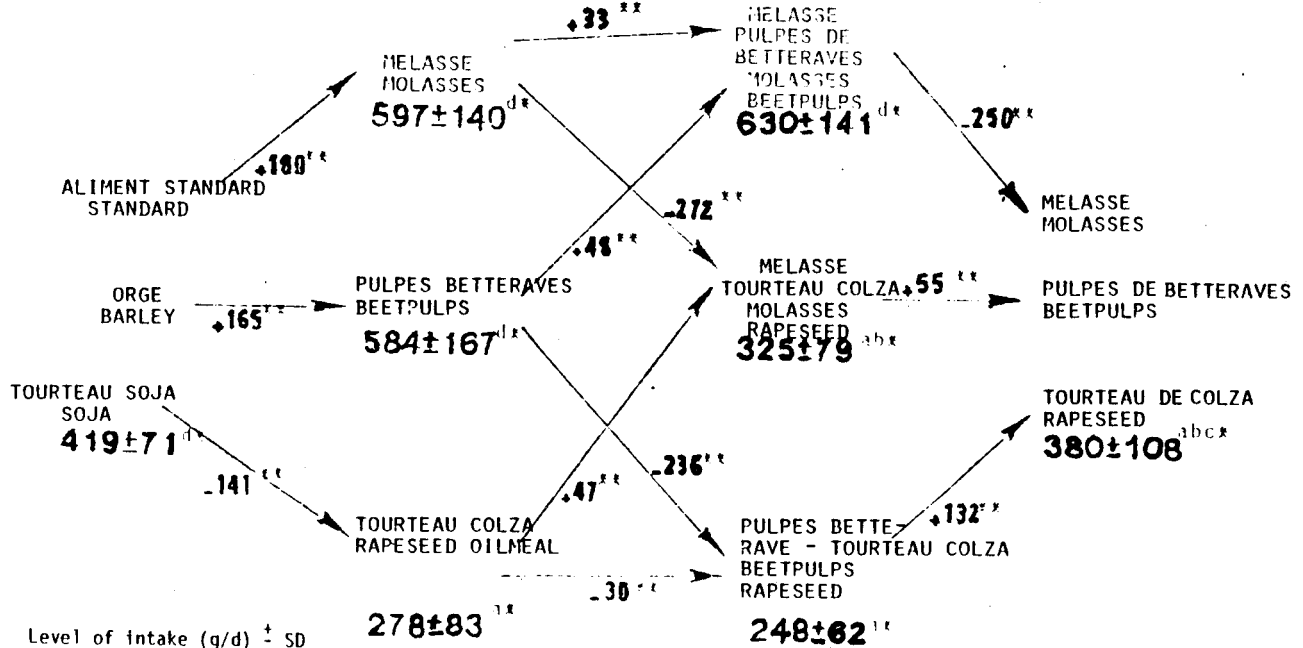
Nous avons voulu savoir si la faible acceptabilité du tourteau de colza peut être réduite partiellement ou totalement par la présence d'aliments tels que la mélasse, qui généralement augmente l'acceptabilité de l'aliment composé et des pulpes de betteraves qui a la même propriété mais de façon nettement moins marquée.

Huit chèvres placées dans les mêmes conditions que dans le premier essai, reçoivent suivant un dispositif expérimental de carré latin, 8 aliments successivement pendant 8 périodes de 4 jours à raison de 2 repas de 300 g par jour. L'aliment standard était composé de 80 % d'escourgeon et de 20 % de tourteau de soja ; il était de moyenne acceptabilité. A ce mélange, on incorporait soit séparément 10 % de tourteau de colza (aliment colza), 6 % de mélasse de betteraves (aliment mélasse), 30 % de pulpes de betteraves déshydratées (aliment pulpes), soit 2 à 2 (aliments Mélasse-Colza, Pulpes-Colza, Mélasse-Pulpes) soit les 3 en même temps (aliment Mélasse-Pulpes-Colza).

ACCEPTABILITE DES ALIMENTS COMPOSES CONTENANT DU TOURTEAU DE COLZA CONTENANT OU NON DE LA MELASSE ET DES PULPES DE BETTERAVES DESHYDRATEES CHEZ LA CHEVRE.

PALATABILITY OF CONCENTRATES INCLUDING RAPE OILMEAL WITH OR WITHOUT MOLASSES AND BEETPULPS IN GOATS.

FIGURE 1




Level of intake (g/d) ± SD

* NIVEAUX D'INGESTION en g/j ± ECART-TYPE - toutes les valeurs d'ingestion n'étant affecté d'aucune lettre identique sont significativement différentes au seuil de 5 %. Two values of LEVEL OF INTAKE without same letters are significantly different (P ≤ 5%).

** Différence de niveau d'ingestion entre 2 aliments. Difference of intake level between 2 feeds.

Les résultats sont présentés à la figure 1. Le tourteau de colza réduit l'acceptabilité de l'aliment. La présence simultanée de mélasse ou de pulpes avec le tourteau de colza n'a pas d'effets améliorateurs alors que la présence simultanée de mélasse et de pulpes réduit partiellement l'effet négatif du tourteau de colza. Ainsi cet effet semble prépondérant par rapport à l'effet favorable de la mélasse et des pulpes qui se manifeste surtout en l'absence de tourteau de colza.

## V - ACCEPTABILITE DE DIFFERENTS TOURTEAUX DE COLZA

Nous avons voulu savoir si les tourteaux de colza commercialisés en France présentaient des acceptabilités variables. Pour cela, nous avons testé trois tourteaux de colza provenant de trois unités de fabrication différentes. Ils sont incorporés à raison de 10 % dans l'aliment composé. Ces aliments ont d'abord été comparés, distribués seuls dans les conditions du premier essai. Les résultats étant non significatifs malgré une tendance à ce qu'un tourteau de colza (tourteau A) soit plus aisément accepté que les 2 autres, nous avons utilisé la méthode de la cafetéria.

A 10 chèvres, les trois aliments étaient présentés simultanément pendant 4 minutes (2 coupelles par aliment, 200 g par coupelle).

Résultats :

| Aliment | Tourteau colza A 10 % | Tourteau colza B 10 % | Tourteau colza C 10 % |
|---|---|---|---|
| - Niveau d'ingestion g/chèvre - Moyenne + écart-type 10 chèvres | $208^{+}_{-}48$ | $147^{+}_{-}50$ | $139^{+}_{-}16$ |
| - Durée d'ingestion (en secondes) | $66,8^{+}_{-}23$ | $50^{+}_{-}18$ | $43^{+}_{-}10$ |
| - Quantité ingérée par seconde g/s | 3,11 | 2,94 | 3,23 |

L'aliment avec le tourteau de colza A est significativement mieux accepté par les chèvres que les autres aliments. La durée d'ingestion pour cet aliment est significativement plus longue mais les vitesses d'ingestion sont très voisines pour les 3 aliments.

Ainsi l'acceptabilité d'un tourteau de colza semble supérieure à celle des 2 autres tourteaux. Mais cette meilleure acceptabilité ne semble pas modifier la vitesse d'ingestion.

## CONCLUSION

D'après cette expérience, il semble bien que la présence de tourteau de colza dans l'aliment composé réduit assez nettement l'acceptabilité de celui-ci. La présence simultanée de mélasse ou de pulpes de betteraves ne peut masquer que partiellement la médiocre acceptabilité du colza.

Ces premiers résultats montrent que la chèvre peut discriminer des aliments contenant différents taux de tourteau de colza ou des tourteaux d'origine différente s'ils sont distribués simultanément. Distribués seuls, les mêmes tendances apparaissent mais aucune différence significative n'a pu être mise en évidence.

La chèvre s'est révélée un animal très intéressant pour comparer des aliments à appétibilité variable . Si leur appétibilité n'est pas très différente, la méthode de la cafetéria est à préconiser. Il est encore possible d'améliorer l'efficacité de ces méthodes.

Ces travaux devront être poursuivis sur les chèvres à haut pouvoir de discrimination de l'acceptabilité des aliments pour tester les tourteaux de colza ayant subi diverses technologies ou les nouvelles variétés qui sont ou seront susceptibles d'être commercialisées.

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MORAND-FEHR P., HERVIEU J. et SAUVANT D., 1982. Intern. Goat and Sheep Res, 2 (2) in press.

# COMPARAISON DE LA DIGESTIBILITE DANS LE RUMEN DES CONSTITUANTS PARIETAUX ET DU CONTENU CELLULAIRE DU TOURTEAU DE COLZA AVEC LES PRINCIPALES AUTRES SOURCES PROTEIQUES.

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## I. INTRODUCTION

Plusieurs études ont permis de caractériser la composition glucidique, de la paroi végétale en particulier, du colza et de la comparer à celle d'autres sources protéiques comme le tourteau de soja. (Nehring, 1966 ; Hoffman et Nehring, 1969 ;Rinaudo et Chambat, 1976 ; Borgida et Tollier, 1976 ; Guilbot et Tollier, 1976 ; Theander et Anan, 1977). Par contre, aucune étude n'a cherché, à notre connaissance, à comparer la dégradation dans le rumen des composés de la paroi végétale du colza à celles des principaux autres aliments riches en protéines utilisés pour les ruminants. Ce thème est l'objet de la présente communication.

## II. MATERIEL ET METHODES

Le travail a été conduit selon la méthode des sachets de nylon (Demarquilly, Chenost, 1969 ; modifiée par Bertrand, Gueneau, 1982), placés pendant 6, 12, 24 et 48 h dans le rumen de chèvres fistulées recevant un régime mixte de foin de graminées et d'aliment concentré qui permet d'assurer une couverture suffisante de leurs besoins nutritifs et de ceux des micro-organismes de la panse.

Neuf aliments fréquemment utilisés comme sources de protéines chez les ruminants ont été considérés dans ce travail (Tableau 1). Chaque aliment est l'objet de 3 répétitions de cinétiques ; à chaque cinétique, un sachet contenant la paille comme témoin

est retiré après douze heures d'incubation pour permettre de corriger les résultats par analyse de covariance.

Les teneurs en cellulose brute, C.B., N.D.F., A.D.F. et A.D.lignine ont été mesurées sur les aliments et les résidus recueillis dans les sachets aux différents temps d'incubation dans le rumen.

## III RESULTATS ET DISCUSSION

Les données du Tableau 1 indiquent que les teneurs en cellulose brute et NDF, ADF et ADL, selon Van Soest, sont assez comparables à ce qui est généralement observé pour ces matières premières (Sauvant, 1981) ; la teneur en A.D.L. du tourteau de coprah est cependant nettement supérieure à celle proposée dans la référence précédente ou citée par Rinaudo et Chambat (1976). La teneur en lignine de la paroi végétale, estimée par le rapport A.D.L./N.D.F., est de 27,9% pour le colza, -cette valeur étant conforme aux données de la bibliographie, et la plus élevée des 9 aliments considérés.

Les comparaisons des cinétiques de dégradation dans le temps des constituants des aliments sont effectuées à partir de graphiques à ordonnée logarithmique de manière à pouvoir apprécier, par les pentes, les vitesses de dégradation.

Les figures 1 et 2 rapportent respectivement les cinétiques de disparition des fractions N.D.F. et A.D.F. Les allures des cinétiques sont comparables entre les différentes matières premières pour ces deux composants. Deux groupes peuvent être distingués au sein des aliments :
- les aliments à composés pariétaux lentement dégradables qui se caractérisent en général par un ralentissement de la vitesse de dégradation au fur et à mesure que la durée d'incubation augmente. Ainsi le taux horaire de dégradation de la fraction N.D.F. du tourteau de colza est respectivement de 2,0, 5,7, 0,7 et 1,0%/h pour lesquatre intervalles de temps considérés successivement. Outre le tourteau de colza, la luzerne déshydratée, le tourteau de tournesol et d'arachide appartiennent à ce groupe. La dégradabilité en 48 heures des composés de la paroi (N.D.F. et A.D.F.) de ces ingrédients est faible : 49 et 29% pour le tourteau de colza ; 51 et 47% pour la luzerne déshydratée ; 39 et 20% pour le tourteau de tournesol ; 57 et 37% pour le tourteau d'arachide.

- pour les autres aliments, la dégradabilité des constituants de la paroi (N.D.F. et A.D.F.) en 48 heures, ou même dans un plus court laps de temps pour le lupin et le tourteau de soja, est plus

importante : 75 et 65% pour le tourteau de palmiste ; 79 et 62% pour le tourteau de coprah ; 85 et 78% pour le tourteau de germe de maïs. Les matières premières de ce groupe se caractérisent par une très faible teneur en lignine (Tableau 1), sauf pour les tourteaux de palmiste et de coprah qui se caractérisent par des temps de latence importants (6 à 12 h) avant la mise en place des processus de dégradation de la paroi végétale.

La Figure 3 traduit les cinétiques de dégradation des contenus cellulaires estimés par les fractions solubles dans le détergent neutre de Van Soest (N.D.S.) Les taux horaires de disparition, entre 0 et 6 heures, de la fraction N.D.S. varient entre 10 et 30% ; ils semblent être largement déterminés par les différences connues pour ces aliments de solubilité et de dégradabilité de l'azote (Fig.4). Après 6 heures d'incubation, la fraction N.D.S. du tourteau de colza s'effectue à une vitesse horaire plus faible que celle des autres aliments considérés de sorte qu'après 24 ou 48 h d'incubation, le colza est l'aliment qui présente la fraction N.D.S. non dégradée la plus importante. Lindberg et Varvikko (1982) ont déjà remarqué que la protéine du tourteau de colza se dégradait à court terme plus rapidement, mais à long terme plus lentement que celle du tourteau de soja. Ces résultats de dégradation de la fraction N.D.S. doivent être considérés avec prudence dans la mesure où le traitement technologique l'influence vraisemblablement fortement. Lindberg et coll. (1982) ont ainsi montré que la dégradation de la fraction protéique du colza dans le rumen dépendait largement du traitement technologique qui lui était appliqué.

## IV CONCLUSION

Cette étude a confirmé la richesse en lignine de la paroi végétale du tourteau de colza. Ce degré de lignification important est à l'origine de la faible dégradabilité de cette paroi végétale. Les composés cellulaires du tourteau de colza étudié sont également lentement dégradés en raison, peut-être, de la faible dégradabilité de la protéine, du rôle de barrière joué par les parois végétales peu dégradables et éventuellement en relation avec d'autres caractéristiques de cet aliment non mesurées dans ce travail.

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TABLEAU 1. Teneur en constituants pariétaux des différentes matières premières étudiées (en % de la matière sèche).

| | cellulose brute | N.D.F. | A.D.F. | A.D. Lignine |
|---|---|---|---|---|
| T. de soja | 4,2 | 6,7 | 3,7 | 0,2 |
| T. de germe de maïs | 10,1 | 38,2 | 11,4 | 1,0 |
| T. de colza | 10,2 | 30,5 | 18,8 | 8,5 |
| T. d'arachide | 13,0 | 18,5 | 14,0 | 4,0 |
| T. de palmiste | 18,0 | 78,2 | 44,4 | 10,6 |
| T. de coprah | 18,1 | 60,1 | 34,3 | 9,5 |
| T. de tournesol | 22,6 | 36,3 | 23,7 | 6,9 |
| Lupin | 12,8 | 20,6 | 14,5 | 0,7 |
| Luzerne déshydratée | 31,2 | 48,7 | 33,6 | 8,4 |

FIGURE 1.

# CINETIQUES DE LA DISPARITION DE LA FRACTION N.D.F. DANS LE RUMEN

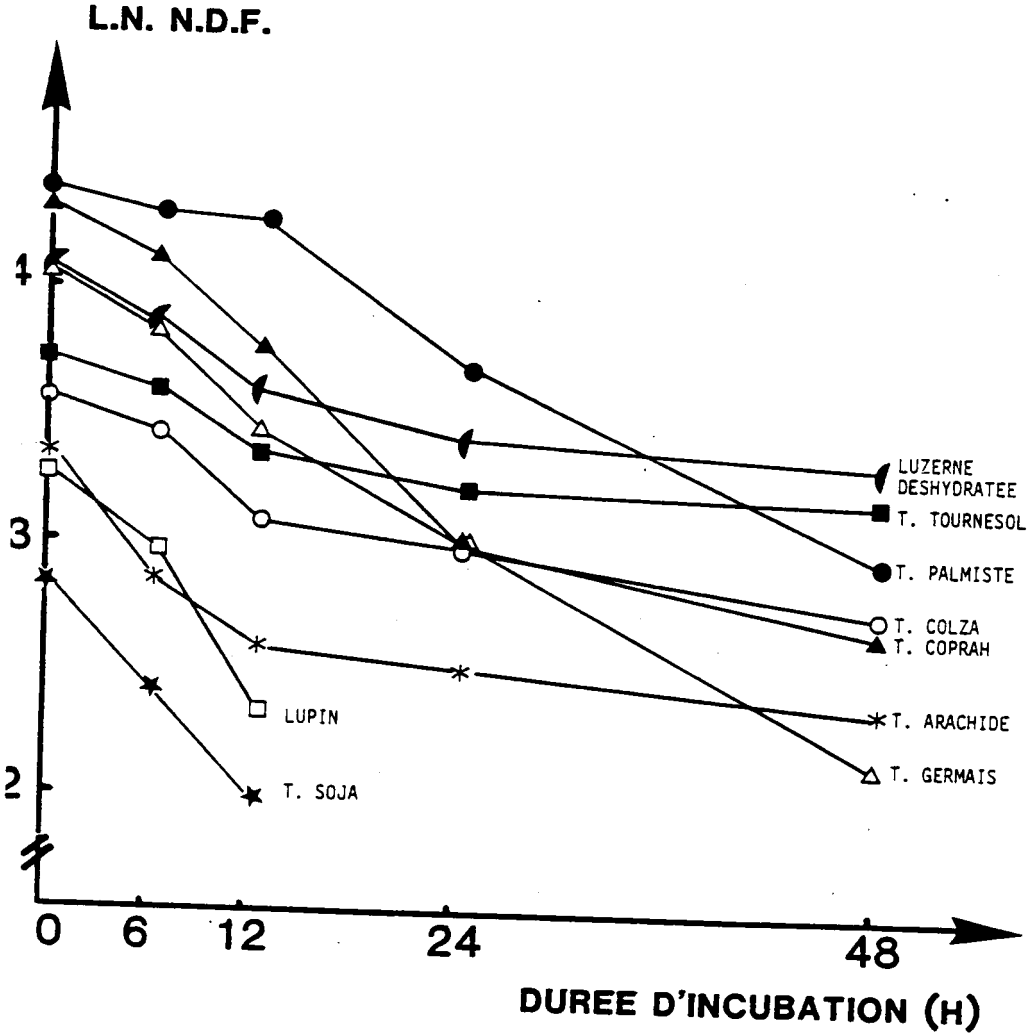


FIGURE 2.

## CINETIQUES DE LA DISPARITION DE LA FRACTION A.D.F. DANS LE RUMEN

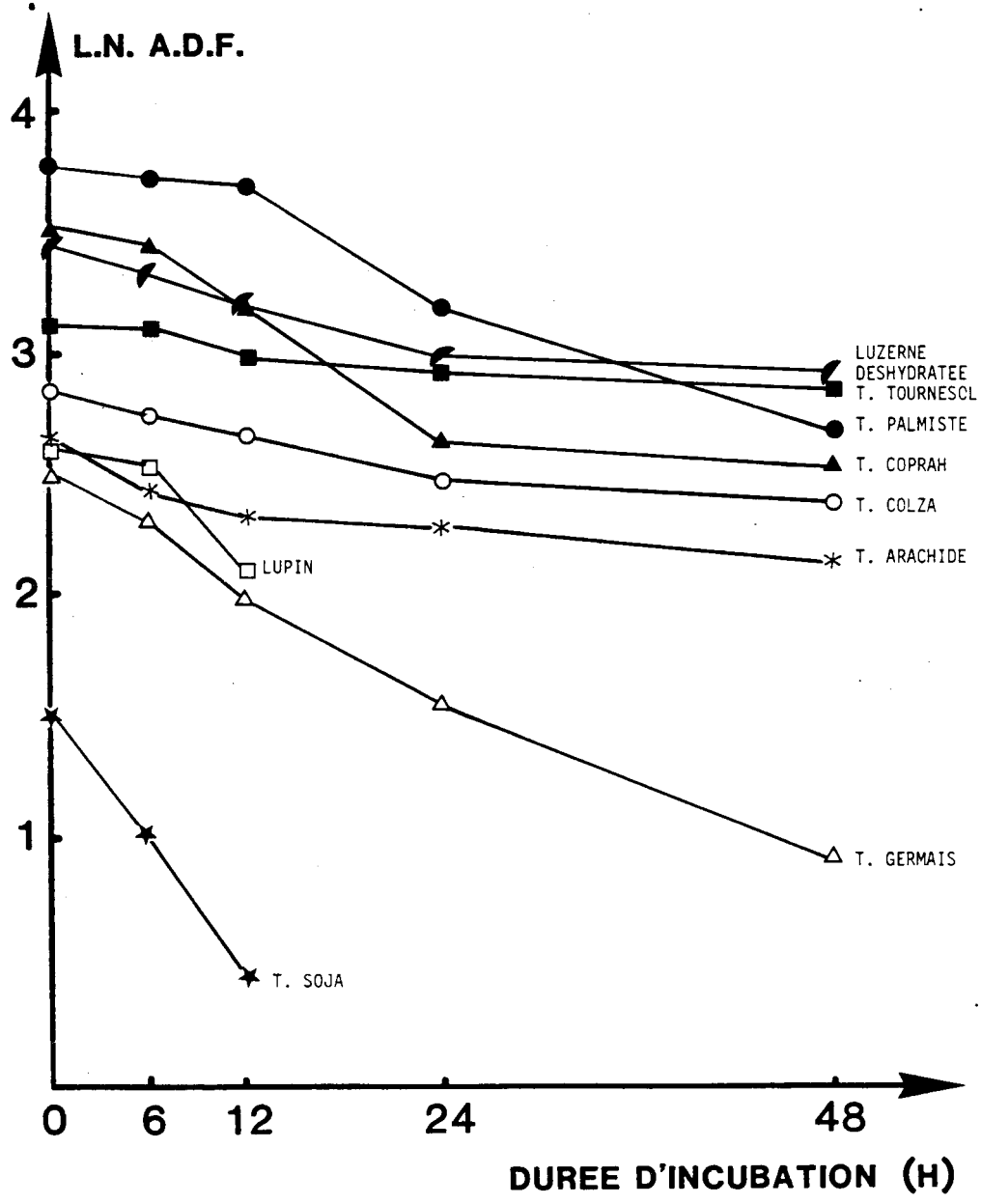


FIGURE 3.

# CINETIQUE DE DISPARITION, DANS LE RUMEN, DE LA FRACTION SOLUBILISEE PAR LE DETERGENT NEUTRE

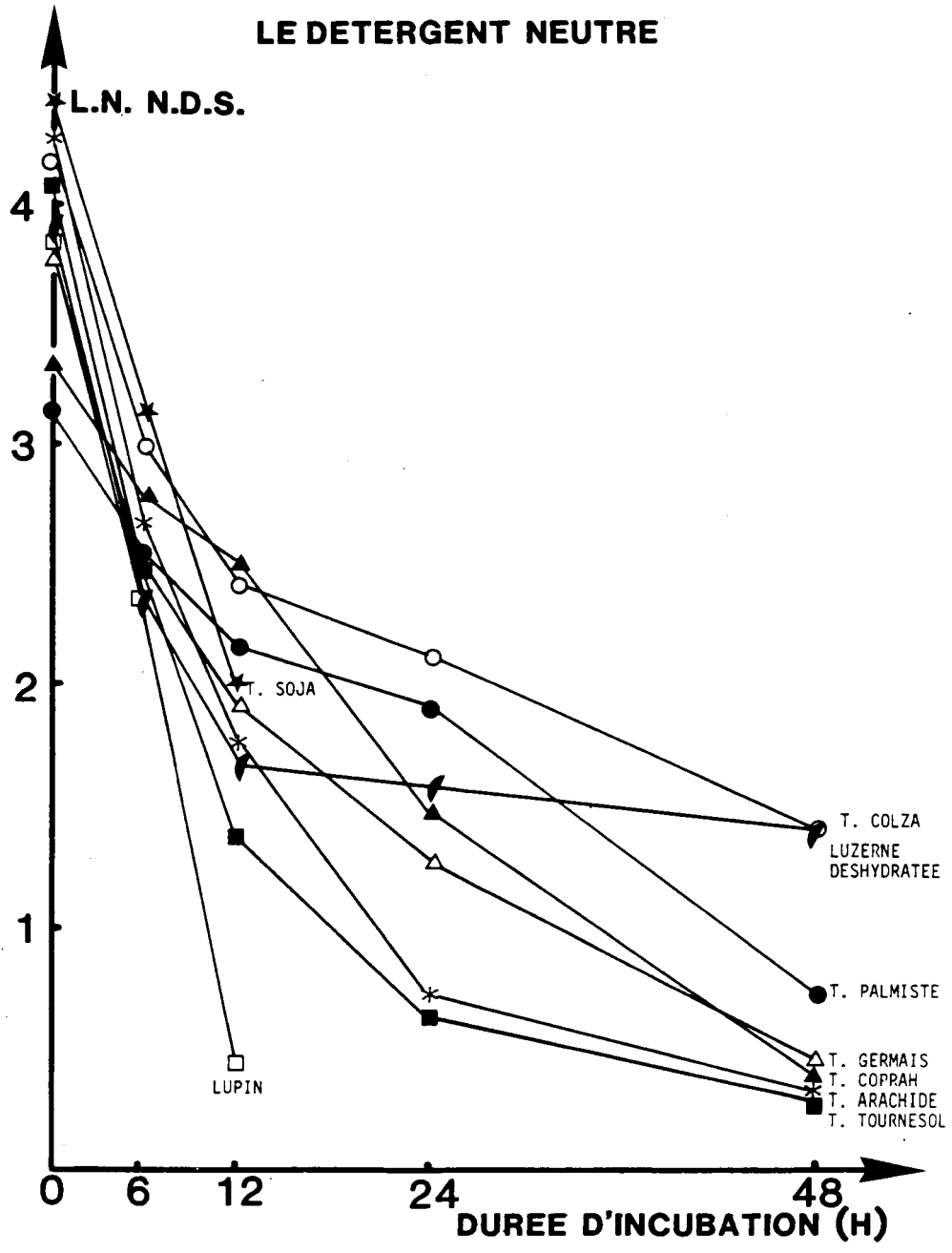
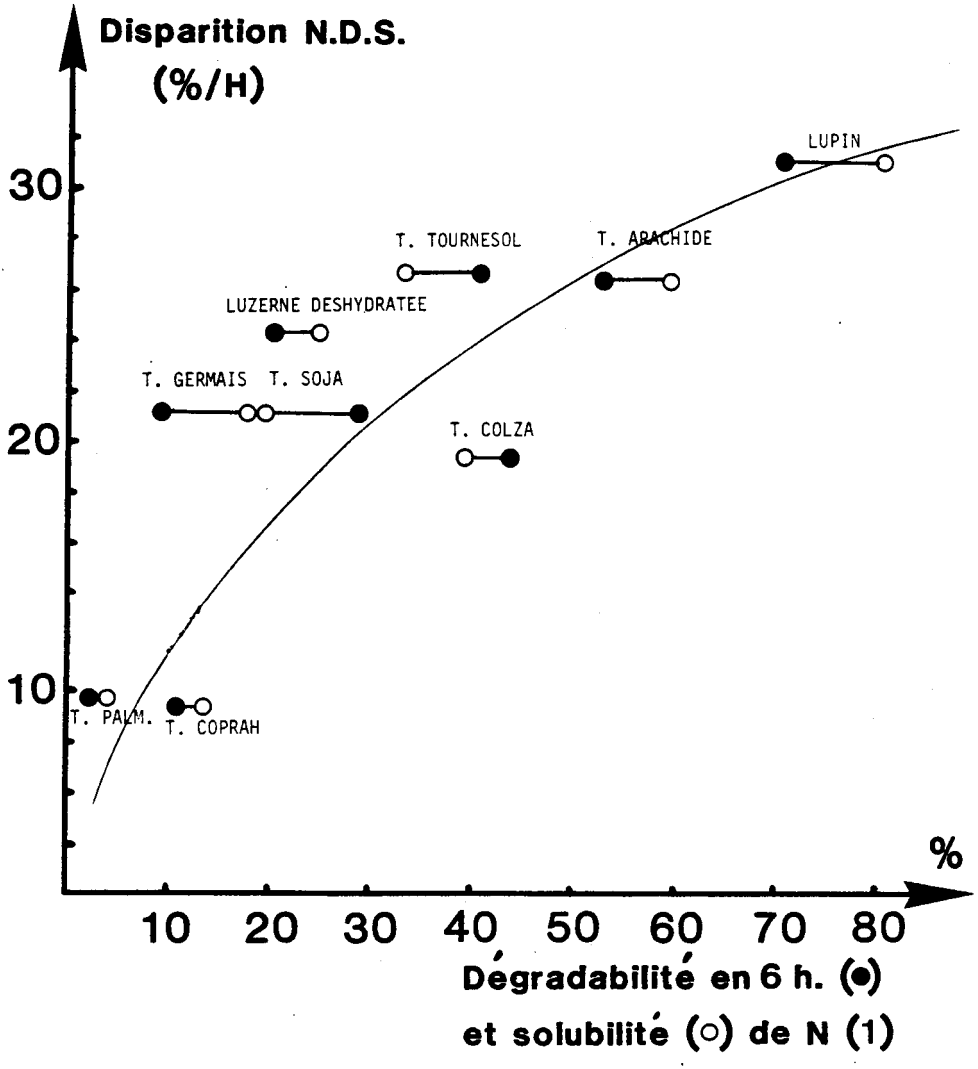


FIGURE 4.

**RELATION, DE 0 ET 6 HEURES, ENTRE LE TAUX DE DISPARITION DU N.D.S. ET LA DEGRADABILITE OU LA SOLUBILITE DE L'AZOTE**




(1) Valeurs citées par Vérité , Sauvant (1981 )

## SESSION K - SESSION K - SITZUNG K

# VALEUR NUTRITIONNELLE DE L'HUILE
# NUTRITIONAL VALUE OF THE OIL
# ERNÄHRUNGSWERT DES ÖLS

Président - Chairman -Vorsitzender : J. FLANZY

Vice-Président - Co-Chairman - Zweiter Vorsitzender : R. VLES

# EFFETS A LONG TERME DE PLUSIEURS GRAISSES ALIMENTAIRES (DONT L'HUILE DE COLZA) SUR LES LIPIDES SERIQUES D'UNE POPULATION DE RELIGIEUSES BENEDICTINES

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L'objectif de notre étude était de modifier la composition en acides gras de la ration alimentaire, et de mesurer les effets sur les lipides sériques.

## POPULATION

Cette étude porte sur 62 sujets de sexe féminin, vivant en Communauté fermée dans un monastère rural. L'âge moyen était de 52 ± 6 ans (âges extrêmes 23 et 70 ans).

Cette population institutionnalisée permettait :

- d'obtenir une adhésion totale au régime
- de limiter notre action aux seules normes diététiques
- d'éviter une interaction des autres facteurs de risque (alcool), tabac hygiène de vie...).

L'étude comportait des régimes successifs de 5 mois chacun, avec un apport isocalorique ne différant que par la composition en acides gras des lipides visibles de la ration calorique journalière. Cette partie variable de chaque période de régime représentait 20% des calories totales de la ration, apportée soit sous forme d'une huile végétale, soit sous forme de graisses du lait.

## RATION CALORIQUE

L'apport calorique moyen était de 2100 calories par jour et par personne.

Avec un apport de 81 g, les protéines représentaient 16%

de l'apport calorique quotidien. Les deux tiers des protéines étaient d'origine animale.

Avec un apport de 281 g, les hydrates de carbone représentaient 54% des calories de la ration. Le rapport entre les sucres d'assimilation lente et rapide était de 38/16.

Les lipides alimentaires fournissaient 30% des calories de la ration. Ils comportaient une part constante (un tiers de l'apport lipidique soit 24 g) invariable pendant toute la durée de l'étude, et une partie variable (2/3 de l'apport lipidique soit 48 g).

Cinq périodes ont été consacrées à des huiles et une aux graisses du lait. Pour les périodes où une huile était testée, les graisses visibles étaient composées de l'huile étudiée et d'une margarine correspondante. La phase grasse de cette margarine était constituée de 82% de l'huile étudiée et d'un concret de palme, identique pour toutes les margarines.

## APPORTS QUOTIDIENS EN ACIDES GRAS FOURNIS PAR LES LIPIDES VISIBLES

Les six graisses alimentaires qui ont été étudiées étaient dans l'ordre chronologique : l'huile de tournesol, le fluide de palme, l'huile d'arachide, les graisses du lait, l'huile de colza pauvre en acide érucique, enfin l'huile de maïs.

Les apports journaliers en acides gras essentiels étaient très variables selon la période envisagée : ainsi l'apport d'acide linoléique variait de 1 g/jour (période graisses du lait) à plus de 30 g/jour (période huile de tournesol).

Pour l'acide alpha-linolénique, tous les régimes avaient un apport très inférieur à 1 g/jour sauf pour la période colza où l'apport était de 3,6 g/jour.

Notons que pendant la période huile de tournesol, avec un apport moyen de 30,5 g/jour/personne, l'acide linoléique représentait 13,7% des calories totales de la ration, alors que l'acide alpha-linolénique, avec un apport de 0,1 g, représentait 0,05% des calories totales.

Pendant la période fluide de palme, l'apport journalier d'acide linoléique était de 6,5 g/personne (2,9% des calories totales)

et l'apport d'acide alpha-linolénique de 0,1 g/personne (0,05% des calories totales).

Dans la période consacrée aux graisses du lait, l'apport d'acide linoléique était de 0,23 g, celui d'acide alpha-linolénique de 0,54 g. Ils représentaient respectivement 0,6% et 0,24% des calories totales de la ration.

Avec la période colza, l'acide linoléique représentait 4,8% des calories totales de la ration avec un apport de 10,7 g, et l'acide alpha-linolénique représentait 1,6% des calories totales avec un apport de 3,6 g.

Le régime maïs fournissait 28,8 g/personne d'acide linoléique soit 12,9% des calories totales de la ration, et 0,42 g/personne d'acide alpha-linolénique soit 0,19% des calories totales.

## RESULTATS

### Poids :

Les valeurs moyennes de poids étaient identiques pour les six régimes étudiés.

### Cholestérol total sérique :

Il y avait des variations significatives du cholestérol total sérique. Le test F était significatif (p 0,01).

Le cholestérol total sérique le plus faible était observé après la période tournesol (1,95 g/l). Il n'y avait pas de différence significative avec le taux de cholestérol total noté à la fin de la période colza (2,00 g/l).

Après les périodes fluide de palme et arachide, la cholestérolémie était de 2,10 g/l et différait significativement (p 0,001) par rapport à la période tournesol.

Après le régime maïs, le cholestérol total sérique était de 2,20 g/l (différence significative avec celui des périodes colza et tournesol).

C'est après le régime graisses du lait que la cholestérolémie totale moyenne était la plus élevée (2,55 g/l). Elle différait significativement avec toutes les autres périodes.

Cholestérol des HDL sériques :

Sur les six régimes, cinq déterminaient un taux de cholestérol des HDL pratiquement semblable. Seule la période fluide de palme entraînait un taux de cholestérol des HDL supérieur et qui différait significativement de celui des autres périodes.

Acides gras des phospholipides sériques

Les résultats des acides gras des phospholipides permettent d'isoler deux groupes de régimes :

1er groupe : les régimes colza, fluide de palme, arachide déterminaient une composition en acides gras des phospholipides sériques très proche. Il existait une seule différence concernant l'acide eicosapentaénoïque, dont le taux était beaucoup plus élevé avec le régime colza.

2ème groupe : les trois autres régimes entraînaient des différences importantes par rapport aux trois régimes précédents et entre eux.

La teneur en acide linoléique était élevée pour les deux régimes polyinsaturés (tournesol et maïs) et faible pour le régime graisses du lait. Par contre, les dérivés supérieurs de la famille W6 (di-homo-gamma-linolénique et arachidonique) apparaissaient à des taux significativement plus bas après le régime tournesol par rapport au régime graisses du lait.

Quant au régime maïs, il entraînait une composition des phospholipides très proche de celle du régime graisses du lait.

Les régimes tournesol et maïs entraînaient des taux semblables d'acide éicosapentaénoïque proches de ceux des régimes fluide de palme et arachide ; c'est après le régime colza et graisses du lait que ce taux était le plus élevé.

COMMENTAIRES

Période de régime tournesol :

Avec un régime isocalorique, dont 20% des calories sont fournies par l'huile de tournesol, l'apport journalier moyen de 30 g

d'acide linoléique par personne pendant cinq mois entraînait une diminution des métabolites de l'acide linoléique : acide di-homo-gamma-linolénique et arachidonique. Nous sommes en présence d'un système qui paraît se situer en dehors des voies de régulation métabolique normale.
On peut suggérer qu'avec 13,7% des calories totales sous forme d'acide linoléique, il semble exister une compétition au niveau de l'acide gras en position 2 des phospholipides, ce qui entraînerait un blocage de la voie métabolique de la famille W6.

Période de régime maïs :

Sous l'effet du régime maïs, où l'acide linoléique représentait 12,9% des calories, on constate un métabolisme normal de l'acide linoléique. Il y a alors probablement un effet de l'apport alimentaire d'acide palmitique (double de celui du régime tournesol) et de l'acide alpha-linolénique (dont l'apport est 4 fois plus élevé).

Période graisses du lait :

Dans ce régime, il existait une carence d'apport en acide linoléique (0,6% des calories totales de la ration). On peut concevoir que les systèmes enzymatiques sont stimulés pour pallier à l'insuffisance du substrat : l'acide linoléique.

Période colza :

L'apport d'acide alpha-linolénique de 3,6 g entrainaît un métabolisme plus important des acides gras de la famille W3. Seul, le régime graisses du lait présentait un métabolisme des acides gras de la famille W3 aussi actif.

Avec un apport d'acide linoléique de 10,7 g/jour (4,5% des calories), nous nous retrouvions dans un système métabolique normal des acides gras de la famille W6 et cela malgré l'apport de 3,6 g d'acide alpha-linoléique (soit 1,6% des calories totales de la ration).

On peut constater qu'avec un apport de 1,6% des calories de la ration sous forme d'acide alpha-linolénique, on n'a pas dépassé les limites de capacité de régulation métabolique au delà desquelles il existe une compétition entre les deux voies métaboliques : acides gras de la famille W6 et acides gras de la famille W3.

CONCLUSION

Dans les conditions particulières de cette études chez les humains, l'huile de colza assure un meilleur apport des acides gras essentiels :

1/ 10,7 g/jour/ personne d'acide linoléique nécessaires au métabolisme des acides gras de la famille W6 dont un métabolite essentiel est l'acide arachidonique.

2/ 3,6 g/jour/personne d'acide alpha-linolénique nécessaires au métabolisme des acides gras de la famille W3 (notamment synthèse de l'acide éisosa-pentaénoïque), mais sans provoquer de compétition entre ces deux grandes familles d'acides gras polyinsaturés.

ACIDES GRAS DES PHOSPHOLIPIDES DU SERUM

| | TOURNESOL | MAIS | GRAISSES DU LAIT | | COLZA | FLUIDE DE PALME | ARACHIDE |
|---|---|---|---|---|---|---|---|
| nombre de sujets | 23 | 22 | 23 | | 21 | 22 | 22 |
| C16 : 0 | 28,3 ± 2,8 | 27,4 ± 3,5 | 30,4 ± 3,5 | | 26,7 ± 2,6 | 27,5 ± 3,4 | 26 ± 1,4 |
| C18 : 0 | 19,8 ± 1,8 | 15,8 ± 1,7 | 16 ± 1,5 | | 14,6 ± 1,2 | 14,7 ± 2,1 | 14,8 ± 0,9 |
| C18:1W9 | 8,7 ± 1,1 | 10,5 ± 0,8 | 10,2 ± 1,4 | | 13,5 ± 1,9 | 13,8 ± 1,6 | 11,6 ± 0,8 |
| C18:2W6 | 26,1 ± 4 | 27,2 ± 4 | 16,3 ± 2,3 | | 22,4 ± 2,6 | 21,9 ± 4 | 24,3 ± 2,9 |
| C20:3W6 | 1,97 ± 0,6 | 3,46 ± 1,4 | 3,97 ± 0,9 | | 2,83 ± 0,7 | 2,76 ± 0,9 | 3,33 ± 0,8 |
| C20:4W6 | 8,9 ± 2,3 | 13,8 ± 2,3 | 12,22 ± 1,7 | | 10,5 ± 2,3 | 0,85 ± 2,7 | 11,11 ± 1,8 |
| C20:5W3 | 0,8 ± 0,3 | 1,04 ± 0,3 | 2,08 ± 0,9 | | 2,01 ± 1,0 | 0,7 ± 0,3 | 0,8 ± 0,24 |

Répartition (en %) des principaux acides gras des phospholipides sériques à la fin de six périodes de régime comportant 20% de l'apport calorique total sous forme de l'une des graisses visibles suivantes: huiles de tournesol, de maïs, d'arachide, nouvelle huile de colza, fluide de palme et graisses du lait

# ETUDE DES BESOINS QUALITATIFS ET QUANTITATIFS EN ACIDES GRAS ESSENTIELS EN FONCTION DU STADE DE DEVELOPPEMENT CHEZ LE RATON.

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La plupart des recherches centrées sur les acides gras essentiels ont donc été axées sur l'étude des acides gras en n-6, les travaux concernant le rôle nutritionnel de l'acide linolénique sont encore très fragmentaires. Cet acide gras est certes essentiel puisqu'il n'est pas synthétisé pour les mammifères, mais est-il réellement indispensable au maintien de l'organisme en bonne santé ? Cette incertitude est liée à deux types d'observations : d'une part au fait que le 18:3 n-3 peut restaurer une croissance normale chez le rat dont le régime est carencé en acides gras essentiels sans toutefois faire disparaitre les autres symptômes de carence et d'autre part à l'effet inhibiteur exercé par le 18:3 n-3 sur les conversions de la famille des n-6. La plupart des travaux concernant le métabolisme des acides gras essentiels ont été réalisés au niveau hépatique, or si à ce niveau "l'indispensabilité" de l'acide linolénique est très contreversée, il apparait actuellement que cet acide gras joue un rôle important au cours du développement cérébral.

Or peu d'huiles alimentaires de consommation courante apportent cet acide linolénique en quantité appréciable, les plus courantes sont l'huile de soja et l'huile de colza. L'huile de colza dépourvue d'acide érucique (huile de Primor) présente l'avantage d'apporter 8 à 9 % de 18:3 n-3 et 20 à 25 % de 18:2 n-6 c'est-à-dire une quantité d'acide linoléique très voisine de l'huile d'arachide.

L'utilisation comparée de ces deux huiles nous a donc paru très intéressante pour tenter d'apprécier l'incidence de l'acide linolénique sur la répartition des acides gras polyinsaturés (AGPI) tissulaires en période périnatale chez le raton.

Sans avoir la prétention de définir avec précision le besoin optimal en acides gras polyinsaturés de la série n-3 chez la femelle en gestation, nous pouvons toutefois à la lumière des résultats obtenus, confirmer l'importance du 18:3 n-3 alimentaire sur la composition des AGPI du tissu nerveux et définir un taux maximal à partir duquel la répartition des AGPI est constante. Couplées à des tests physiologiques, ces études analytiques nous permettrons une

meilleure approche dans la définition des besoins qualitatifs et quantitatifs en acides gras essentiels du cerveau en voie de développement.

Résultats et discussion

A partir de mélanges adéquants d'huile de Primor et d'huile d'arachide, nous avons utilisé huit régimes semi-synthétiques différents dans lesquels les lipides représent environ 20 % de l'apport énergétique global. Les différents régimes se caractérisent par des teneurs identiques en acide linoléique et des taux variables en acide linolénique. Ce dernier acide gras représente respectivement 0,2 % - 0,5 % - 1 % - 1,5 % - 2 % - 3 % - 6 % et 9 % des acides gras totaux. Les différents régimes sont administrés aux femelles dès l'accouplement et pendant les périodes de gestation et de lactation. Après sevrage, les jeunes rats continuent à recevoir ces régimes jusqu'à l'âge de 60 jours.

Que ce soit au niveau des reins, des testicules ou du cerveau entier, nous nous limiterons dans la présentation des résultats à l'étude de la composition en acides gras de la fraction la plus riche en acides gras polyinsaturés, c'est-à-dire la phosphatidyl-éthanolamine (PE). Nous ferons une exception pour le tissu hépatique puisque, à ce niveau la répartition des acides gras au sein de la PE ne reflète pas les observations faites dans l'extrait lipidique total. Que ce soit au niveau du foie, des reins, des testicules, les modifications induites par le régime sur la répartition des acides gras polyinsaturés à l'âge de 15 jours persistent chez l'adulte.

Dans tous les tissus étudiés, la supplémentation du régime en acide linolénique conduit à une augmentation du taux des AGPI en n-3 (22:5 n-3 et 22:6 n-3) et une diminution du taux de 22:5 n-6 (Figures 1 et 2). Les modifications de la répartition des AGPI observées à l'âge de 15 jours persistent chez l'adulte toutefois, l'amplitude des variations dépend du tissu considéré.

En accord avec Crawford et coll., nous remarquons que parmi tous les tissus retenus dans cette étude, c'est le foie dont la composition en AGPI est la plus sensible à la nature et aux taux des acides gras essentiels du régime.

Des apports en 18:3 n-3 dans le régime maternel compris entre 0,2 et 9 % ne semblent pas induire d'inhibition de l'activité des premières étapes d'élongation-désaturation des acides gras essentiels en n-6 dans le cerveau, les reins et les testicules puisque nous n'avons pas pu mettre en évidence de modifications significatives de la proportion de 20:4 n-6 au sein de la PE mais également des lipides totaux dans ces organes. Par contre, si au sein de la PE du

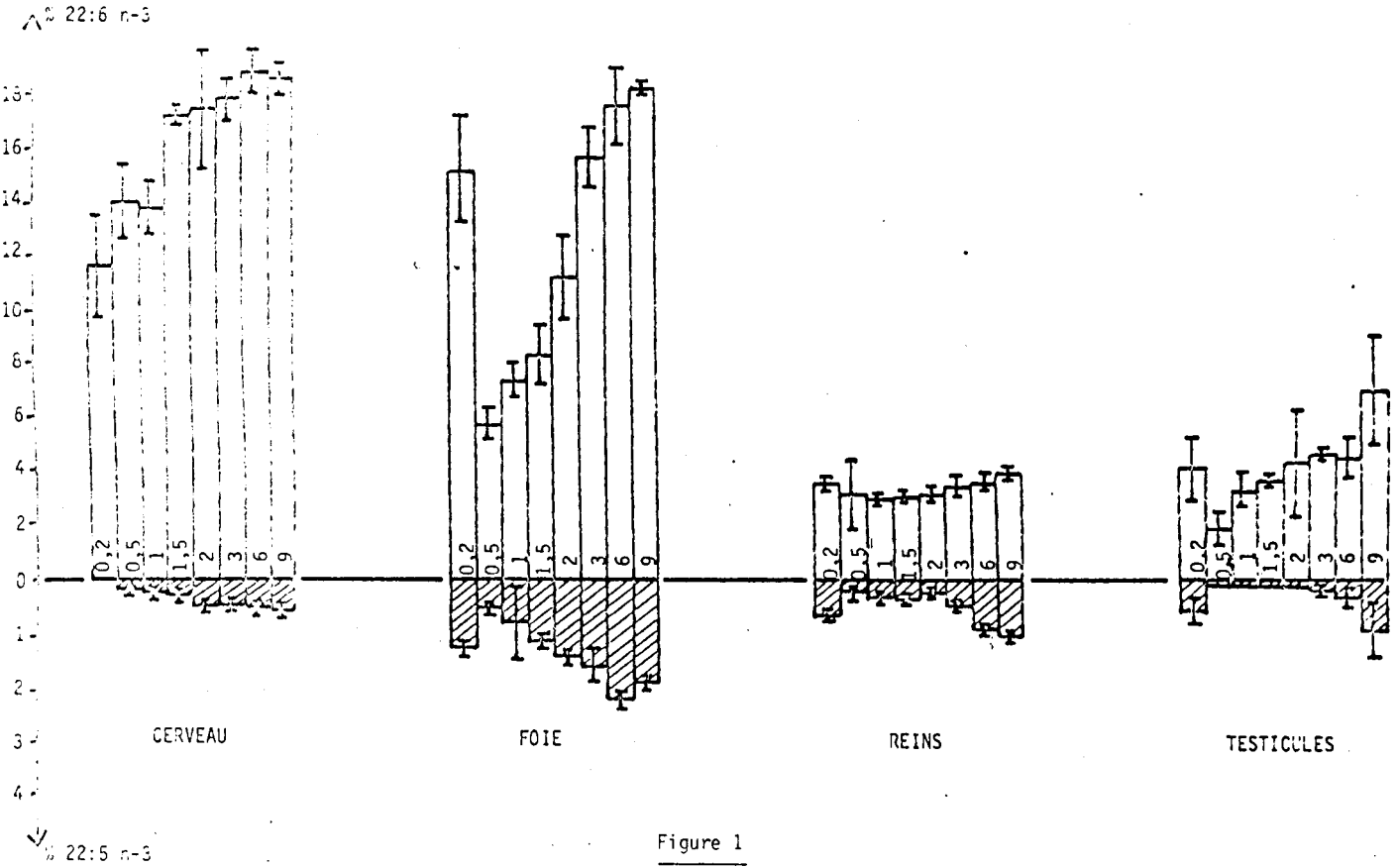


Figure 1

REPARTITION DES ACIDES GRAS TERMINAUX DE LA SERIE n-3 DANS LA P.E. DES DIFFERENTS TISSUS A $J_{15}$

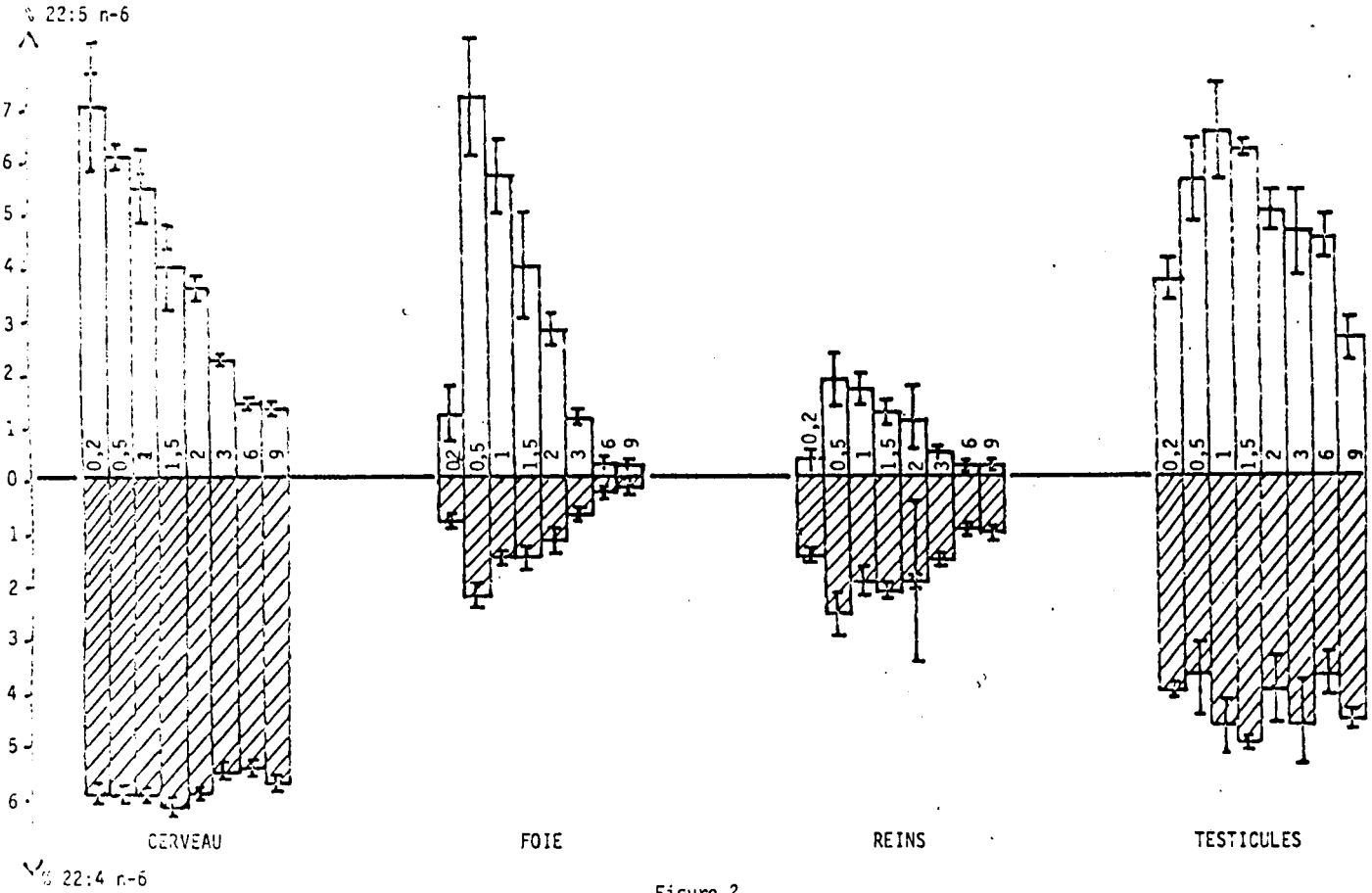


Figure 2

REPARTITION DES ACIDES GRAS TERMINAUX DE LA SERIE n-6 DANS LA P.E. DES DIFFERENTS TISSUS A $J_{15}$

foie, le taux d'acide arachidonique est constant, il n'en est pas de même dans l'extrait lipidique total. La répartition des acides dans les lipides totaux hépatiques chez les animaux âgés de 15 jours montre que la teneur en 20:4 n-6 est très différente entre les deux régimes extrêmes. Lorsque l'on compare deux à deux les valeurs obtenues pour cet acide gras dans chacun des lots, on remarque que jusqu'à 1,5 %, les apports en 18:3 n-3 sont sans effet, alors que la chute du taux de 20:4 n-6 entre les lots 1,5 % et 2 % est significative (Figure 3). Le taux d'acide linoléique a par opposition tendance à s'accumuler avec là aussi un accroissement brusque entre les lots 1,5 % et 2 %.

Au niveau hépatique, pour des régimes où le taux de 18:3 n-3 représente 2 % et plus des acides gras totaux, la diminution d'activité de la Δ6 désaturase dans la série n-6 est assez évidente (l'augmentation du taux de 18:2 n-6 et la chute du 20:4 n-6 en sont les témoins). Ceci permettrait également d'expliquer que dans ces conditions la proportion des acides gras terminaux (22:4 n-6 et 22:5 n-6) est également plus faible. L'inhibition de la première étape métabolique conduit bien évidemment à une réduction de la synthèse de tous les acides gras de cette série.

Toujours au niveau hépatique, lorsque le régime s'appauvrit en 18:3 n-3 (taux de 18:3 n-3 inférieur à 1,5 %), la stabilité de la teneur en 20:4 n-6 des lipides totaux peut s'expliquer par le fait que l'activité Δ6 désaturase du 18:2 n-6 n'est pas modifiée mais que par contre les activités d'élongation désaturation en aval du 20:4 n-6 sont limitées puisque même dans ces conditions les taux de 22:4 n-6 et 22:5 n-6 chutent notamment dans la PE qui est le phospholipide le plus riche en acides gras longs et polyinsaturés. Compte tenu des travaux antérieurs sans préjuger de l'activité de la Δ6 désaturase dans ces conditions nutritionnelles, la stabilité du 20:4 n-6 est très certainement due à l'intensité des phénomènes de rétroconversion du 22:4 n-6 et du 22:5 n-6.

Au niveau cérébral, que ce soit dans l'extrait lipidique total ou au niveau de la PE, les taux de 20:4 n-6 et de 22:4 n-6 sont indépendants du régime utilisé. L'inhibition de l'activité de la Δ4 désaturase du 22:4 n-6 en 22:5 n-6 pourrait être à l'origine de la chute du taux de ce dernier dans les lipides cérébraux. Sprecher a en effet montré que contrairement au foie, l'activité de la Δ4 désaturase est importante dans le cerveau. Afin d'expliquer le comportement des acides gras polyinsaturés au niveau du cerveau, il faut imaginer une régulation à deux niveaux : d'une part, par rétrocontrôle des acides gras terminaux de la série n-3 sur les activités enzymatiques des deux séries n-3 et n-6 et d'autre part, par une sélectivité de captation des acides gras au niveau de la barrière hémato-encéphalique qui deviendrait prépondérante lorsque les besoins en acides gras de la série n-3 sont assurés.

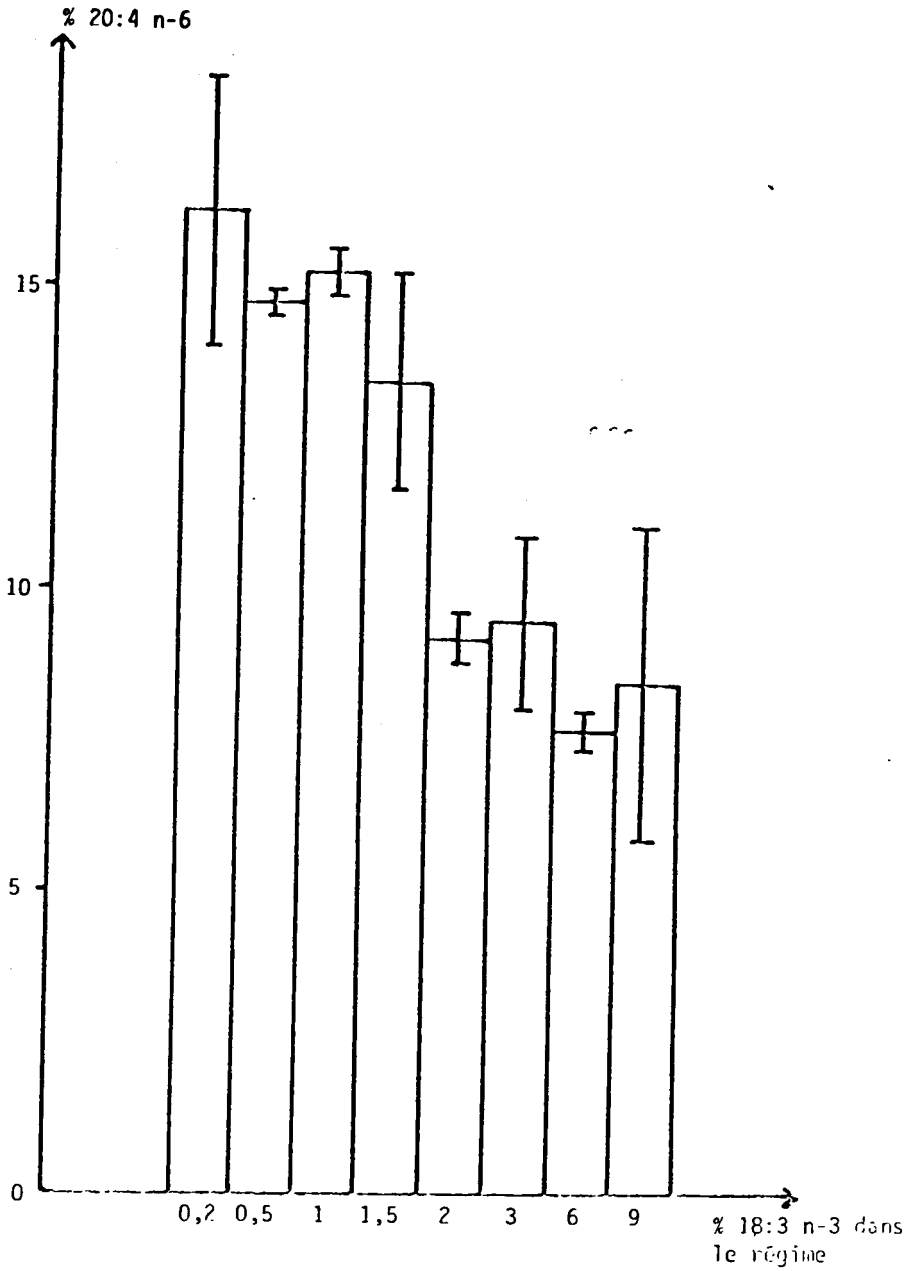


Figure 3

VARIATIONS DU TAUX DE 20:4 n-6 DANS LES LIPIDES DU FOIE EN FONCTION DE L'APPORT EN 18:3 n-3 DANS LE REGIME MATERNEL

Nous venons de voir que la répartition en acides gras polyinsaturés chez le raton en voie de développement dépendait très étroitement de la nature et du taux d'acides gras essentiels du régime maternel. Il ne faut pourtant pas négliger l'importance de la lactation puisqu'au cours de cette étape nutritionnelle intermédiaire, il y a au niveau des glandes mammaires une synthèse importante d'acides gras. Cette synthèse endogène conduit à une dilution des acides gras essentiels du régime maternel captés par les glandes mammaires. Dans le lait maternel, le taux de 18:3 n-3 reste très dépendant des apports maternels.

On remarque alors que les modifications obtenues entre les lots 1,5 et 2 % correspondent en fait à des régimes où le taux d'acide linolénique varie entre 0,5 et 0,7 % des acides gras totaux.

La très grande sensibilité du tissu hépatique aux apports lipidiques exogènes n'est certes plus à démontrer, la teneur en acides gras polyinsaturés de ce tissu est le reflet assez fidèle des manipulations diététiques. A l'inverse, le tissu cérébral est le plus indépendant de la nature et du taux des acides gras polyinsaturés exogènes à partir d'un certain seuil d'apport.

Ces données analytiques représentent les premiers éléments de référence quant à la définition du besoin en acides gras essentiels de la série n-3 pour le cerveau en voie de développement.

Cette hypothèse est sinon confirmée, du moins renforcée par les résultats préliminaires obtenus lors de la mesure de l'électrorétinogramme chez les animaux soumis à des régimes différents quant à l'apport en acide linolénique.

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# EFFETS DE L'ACIDE LINOLENIQUE ALIMENTAIRE SUR LES CARACTERISTIQUES DE LA MEMBRANE PLASMIQUE DE L'HEPATOCYTE DE RAT.*

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## INTRODUCTION.

Le travail qui fait l'objet de cette communication a permis d'étudier l'influence qu'un apport alimentaire, plus ou moins riche en acide linolénique et plus ou moins prolongé, était susceptible d'avoir sur les caractéristiques chimiques, physiques et fonctionnelles de la membrane plasmique de l'hépatocyte de de rat. La réversibilité des modifications éventuellement observées a été recherchée.

## METHODES.

Trois lots de rats mâles Wistar ont été préalablement équilibrés sur régime complet pour rats, contenant 3,5% de lipides dont les acides gras renfermaient 3% d'acide linolénique ("régime témoin "). Les rats ont ensuite été maintenus (1 à 17 semaines) sur régime à 10 ou 20%, soit d'huile de lin utilisée comme "modèle hyperlinolénique"(18:3 = 55 % des acides gras de l'huile), soit de nouvelle huile de colza (18:3 = 8,5%), soit enfin d'une huile non linolénique (huile d'olive). Dans tous les cas, l'huile était ajoutée à un régime de base apportant tous les constituants nécessaires au développement des rats, mais totalement dépourvu de lipides. Après 17 semaines sur régime huile de lin, certains rats ont été placés (1 à 17 semaines) sur le régime témoin. A partir du foie, des coupes ont été préparées en vue d'examens histologiques en micros-

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copie optique. Une autre partie aliquote du foie a été utilisée pour purifier la membrane plasmique des hépatocytes. Les activités de certaines enzymes associées à cette membrane ont été mesurées. Les lipides extraits de la membrane ont été analysés. Enfin des mesures de microviscosité membranaire ont été réalisées par la technique de dépolarisation de fluorescence.

RESULTATS ET DISCUSSION.

1/ Examens histologiques.

Les microphotographies obtenues en microscopie optique ne permettent pas de mettre en évidence de différence dans l'aspect des parenchymes hépatiques des rats témoins et des rats ayant reçu le régime à 10% d'huile de colza. En particulier, l'élargissement des sinusoïdes sanguins qui est très apparent dans le parenchyme hépatique des rats ayant ingéré pendant 15 semaines le régime à 10% d'huile de lin n'est pas observé chez les rats nourris 15 semaines avec un régime renfermant 10 ou même 20% d'huile de colza.

2/ Composition des phospholipides de la membrane plasmique des hépatocytes.

Les proportions relatives des divers phospholipides sont les suivantes pour la membrane des rats témoins:

| | |
|---|---|
| phosphatidyléthanolamine (PE) | 23,8 ± 1,0 % |
| phosphatidylcholine (PC) | 42,0 ± 2,5 % |
| phosphatidyl sérine (PS) | 9,3 ± 1,0 % |
| phosphatidylinositol (PI) | 5,9 ± 0,5 % |
| sphingomyéline (SM) | 16,2 ± 0,6 % |

Au cours des régimes lin et colza, on note une augmentation régulière du taux de PI, qui de 5,9 % (témoin) passe à 7,5% et 8,3% après 17 semaines d'un régime à 10% et à 20% d'huile de lin. Pour les régimes (10% et 20%) colza, les valeurs correspondantes sont 7,0% et 7,5%. La sphingomyéline, par contre, diminue au cours des régimes lin et colza: 12% et 11% après 17 semaines de régimes renfermant respectivement 10% d'huile de lin et d'huile de colza, contre 16% pour les témoins. Les proportions des autres phospholipides ne sont pas affectées par les huiles étudiées.

3/ Compositions en acides gras des phospholipides membranaires.

L'analyse a été limitée au deux principaux phospholipides (PC et PE) qui à eux deux représentent près des 2/3 des phospholipides totaux.

3.1/ Acides gras saturés: Comparativement aux rats témoins, chez les animaux alimentés avec les régimes lin et colza, le taux d'acide 16:0 diminue tandis que celui d'acide 18:0 augmente; ceci aussi bien pour PC que pour PE. Il en résulte que la somme des acides gras saturés est inchangée par rapport à PC et PE témoins. Les changements mis en évidence dans les proportions relatives des acides 16:0 et 18:0, traduisent l'action inhibitrice que les acides polyinsaturés alimentaires sont connus exercer sur l'activité de l'acide gras synthétase (d'où diminution de 16:0) et sur l'activité de la $\Delta 9$ désaturase (d'où diminution de 18:0).

3.2/ Acides gras insaturés n-6: Les acides linoléique (18:2) et arachidonique (20:4) constituent 13 % et 21 % des acides totaux de PC et 9 % et 19 % de ceux de PE pour la membrane des rats témoins. Les animaux nourris avec le régime à 10% d'huile de lin présentent une diminution importante de 20:4 et une augmentation de 18:2, dans PE et PC. Le rapport molaire 20:4/18:2 diminue de ce fait de moitié après 4 semaines de régime à 10% en huile de lin et d'un facteur 3 dans le cas du régime lin à 20%. Ces variations sont le reflet de la compétition qui se manifeste au niveau de la $\Delta 6$ désaturase entre les acides linoléique et linolénique. Dans la mesure où il a été démontré que cette compétition est en faveur de ce dernier, on conçoit, qu'en raison de la richesse toute spéciale de l'huile de lin en acide linolénique par rapport à l'acide linoléique (18:3/18:2 = 3,0), la biosynthèse de l'acide arachidonique soit particulièrement perturbée. Par contre, nous avons démontré que, dans PC et PE, les taux d'acides linoléique et linolénique ne sont pas modifiés à la suite d'ingestion pendant 17 semaines consécutives d'un régime à 10% et même à 20% d'huile de colza. En d'autres termes, pour un rapport molaire 18:3/18:2 égal à 0,3 (huile de colza) la biosynthèsedes acides polyinsaturés n-6 n'est pas altérée. Enfin, nous avons pu montrer que les modifications entraînées par l'huile de lin régressent lorsque le régime lin est remplacé par le régime témoin. Les proportions de

18:2 et de 20:4 reprennent leurs valeurs initiales.

3.3/ Acides gras insaturés n-3: En dépit de la richesse de l'huile de lin en acide linolénique, celui ci n'est que très faiblement incorporé dans PE et PC (respectivement au plus 3% et 4% des acides gras totaux du phospholipide considéré). Dans le cas des régimes apportant de l'huile de colza, PC et PE renferment moins de 2% de 18:3 après 17 semaines d'une alimentation à 20% d'huile. Par contre, dans les deux phospholipides, on note une très nette augmentation de l'acide 20:5 (10 fois pour PC comme pour PE) aux dépens de l'acide 22:6 , lorsque les rats reçoivent le régime à 10% d'huile de lin pendant 17 semaines. Pour le régime correspondant d'huile de colza, l'accroissement du taux de 20:5 est seulement de trois fois en moyenne pour PC et PE, également aux dépens de l'acide 22:6.

## 4/ Influence de l'acide linolénique sur l'activité des enzymes associées à la membrane.

4.1/ 5'Mononucléotidase: L'activité spécifique de cette enzyme augmente de près de deux fois après 10 semaines de régimes à 10% d'huile de lin ou d'huile de colza . Toutefois l'essentiel de cette augmentation est lié non pas à l'apport alimentaire en acide linolénique mais à la surcharge lipidique des régimes. Nous avons en effet observé que l'huile d'olive, pour une même quantité dans le régime, provoque aussi une élévation d'activité de l'enzyme, mais qui représente seulement 75% de celle entraînée par les régimes correspondants lin ou colza.

4.2/ ATPase $Na^+K^+$ dépendante: L'ingestion par des rats d'un régime lin, colza ou olive est suivie d'une diminution de deux fois de l'activité de l'enzyme ( pour 10% d'huile dans l'aliment). La perturbation est donc associée à la nature hyperlipidique des régimes et non à la présence d'acide linolénique.

4.3/ ATPase $Mg^{2+}$ dépendante: Des trois enzymes examinées, celle-ci est la seule qui soit particulièrement sensible à l'acide linolénique alimentaire, du moins lorsqu'il est ingéré en quantités importantes. En effet, pour 10% d'huile de lin dans le régime,

l'activité enzymatique diminue de trois fois après 17 semaines d'alimentation et de presque deux fois après seulement deux semaines. Toutefois , dès que l'huile de lin est supprimée du régime, on assiste immédiatement à un retour à l'activité normale. Enfin, en ce qui concerne l'huile de colza ( ainsi d'ailleurs que l'huile d'olive) il n'y a pas d'effet sur l'activité de l'enzyme , même pour 20% d'huile de colza dans le régime.

5/ Microviscosité membranaire.

Les mesures de dépolarisation de fluorescence que nous avons pratiquées sur les préparations de membrane purifiée n'ont pas permis de mettre en évidence de perturbations dans la microviscosité membranaire ( pour une température donnée).

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# CARDIAC LIPID CHANGES IN RATS AND HOW THESE CORRELATE TO MYOCARDIAL NECROSIS

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Extensive experimentation in the past decade on the safety of vegetable oils showed characteristic necrotic heart lesions in male rats related to the level and kind of oil in the diet (1-6). Certain dietary fatty acids such as linolenic (18:3n-3) (2, 7) and erucic acid (8) were identified as being involved in myocardial necrosis, while saturates (9) and linoleic acid (18:2n-6) (2) were associated with a low incidence of heart lesions. A statistical evaluation of much of the published data (23 experiments) on heart lesions in male rats fed high levels of fats and oils identified dietary 18:3n-3 and saturated fatty acids as most closely associated with differences in incidence of myocardial lesions among diets within experiments (10). In fact, 73% of the variation was explained by these fatty acids, which is almost all of the variation that can be explained by this model considering the fact that most of the residual source of variation (about 25%) is due to binomial sampling which is a function of the underlying incidence levels and sample size in each experiment (11). These results provided a model for predicting the relative cardiotoxicity of vegetable oils based on their fatty acid composition.

This experiment was designed to test the relationship of dietary fatty acids to heart lesions in male rats and determine if the cardiac lipids showed evidence of change which could be correlated to myocardial damage.

## MATERIALS AND METHODS

The compositions of the dietary fats fed at 20% by weight are shown in Table 1. Soybean and LEAR (low erucic acid rapeseed) oils were selected because they contain 18:3n-3, which based on the statistical evaluation, should result in a relatively high incidence of heart lesions. The saturated fatty acid content of the two oils was increased by mixing in cocoa butter. Triolein was mixed with the oils in proportions similar to that with cocoa

butter to assure that the cardiopathogenic results were not due to dilution of other factors in the oils. In some oil mixtures the 18:3n-3 content was restored by adding an appropriate amount of linseed oil.

The semi-synthetic diets (12) were fed for 1, 2, 3, 4 and 16 weeks to male Sprague-Dawley rats. The hearts of 44 rats per diet were examined histopathologically after 16 weeks as described previously (13). The cardiac lipids of rats were analyzed at all time periods by procedures described elsewhere (14, 15). Tissue samples were also obtained from weanling rats (zero time).

Table 1. Dietary fats and their fatty acid composition.

| | % by wt of the diet | | | | | | |
|---|---|---|---|---|---|---|---|
| LEAR oil | 20 | 16 | 16 | – | – | – | – |
| Soybean oil | – | – | – | 20 | 16 | 9.6 | 9.5 |
| Cocoa butter (CB) | – | 4 | – | – | 4 | – | – |
| Triolein (18:1) | – | – | 4 | – | – | 9.6 | 9.5 |
| Linseed oil | – | – | – | – | – | 0.8 | 0.8 |
| Erucic acid (22:1) | – | – | – | – | – | – | 0.2 |
| Fatty acids (% by wt) | | | | | | | |
| Total saturates | 7.2 | 16.6 | 5.4 | 16.2 | 27.6 | 8.7 | 9.1 |
| Total monoenes | 60.2 | 57.6 | 68.7 | 25.1 | 27.4 | 56.3 | 56.7 |
| 18:2n-6 | 22.0 | 17.8 | 17.9 | 51.9 | 40.1 | 28.3 | 27.5 |
| 18:3n-3 | 10.3 | 7.9 | 7.8 | 6.7 | 4.9 | 6.7 | 6.7 |

## RESULTS AND DISCUSSION

Both soybean oil and LEAR oil fed to male Sprague-Dawley rats for 16 weeks gave a high incidence of heart lesions (Table 2). The incidence of heart lesions was significantly reduced by increasing the saturated fatty acid content of the oils by about 10%. Substitution of triolein for cocoa butter in these oils resulted in no change in the incidence of heart lesions. This indicated that changes in certain dietary fatty acids (saturates), and not dilution of factors (toxin?) in vegetable oils, influenced the incidence of heart lesions. These results are consistent with previous studies where no cardiotoxins could be removed from LEAR and soybean oils by exhaustive fractionation (16-18), and agree well with those predicted by the model (10) (Table 2).

An analysis of the heart lipids showed no significant diet differences in any of the lipid classes at any time period except in the triglyceride level (Table 3). On the other hand, the

significant time effect for all lipid classes appears to reflect only differences in water content of heart tissue, since the values were expressed as mg per g of wet heart. In any case, the cardiac lipid class changes could not be related to the observed cardiopathogenic response.

Table 2. Observed and predicted incidence of myocardial lesions in male rats fed the experimental diets for 16 weeks.

| Diets | Incidence (%) (n=44) Observed | Predicted | Comparisons (d.f.) | $X^2$ |
|---|---|---|---|---|
| LEAR | 61 | 64 | | |
| LEAR + CB | 36 | 47 | All diets (6) | 13.0* |
| LEAR + 18:1 | 55 | 62 | Effect of saturates (1) | 10.2** |
| Soybean | 57 | 46 | Effect of triolein (1) | 0.1 |
| Soybean + CB | 34 | 27 | | |
| Soybean + 18:1 | 59 | 55 | | |
| Soybean + 18:1 + 22:1 | 55 | 55 | | |

Table 3. Heart, lipid and cardiac lipid class weights pooled over all 7 diets.

| Description | Time on diet (weeks) 0 | 1 | 2 | 3 | 4 | 16 | P < 0.01 Diets | Time |
|---|---|---|---|---|---|---|---|---|
| Heart | 205[a] | 414 | 569 | 666 | 883 | 1107 | NS | S |
| Lipids | 35.1[b] | 33.2 | 32.7 | 34.8 | 38.3 | 36.9 | NS | S |
| CE | 0.6[b] | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | NS | S |
| TG | 4.6 | 7.7 | 6.5 | 8.2 | 8.6 | 9.9 | S | S |
| C | 3.5 | 3.2 | 2.6 | 2.8 | 3.0 | 2.2 | NS | S |
| DPG | 3.7 | 3.1 | 3.1 | 3.4 | 4.1 | 2.7 | NS | S |
| PE | 5.9 | 5.6 | 6.1 | 6.7 | 7.6 | 6.4 | NS | S |
| PS + PI | 0.9 | 0.6 | 1.2 | 1.0 | 0.8 | 0.9 | NS | S |
| PC | 12.5 | 9.9 | 10.4 | 10.6 | 11.5 | 9.3 | NS | S |
| SP | 1.9 | 1.4 | 1.6 | 1.0 | 1.3 | 0.8 | NS | S |

[a] mg [b] mg/g wet weight

The fatty acid composition of the cardiac lipid classes were also investigated in hopes of locating changes which would correlate to the observed cardiopathogenic response in rats. The cardiac phospholipids, generally maintain a characteristic fatty acid composition, and as membrane components, were of great interest since compositional changes might correlate to changes in heart lesion incidence. Table 4 shows that the relative concentration of cardiac arachidonic acid (20:4n-6) and the Σ saturates and ΣC22 PUFA (polyunsaturated fatty acids) remained constant throughout the experimental period. Of these, only the sum of saturates was affected by diet. It is noteworthy, that the level of saturated fatty acids in the phospholipid classes at 1 to 4 weeks and 16 weeks were significantly correlated, positively to the level of dietary saturates, and negatively to heart lesions (Table 5). This completes a rather significant correlation between dietary saturates : level of saturates in cardiac phospholipids : and heart lesions. The correlation of dietary saturates to heart lesions in this experiment was -0.81. Therefore, the results of this study suggest that the level of dietary saturates as well as the level of saturates in cardiac phospholipids could be used to predict heart lesion incidence.

Table 4. Relative concentration (%) of fatty acids and groups of fatty acids in cardiac phospholipids. Values are means of 7 diets and 5 time periods.

| Description | PC | PE | DPG | PS + PI | SP | P < 0.01 Time | P < 0.01 Diet |
|---|---|---|---|---|---|---|---|
| 20:4n-6 | 26 | 22 | 3 | 21 | 0 | NS | NS |
| Σ Saturates | 46 | 37 | 5 | 54 | 83 | NS | S |
| Σ C22 PUFA | 6 | 23 | 3 | 9 | 0 | NS | NS |

The C22 n-3 PUFA (i.e., 22:6n-3 and 22:5n-3) have also been considered as correlating to heart lesions, since diets which gave a high incidence of heart lesions generally contained 18:3n-3 (2, 6, 7, 10), and rats fed these diets had high levels of cardiac C22 n-3 PUFA (6, 15, 19, 20). However, as pointed out previously, the level of C22 n-3 PUFA is related to the dietary level of 18:3n-3 irrespective of the source (14). The results of this experiment support this finding as seen in Table 6. Most of the C22 PUFA (about 90-95%) in the cardiac phospholipids were derived from 18:3n-3, and only when the dietary 18:2n-6 content was greater

than 40% was the content of the C22 n-3 PUFA reduced slightly (to about 80-85%). As a comparison, a corn oil containing diet with ≤1% 18:3n-3 and 56% 18:2n-6 gave a 35 to 40% content of C22 n-3 PUFA (Table 6). It is quite evident from the results in Table 6 that the relative proportion of C22 n-3 PUFA (22:5n-3 and 22:6n-3) was not affected by the addition of dietary saturates which had resulted in a decrease in the incidence of myocardial lesions. Therefore, the results suggest that the content of the C22 n-3 PUFA in the heart phospholipids may not be a reliable indicator of heart lesions, although it appears to be a necessary component, since no dietary oils have been observed to give a high incidence of heart lesions when the content of the C22 n-3 PUFA is low. The results of this study indicate that the incidence of heart lesions in male rats can be lowered by dietary saturates even in the presence of high levels of C22 n-3 PUFA (21).

Table 5. Correlation of cardiac phospholipid saturates to dietary saturates and heart lesions.

| Phospho-lipid | Time (weeks) | Dietary saturates | Heart lesions |
|---|---|---|---|
| PE | 1-4 | 0.91 | |
| | 16 | 0.97 | -0.80 |
| PC | 1-4 | 0.84 | |
| | 16 | 0.93 | -0.77 |
| PS + PI | 1-4 | 0.85 | |
| | 16 | 0.89 | -0.74 |

Table 6. Relative concentration (%) of C22 n-3 PUFA in the C22 PUFA in cardiac phospholipids of rats. Values are means over 5 time periods.

| | Dietary | | % (Σ C22 n-3/Σ C22 PUFA) | | |
|---|---|---|---|---|---|
| Diet | 18:3 | 18:2 | PE | PC | PS + PI |
| LEAR | 10.3 | 22.0 | 96 | 94 | 91 |
| LEAR + CB | 7.9 | 17.8 | 95 | 94 | 92 |
| LEAR + 18:1 | 7.8 | 17.9 | 96 | 94 | 92 |
| Soybean + 18:1 | 6.7 | 28.3 | 94 | 91 | 85 |
| Soybean + 18:1 + 22:1 | 6.7 | 27.5 | 94 | 91 | 88 |
| Soybean + CB | 4.9 | 40.1 | 84 | 81 | 73 |
| Corn | 0.7 | 56.1 | 42 | 35 | 34 |

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# Quality Evaluation of Canola Frying Fats

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## Introduction

Canola oil, from low erucic acid, low glucosinolate rapeseed, is now our major source of edible oil in Canada. It is processed into salad and cooking oils, margarines, and shortenings for baking and frying. However, little information has been reported on the use and performance of canola frying fats as compared to other more commonly used fats and oils. A study by Dobbs (1975) identified off odours in heated rapeseed oil which may have limited its acceptance as a frying oil. However, since that time many improvements have been made in the chemical composition of the oil, and also in processing techniques. Thus the quality of canola fats and oils for frying is much improved, but there are few studies which have assessed the quality. Thus, one aim of this study was (1) to gain information on the performance of canola frying fats and oils. Another objective was (2) to evaluate newer methods for the determination of frying fat quality. Concern has been expressed regarding the nutritional quality of heated fats and oils; Germany has set limits on the amounts of degradation products allowed in frying fats. In Canada as yet there are no regulations concerning the quality of used frying fats, but it was considered useful to have a rapid method for monitoring fat quality.

## Experimental

The project was set up to simulate commercial frying using a small commercial electric deep-fryer such as is used in a small restaurant. Two separate frying studies were carried out using frozen French fry potatoes in institutional pack. In the first study, two solid hydrogenated canola frying fats (I.V.=78; 76) from different processors were compared to a solid hydrogenated soy fat (I.V.=83). In the second study, a liquid or pourable canola frying

fat (I.V.=94) was compared with a liquid soy fat (I.V.=97). In the first study frying was done for 5 consecutive days with each fat; in the second study frying time was increased to 10 days for each fat. In other aspects the conditions of the two studies were similar. Each day, a lot of frozen potatoes was fried every 15 min throughout a 7½ hour day. Samples of both the frying fat and the cooked French fries were taken periodically and were stored at -20°C for later analysis. The fryer was topped up twice daily with freshly melted fat, and was turned off overnight.

The frying fats and the fats extracted from the cooked French fries were analysed for thiobarbituric acid (TBA) value, iodine value (I.V.), peroxide value, hydroperoxide value, fatty acid composition, colour, viscosity, smoke point, free fatty acids and contents of polar components.

## Results and Discussion

Frying Fat Quality. The results of all tests were correlated with hours of frying time (Table 1), as reported by Stevenson et al. (1983). In brief, the highest correlations with hours of frying time were observed with the free fatty acid values, and with the contents of polar components as determined by a modified column chromatographic method of Billek et al. (1978). The free fatty acid values for the solid frying fats ranged from 0.02% initially to 1.2% at the end of 5 days of frying. The two canola fats gave similar values, slightly lower than the values for the soy fat. Fatty acid values for the fats extracted from the cooked French fries were very similar to the values for the actual frying fats.

The content of polar components in frying fats has been reported to be a more useful indicator of deterioration as it gives an estimate of the total amount of breakdown products in the fat. An actual value of 30% polar components was determined to be the practical end point of use of a commercial frying fat (Billek, 1979).

Analytical Methods. Methods for determining polar components were evaluated using a Food oil Sensor (Northern Instruments Corp.), column chromatography and thin-layer chromatography. The Food oil Sensor, a small portable instrument (Graziano, 1978) gives an estimation of the polarity of a fat by measuring its dielectric constant relative to a fresh fat sample. However, initial testing of the instrumentgave inconsistent results and the method was abandoned.

Table 1: Correlation (r) of hours of frying time with physical and chemical changes in frying fats.[a]

| | Physical | | | Chemical | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fat | Colour | Smoke point | Viscosity[b] | Peroxide value | TBA value | Hydroperoxide value | Iodine number | Free fatty acids | Polar components |
| Solid Fats | | | | | | | | | |
| Canola I | - | -0.80 | 0.76 | 0.34 | 0.54 | - | 0.50 | 0.99 | 0.95 |
| Canola II | - | -0.78 | 0.80 | 0.77 | 0.26 | - | 0.28 | 0.99 | 0.97 |
| Soy | - | -0.70 | 0.93 | 0.69 | -0.31 | - | 0.38 | 0.99 | 0.96 |
| Liquid Fats | | | | | | | | | |
| Canola I | 0.96 | -0.90 | 0.92 | 0.86 | 0.89 | 0.67 | -0.36 | 0.99 | 0.96 |
| Soy | 0.97 | -0.92 | 0.95 | 0.88 | 0.05 | 0.34 | -0.19 | 0.98 | 0.96 |

[a] From Stevenson et al. (1983).

[b] 12 rpm at 60°C for solid fats and at 21°C for liquid fats.

A column chromatographic method (Billek et al., 1978) was evaluated for reproducibility and was subsequently modified to improve its efficiency. The original 25 g silica gel column was reduced to a 6.25 g column packed in a smaller diameter water-jacketed glass column. A 250 mg sample of the used fat was chromatographed first with 50 ml of mixed solvent (petroleum ether:ether 87:13 v/v) to elute the non-polar or triglyceride fraction and then with 50 ml of diethyl ether to elute the polar fraction (Fig. 1). The contents of triglyceride and polar lipid in each fraction were determined gravimetrically. The resolution between the two fractions was slightly improved over the large column, and considerable savings in column packing, solvents and time to run each column were realized.

Using the small column method, the contents of polar components were determined for each of the solid and liquid fats at the beginning and end of frying (Table 2). In the first study of solid frying fats, the contents of polar components reached only 12-13% after 5 days of frying. Although no formal sensory evaluation was done on the French fries, comments from operators indicated that the fries were still of good quality. In the second study, using the liquid frying fats, the total frying time was extended to 10 days to reach a higher level of deterioration in the fats. However, chemical tests still revealed only 15% of polar components in either fat.

Table 2: Contents of polar components in frying fats at the beginning and end of each frying study (%).

| | Days of frying | | |
|---|---|---|---|
| Fat Source | 0 | 5 | 10 |
| Solid hydrogenated | | | |
| Canola I | 4.31 | 13.10 | |
| Canola II | 4.51 | 13.42 | |
| Soy | 2.51 | 12.70 | |
| Liquid hydrogenated | | | |
| Canola | 3.41 | | 15.12 |
| Soy | 4.84 | | 15.33 |

In this study formal sensory evaluation panels were conducted on days 1, 2, 4, 6, 8 and 10 of frying. The testing was done in the Food Science Department in individual booths, using staff and

students as members of the panel. On each day of testing, a sample of French fries was compared to a control sample of the same potatoes cooked in fresh frying fat. There was no significant decrease observed in the quality of the French fries cooked in either canola or soy fat after 10 days of frying, which would indicate that a content of 15% polar components does not cause noticeable deterioration in the quality of the fat. It would be interesting in future experiments to continue frying over an extended period and to correlate the content of polar components with a decrease in sensory quality of the fat. However, results did show that liquid frying fats stand up well under extended periods of frying and that canola fat performed as well as soy fat.

Although the modified column chromatographic method could be used for monitoring frying fat quality, it was felt to be somewhat tedious for routine analysis. A thin-layer chromatographic technique using the Iatroscan Analyzer was investigated as a possible method for routine analysis of polar components in frying fats. This instrument, manufactored by Iatron Laboratories of Japan and described in detail by Ackman (1981), uses a flame ionization detector to quantitatively estimate the material after thin layer chromatographic separation on glass chromarods coated with silica gel. Although the Iatroscan system has been used for analysis of blood lipids and fish lipids, it has not reportedly been used for analysis of food lipids. Representative standards for a triglyceride and a polar fraction were obtained by column chromatography of a used frying fat. These were individually spotted, developed and scanned on the chromarods and were used to establish standard curves relating the amount of material spotted to the detector response. The detector response was linear over a range of sample size from 1-30 μg. The response was also slightly greater for the triglyceride standard than for the same amount of polar lipid standard.

Although it was possible to obtain good resolution of mixtures of the above standards (Fig.2), the precision and accuracy of the analysis were not sufficiently high to recommend this method at the present time. A recent paper (Crane et al., 1983) discusses some of the factors which affect the reproducibility of results with the Iatroscan instrument.

## Summary and Conclusions

A number of chemical and physical tests were done to evaluate the quality of canola fats during extended deep frying. Quantitative thin-layer chromatography using the Iatroscan Analyser was not as accurate as a modified column chromatography method for determining total polar components. The free fatty acid values and the

contents of polar components, as determined by column chromatography, gave high correlations with hours of frying. The results showed that the canola frying fats were of good quality throughout 5-10 days of frying.

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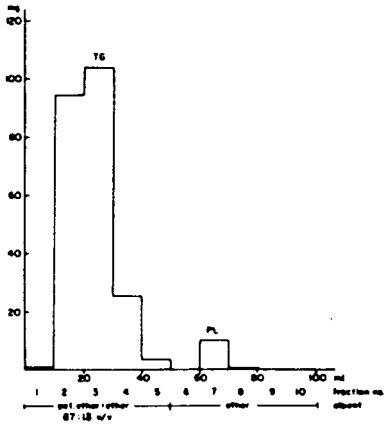


FIGURE 1: COLUMN CHROMATOGRAPHY OF 250 MG OF USED FRYING FAT ON 6.25 G SILICA GEL.

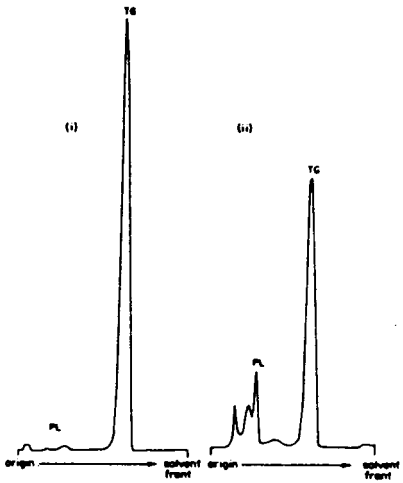


FIGURE 2: TYPICAL CHROMATOGRAMS OF LIPID MIXTURES CONTAINING (I) 4.79% POLAR COMPONENTS AND (II) 34.43% POLAR COMPONENTS, DEVELOPED AND SCANNED ON CHROMARODS.

# EFFET DE L'INGESTION D'HUILE DE COLZA PRIMOR CHAUFFEE SUR CERTAINES CARACTERISTIQUES DES LIPIDES TISSULAIRES ET SUR L'EXCRETION URINAIRE DE GLUCURONIDES, CHEZ LE RAT (INFLUENCE DE LA TEMPERATURE ET DE LA DUREE DU CHAUFFAGE)

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Des expériences précédentes (1) nous ont montré que l'ingestion, par le Rat, d'huile de lin thermopolymérisée, permettait de mettre en évidence un lien entre les monomères cycliques de l'huile, ceux des tissus et la détoxification par la voie de l'acide glucuronique conjugué dans l'urine. Nous avons voulu vérifier si, avec une huile alimentaire, on retrouvait les mêmes phénomènes. Par ailleurs, nous avons cherché à savoir quels étaient les effets respectifs de la température et de la durée du chauffage.

## MATERIEL ET METHODES

De l'huile de colza primor a été chauffée dans diverses conditions : 200°C pendant 10 h, 200°C pendant 40h,240°C pendant 10 h, 240°C pendant 40 h. Ces huiles chauffées, ainsi que l'huile fraîche correspondante,ont été introduites comme seule source de lipides, au taux de 15 p.100, dans un régime purifié équilibré administré depuis l'âge de 4 semaines à des rats mâles WISTAR EOPS, provenant de l'élevage de la Station. 6 lots de 12 rats ont été constitués : 4 lots ont reçu les différentes huiles chauffées, tandis que 2 lots ont reçu l'huile fraîche. La consommation de nourriture d'un de ces 2 lots a été alignée, rat par rat (pair feeding) sur celle du lot recevant l'huile surchauffée (240°C, 40 h).

Les 5 autres lots ont consommé leur nourriture ad libitum. Pour ces 6 lots, nous avons utilisé la nomenclature suivante, qui sera employée dans tout ce rapport.

| Lot | Huile incorporée dans le régime | Consommation |
|---|---|---|
| A | 240° C, 40 h | ad libitum |
| B | 240° C, 10 h | ad libitum |
| C | 200° C, 40 h | ad libitum |
| D | 200° C, 10 h | ad libitum |
| E | Huile fraîche | ad libitum |
| F | Huile fraîche | alignée sur celle des rats du lot A |

La durée de l'administration a été de 13 semaines. L'urine de chaque animal a été recueillie quotidiennement sur HCl N pendant 10 jours consécutifs à 2 reprises : du 8ème au 18ème jour, ainsi que les 10 derniers jours de l'expérience. Cette urine a été congelée immédiatement après le prélèvement ; les urines des 10 jours consécutifs ont été rassemblées pour le dosage des glucuronides. Lors du sacrifice des animaux, le foie et le tissu adipeux épididymaire droit ont été prélevés pour l'analyse des acides gras et des monomères cycliques.

L'acide glucuronique conjugué a été dosé par la méthode de NIR (2), après que l'acide glucuronique libre ait été éliminé selon le procédé de FISHMAN et GREEN (3). Les lipides du foie et du tissu adipeux ont été extraits par la méthode de FOLCH et al. (4). Les esters méthyliques d'acides gras ont été préparés selon la méthode de MORRISON et SMITH (5). Les monomères cycliques ont été déterminés selon la technique décrite par POTTEAU (6). Ces monomères cycliques, ainsi que les acides gras normaux ont été dosés par chromatographie en phase gazeuse sur des colonnes capillaires en verre imprégnées de Carbowax 20 M. AT, à 190°C, en utilisant l'hélium comme gaz vecteur. L'identification des isomères géométriques de l'acide linolénique a été effectuée en utilisant la méthodologie décrite par SEBEDIO et ACKMAN (7).

RESULTATS

Les éléments les plus intéressants de la composition des huiles figurent dans le tableau 1.

On y constate d'abord que les monomères cycliques ne sont présents en teneur élevée que dans l'huile chauffée à 240°C pendant 40 heures. Leur teneur n'est cependant pas négligeable dans l'huile chauffée à 200°C pendant 40 heures ainsi que dans celle qui l'a été à 240°C pendant 10 heures.

Par ailleurs, on note la diminution considérable des teneurs en acides linoléique et linolénique corrélativement avec l'intensité du chauffage, tandis que des isomères géométriques du 18 : 2 ω 6 et du 18 : 3 ω 3 apparaissent en quantités notables.

On retrouve ces monomères cycliques et ces isomères géométriques dans le tissu adipeux des rats (tableau 2).

Dans le foie, les monomères cycliques ne sont présents qu'à des teneurs assez faibles, sauf dans le lot A (tableau 3). Les isomères géométriques des acides linoléique et linolénique sont également présents et en quantités parfois importantes; on observe à ce sujet deux phénomènes intéressants : d'abord les teneurs du 18 : 2 c9, t12 sont environ la moitié de celles du 18 : 2 t9, c12, alors que dans les huiles, les teneurs étaient équivalentes. PRIVETT et al. (8) avaient observé qu'une double liaison de configuration cis était nécessaire en position 9 pour la conversion du 18 : 2 en acides gras polyinsaturé. Ceci expliquerait que le 18 : 2 c9, t12 ayant pu être transormé, au moins partiellement, est moins abondant, dans le foie que le 18 : 2 t9, c12 qui, lui,n'aurait pu être métabolisé en acides gras polyinsaturés longs.

L'autre phénomène intéressant à noter est que l'on décèle des métabolites polyinsaturés longs provenant des isomères géométriques de l'acide linolénique (X, Y et Z sur le tableau 3). Ces composés sont en cours d'identification.

En ce qui concerne l'acide glucuronique conjugué urinaire, on constate (tableau 4) un effet notable du chauffage de l'huile. Les effets du niveau de température, ainsi que de la durée du chauffage sont également très significatifs. On retrouve donc bien ici les phénomènes décrits (1) avec une huile modèle. Cependant, compte tenu des niveaux assez faibles d'excrétion observés, ainsi que des teneurs peu importantes de monomères cycliques dans le tissu adipeux, il est vraisemblable que la détoxification par la voie de l'acide glucuronique n'est sans doute pas la seule voie de détoxification des produits formés dans l'huile au cours du chauffage.

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Tableau 1

Composition des huiles de colza primor utilisées en quelques acides gras et en monomères cycliques (en pourcentage des acides totaux de l'huile).

| | H. fraîche | H. chauffée à 200°C,10 h | H. chauffée à 200°C,40 h | H. chauffée à 240°C, 10h | H. chauffée à 240°C, 40h |
|---|---|---|---|---|---|
| 18 : 1 c9 | 49,08 | 49,25 | 47,20 | 48,07 | 43,56 |
| 18 : 2 c9,c12 | 20,28 | 18,68 | 13,61 | 15,03 | 5,52 |
| 18 : 2 c9,t12 | 0,14 | 0,18 | 0,30 | 0,84 | 1,27 |
| 18 : 2 t9,c12 | 0,13 | 0,12 | 0,18 | 0,67 | 1,03 |
| 18 : 3 monotrans dicis(1) | tr. | 0,16 | 0,51 | 1,36 | 0,22 |
| 18 : 3 c9,c12, c15 | 9,22 | 7,27 | 3,18 | 1,77 | 0,08 |
| 18 : 3 ditrans, monocis(1) | | | 0,05 | 0,67 | 0,44 |
| 18 : 3 monotrans dicis (1) | 0,17 | 0,35 | 0,49 | 1,47 | 0,27 |
| Monomères cycliques | 0,04 | 0,18 | 0,41 | 0,59 | 2,08 |

1) L'identification précise de ces composés est en cours.

Tableau 2

Composition du tissu adipeux en quelques acides gras et en monomères cycliques

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 18 : 2 c9,c12 | 4,88 | 12,15 | 11,43 | 15,65 | 17,05 | 16,64 |
| 18 : 2 c9,t12 | 0,98 | 0,76 | 0,35 | 0,22 | 0,13 | 0,11 |
| 18 : 2 t9,c12 | 1,03 | 0,64 | 0,22 | 0,15 | 0,14 | 0,11 |
| 18 : 3 monotrans dicis (1) | 0,18 | 0,91 | 0,38 | 0,25 | 0,10 | tr. |
| 18 : 3 c9,c12, c15 | 0,13 | 0,94 | 1,87 | 3,86 | 4,87 | 4,45 |
| 18 : 3 ditrans monocis(1) | 0,24 | 0,45 | 0,05 | tr. | | |
| 18 : 3 monotrans dicis (1) | 0,19 | 0,75 | 0,30 | 0,15 | 0,05 | tr. |
| Monomères cycliques | 0,43 | 0,14 | 0,14 | 0,07 | 0,04 | 0,04 |

(1) L'identification précise de ces composés est en cours.

Tableau 3

Composition du foie en quelques acides gras et en monomères cycliques

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 18 : 2 c9,c12 | 4,88 | 8,76 | 8,94 | 11,02 | 12,06 | 11,31 |
| 18 : 2 c9,t12 | 0,31 | 0,16 | 0,04 | 0,02 | 0,02 | 0,03 |
| 18 : 2 t9,c12 | 0,76 | 0,42 | 0,09 | 0,08 | 0,06 | 0,08 |
| 18 : 3 monotrans dicis(1) | 0,05 | 0,37 | 0,12 | 0,06 | tr. | 0,03 |
| 18 : 3 c9,c12,c15 | 0,06 | 0,27 | 0,40 | 0,98 | 1,47 | 1,13 |
| 18 : 3 ditrans monocis(1) | 0,07 | 0,17 | tr. | | | |
| 18 : 3 monotrans dicis(1) | 0,07 | 0,50 | 0,17 | 0,10 | 0,02 | 0,02 |
| X (1) | 0,27 | 0,56 | 0,25 | 0,08 | tr. | 0,02 |
| 20 : 5 ω 3 | 0,03 | 0,22 | 0,62 | 0,83 | 0,98 | 1,03 |
| Y (1) | 0,04 | 0,02 | | | | |
| 22 : 5 ω 3 | 0,12 | 0,35 | 0,70 | 0,80 | 0,96 | 0,97 |
| Z (1) | 0,41 | 0,14 | | | | |
| 22 : 6 ω 3 | 1,87 | 3,29 | 4,90 | 4,82 | 4,04 | 4,61 |
| Monomères cycliques | 0,17 | 0,08 | 0,07 | 0,05 | 0,04 | 0,04 |

(1) L'identification précise de ces composés est en cours.

Tableau 4

Acide glucuronique conjugué dans l'urine

| | Bilan 1 | | Bilan 2 | |
|---|---|---|---|---|
| | Consommation pendant le bilan (g/j) | Acide glucuronique conjugué urinaire (mg/j) | Consommation pendant le bilan (g/j) | Acide glucuronique conjugué urinaire (mg/j) |
| A | 13,95 | 8,76 | 16,11 | 10,34 |
| B | 13,30 | 5,28 | 14,84 | 7,29 |
| C | 13,57 | 5,16 | 15,30 | 7,91 |
| D | 13,36 | 3,41 | 15,07 | 5,64 |
| E | 13,51 | 3,15 | 14,75 | 5,55 |
| F | 13,74 | 2,45 | 15,39 | 5,56 |
| Ecart-type commun sur les moyennes | 0,261 | 0,366 | 0,624 | 0,332 |
| F de l'analyse de variance | 0,88 | 39,02 | 3,27 | 32,74 |
| Significativité | NS | ++ | + | ++ |
| Résultats des comparaisons multiples des moyennes par la méthode des contrastes : | | | | |
| - Effet de l'alignement de nourriture | | NS | NS | NS |
| - Effet du chauffage de l'huile | | ++ | NS | ++ |
| - Effet du niveau de la température de chauffage | | ++ | NS | ++ |
| - Effet de la durée du chauffage | | ++ | ++ | ++ |
| - Interaction température x durée | | + | NS | NS |

# Toxicological and nutritional evaluation of five different heated oils in rats

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## Abstract

Five different vegetable oils, viz. peanut oil (PO), sunflower oil (SFO), soybean oil (SBO), low-erucic-acid rapeseed oil (LEAR) and a mixture of palm oil, sunflower oil and rapeseed oil (PSRO; C 18:3 < 2.0 %) were subjected to a heat-treatment comparable to that of deep-fat frying. The oils were heated on 20 consecutive days. The temperature was raised to 220 °C and kept above 200 °C for about 12 hours in total. The oils subjected to 20 heating cycles and the non-heated oils were fed to rats at a dietary level of 15 % for a period of 19 weeks. After 10 weeks the rats were mated within each diet group in order to examine possible effects of the heated oils on reproduction.

The results of the chemical characterization of the heated and non-heated oils indicate that PO and LEAR were the most stable ones towards heat-treatment.

No outstanding differences in growth, food intake, food efficiency, plasma transaminase activity, plasma cholesterol, retinol, lipoprotein content and microscopy of several organs were observed between the heated and non-heated oil groups. Fat digestibility was slightly lower and plasma α-tocopherol levels were considerably lower in the heated oil groups than in the non-heated controls. There was an increase in the relative weight of the liver in females fed heated PO, heated SFO or heated PSRO. Relatively high liver weights were also observed in the groups fed LEAR, both heated and non-heated.

The reproduction study did not reveal any adverse effects of any of the heated oils on fertility, litter size at birth or mortality of the pups. Body weight gain of the pups during lactation was significantly depressed in all groups fed heated oil, except in the group fed heated PO.

In general, the results obtained with LEAR were not distinguishable from those obtained with the other oils.

## INTRODUCTION

In deep-fat frying, often the fat or oil is kept hot for long periods at temperatures which may exceed 200 °C. Heating causes measurable changes in the chemical and physical characteristics. These changes include the formation of low molecular oxidation products such as peroxides, carbonyls etc. or high molecular weight compounds such as dimeric- and polymeric triglycerides, which increase the viscosity of

the frying fat. Many reports of experimental studies with animals show that the biological properties of heated fats or oils are closely related to their chemical properties. Growth retardation, reduced food efficiency, decreased haemoglobin content of the blood, increased liver size and microscopical abnormalities of the liver are common findings upon feeding heated oil.

In many countries, for instance in France, especially oils are used for frying. Since oils are more susceptible to the damaging effect of heat-treatment than fats - because of the higher content of unsaturated fatty acids - the health risk involved in the ingestion of products fried in oils might be correspondingly higher than that associated with consuming products fried in fats or hydrogenated oils.

Some countries have attempted to regulate to what degree an oil or fat is allowed to be deteriorated by heating before it should be condemned. In The Netherlands, the content of polymeric triglycerides should not exceed 10 per cent (Van der Heide, R.F. and J. van der Veen, 1977). In the Federal Republic of Germany a level of 27 per cent of polar compounds is considered the upper limit (Billek, G. et al., 1979). In France, there is an ordinance of 1973 saying that oils with a linolenic acid level higher than 2 per cent should not be used for deep-fat frying.

In low-erucic-acid rapeseed oil usually a linolenic acid content of approximately 9 per cent is found and should, therefore, like soybean oil with about 6 per cent linolenic acid, not be used for frying. In order to obtain more information on the effect of heat-treatment on the chemical and biological properties of a commercial low-erucic-acid French rapeseed oil (LEAR), we undertook a study in which heated and non-heated LEAR was fed to rats at a dietary level of 15 per cent for 19 weeks. Heated and non-heated peanut oil (PO), sunflower oil (SFO), soybean oil (SBO) and a mixture of palm oil, sunflower oil and low-erucic-acid rapeseed oil (51/29/20; PSRO) were tested simultaneously for comparative purposes. The study was sponsored by CETIOM, Paris, France, under the supervision of CNERNA, Paris, France.

## HEAT-TREATMENT AND CHEMICAL ANALYSES OF THE OILS

The fatty acid composition of the five non-heated oils, determined by capillary GLC, is given in table 1.

TABLE 1 - FATTY ACID COMPOSITION OF THE NON-HEATED OILS IN WEIGHT % OF THE FATTY ACID METHYL ESTERS

| Fatty acid | peanut oil | sunflower oil | soybean oil | rapeseed oil | palm/ sunflower/ rapeseed |
|---|---|---|---|---|---|
| 12:0 | - | - | - | - | 0.2 |
| 14:0 | - | - | 0.1 | - | 0.5 |
| 16:0 | 10.7 | 5.7 | 10.7 | 5.2 | 21.7 |
| 16:1 | 0.1 | - | 0.1 | 0.3 | 0.1 |
| 17:0 | 0.1 | - | 0.1 | - | 0.1 |
| 18:0 | 3.4 | 4.5 | 4.0 | 1.6 | 4.2 |
| 18:1 | 46.9 | 16.6 | 23.9 | 57.3 | 38.2 |
| 18:2 | 31.7 | 71.5 | 53.3 | 21.8 | 31.7 |
| 18:3 | 0.1 | 0.1 | 6.0 | 8.7 | 1.8 |
| 20:0 | 1.5 | 0.3 | 0.4 | 0.6 | 0.4 |
| 20:1 | 1.1 | 0.1 | 0.2 | 1.8 | 0.5 |
| 22:0 | 3.1 | 0.7 | 0.4 | 0.2 | 0.2 |
| 22:1 | - | - | - | 1.9 | 0.3 |
| 24:0 | 1.3 | - | - | - | - |

The following remarks can be made: The linoleic acid content of PSRO was similar to that of PO, but differs in that it has a higher linolenic acid content of 1.8 %, which is, however, lower than the highest permittable level of 2 % mentioned in the French ordinance. The rapeseed oil was low in erucic acid (1.9 %) and high in oleic acid (57.3 %). Relatively high levels of linolenic acid were found both in SBO and LEAR.

30 l of each type of oil were heated simultaneously in deep fat fryers (type: Becuwe Thomselle) on 20 consecutive days. Every day the following temperature cycle was applied: Ambient - 220 °C - 200 °C - 220 °C - ambient. In total (20 days) the temperature was kept above 200 °C for about 12 hours. No foods were fried. To follow the degree of deterioration of the oil, samples were taken after 0, 5, 10, 15 and 20 days of heat-treatment and analyzed for a.o. polar material (Guhr, G. and J. Waibel, 1978) and polymeric triglycerides (by High Pressure Gelpermeation Chromatography). These two parameters can be considered as good indicators of the heat-damage of the oils.

The results obtained are given in figures 1 and 2.

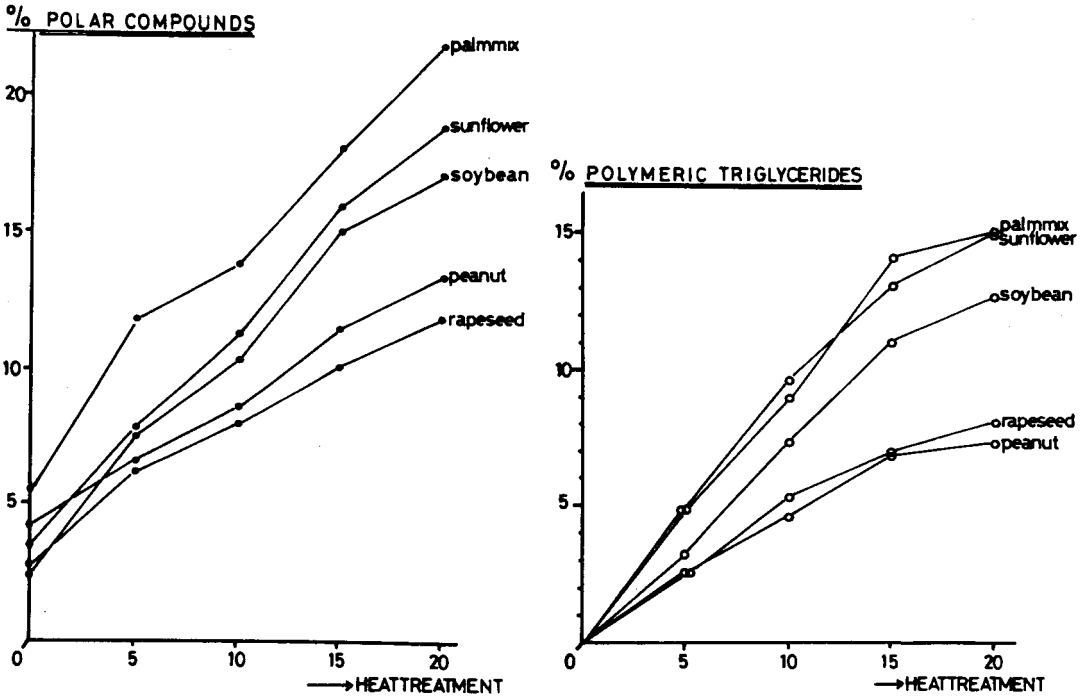


Fig. 1 and 2 - EFFECT OF HEAT-TREATMENT ON THE PERCENTAGE OF POLAR COMPOUNDS AND POLYMERIC TRIGLYCERIDES OF FIVE DIFFERENT OILS

The contents of polar material and polymeric triglycerides showed a gradual increase with an increasing number of heat-treatments. The highest levels after 20 days of heat-treatment were observed with PSRO, SFO and SBO, whereas PO and LEAR showed the lowest levels, indicating that the latter two oils were the most stable ones towards heat-treatment.

FEEDING STUDY IN RATS

The oils obtained after 20 heating cycles were used for a combined subchronic toxicity and reproduction study in rats. For that purpose, the oils, both heated and non-heated, were incorporated in a, nutritionally well-balanced, semi-purified diet at a level of 15 % and fed to groups of 20 male and 20 female Wistar rats (Cpb:WU) for a period of 19 weeks. For the composition of the diets, see table 2.

TABLE 2 - COMPOSITION OF THE DIETS

| Ingredient | Weight % |
|---|---|
| Casein | 22 |
| Dl-methionine | 0.2 |
| Wheat starch | 26.5 |
| Saccharose | 26.5 |
| Cellulose | 5 |
| Vitamin ADEK mixture[1)] | 0.35 |
| Vitamin B mixture[2)] | 0.25 |
| Mineral mixture[3)] | 4.20 |
| Heated or non-heated oil | 15 |
| Total | 100.00 |

1) Per one g mixture: vitamin A 2215 IU, vitamin $D_3$ 705 IU, vitamin E 15 mg, vitamin $K_3$ 1 mg.

2) Per one g mixture: vitamin $B_1$ 3 mg, vitamin $B_2$ 2.25 mg, vitamin $B_6$ 4.5 mg, niacin 15 mg, Ca-panthothenate 6 mg, biotin 0.075 mg, folic acid 0.75 mg, vitamin $B_{12}$ (0.1 %) 37.5 mg, choline chloride (50 %) 931 mg.

3) Per one g mixture: $KH_2PO_4$ 399 mg, $CaCO_3$ 389 mg, NaCl 142 mg, $MgSO_4$ 58 mg, $FeSO_4.7\ H_2O$ 5.7 mg, $ZnCl_2$ 0.9 mg, $CuSO_4.5\ H_2O$ 0.8 mg, $MnSO_4.2\ H_2O$ 4.6 mg, $CoCl_2.6\ H_2O$ 0.02 mg, KJ 0.07 mg, $KCr(SO_4)_2.12\ H_2O$ 0.08 mg.

The rats were housed under conventional conditions, five per sex per cage, in suspended stainless steel cages, fitted with wire mesh floors and fronts, in a room which was controlled with respect to ventilation (c. 10 air changes per hour), temperature (23 $\pm$ 1 °C) and relative humidity (50 $\pm$ 10 %). A 12-hour light/dark cycle was maintained.

At week 10 of the study, males and females within each group were allowed to mate, one male to one female, for a period of three weeks, in order to examine possible effects of the heated oils on reproduction.

Observations were made of behaviour and with respect to general health. In the parent animals the following determinations were carried out:

body weight, food intake and food efficiency: weekly during the first 10 weeks of the study; digestibility of fat and protein: week 10; in terminal blood samples: GOT and GPT (Lippi and Guidi-method), vitamin A and E (high speed liquid chromatography and HPLC respectively), cholesterol and lipoproteins (enzymatic method and electrophoresis on agarose gel). After 19 weeks of feeding the various diets, the parent rats were killed, the liver, heart, kidneys, thymus, adrenals and gonads were weighed and subjected to microscopical examination.

In the reproduction study observations were made as well as calculations of the fertility index of the females, the mean litter size at birth, the mean pup weight at birth and on day 4, 14 and 21 after birth, pup mortality at birth and during lactation. From the number of implantation sites found in the uterus of the mothers and the total number of pups born, the resorption quotient was calculated as the total number of implantations/total number of pups born.

Most of the data were analyzed statistically by a two-way analysis of (co)variance, whereby the effect of heating, type of oil and interaction between heating and type of oil were examined.

## RESULTS

Data on body weight gain, food consumption and food efficiency are summarized in table 3.

TABLE 3 - BODY WEIGHT GAIN, FOOD INTAKE AND FOOD EFFICIENCY. MEAN VALUES OF GROUPS OF 20 RATS

| Diet group | Body weight gain (g/rat) week 0-10 | Food intake (g/rat) week 0-10 | Food efficiency (gain/food) week 0-10 | Body weight gain (g/rat) week 0-10 | Food intake (g/rat) week 0-10 | Food efficiency (gain/food) week 0-10 |
|---|---|---|---|---|---|---|
| | MALES | | | FEMALES | | |
| PO-control | 274.7 | 1030 | 0.267 | 136.6 | 739 | 0.185 |
| PO-heated | 291.3 | 1086 | 0.268 | 136.3 | 738 | 0.185 |
| SFO-control | 283.1 | 1031 | 0.275 | 128.7 | 717 | 0.179 |
| SFO-heated | 277.3 | 1045 | 0.265 | 135.2 | 714 | 0.189 |
| SBO-control | 292.4 | 1052 | 0.278 | 132.9 | 700 | 0.190 |
| SBO-heated | 286.4 | 1023 | 0.280 | 142.0 | 741 | 0.192 |
| LEAR-control | 266.4 | 1017 | 0.262 | 128.2 | 705 | 0.182 |
| LEAR-heated | 264.9 | 1015 | 0.261 | 136.9 | 720 | 0.190 |
| PSRO-control | 286.4 | 1045 | 0.274 | 133.6 | 704 | 0.190 |
| PSRO-heated | 266.7 | 1010 | 0.264 | 136.4 | 732 | 0.186 |

There were no outstanding differences in body weight gain amongst the various groups. Only in males fed LEAR, both heated and non-heated, body weight gain was less than in the other groups. The differences with the other groups were statistically significant in most of the weeks examined. Food intake figures were generally comparable amongst the groups. Males fed heated SBO or heated PSRO tended to eat less than the corresponding controls, but in females the opposite phenomenon occurred. Food efficiency was slightly lower in males fed LEAR, both heated and non-heated, but no such an effect was noticeable in females.

The results of determinations of the digestibility of fat and protein are given in table 4.

TABLE 4 - DIGESTIBILITY OF FAT AND PROTEIN DETERMINED DURING WEEK 10 OF THE STUDY

| Diet group | Males | | Females | |
|---|---|---|---|---|
| | Digestibility coefficient (%) for | | Digestibility coefficient (%) for | |
| | Fat | Protein | Fat | Protein |
| PO-control | 93.5 | 78.2 | 95.9 | 84.8 |
| PO-heated | 93.1 | 81.3 | 94.9 | 75.4 |
| SFO-control | 96.0 | 79.2 | 97.6 | 83.7 |
| SFO-heated | 94.1 | 76.2 | 94.7 | 82.8 |
| SBO-control | 96.1 | 74.4 | 97.7 | 84.6 |
| SBO-heated | 95.0 | 80.7 | 97.3 | 85.8 |
| LEAR-control | 94.8 | 82.6 | 96.8 | 87.4 |
| LEAR-heated | 94.4 | 78.2 | 95.7 | 89.0 |
| PSRO-control | 95.3 | 79.1 | 97.0 | 86.0 |
| PSRO-heated | 93.8 | 78.6 | 94.2 | 84.3 |

The digestibility of fat and protein was high in all groups. However, the digestibility of fat in the diets with heated oil was consistently slightly lower than that of the corresponding diet with non-heated oil. The differences were greatest with the oils containing the highest content of polymeric triglycerides viz. heated SFO and heated PSRO. The digestibility of protein was not noticeably affected by the heat treatment of any of the oils.

The levels of vitamin A and E determined in pooled plasma samples at the end of the study are presented in table 5, together with the content of vitamin A and E in the diets.

TABLE 5 - RESULTS OF VITAMIN A AND E DETERMINATIONS IN FOOD AND PLASMA OF RATS

| Diet group | Vitamin A in diet (IU/g) | Retinol content (µ mol/l) of plasma[1] | | α-tocopheryl acetate in diet (µg/g) | α-tocopherol content (µ mol/l) of plasma[1] | |
|---|---|---|---|---|---|---|
| | | males | females | | males | females |
| PO-control | 12.9 | 1.50 | 0.80 | 85 | 33.4 | 31.4 |
| PO-heated | 12.8 | 1.45 | 0.83 | 65 | 22.4** | 23.9** |
| SFO-control | 12.8 | 1.35 | 0.85 | 160 | 35.4 | 30.7 |
| SFO-heated | 12.8 | 1.28 | 0.98 | 100 | 21.3*** | 20.6** |
| SBO-control | 14.0 | 1.40 | 0.85 | 75 | 31.2 | 30.0 |
| SBO-heated | 13.2 | 1.28** | 0.93 | 70 | 18.8*** | 22.7* |
| LEAR-control | 12.8 | 1.48 | 0.68 | 110 | 36.9 | 31.6 |
| LEAR-heated | 13.0 | 1.50 | 0.73 | 80 | 22.1** | 22.4*** |
| PSRO-control | 13.0 | 1.20 | 0.60 | 110 | 38.3 | 32.8 |
| PSRO-heated | 13.0 | 1.18 | 0.70* | 70 | 17.6* | 20.6** |

1) Values are the mean of 4 pooled samples of 5 rats/sex/group.

Degrees of significance (Student t test): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The plasma levels of vitamin E (α-tocopherol) of the groups fed the heated oils were considerably lower than those of the controls fed the corresponding non-heated oils, both in males and females. It is most likely that the decreased plasma levels are due to the lower vitamin E contents of the diets containing heated oil as compared with the corresponding diets containing non-heated oil. The relatively low α-tocopherol levels of the diets with heated oil can probably be accounted for by losses of vitamin E, naturally present in the oils, during the heat-treatment. The vitamin A levels of both the diets and the plasma samples were rather uniform amongst the groups.

The determinations of cholesterol, lipoproteins, glutamic-oxalacetic transaminase- and glutamic-pyruvic transaminase activity did not reveal any outstanding differences amongst the groups.

The mean relative weight of the liver, determined at autopsy after a 19-week feeding period, is given in table 6.

TABLE 6 - MEAN RELATIVE LIVER WEIGHT OF GROUPS OF 20 MALE AND 20 FEMALE RATS AFTER FEEDING THE VARIOUS DIETS FOR 19 WEEKS

| Diet group | Mean relative liver weight (g/kg) ± SEM | |
|---|---|---|
| | Males | Females |
| PO-control | 29.3 ± 0.5 | 32.6 ± 0.6 |
| PO-heated | 29.3 ± 0.6 | 35.1 ± 0.9 |
| SFO-control | 30.5 ± 0.5 | 31.6 ± 0.6 |
| SFO-heated | 29.9 ± 0.4 | 35.0 ± 0.8 |
| SBO-control | 30.1 ± 0.6 | 34.5 ± 0.5 |
| SBO-heated | 30.0 ± 0.6 | 33.3 ± 0.6 |
| LEAR-control | 32.4 ± 0.5 | 35.6 ± 0.7 |
| LEAR-heated | 32.9 ± 0.4 | 35.1 ± 0.5 |
| PSRO-control | 30.5 ± 0.5 | 32.7 ± 0.6 |
| PSRO-heated | 31.6 ± 0.5 | 35.5 ± 0.7 |

There was no effect of heat-treatment on the relative weight of the liver in males. In females, however, there was a statistically significant increase in liver weight in the groups fed heated PO, SFO or PSRO. The values found in these groups (c. 35 g/kg) were of the same magnitude as those found in the groups fed heated or non-heated LEAR. Relatively high liver weights were also observed in males fed heated or non-heated LEAR.

Gross examination at autopsy and microscopical examination of kidneys, liver, heart, testes, adrenals, pancreas, ovaries and thymus did not reveal any abnormality attributable to the feeding of the heated or non-heated oils. The macroscopical as well as microscopical observations in the groups fed LEAR were not essentially different from those in the other groups.

In table 7 a summary of the reproduction data is given.

TABLE 7 - SUMMARY OF THE REPRODUCTION DATA

| Diet group | Fertility[1] index (%) | Mean litter size at birth | Mean pup weight at birth (g) | Mean pup weight at day 21 (g) | Pup mortality at birth (%) | Pup mortality during lactation (%) | Resorption[2] quotient |
|---|---|---|---|---|---|---|---|
| PO-control | 95 | 10.9 | 5.6 | 45.5 | 5.3 | 10.0 | 1.11 |
| PO-heated | 100 | 9.6 | 5.9 | 44.9 | 3.1 | 1.5 | 1.85 |
| SFO-control | 95 | 8.3 | 5.2 | 45.9 | 22.9 | 24.3 | 2.10 |
| SFO-heated | 89 | 10.2 | 5.1 | 38.1 | 21.3 | 21.8 | 1.33 |
| SBO-control | 100 | 11.4 | 5.5 | 44.7 | 9.7 | 16.0 | 1.10 |
| SBO-heated | 95 | 10.9 | 5.4 | 38.3 | 11.1 | 21.5 | 1.12 |
| LEAR-control | 100 | 10.9 | 5.2 | 43.1 | 4.1 | 18.5 | 1.13 |
| LEAR-heated | 100 | 11.6 | 5.5 | 38.2 | 1.3 | 6.3 | 1.09 |
| PSRO-control | 100 | 10.0 | 5.5 | 47.6 | 4.0 | 15.9 | 1.17 |
| PSRO-heated | 95 | 10.2 | 5.8 | 38.9 | 9.3 | 11.9 | 1.15 |

1) Fertility index = no. of pregnancies/no. of matings x 100 %.
2) Resorption quotient = total no. of implantations/total no. of pups born.

The number of successful matings, as expressed by the fertility index, was high in all groups and did not appear to be affected by the heat-treatment of any of the oils. Mean litter size at birth was relatively low in the group fed non-heated SFO. A consistent difference between the groups fed heated oil and the corresponding controls was, however, not apparent. Body weight gain of the pups during lactation was significantly depressed in all groups fed heat-treated oil, except in the group fed heated PO. The growth retardation was noticeable already on day 14 and was more marked on day 21, resulting in statistically significant differences between the heated and non-heated oil groups. In the groups fed SFO, both heated and non-heated, the mean pup weight at birth was decreased in a statistically significant way as compared with most other groups. Mortality of pups at birth and during lactation was extremely high in both SFO-fed groups. In the LEAR-fed groups mortality figures were not distinguishable from those of the other groups. The resorption quotient was unusually high in the group fed heated PO and especially in the group fed non-heated SFO. There was, however, a large variation amongst the resorption quotient of the individual animals in these groups. An effect of the heat-treatment of the oils on the resorption quotient was not apparent.

The groups fed heated LEAR showed the most favourable reproduction data: the highest number of pups at birth with a relatively high body weight, low mortality figures at birth and during lactation and the lowest resorption quotient.

## DISCUSSION AND CONCLUSION

Feeding studies in rats conducted at our institute with oils and fats that had been submitted to severe heat-treatment revealed growth depression accompanied with decreased food intake and food efficiency, decreased haemoglobin content of the blood and increases of the relative weight of the liver and occasionally also of the kidneys (Van der Heide, R.F. and J. van der Veen, 1977). In the present study, the parent rats did not exhibit decreased growth rate, food intake or food efficiency in any of the groups fed heated oil. The heat-treatment applied was probably not sufficiently severe to induce the above mentioned changes.

The digestibility of the fat in the diets with heated oil was consistently slightly lower than that in the diets with the corresponding non-heated oil, both in males and females. The differences were greatest with the oils containing the highest content of polymeric triglycerides viz. heated SFO and heated PSRO. This relationship suggests that the decreased digestibility of the heated oil had possibly been caused by the presence of polymeric triglycerides.

The slightly increased liver weights observed in some groups of females fed heated oil were not accompanied with histopathological changes or with increases in transaminase activity, which usually indicate liver damage. The increased liver weights are therefore considered a reflection of altered metabolic processes in the liver, rather than of hepatotoxic properties of heated oils. The relatively high liver weight in the groups fed both heated and non-heated LEAR may likewise be accounted for by altered metabolic processes in the liver due to xenobiotic substances in LEAR.

The only effect on reproduction which can be related to the feeding of the heat-treated oils was a decrease in growth rate of the pups during the third week of lactation. This phenomenon was hardly noticeable in the group fed heated PO, but in the other groups fed heated oil, the mean pup weight was 11-18 % lower than in the corresponding group fed non-heated oil. Lower pup weights at day 21 were also observed in a comparable study of Coquet, B. et al. (1977), although the differences between the groups fed heated or non-heated oil were less pronounced than in the present study. The growth depression mainly occurred during the last week of lactation, when the pups are no longer solely dependent on breast milk, because they eat also from the feeders. In palatability tests it has been established that the rats showed a dislike for the diets with heated oil (Sinkeldam, E.J., unpublished observations). Therefore, a reduced food intake might have been the cause of the decreased growth rate.

The relatively high number of pups born dead in the groups fed heated or non-heated SFO was remarkable. In the group fed non-heated SFO this finding was accompanied with a relatively small litter size, a low pup weight at birth and a high number of resorptions in utero. A relatively high pup mortality with SFO has also been observed by Guillaumin, R. et al. (1980). The cause of the phenomenon with SFO remains unclear.

From the results obtained it seems justified to conclude that the feeding of the five heated oils to rats at a dietary level of 15 % for 19 weeks did not induce effects of obvious toxicological significance. With respect to the deteriorating effect of heat-treatment the two oils with a relatively high linolenic acid content, viz. LEAR and SBO, were not worse than the other oils. On the contrary LEAR was, with PO, the oil most stable towards heat-treatment.

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# Effets comparatifs de l'huile de tournesol et de l'huile de colza à faible teneur en acide érucique dans le traitement de l'hypercholestérolémie familiale hétérozygote.

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Résumé

On a démontré que l'huile de colza faible en acide érucique abaissait la cholestérolémie totale chez des sujets normaux. Avec un rapport acides gras polyinsaturés sur acides gras saturés (P/S) de 5, cette huile pourrait être utilisée avantageusement comme supplément diétothérapeutique chez des patients hypercholestérolémiques.

Une étude a été menée chez des sujets féminins porteurs de la forme hétérozygote de l'hypercholestérolémie familiale traités dans une clinique de dépistage. Une étude à double insu avec permutation de traitements a permis de comparer les effets de l'ingestion et du retrait de suppléments d'huile de tournesol ou d'huile de colza. Les sujets ont été divisés au hasard en deux groupes soumis à deux périodes expérimentales de quatre semaines précédées de périodes d'épuration de trois semaines. Le supplément d'huile administré (55 ml/jour) accompagnait un régime bas en cholestérol.

Nous n'avons pas remarqué de différence significative entre les effets de l'huile de tournesol et ceux de l'huile de colza sur les paramètres plasmatiques. L'ingestion de suppléments d'huile entraînait des réductions du cholestérol total (7%), du C-LDL (10%), de l'apo-B-totale et de l'apo-B-LDL (11%). Les résultats montrent une hausse significative des taux de C-HDL (34%) et de l'apo-A-totale (30%). Ces paramètres diminuaient respectivement de 42% et 36% au cours de la période d'épuration. Finalement, on a aussi noté une baisse importante (29%) de l'indice athérogène et une augmentation (45%) de ce paramètre lors du retrait des suppléments. Cette étude préliminaire permet de conclure au potentiel de l'huile de colza comme supplément polyinsaturé dans le traitement de l'hypercholestérolémie familiale.

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## INTRODUCTION

L'hypercholestérolémie familiale monogénique est caractérisée par des taux élevés de cholestérol sérique, particulièrement des LDL. Le traitement recommandé est un régime alimentaire pauvre en cholestérol (< 300 mg/jour) avec un apport en acides gras polyinsaturés élevé (P/S = 2). On recommande à cet effet des suppléments d'huiles végétales (huile de carthame, de tournesol ou de maïs). L'huile de colza à faible teneur en acides érucique a un rapport acides gras polyinsaturés/acides gras saturés (P/S) de 6 légèrement inférieur à celui de l'huile de tournesol (P/S = 7.0) mais plus élevé que celui de l'huile de maïs (P/S = 4). Chez des sujets normolipidémiques, on a observé une diminution de 15 à 20% de la cholestérolémie quand l'huile de colza constituait la totalité des graisses alimentaires (1, 2). L'objectif de cette étude préliminaire est de vérifier l'effet de l'huile de colza sur la cholestérolémie d'individus atteints d'hypercholestérolémie familiale hétérozygote.

## MATERIEL ET METHODES

L'étude a été menée avec 9 sujets féminins de poids normal, âgés de 26 à 46 ans, porteurs de la forme hétérozygote de l'hypercholestérolémie familiale. Les sujets étaient traités en clinique externe depuis au moins 1 an. Toute médication hypocholestérolémiante a été suspendue mais ils ont continué de s'astreindre au régime thérapeutique conventionnel.

L'étude à double insu avec permutations de traitements a duré 15 semaines. Pendant la 1ère période (4 semaines de standardisation), les sujets divisés au hasard (Groupe A: 4 sujets, Groupe B: 5 sujets) ne recevaient aucun supplément d'huile végétale. Durant la 1ère période expérimentale de 4 semaines, les sujets du groupe A ingéraient un supplément d'huile de colza et ceux du groupe B, d'huile de tournesol. Après une période d'épuration de 3 semaines sans supplément d'huile, l'étude a été complétée par une deuxième période expérimentale (4 semaines) avec inversion des suppléments. La quantité recommandée quotidiennement (45 g/jour) a été calculée selon l'apport moyen en acides gras d'un premier bilan alimentaire à l'exclusion des suppléments que les sujets prenaient déjà, de façon à obtenir un P/S global de 2.0.

Lors de la première et de la dernière visite, on établissait le bilan alimentaire pour chaque sujet et le P/S moyen de l'ensemble. Au début et à la fin de chaque période du protocole, un échantillon sanguin a été prélevé pour le dosage du cholestérol (Autoanalyseur Technicon). Les VLDL ont été séparées par ultracentrifugation et la fraction de densité supérieure à 1.006 g/ml a été utilisée pour la mesure des HDL. Les données ont été

évaluées par analyse de variance et analyse statistique de plan avec permutations de traitements(4).

## RESULTATS

Les deux bilans alimentaires n'étaient pas différents et correspondaient aux objectifs du traitement diététique (chol. < 300 mg/j. et P/S = 1.8-2.0). De même, les consommations quotidiennes moyennes de suppléments d'huile atteignaient la quantité recommandée (45 g/jour).

Comme le démontrent les résultats du Groupe A (tableau 1), l'élimination de toute médication et du supplément d'huile a entraîné une augmentation des taux de l'apo-B totale (22%), de l'apo-B-LDL (12%) et de l'indice athérogène (19%) et une diminution du Chol-HDL (13%) et de l'apo-A totale (16%: $P < 0.05$). Après 2 semaines de supplémentation avec l'huile de colza, on a remarqué une diminution des taux de cholestérol total (8%), du Chol-LDL, de l'apo-B totale et de l'apo-B-LDL (de 8 à 13%). Par contre, les taux d'apo-A augmentaient après 4 semaines de traitement ($P < 0.005$) et reflètaient l'accroissement de chol-HDL (27%; $P > 0.001$). L'indice athérogène a diminué graduellement (35%) avec le supplément d'huile de colza. La période d'épuration a supprimé les effets de la supplémentation en entraînant une hausse des taux d'apo-B (34%) et d'apo-B-LDL (24%) et des baisses significatives des concentrations en apo-A (36%; $P < 0.001$) et en Chol-HDL (42%; $P < 0.005$). Cette diminution a amené une augmentation importante (71%) de l'indice athérogène. Quand les sujets retournaient à un supplément d'huile de tournesol, on a remarqué une tendance à la baisse des taux de cholestérol total, du Chol-LDL, de l'apo-B totale et de l'apo-B-LDL. Les concentrations des paramètres reliés aux HDL augmentaient graduellement avec une baisse concomitante de l'indice athérogène (31%).

Chez les sujets soumis à une séquence inverse de suppléments (Groupe B, tableau 2), l'huile de tournesol a produit une baisse du cholestérol total, du Chol-LDL, de l'apo-B totale et de l'apo-B-LDL (de 8 à 12%). Au cours de la période d'épuration, on a remarqué une baisse significative du taux d'apo-A ($P < 0.05$) et du Chol-HDL ($P < 0.01$) avec une augmentation significative (22%; $P < 0.01$) de l'indice athérogène. La supplémentation avec l'huile de colza a entraîné une hausse graduelle des taux de Chol-HDL.

L'analyse statistique démontre qu'il n'y a pas de différence significative entre les effets des deux huiles ou leur séquence d'administration sur les paramètres biochimiques évalués.

## DISCUSSION

Une étude à double insu avec permutations de traitements permet de supprimer l'effet de séquence (4). De même,

TABLEAU 1: Influence des suppléments polyinsaturés sur les paramètres lipidiques sanguins.

GROUPE A[1]

| Paramètres sanguins mg/100 ml | Périodes (semaines) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 6 | 8 | 11 | 13 | 15 |
| | | Stand. | 1ère Pér. expériment. | | Epuration | 2e Pér. expériment. | |
| Chol total | 352 ± 73[2] | 371 ± 50 | 340 ± 35 | 364 ± 76 | 343 ± 45 | 325 ± 70 | 371 ± 91 |
| Apo-B | 156 ± 38 | 190 ± 45 | 169 ± 30 | 170 ± 49 | 228 ± 73 | 203 ± 57 | 223 ± 71 |
| Apo-A | 225 ± 21 | 190 ± 16 | 238 ± 19 | 257 ± 23 | 165 ± 37 | 176 ± 32 | 214 ± 35 |
| Chol-LDL | 291 ± 67 | 320 ± 53 | 280 ± 34 | 292 ± 77 | 292 ± 34 | 276 ± 68 | 314 ± 87 |
| Apo-B-LDL | 153 ± 41 | 172 ± 41 | 155 ± 37 | 161 ± 41 | 200 ± 58 | 178 ± 45 | 206 ± 83 |
| Chol-HDL | 47 ± 10 | 41 ± 6 | 50 ± 9 | 60 ± 4 | 35 ± 9 | 45 ± 8 | 45 ± 5 |
| Indice athérogène[3] | 7.9 ± 3.1 | 9.4 ± 2.6 | 7.1 ± 1.8 | 6.2 ± 1.4 | 10.6 ± 3.6 | 7.4 ± 3.0 | 8.3 ± 2.4 |

[1] Séquence de la supplémentation d'huile (huile de colza - huile de tournesol).

[2] Moyenne ± écart-type.

[3] Rapport $\frac{\text{cholestérol total}}{\text{cholestérol-HDL}}$

TABLEAU 2: Influence des suppléments polyinsaturés sur les paramètres lipidiques sanguins.

GROUPE B[1]

| Paramètres sanguins mg/100 ml | Périodes (semaines) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 6 | 8 | 11 | 13 | 15 |
| | | Stand. | 1ère Pér. expériment. | | Epuration | 2e Pré. expériment. | |
| Chol total | 300 ± 26[2] | 319 ± 39 | 287 ± 39 | 327 ± 35 | 285 ± 20 | 309 ± 54 | 310 ± 49 |
| Apo-B | 137 ± 14 | 157 ± 31 | 140 ± 26 | 151 ± 16 | 165 ± 14 | 187 ± 35 | 173 ± 39 |
| Apo-A | 201 ± 19 | 210 ± 20 | 225 ± 29 | 229 ± 24 | 178 ± 37 | 188 ± 35 | 190 ± 36 |
| Chol-LDL | 244 ± 31 | 262 ± 40 | 233 ± 32 | 262 ± 26 | 237 ± 17 | 260 ± 47 | 257 ± 39 |
| Apo-B-LDL | 129 ± 17 | 144 ± 26 | 133 ± 18 | 146 ± 15 | 153 ± 16 | 171 ± 23 | 158 ± 29 |
| Chol-HDL | 40 ± 4 | 44 ± 3 | 44 ± 4 | 51 ± 9 | 36 ± 3 | 39 ± 13 | 41 ± 7 |
| Indice athérogène[3] | 7.5 ± 0.7 | 7.3 ± 0.7 | 6.5 ± 0.8 | 6.5 ± 0.1 | 7.9 ± 0.5 | 8.3 ± 2.4 | 7.5 ± 0.6 |

[1] Séquence de la supplémentation d'huile (huile de tournesol - huile de colza).

[2] Moyenne ± écart-type.

[3] Rapport $\frac{\text{cholestérol total}}{\text{cholestérol-HDL}}$

l'inclusion d'une étape de standardisation sans médication ni supplément d'huile et une période d'épuration nous apparaît primordiale. Les baisses des taux de cholestérol total observées sont comparables à celles rapportées au cours d'autres études cliniques (5, 6). De plus, le supplément d'huile de colza produit une diminution du taux de Chol-LDL (lipoprotéines athérogènes) plus importantes. L'élimination des suppléments insaturés fait augmenter jusqu'à 35% les taux de l'apo-B totale. Nos résultats sont comparables à ceux de Boberg (10).

La plupart des études cliniques à court terme (5, 6, 7,) montrent une diminution de l'ordre de 10 à 25% du taux de Chol-HDL quand des sujets hyperlipidémiques sont soumis au régime hypocholestérolémiant riche en graisses polyinsaturés. Nos résultats s'accordent plutôt avec l'étude d'Oslo (8) puisque nous avons observé des hausses significatives (jusqu'à 40%) avec la supplémentation à l'huile de colza. De plus, quand nous avons retiré ces suppléments, les taux de Chol-HDL ont chuté pour atteindre les niveaux les plus bas de l'étude. Ces variations se reflètent invariablement sur les valeurs de l'indice athérogène. Comme les HDL protègent l'individu contre l'athérosclérose et qu'une diminution de la valeur de l'indice athérogène indique une baisse du risque coronarien, nous pouvons affirmer que le traitement avec le supplément d'huile de colza a été bénéfique pour les sujets. Cependant, nous sommes conscients que d'autres facteurs alimentaires tels que les lipides et les glucides du régime peuvent influencer les taux de Cho-HDL. Il serait donc intéressant, dans une étude plus élaborée, d'établir la répartition calorique des lipides, protéines et glucides à chaque étape du protocole.

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# Comportement de l'huile de colza et de quelques autres huiles lors du chauffage - Aspects chimiques

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L'étude porte sur 4 huiles chauffées : arachide, tournesol, soja et colza, portées 20 fois à 220°C. Elle consiste en des dosages complémentaires à ceux effectués par le T.N.O. (Hollande). Son objet est de définir la nature et le niveau des transformations d'ordre chimique supportées par les glycérides et les acides gras.

Ainsi ont été obtenues les teneurs en :

- glycérides altérés (tableau 1) ;
- polymères de glycérides (dimères et oligomères) (tableau 2) ;
- esters altérés (ou E.C.N.) (tableau 3) ;
- esters polymérisés (tableau 3) ;
- oxyesters (oxymonomères et oxypolymères (tableaux 1 et 3) ;
- esters altérés non oxydés et oxydés non polymérisés ;
- monomères cycliques (tableau 4).

Les méthodes choisies pour ce faire correspondent à l'état actuel de nos connaissances en la matière.

## Résultats

Tous les acides insaturés participent à l'élaboration de produits de transformation thermooxydative, d'autant plus que les acides sont plus insaturés et que ceux-ci sont plus souvent représentés.

L'ensemble des données d'ordre chimique recueillies reflète la bonne tenue générale des huiles de colza et d'arachide, qui offrent un comportement tout à fait comparable. Ces deux huiles chauffées sont en effet les mieux placées pour :

- les glycérides altérés (tableau 2) ;
- les polymères de triglycérides (tableau 3) ;

- les dimères et oligomères supérieurs ;
- les esters altérés (E.C.N.) (tableau 3) ;
- les esters polymérisés (tableau 3) ;
- les oxyesters (tableau 1) ;
- les oxypolymères (esters) (tableau 3) ;
- les esters altérés "non oxydés".

En ce qui concerne les monomères cycliques, les valeurs trouvées (tableau 4) sont toujours faibles, et les variations après 20 chauffages peu significatives. Les huiles contenant de l'acide linolénique : colza et soja, fournissent cependant des teneurs en "monomères cycliques" un peu plus élevées après chauffage, que les huiles n'en possédant pas.

Conclusion

L'acide linolénique présent dans l'huile de colza (9 % environ) ne constitue donc pas, au plan des chauffages et pour les critères chimiques examinés de cette étude, un élément valable de différenciation.

La tenue à la chaleur, pour les 4 huiles étudiées, peut, d'une façon simplifiée, se résumer dans le tableau 5 où figurent pour chaque matière les pourcentages en glycérides non altérés et en esters non altérés après 20 chauffages à 220°C maximum. On remarque ainsi le comportement très satisfaisant et comparable des huiles d'arachide (87 % de triglycérides non altérés) et de colza (88 % de glycérides non altérés). La même constatation peut être faite pour les esters des acides gras non altérés.

Ces résultats sont tout à fait comparables à ceux obtenus par l'ITERG en 1977 et 1980 dans des conditions expérimentales très proches.

TABLEAU I - POURCENTAGE EN MASSE, DETERMINES PAR ETALONNAGE INTERNE, DES ESTERS METHYLIQUES DES ACIDES GRAS DE COMPOSITION DES HUILES CHAUFFEES ET NON CHAUFFEES (20 CHAUFFAGES)

| HUILES | ARACHIDE | | TOURNESOL | | SOJA | | COLZA | |
|---|---|---|---|---|---|---|---|---|
| Acides gras | 0 ch. | 20 ch. | 0 ch. | 20 ch. | 0 ch. | 20 ch. | 0 ch. | 20 ch. |
| C 16 | 10,2 | 10,5 | 6,4 | 6,3 | 10 | 10 | 5,1 | 5,3 |
| C 16 : 1 | 0,2 | 0,2 | 0,1 | 0,1 | 0,1 | 0,1 | 0,4 | 0,4 |
| C 18 | 3,3 | 3,3 | 4,4 | 4,3 | 3,8 | 3,7 | 1,8 | 1,6 |
| C 18 : 1 | 46,3 | 45,7 | 16,4 | 16,1 | 23,5 | 22,6 | 57,6 | 55 |
| C 18 : 2 | 32,4 | 29,9 | 71 | 62,5 | 54 | 48,3 | 21,6 | 20,6 |
| C 18 : 3 | 0,5 | 0,2 | 0,2 | 0,3 | 6,5 | 5,1 | 8,5 | 7,2 |
| C 20 | 1,4 | 1,4 | 0,3 | 0,3 | 0,3 | 0,3 | 0,6 | 0,4 |
| C 20 : 1 | 1,1 | 1,1 | 0,1 | 0,1 | 0,2 | 0,3 | 1,6 | 1,7 |
| C 22 | 3 | 3 | 0,7 | 0,7 | 0,4 | 0,4 | 0,3 | 0,4 |
| C 22 : 1 | 0,1 | 0,1 | - | - | - | - | 1,8 | 1,7 |
| C 24 | 1,3 | 1,3 | - | - | - | - | - | - |
| N.I. | 0,2 | 0,4 | 0,4 | 0,5 | 1,2 | 1,1 | 0,7 | 0,8 |

TABLEAU II - MODIFICATIONS CHIMIQUES APPORTEES PAR 20 CHAUFFAGES SUCCESSIFS, JUSQU'A 220°C, AUX TRIGLYCERIDES D'HUILES D'ARACHIDE, DE TOURNESOL, DE SOJA, ET DE COLZA

(résultats exprimés en % par rapport à l'huile totale)

| Type de modification dosée (% par rapport à l'huile totale) | HUILES | | | |
|---|---|---|---|---|
| | Arachide | Tournesol | Soja | Colza |
| Glycérides altérés | 13 | 20,5 | 18,5 | 12 |
| Polymères de triglycérides | 8,1 | 16,6 | 13,6 | 9,1 |
| dont | | | | |
| dimères | 6,1 | 11,7 | 9,8 | 6,7 |
| oligomères supérieurs | 2 | 4,9 | 3,8 | 2,4 |

TABLEAU III - MODIFICATIONS CHIMIQUES APPORTEES PAR 20 CHAUFFAGES SUCCESSIFS, JUSQU'A 220°C, AUX ESTERS METHYLIQUES DES HUILES D'ARACHIDE, DE TOURNESOL, DE SOJA ET DE COLZA

(résultats exprimés en % par rapport aux esters totaux)

| Type de modification dosée (% par rapport aux esters totaux) | HUILES | | | |
|---|---|---|---|---|
| | Arachide | Tournesol | Soja | Colza |
| Esters altérés | 4,9 | 8,5 | 7,6 | 4,8 |
| Esters polymérisés | 3,1 | 6,6 | 5,6 | 3 |
| Oxyesters | 2,1 | 3,3 | 3,2 | 2,4 |
| dont | | | | |
| oxymonomères | 1,5 | 1,9 | 2 | 1,7 |
| oxypolymères | 0,6 | 1,4 | 1,2 | 0,7 |
| Modifications calculées par déduction | | | | |
| Esters altérés, "non oxydés" (non oxyesters) | 2,8 | 5,2 | 4,4 | 2,4 |
| Esters altérés, "non oxydés" polymérisés (non oxyesters polymérisés) | 2,5 | 5,2 | 4,4 | 2,3 |

TABLEAU IV - TENEURS EN "MONOMERES CYCLIQUES" DES HUILES CHAUFFEES ET NON CHAUFFEES (% par rapport à l'huile)

| Huiles | 0 chauffage | 20 chauffages | Accroissement $\Delta = C_{20} - C_0$ |
|---|---|---|---|
| Arachide ..... | 0,09 | 0,08 | - 0,01 |
| Tournesol ..... | 0,035 | 0,10 | 0,06 |
| Soja ..... | 0,08 | 0,21 | 0,13 |
| Colza ..... | 0,10 | 0,25 | 0,15 |

TABLEAU V - POURCENTAGE DE TRIGLYCERIDES "NON ALTERES"* ET D'ESTERS "NON ALTERES"** SUBSISTANT DANS LES HUILES APRES 20 CHAUFFAGES SUCCESSIFS

| Huiles après 20 chauffages | Triglycérides "non altérés" | Esters "non altérés" |
|---|---|---|
| Arachide ..... | 87,0 | 95,1 |
| Tournesol ..... | 79,5 | 91,5 |
| Soja ..... | 81,5 | 92,5 |
| Colza ..... | 88,0 | 95,2 |

* - Méthode UICPA (1)
** - Esters "non altérés" (3)

# THE METHIONINE AND CHOLINE STATUS OF DIETS USED IN RAPESEED OIL FEEDING TRIALS

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## Introduction

This study was undertaken to evaluate the nutritional adequacy of the 20% casein/20% oil diet fed to rats that has routinely been used to test the cardiopathogenicity of vegetable oils (1). These diets containing 20% oil have a caloric content of approximately 5000 Kcal/kg and if casein is the sole source of protein, the methionine content of the diet may be inadequate when expressed on a per calorie basis (2). If the basal diet is low in methionine, then the effects of such a deficient diet on the development of heart lesions needs to be established. Diets were therefore fed to rats, with and without supplemental methionine, to determine if the dietary content of this nutrient affected the growth, health and cardiopathology of the rat. In addition, diets containing graded levels of choline - another lipotropic factor known to spare methionine - were fed to further study the effects of the nutrient status of the 20% casein/20% oil diet.

## Materials and Methods

Six diets containing 20% soybean oil and 20% casein were formulated which contained 0 or 0.15% supplemental L-methionine and 0.0, 0.05 or 0.10% supplemental choline (Table 1). Prior to incorporating casein into the diets the methionine content was determined chemically (3). Six groups of 25 weanling, male Sprague-Dawley rats were housed individually and fed the diets for 16 weeks. After 3, 6 and 12 weeks on the diets, urine was collected from 5 rats per diet and analysed for urea and formiminoglutamate (4) to indicate the status of protein metabolism. At the end of 16 weeks the hearts were removed and examined for myocardial necrosis and fibrosis (5). Liver samples were also removed for lipid analysis and for histological examination of lipid infiltration using Oil red O staining.

TABLE 1

Percent composition of experimental diets

| | Diet (% by weight) | | | | | |
|---|---|---|---|---|---|---|
| Casein | 20 | 20 | 20 | 20 | 20 | 20 |
| Choline | 0 | 0 | 0.05 | 0.05 | 0.10 | 0.10 |
| Methionine | 0 | 0.15 | 0 | 0.15 | 0 | 0.15 |
| Oil | 20 | 20 | 20 | 20 | 20 | 20 |
| Ingredients | | | | | | |
| Casein[1] | 20 | 20 | 20 | 20 | 20 | 20 |
| L-methionine | - | 0.15 | - | 0.15 | - | 0.15 |
| Choline | - | - | 0.05 | 0.05 | 0.10 | 0.10 |
| Soybean oil | 20 | 20 | 20 | 20 | 20 | 20 |
| Vitamin mix[2] | 1 | 1 | 1 | 1 | 1 | 1 |
| Mineral mix[3] | 4 | 4 | 4 | 4 | 4 | 4 |
| Corn starch | 30 | 30 | 30 | 30 | 30 | 30 |
| Sucrose | 20 | 19.85 | 19.85 | 19.80 | 19.9 | 19.75 |
| Alfa floc | 5 | 5 | 5 | 5 | 5 | 5 |

[1]Vitamin free casein, Teklad Test Diets Co.
[2]Contains no choline
[3]USP XVII mix

## Results and Discussion

Chemical analysis of the casein used in the experimental diets indicated that it contained 2.60% methionine and 0.40% cystine. Diets with no added methionine therefore contained 1.33 mg sulfur amino acids/Kcal ME; supplemented diets contained 1.67 mg sulfur amino acids/Kcal ME.

Rats eating the methionine supplemented diets were significantly heavier ($p<0.01$) and consumed more feed than rats eating the unsupplemented diets (Table 2). The choline status of the diet had no effect on these two parameters.

TABLE 2

Experimental data from rats fed diets differing in their methionine and choline content

| | Diet (% by weight) | | | | | |
|---|---|---|---|---|---|---|
| Casein | 20 | 20 | 20 | 20 | 20 | 20 |
| Choline | 0 | 0 | 0.05 | 0.05 | 0.10 | 0.10 |
| Methionine | 0 | 0.15 | 0 | 0.15 | 0 | 0.15 |
| Oil | 20 | 20 | 20 | 20 | 20 | 20 |
| Weight Gain (g/rat) | 526 | 573 | 523 | 555 | 482 | 576 |
| Feed Consumed (g/rat) | 1987 | 2161 | 2096 | 2161 | 1948 | 2190 |
| Liver Weight (g) | 17.42 | 18.19 | 17.57 | 17.91 | 16.06 | 18.34 |
| Liver Lipid (%) | 8.82 | 9.84 | 6.63 | 8.40 | 6.64 | 8.16 |
| Heart Weight (g) | 1.58 | 1.56 | 1.57 | 1.65 | 1.46 | 1.58 |
| Heart Lesion Incidence (%) | 28 | 40 | 28 | 28 | 20 | 28 |

Livers from animals receiving the diets with added methionine were heavier ($p<0.05$) and contained more lipid ($p<0.001$) than livers of rats eating no supplemental methionine (Table 2). Adding choline to the diet significantly reduced the amount of liver lipid ($p<0.001$). These trends for liver lipid were similar when either data from gravimetric analysis (Table 2) or from lipid accumulation scores using Oil red O staining techniques were compared. The range of liver lipid levels found, indicated that no animals had fatty liver syndrome associated with diets deficient in lipotropic factors since the lipid levels were within the normal range reported in rats (6).

Analysis of the hearts of the animals fed the experimental diets indicated that the type of diet had no effect on the size of the heart. Pathological examination of the hearts showed that animals on all diets had myocardial lesions (20-40% incidence) detectable by microscopic examination. However, there was no relationship between methionine or choline status of the diet and the incidence of heart lesions.

Lipid analysis of the liver samples indicated that triglyceride (TG) was the largest class, followed by phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Table 3). Adding methionine to the diet significantly increased TG and cholesterol ester (CE). Choline on the other hand lowered the amounts of hepatic TG, PC and PE. The amounts of none of the other classes (cholesterol, cardiolipin, sphingomyelin, phospahtidylserine/inositol, lyso-PC and lyso-PE) were affected by diet.

TABLE 3

Composition of some liver lipids from rats fed experimental diets for 16 weeks

| | Diet (% by weight) | | | | | |
|---|---|---|---|---|---|---|
| Casein | 20 | 20 | 20 | 20 | 20 | 20 |
| Choline | 0 | 0 | 0.05 | 0.05 | 0.10 | 0.10 |
| Methionine | 0 | 0.15 | 0 | 0.15 | 0 | 0.15 |
| Oil | 20 | 20 | 20 | 20 | 20 | 20 |
| Component (mg/g wet weight) | | | | | | |
| TG[1] | 45.0 | 77.0 | 33.2 | 45.7 | 29.4 | 47.8 |
| PC[2] | 13.7 | 12.8 | 13.0 | 12.3 | 11.5 | 11.2 |
| PE[2] | 8.4 | 6.8 | 6.4 | 7.0 | 5.9 | 6.0 |
| CE[1] | 3.3 | 6.8 | 2.3 | 3.7 | 2.2 | 3.8 |

[1]analysed by Iatroscan (10 rats/diet)
[2]analysed by phosphorous analysis (4 rats/diet)

Urine samples analysed indicated that rats eating the diets with no added methionine were excreting low levels of formiminoglutamate at 3 and 6 weeks, but by 12 weeks formiminoglutamate was non-detectable in all samples. The detection of formiminoglutamate in the urine has been used as an indicator of inadequate dietary methionine (7). The type of diet had no apparent affect on urea excretion.

The results of the present experiment indicate that the 20% casein/20% oil diet fed in this study required additional methionine to promote optimal growth and feed consumption. Adding methionine also increased liver lipid levels. Choline addition to the diet had no effect on growth but reduced liver lipids. It should be emphasized however that all groups

sampled had liver lipid values well within the normal range. The incidence of heart lesions was found not to be affected by the methionine or choline content of the diet. It is concluded therefore that methionine supplementation of the 20% oil/20% casein diet is advisable for nutritional reasons, but the addition of methionine or choline to the diet does not affect the incidence of myocardial lesions.

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# SEANCE DE CLOTURE - CLOSING SESSION - SCHLUSSSITZUNG

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Concluding overview

# Developments in rapeseed genetics and breeding

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## Introduction

It is evident from the papers presented at this Congress that rapeseed breeding and related technologies have continued to advance at a very rapid rate. Quality changes reported at this and the two previous congresses have propelled the Brassica oilseeds to the forefront of interest among the edible oil crops of the world. Recognition of their potential is evident not only in the Canadian, Chinese and European efforts to convert to low erucic, low glucosinolate (canola) quality but also in the resources and interest devoted to their introduction into Russia, Australia, Spain, U.S.A., Egypt, Ethiopia, Kenya and many more countries. In addition, major traditional producers such as China, India and Pakistan are looking to increased yields from these crops to supply their growing populations with a nutritious edible oil.

## The potential for B. juncea

It appears from the papers presented, and from other information available, that the mustard species B. juncea will in time rival in importance the two rapeseed species as a world edible oil source. This annual species, although not well adapted to northern Europe, will gain in favor in other regions because of its drought, disease and seed shattering resistance. A good yellow seedcoat color is also available within the species and normally under arid conditions higher seed yields are obtained than for spring rapeseed. Areas in which this crop may dominate are Australia, southern United States, the Indian subcontinent, Canada and other arid regions. Normally the high erucic, high glucosinolate levels of B. juncea would restrict its

usefulness as an oil crop. However, recent identification of low erucic plants by Kirk and Oram (1981), coupled with either the new patented ammonia meal detoxification (Canada Patent) or the low glucosinolate characteristics recently reported by Cohen et al. (1983), paves the way for the production of canola oil and meal quality B. juncea.

Quality factors

Reported progress toward additional quality improvements in rapeseed meal was minor. The glucosinolate inheritance studies in Brassica and their close relatives suggest that we still have much to learn about the genetic and environmental factors that dictate the presence and amounts of each glucosinolate. However, they did confirm that the quantitative and qualitative glucosinolate values were largely under maternal genetic control.

Swedish studies on oil composition indicated that the potential for extending the range in fatty acid composition in rapeseed may be much greater than previously thought, with values of 10 to 12% palmitic, or over 5% stearic acid reported in high linoleic acid selections. In addition, the long sought after high linoleic, low linolenic oil now appears feasible with linoleic, linolenic acid percentages of 42 and 3 respectively being recorded in B. napus oil. Other studies indicated that the linolenic biosynthesis appeared to follow a common pathway for deposition in the galactolipids and triglycerides.

Although progress is being made toward fixing a desirable yellow seedcoat color in B. napus, breeders have yet to combine good yellow seed color with an acceptable agronomic performance.

Hybrid variety development

Quality aspects have highlighted previous congresses, but this congress will be remembered for ushering in the era of hybrids and biotechnology. It is now certain that within the next few years several countries will begin to commercially exploit the advantage of hybrid vigor in both rape species and in B. juncea. From reports presented, the potential yield increase is in the order of 30 to over 40%, with the hybrids showing the greatest advantage under adverse environmental conditions. Several different approaches have been taken by researchers in developing economic hybrid systems. Some are using genic male steriles (GMS), others the self incompatibility system (SI), and still others cytoplasmic male steriles (CMS) resulting from substitution of foreign cytoplasms through

interspecific crosses.

The most commercially advanced is a Chinese B. napus GMS system which has been under evaluation since 1980. The variety is now grown on over 700 ha, with a yield advantage of 33% over the best commercial varieties. This system requires a large amount of hand labour in the hybrid seed production field, and as such it is not economical in most producing countries. The SI system being pursued in B. napus in England and China can also be effective, but most breeders have sought to perfect CMS genetic restorer systems. CMS plants of B. napus and B. campestris were reported using cytoplasm from radish, Diplotaxis muralis, B. juncea, B. nigra and B. carinata, as well as B. campestris cytoplasm in B. napus. CMS B. juncea plants with genetic restorers were also reported arising from crosses with B. nigra and B. campestris. In examining these cytoplasms, researchers found B. nigra and B. carinata cytoplasm to be virtually identical. Within the second complex of B. juncea, B. campestris, B. napus and B. oleracea, the cytoplasms of B. juncea and B. campestris were almost identical, while B. napus had greater homology with B. oleracea than with B. campestris. However, more than one author cautioned that a single species may encompass more than one cytoplasm.

The exploitation of these CMS systems await either the identification of usable genetic fertility restorers and/or their incorporation into parents with acceptable agronomic and quality characteristics. It is no longer a question of can successful hybrids be produced but rather how soon for each species and form?

## Biotechnology advances

The biochemical, genetic and physiological variability and pliability of the oilseed Brassicas has and continues to be the key to improving their performance in the farm field and market place. This pliability has now been extended to biotechnological manipulations. The oilseed Brassicas are one of the few crops that have responded to anther culture and the production of haploid and doubled haploid plants. Papers presented suggest that for some species and some institutions the haploid production rate has or is approaching the level required for practical use in ongoing breeding programs.

In a similar fashion the B. napus species has also yielded to cell fusion techniques with remarkable results. Normal green CMS plants were obtained from the fusion of protoplasts

containing chlorotic CMS cytoplasm with protoplasts from normal green, fertile plants. Similarly, CMS plants, tolerant to triazine herbicides, were obtained by fusing protoplasts with fertile, triazine-tolerant cytoplasm and CMS protoplasts. Techniques were also reported which permit the direct development of plants from stem and root protoplasts without an intervening callus phase. Such developments greatly expand plant breeding horizons.

## Traditional breeding developments

In the more traditional approach to plant improvement the inheritance of white rust (Albugo candida) resistance in B. napus has been documented. Aphid resistance has also been closely correlated with anatomical features, such as deeply placed vascular bundles and a thick epidermis, while B. juncea was reported to have resistance or partial resistance to the parasite Orobanche. The merits of hybrid, synthetic and pure line varieties were debated, and the relative importance of the various yield components of rape and mustard were examined by several authors. Indian researchers noted that yield stability of a variety under different environments was of greater importance to the Indian farmer than a variety's ability to produce a maximum yield under optimum conditions. A reexamination of varietal stability showed varieties with the greatest plasticity for yield components had the greatest yield stability. Breeders were also reminded that the environment under which seed of a candidate variety is produced can have a major impact on its comparative performance in yield trials.

It is clear from the papers presented at the Genetics and Breeding sessions of this Congress that the potential for improvement in yield, protection and quality is as great today as it has ever been. There is every reason to look to the future with excitement and confidence.

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Monsieur le Président,
Mesdames, Messieurs,
Chers Collègues et Amis,

C'est un exercice toujours périlleux de tenter de faire une synthèse, surtout dans le temps qui m'est imparti.

Il ressemble souvent à celui d'une funambule qui aurait oublié son instrument d'équilibre, mais j'essayerai de ne pas perdre le mien surtout après ce que nous avons vu au cours de la soirée d'hier à la Conciergerie.

La compréhension de l'élaboration de la production de matière sèche et de la formation du rendement en grains de colza nécessite une connaissance précise des différents mécanismes physiologiques au cours des phases successives du développement de la plante.

Ceci dans le but :

- d'aboutir à une modélisation de la production de matière,
- d'atteindre ou de maintenir le potentiel de production du cultivar,
- de mieux valoriser les facteurs de production mis en oeuvre,
- d'élaborer de meilleures techniques de mise en place de la culture,
- de préciser les techniques de rattrapage permettant d'atteindre l'objectif de rendement visé par l'agriculteur dans le cadre des successions culturales envisagées. Et enfin,
- de modifier l'architecture de la plante, soit par la voie génétique, soit par la mise en oeuvre des techniques agronomiques appropriées.

Des efforts importants ont été consentis dans les différents pays producteurs de colza depuis le 5ème Congrès, sur cette approche, puisque 24 communications orales et plus de 50 affiches

ont été consacrées à la physiologie et à l'agronomie du colza.

L'analyse de la croissance et du développement du couvert végétal a été abordée par de très nombreux chercheurs, ce qui a abouti à une meilleure connaissance de :
- la mise en place des capteurs photosynthétiques et de leur fonctionnement en fonction de leur âge (de leur niveau dans la structure de la végétation) du rayonnement intercepté, de la température,
- la translocation des assimilats vers les différents organes et tout particulièrement vers les organes de réserves,
- des tentatives de modélisation ont été proposées pour les différentes phases.
- Les mécanismes de résistance au froid, que ce soit au niveau de la graine, ou à celui de la jeune plante ont été également abordés.
- Les processus de résistance à la sécheresse ont été également largement évoqués.
- Les facteurs controlant le développement des siliques, puis des graines ont été analysés.

L'importance des différents sujets traités dans un temps trop court souvent pour un chercheur avide de faire connaître tous ses travaux doit permettre d'analyser encore plus finement les différentes phases d'élaboration du rendement et de mieux guider l'agronome puis l'agriculteur dans l'optimisation de sa production.

Le raisonnement des itinéraires techniques et l'influence de chacun des facteurs, date de semis, dose de semis, structure du peuplement, fertilisation azotée, fertilisation soufrée ont été étudiés dans le but d'une meilleure valorisation des intrants, d'une optimisation des moyens mis en oeuvre pour que l'agriculteur puisse bénéficier le plus rapidement possible des progrès de la technique dans un monde où l'économie domine et précède les scientifiques.

Je voudrais enfin souligner la qualité des exposés et le niveau scientifique de leurs travaux, les efforts d'ingénuosité dans la présentation des affiches et la richesse des éléments apportés.

Je me réjouis aussi très vivement des rapprochements des différentes disciplines scientifiques dans le domaine très large de l'agronomie et les efforts déployés par chacun pour une meilleure compréhension du fonctionnement d'une culture de colza.

Je remercie les traducteurs pour la qualité de leur travail malgré la cadence parfois rapide mais compréhensive des scientifiques.

Je vous remercie également tous pour votre attention soutenue tout au long de ce Congrès.

Michel ROLLIER

# CONCLUDING OVERVIEW OF PRESENTATIONS ON ANALYSIS AND COMPOSITION OF SEEDS AND PRODUCTS, NUTRITIONAL VALUE OF MEAL AND OIL, AND INDUSTRIAL TECHNOLOGY AND PRODUCTS

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The presentations in these three major areas of interest have been summarized in eleven subgroups: processing, weed seed contamination, hulls, glucosinolates and phenolics, methods of analysis, protein, oil, ruminants, swine, poultry and finally, mustard.

## PROCESSING

The microchemical structure of rapeseed at various stages in the solvent extraction process was studied by fluorescence microscopy. Crushing had no effect on cellular constituents. Cooking fused protein into masses encompassing phytin-containing globoids and lipids. Structural and chemical components of the hull were unchanged by processing.

The removal of solvent from rapeseed meal was studied in relation to the moisture content of the seed before crushing, the presence of hulls and solvent-meal contact time. Adsorption-desorption isotherms of hexane vapor in meal were established over the temperature range 40° to 105°C, in the absence of water vapor, and rates of internal diffusion were measured. It is extremely difficult to remove the last traces of hexane from meal. One presentation reported on aerated silos and a modified desolventization process.

An experimental scale process of rapeseed extraction involving

slurry grinding eliminated cracking or rolling, cooking, prepress and flaking operations. Two mills, each requiring eight seconds of seed residence time in a countercurrent system, followed by three solvent mixing stages, reportedly recovered 99% of the oil.

There were two presentations on weed seeds in rapeseed. One concerned the identification and affects of wild mustard and the other dealt with feeding five rapeseed screenings to pigs, where the dominant weed seeds were stinkweed and lamb's-quarters.

## DEHULLING

Two reports dealt with equipment and processes for dehulling of rapeseed as a means of fiber reduction in the meal and as a step toward the production of food grade protein concentrates. Rapeseed hulls fed to growing rabbits at levels up to 40% of the diet, substituting for dehydrated alfalfa, gave very satisfactory growth and feed utilization performance. The characteristics of body fat were studied and taste panel evaluations were conducted on fresh and frozen meat, with very satisfactory results. In another report the feeding values of dehulled and regular rapeseed meal were compared.

## GLUCOSINOLATES AND PHENOLICS

Comparisons of unsaturated nitrile with glucosinolates were made with rats. Untreated rapeseed meal and isolated intact glucosinolates both resulted in reduced gain and enlargement of

thyroid and kidneys whereas nitrile-containing diets were similar to the casein control.

Low glucosinolate rapeseed meal was fed in a two-generation reproduction experiment with rats, using over 50% rapeseed meal to provide 20% dietary protein. Female and pup weight gains were reduced and there was evidence of low zinc availability but there were no effects on number of live pups born or on 26-day weights of pups.

Inactivation of myrosinase in whole seed by hydrothermic, microwave and gamma radiation methods was examined in order to find ways of reducing corrosion of equipment and diffusion of sulphur compounds into the oil.

While sinapine in rapeseed is well known, it is possible that several other phenolic choline esters occur in rapeseed. Two new minor polyphenols have been isolated from low glucosinolate rapeseed, comprising 0.1 and 0.04% of the meal dry matter and identified as rather unstable esters of sinapine.

Isolated potassium salts of different glucosinolates were added singly and in graded amounts, with and without myrosinase added, to rat diets used in N-balance trials. Intact glucosinolates, without myrosinase, showed toxic effects which in some cases were enhanced by myrosinase.

## METHODS

A short term rat growth test using autoclaved full-fat rapeseed

was reported and high negative correlation coefficients were found between glucosinolate level in the diet and certain rat responses, including weight gain, PER, efficiency of feed utilization and thyroid weight.

Analytical methods for determining glucosinolates permit selection of methods of analysis differing with regard to simplicity, instrumentation and cost. Using HPLC techniques, all known types of glucosinolates can be assayed quantitatively without prior enzymatic hydrolysis to aglucon products.

A rapid polarographic method for simultaneous determination of total glucosinolates and free glucose, using combinations of enzymes, was discussed.

For the rapid measurement of oil content in rapeseed the NMR (nuclear magnetic resonance) technique is often used. The various sources of error with the NMR were examined in detail and temperatures of magnet and sample and the system of standard preparation were identified as the most important sources of error.

Methods for detecting and measuring residual solvent (hexane) in rapeseed meal were compared, and collaborative tests on a promising method have been conducted in 21 laboratories.

## PROTEIN AND PROTEIN CONCENTRATES

One of the principal proteins of rapeseed is 12S globulin, saline extractable and of molecular weight 300,000. Its amino acid composition

and structure were described. Elsewhere the saline soluble protein fractions of "double-low" and low erucic acid cultivars were compared and found to be very similar.

An extraction method of pilot plant scale employing methanol, ethanol and isopropanol applied to dehulled, oil-extracted rapeseed meal resulted in a product containing 60 to 65% protein and very low levels of glucosinolates, polyphenols and phytates.

Success in improving cooked, dehulled, oil-extracted meal by subsequent ethanol extraction was reported. Removal of antinutritional and unpalatable compounds occurred.

Rapeseed and soybean isolates prepared by acid precipitation were heated to 105, 120 and 145°C and their functional properties were compared. Heating of rapeseed isolates improved water absorption and gelation properties.

OIL

The synthesis, nature and distribution of lipids and fatty acids during the various stages of development of rapeseed were reported and the biosynthesis pathway was identified. Initially lipids are mainly functional phospho- or gluco- lipids. When deposition begins, this occurs rapidly and oil and protein are deposited in fixed proportions.

Several reports were presented on rapeseed oil processing and use. The use of surfactants, especially sorbitan tristearate,was

found effective in reducing polymorphic transition to the beta form. The effect was most pronounced in selectively hydrogenated canola (low erucic) oil.

The effects of various processing variations and of impurities present in refined rapeseed oil on hydrogenation were discussed. Recrystallization of margarines, containing low erucic acid oil, from β' to β form was studied using a rapid test with a small stirrer-cooler to measure changes during storage. Diglycerides retarded recrystallization.

Changes in fatty acid composition of low erucic rapeseed oil used under simulated deep frying conditions were studied. Pronounced decreases in linoleic and linolenic acids were observed after 30 hr at 170°C. In another study it was reported that free fatty acids and contents of polar components gave the best correlations with frying time. Two chromatographic methods were compared.

Low erucic oil was heated at temperatures of 200 and 240°C for 10 and 40 hr, then fed to rats. Cyclic monomers, and geometric isomers of linoleic and linolenic acids were found in significant quantities in the heated oil and were also found in the adipose tissue and livers of rats.

A comparison of rapeseed and sunflower oils showed that rapeseed oil could be used effectively in the treatment of hypercholesterolemia of the familial heterozygote type.

Low erucic oil was compared with sunflower, corn (maize), peanut (groundnut), palm oils and milk fats in long term dietary comparisons with adult human females living in a closed community. The consumption

of rapeseed oil was associated with the lowest blood cholesterol, tryglyceride and apoprotein A values.

Cardiac lipid changes and myocardial necrosis in rats fed various oils and fat-oil mixtures, were examined to determine possible correlations between incidence of lesions and lipid classes or fatty acid changes. None of these, including erucic acid, proved to be a reliable indicator of lesions but it was found that dietary saturated fatty acids reduced heart lesions. The same researchers demonstrated that dietary levels of methionine and choline had no effect on the incidence of myocardial lesions in rats fed rapeseed oil and that saturated dietary fatty acids were beneficial.

## FEEDING RUMINANTS

Treatment of canola rapeseed meal with formaldehyde to reduce rumen breakdown of protein in lactating dairy cows failed to affect milk yield, milk composition, feed intake or digestibility but rumen ammonia levels were reduced.

The digestibility of rapeseed hulls and oil-free hulls was determined with growing and adult sheep. Organic matter was 40-49% and 54 to 61% digestible in oil-free hulls and hulls, respectively. Hulls have been used successfully in rations for lactating cows at 27% of the diet and for lambs at 15-30%.

## FEEDING SWINE

Starter pigs (4-5 wk age) were fed various combinations of canola meal (LG) and soybean meal. For every 1% addition of canola meal,

daily feed intake and gain were reduced by 4 and 2 gm, respectively. Data on biological value and net protein utilization were also presented. Other research with heavier pigs (20-105 kg) fed low glucosinolate meal indicated that pig performance was not influenced by replacing soybean meal with rapeseed meal.

The dehulling of rapeseed improved the digestibility of energy in meal by 10% for pigs, of protein by 5% and increased the protein level by 20%.

An attempt to improve the feeding value of canola meal by acid hydrolysis indicated no value in this process.

## FEEDING POULTRY

Canola meal fed at 10 or 20% in isonitrogenous, isocaloric diets to turkey broilers allowed performance equal to that obtained with soybean meal.

Ammonia and steam treatment of canola meal significantly reduced the level of sinapine in canola meal and the incidence of fishy odor in eggs of selected brown-egg layers but did eliminate the problem. Other investigators observed inhibition of hepatic trimethylamine oxidase by oxazolidinethione.

Hemorrhagic liver in laying hens, a condition aggravated by including rapeseed meal in the diet, was shown not to be caused specifically by progoitrin glucosinolate, but seemed to be related to total glucosinolate content of the diet.

In experiments involving four genotypes of meat birds, both juvenile and adult, and comparing canola meal with soybean meal,

the general performance was similar. However, some differences in sensitivity to rapeseed meal were observed among genotypes and better performance tended to result from feeding combinations of soybean and canola meals.

Five genetic lines of laying hens, in comparisons of low glucosinolate rapeseed meal and soybean meal, were used to study mortality, laying percentage, egg weight, egg shell, efficiency of feed conversion, thyroid weights, plasma enzymes and plasma bile acids. Rapeseed meal at 12% of the diet showed inferior results compared to soybean meal and hen strain differences were observed.

Chicks were fed diets containing rapeseed meal from rapeseed cultivars varying widely in glucosinolate content (7-60 μmol/g, oil-free basis). Effects on the thyroid were highly repeatable from the same seed source but not with mixtures or with over 30 μmol levels of glucosinolates. Iron sulfate dietary supplementation was effective with some of the rapeseed meals.

MUSTARD

Three reports were presented on mustard. One indicated the high nutritional value of mustard protein concentrate, as a potential weaning food, in terms of PER, NPR and NPU, which were comparable to casein. The second report concerned reduction of the glucosinolates and sinapine in Brown or Oriental mustard meal in order to enhance its value as an animal feed. The third report indicated the apparent presence in Indian mustard of two factors, other than phytic acid,

that influence the nutritional availability of zinc.

In conclusion, time did not permit mention of all presentations and some important findings may have been overlooked. However, it is obvious that significant advances have been made in most, if not all, of the areas covered.

# ASPECTS PHYTOSANITAIRES

A. COLENO - Chef du Département de Pathologie Végétale
et Malherbologie - INRA

Les thèmes phytosanitaires ont rassemblé une soixantaine de communications partagées de façon équitable entre les parasites et les prédateurs. La qualité en était excellente et ce sont plutôt des considérations tactiques qui ont fait préférer telle ou telle pour une présentation orale. L'analyse que nous nous proposons maintenant se veut synthétique, elle s'affranchira donc du mode de présentation et portera sur l'ensemble des textes proposés. Nous pouvons y dégager trois sujets de réflexion :

## I - Importance des problèmes phytosanitaires

A l'évidence il y a là des facteurs limitants de la culture, ressentis parfois comme de véritables catastrophes. L'importance de tel ou tel agent est évidemment fonction des conditions climatiques et édaphiques particulières à une contrée.

En ce qui concerne les parasites, Sclerotinia sclerotiorum et Alternaria brassicae sont à la fois les plus cités et les plus travaillés. Selon les situations d'autres parasites potentiels ou déjà bien connus attirent également l'attention : Phoma lingam, Peronospora parasitica, Albugo candida, Plasmodiophora brassicae, des mycoplasmes et quelques virus.

Pour les prédateurs la diversité est plus grande, l'importance variant avec les saisons : Psylliodes, Brevicoryne, Ceutorrhynchus, Lipaphis...

## II - Orientations des recherches

La majorité des travaux portent sur l'étiologie des maladies et sur la biologie du développement (pour les parasites et les prédateurs). Trois thèmes ont été particulièrement enrichis au cours de ce congrès :

a) Mise au point de critères de sélection faciles à utiliser, c'est alors l'interaction plante-microorganisme qui fait l'objet de l'étude en s'intéressant aux composantes du pouvoir pathogène : virulence et agressivité, variabilité au niveau de l'espèce ; mise en oeuvre de techniques d'évaluation simples, rapides, performantes et différentielles ; sans que l'on cherche à aborder au fond les mécanismes impliqués au plan cellulaire.

Pour les prédateurs l'approche est un peu semblable. On s'est intéressé à l'influence de l'hôte sur le développement des insectes en considérant les différents stades de ce développement et également à la nutrition et à la sensibilité des hôtes en fonction des niveaux d'infestation (exemple de la mouche du chou : D. brassicae).

b) Meilleure définition des techniques culturales susceptibles de réduire l'incidence de la maladie. C'est en ces termes par exemple qu'est abordée l'étude de Plasmodiophora brassicae pour lequel au travers de deux communications sont présentées les conditions culturales favorisantes, les corrélations avec la carence en bore, la nuisibilité et les possibilités offertes par la sélection.

c) Mise en oeuvre d'un système de prognose - Pour les parasites, c'est à la prévision épidémique que les chercheurs s'attachent. A travers les communications et les posters on note l'intérêt pour la cinétique de l'infection en définissant les différents stades du développement et les conditions qui y sont chaque fois associées. Pour le Sclerotinia sclerotiorum les études ont porté sur le rôle des exsudats racinaires dans la germination des sclérotes, les conditions d'hygrométrie nécessaires à la pénétration, le rôle des différents organes (principalement les pétales) pour l'initiation de l'infection, les conditions de germination des ascospores et les modalités de leur transport. Dans l'immédiat une telle approche peut permettre de manière parfois empirique de définir des recettes valables ici ou là et qui ne sont pas forcément transposables ; mais il s'agit surtout d'une approche informative qui pourra déboucher sur des modèles descriptifs du développement des épidémies qui offriront alors des possibilités de généralisation.

Pour les prédateurs, les thématiques sont analogues et débouchent sur des prévisions d'infestation. C'est alors les études de

dynamique des populations qui sont entreprises conduisant :

- A la connaissance du cycle, de l'influence de l'hôte et de son stade phénologique, de l'importance des facteurs climatiques ;

- A la mise en évidence des caractéristiques de l'infestation (gradient, effets de bords...). Un bon exemple de cette approche est celle pratiquée sur Psylliodes chrysocephala).

## III - Possibilités de lutte

D'une manière générale le recours à la lutte chimique est important et nous avons pu noter de nombreuses possibilités efficaces tant pour les parasites que pour les prédateurs. Plusieurs questions ont été soulevées : problèmes liés à la rémanence et à la résistance, ceci interroge largement la recherche (nature de la résistance, mécanisme impliqué, valeur sélective...), problèmes de stratégies d'emploi.

La lutte génétique est également une préoccupation majeure. Enfin nous avons noté une proposition originale en matière de lutte biologique avec l'utilisation de nématodes entomopathogènes.

En conclusion l'apport international sur les problèmes phytosanitaires du colza a été important. Il amène les éléments d'une lutte raisonnée. Que peut-on suggérer de plus pour l'avenir : une coordination informelle mais ample ; une étude plus particulière des conditions d'emploi,des méthodes de lutte (contraintes économiques, raisonnement à l'échelle de l'exploitation et de la région), une approche physiologique de la sensibilité (mécanismes impliqués, régulation, influence des stress, de la nutrition...).

Nul doute que le dynamisme scientifique de nos organismes saura répondre à ces interpellations.

MONSIEUR LE PRÉSIDENT,

MESDAMES, MESSIEURS,

JE SUIS PARTICULIÈREMENT HEUREUX, AU TERME DE VOTRE CONGRÈS, DE VENIR VOUS APPORTER LE SALUT ET LES ENCOURAGEMENTS DU PREMIER MINISTRE. LE GOUVERNEMENT FRANÇAIS - ET PLUS SPÉCIFIQUEMENT LE MINISTRE DE L'INDUSTRIE ET DE LA RECHERCHE, AINSI QUE LE MINISTRE DE L'AGRICULTURE - ONT SUIVI AVEC UN INTÉRÊT CONSIDÉRABLE LES TRAVAUX QUI SE SONT DÉROULÉS DANS LE CADRE DU 6ÈME CONGRÈS INTERNATIONAL SUR LE COLZA. CE CONGRÈS QUI N'A PU AVOIR LIEU EN POLOGNE L'AN DERNIER, COMME CELA AVAIT ÉTÉ INITIALEMENT PRÉVU, S'EST DONC TENU À PARIS GRÂCE AU CONCOURS ET AUX EFFORTS CONJOINTS DE L'INRA ET DU CETIOM (CENTRE TECHNIQUE INTERNATIONAL DES OLÉAGINEUX MÉTROPOLITAINS) ET IL EST PLACÉ SOUS L'ÉGIDE DU GROUPE CONSULTATIF INTERNATIONAL DE RECHERCHE SUR LE COLZA.

NOTRE PREMIER DEVOIR SERA DE REMERCIER LES QUELQUES 600 PARTICIPANTS DES 30 PAYS REPRÉSENTÉS ICI, PARMI LESQUELS FIGURENT NOTAMMENT LES PLUS IMPORTANTS PRODUCTEURS DE COLZA ET EN PARTICULIER LA CHINE POPULAIRE, L'INDE, LE CANADA. LES 120 COMMUNICATIONS ORALES PRÉSENTÉES EN 3 JOURS, SANS PARLER DES 150 POSTERS QUI SONT VENUS COMPLÉTER CES PRÉSENTATIONS ; L'ARDEUR DES DÉBATS, LA QUALITÉ DES INTERVENANTS NOUS AUTORISENT À DIRE QUE CE 6ÈME CONGRÈS VENANT APRÈS CELUI DE MALMOË (EN 1978) A RENCONTRÉ UN TRÈS VIF SUCCÈS. JE VOUDRAIS DONC EN REMERCIER TRÈS CHALEUREUSEMENT LES ORGANISATEURS.

JE SUIS PERSUADÉ QU'UN CONGRÈS DE CETTE NATURE EST D'UNE GRANDE UTILITÉ PUISQUE SUR UN THÈME VOIRE MÊME UN OBJET PRÉCIS, IL RASSEMBLE DES POINTS DE VUE ET DES INFORMATIONS SE RÉCLAMANT DE DOMAINES AUSSI VARIÉS QUE L'ÉCONOMIE, LA PHYSIOLOGIE DE LA

PLANTE, LA GÉNÉTIQUE ET LA SÉLECTION, LA PHYTOTECHNIE, LES TECHNOLOGIES INDUSTRIELLES ET LES PRODUITS NOUVEAUX, LA COMPOSITION DES GRAINES, HUILES ET TOURTEAUX, LEUR VALEUR ALIMENTAIRE, ETC...

A MON SENS ÉGALEMENT, L'IMPACT DE CETTE RENCONTRE DÉPASSE EN IMPORTANCE LA SEULE MISE EN COMMUN DES INFORMATIONS CONCERNANT LE COLZA. ELLE POSSÈDE EN EFFET UNE VALEUR TOUT À FAIT EXEMPLAIRE QUANT À LA MODERNITÉ ET À L'ORIGINALITÉ DE LA DÉMARCHE QU'ELLE SOUS-TEND POUR LA POLITIQUE AGRICOLE MONDIALE D'AUJOURD'HUI ET DE DEMAIN PUISQU'ELLE INTÈGRE LES DIMENSIONS SOCIOÉCONOMIQUES DES PROBLÈMES AUX CONSIDÉRANTS SCIENTIFIQUES ET TECHNIQUES LES PLUS AVANCÉS.

EN EFFET, LA RÉVOLUTION VERTE N'EST PAS ET NE SERA PAS QUE LE FRUIT D'ACCORDS ENTRE NATIONS OU ENTRE CONTINENTS SI IMPORTANTS SOIENT ICI LES FACTEURS GÉOPOLITIQUES ELLE SERA AVANT TOUT LA RÉSULTANTE DES PROGRÈS FULGURANTS QUE CONNAIT AUJOURD'HUI LA RECHERCHE SCIENTIFIQUE TOURNÉE VERS LE MONDE VÉGÉTAL ET SES APPLICATIONS.

A CET ÉGARD, LE 6ÈME CONGRÈS MARQUE, IL N'EST PAS EXAGÉRÉ DE LE DIRE, UN VÉRITABLE TOURNANT TECHNOLOGIQUE QUI AURA À COUP SÛR DES CONSÉQUENCES IMPORTANTES POUR L'UTILISATION DU COLZA ET DE SES DÉRIVÉS MAIS AUSSI ET PAR EXTENSION POUR CERTAINS ASPECTS PLUS GÉNÉRAUX DE LA RECHERCHE AGRONOMIQUE.

DES DÉCOUVERTES IMPORTANTES Y ONT ÉTÉ RAPPORTÉES TANT AU PLAN FONDAMENTAL QU'À CELUI DES APPLICATIONS ET L'ON PEUT DÉJÀ AFFIRMER AU VU DE CES RÉSULTATS QUE LE COLZA EST UN BON MODÈLE POUR ÉTUDIER LA BIOLOGIE CELLULAIRE EN GÉNÉRAL ET LA BIOLOGIE FLORALE EN PARTICULIER.

Au plan fondamental, elles s'inscrivent plus particulièrement dans les domaines de la génétique, de la Biologie cellulaire et de la nutrition. La génétique a d'abord marqué des progrès décisifs quant aux propriétés qualitatives du colza, c'est-à-dire, pour l'essentiel à ses caractéristiques biochimiques. Dans la plupart des grands pays producteurs des variétés sans acide érucique et sans glucosinolates ont en effet été obtenues ce qui met désormais cet oléagineux à l'abri des critiques dont il fut l'objet il y a quelques années du fait des effets secondaires que risquaient d'entrainer ses composés. C'est là une très belle victoire de la génétique végétale. Elle devrait constituer un véritable virage dans l'économie mondiale du colza.

Mais les recherches génétiques ont conduit dans ces dernières années à des améliorations qui touchent également aux aspects quantitatifs du problème puisque l'on assiste depuis 1979 à des accroissements graduels de la productivité des plants résultant de la création de nombreuses variétés hybrides. Ici les études sur l'hétérozis du colza, sur la stérilité male ont permis de surmonter de nombreux obstacles.

Mais à côté des résultats déjà considérables qu'autorise la génétique classique, ceux que permet aujourd'hui la Biologie cellulaire et la Biotechnologie à travers les tentatives de cultures <u>in vitro</u> pourraient s'avérer plus décisifs encore. Il faut citer ici les spectaculaires progrès enregistrés grâce à la culture des gamètes et à la fusion des protoplastes. Je ne veux point refaire ici l'historique des travaux entrepris dans ces domaines. Si la culture des tissus végétaux trouve son origine dans les travaux français de Roger GAUTHERET en 1937, la culture de cellules isolées a largement bénéficié des recherches de

MUIR, HILDEBRANDT ET RIKER EN 1954 ET ELLE FUT PERFECTIONNÉE PAR ALBERT LUTZ EN FRANCE. A QUOI, IL FAUT AJOUTER LES TRAVAUX SYSTÉMATIQUES DE BERGMANN ET DE STREET, NOTAMMENT EN ANGLETERRE.

QUANT AUX CULTURES DE GAMÈTES VÉGÉTAUX, ON S'ACCORDE À PENSER QUE MAHESHWARI ET GUYA FURENT SANS DOUTE LES PREMIERS EN INDE À DÉMONTRER QUE LES CELLULES GERMINALES DU POLLEN POUVAIENT SE MULTIPLIER ET DONNER DES EMBRYONS HAPLOÏDES, TRAVAUX QUI FURENT REPRIS PAR NITSCH EN 1967 ET CONDUISIRENT À L'OBTENTION DE PLANTES ENTIÈRES HAPLOÏDES.

S'AGISSANT DU COLZA, ON RÉUSSIT AUJOURD'HUI DES CULTURES À PARTIR DES ANTHÈSES DES OVULES OU ENCORE DES EMBRYONS CE QUI PERMET NOTAMMENT D'ANALYSER L'IMPORTANCE RELATIVE DE L'ORGANISME NATUREL.

LA FUSION DES PROTOPLASTES RENDUE POSSIBLE DEPUIS QUE COCKING EN 1960 A INAUGURÉ LES TECHNIQUES PERMETTANT D'OBTENIR DES PROTOPLASTES VÉGÉTAUX EN GRANDE QUANTITÉ AUTORISE DES CROISEMENTS INTERSPÉCIFIQUES, TEL QUE CELUI DU COLZA ET DU RADIS. CETTE TECHNIQUE OUVRE DES PERSPECTIVES PRATIQUES CONSIDÉRABLES MAIS ELLE NOUS ÉCLAIRE ÉGALEMENT SUR CERTAINS MÉCANISMES FONDAMENTAUX TELLE QUE PAR EXEMPLE LA RÉPRESSION DES CHLOROPLASTES PAR CERTAINS ÉLÉMENTS CYLOPLASMIQUES, APPORTÉS PAR L'ESPÈCE ÉTRANGÈRE. PAR FUSION DE PROTOPLASTES, ON PEUT ALORS MODULER À DESSEIN, LA RICHESSE EN CHLOROPHYLLE AINSI QUE BON NOMBRE DE PROPRIÉTÉS TELLE QUE LA RÉSISTANCE AUX PESTICIDES.

LA POSSIBILITÉ ENFIN, DE FAIRE SE RÉGÉNÉRER DES PLANTULES DE COLZA COMPLÈTES À PARTIR DE CULTURES IN VITRO MONTRE QUE DES PROMESSES CONSIDÉRABLES S'OUVRENT DÉSORMAIS À LA CULTURE DE CET OLÉAGINEUX.

PARMI LES PERCÉES SCIENTIFIQUES QUI SERONT ÉGALEMENT DE NATURE À IMPULSER L'UTILISATION DU SOJA FIGURE ÉGALEMENT LA CONNAISSANCE APPROFONDIE DES RELATIONS ENTRE LA PLANTE ET SES PARASITES (QU'IL S'AGISSE DES MALADIES OU DES RAVAGEURS). EN EFFET, UNE MEILLEURE COMPRÉHENSION DU CYCLE DES AGENTS PATHOGÈNES, DE LEURS PHASES SENSIBLES EN RELATION AVEC LE DÉVELOPPEMENT DE LA PLANTE ET LES CONDITIONS DE SOL ET DE CLIMAT EST DE NATURE À AMÉLIORER LES SYSTÈMES DE PRÉVISION DES ATTAQUES ET DE FAIRE RÉALISER UNE ÉCONOMIE DES MOYENS DE TRAITEMENT.

MAIS À CÔTÉ DE CES RECHERCHES FONDAMENTALES, D'AUTRES, DE CARACTÈRE PLUS APPLIQUÉ, SONT VENUES RENFORCER L'IDÉE QU'UN POTENTIEL ENCORE INSOUPÇONNÉ RÉSIDE DANS LES COMPOSANTES LIPIDIQUES ET PROTÉIQUES DU COLZA. JE N'AI NULLEMENT L'INTENTION DE VISER À L'EXHAUSTIVITÉ MAIS JE SAIS QU'AU COURS DE CE CONGRÈS ONT ÉTÉ DÉCRITES LES PATIENTES RECHERCHES MENÉES SUR LES EFFETS DE L'ACIDE LINOLÉNIQUE QUI EST UNE IMPORTANTE COMPOSANTE DE L'HUILE DE COLZA. NON SEULEMENT CET ACIDE GRAS NE SEMBLE PAS PRÉSENTER LES EFFETS NOCIFS QUE CERTAINS AVAIENT PU REDOUTER (IL N'A PAS D'EFFET PERNICIEUX SUR LE SYSTÈME NERVEUX ET EXERCE DES EFFETS FAVORABLES SUR LES CONSTANTES SANGUINES CHEZ L'HOMME) MAIS LA CONSOMMATION D'HUILE RICHE EN ACIDE LINOLÉNIQUE ET DÉPOURVUE D'ACIDE ÉRUCIQUE ASSURE UN MÉTABOLISME DES ACIDES GRAS QUI EST TOUT À FAIT SATISFAISANT.

DES ÉTUDES POUSSÉES SUR LA RÉSISTANCE THERMIQUE DE L'HUILE DE COLZA (CELLES NOTAMMENT EFFECTUÉES EN HOLLANDE OU ORGANISÉES PAR LE CNERNA) RÉVÈLENT UN COMPORTEMENT ÉGAL À CELUI DES HUILES D'ARACHIDE OU DE SOJA SINON PARFOIS MEILLEUR. DES RECHERCHES MENÉES SUR LA REPRODUCTION DES MURINS SOUMIS À UN RÉGIME ENRICHI EN HUILE DE COLZA RÉVÈLENT, ICI ENCORE, DES

CARACTÉRISTIQUES AU MOINS IDENTIQUES, SINON SUPÉRIEURES, À CELLES DES HUILES PROVENANT D'AUTRES OLÉAGINEUX.

SANS VOULOIR ABUSER DE VOTRE TEMPS, JE CROIS QU'ON NE PEUT MANQUER DE MENTIONNER PARMI LES ACQUITS RÉCENTS DE LA RECHERCHE AGROALIMENTAIRE SUR LE COLZA CEUX QUI CONCERNENT LES COMPOSANTES PROTÉIQUES. JE N'INSISTERAI PAS FAUTE DE TEMPS. IL EST CLAIR QUE LE FUTUR DU COLZA COMME SOURCE DE PROTÉINES ANIMALES ET HUMAINES EST DES PLUS PROMETTEUR. J'Y REVIENDRAI DANS MA CONCLUSION. L'OBTENTION DE TOURTEAUX EN PROVENANCE DE GRAINES SANS THIOGLUCOSIDES, OU DE GRAINES DÉPELLICULÉES FAIT QUE LES TOURTEAUX AINSI PRÉPARÉS ONT UNE VALEUR ÉNERGÉTIQUE ÉQUIVALENTE À CELLE DU TOURTEAU DE SOJA AVEC UNE COMPOSITION TRÈS BIEN ÉQUILIBRÉE EN ACIDES AMINÉS. IL SEMBLE QUE L'ON PUISSE DONC AUJOURD'HUI MULTIPLIER PAR 4 LE TAUX D'INCORPORATION DES TOURTEAUX DE COLZA DANS L'ALIMENTATION DES ANIMAUX. À CÔTÉ DE LA FILIÈRE PROTÉIQUE POUR LE BÉTAIL, S'OUVRENT DES PERSPECTIVES IMPORTANTES POUR LA CONSOMMATION DE PROTÉINES ISOLÉES, DÉBARRASSÉES DE CONTAMINANTS CELLULOSIQUES EN ALIMENTATION HUMAINE, ENCORE QUE DES PROGRÈS RESTENT À ACCOMPLIR EN CE DOMAINE.

LE COLZA REPRÉSENTE DONC, EN CONCLUSION, UNE "CARTE INTELLIGENTE" DU MONDE DE DEMAIN POUR LUTTER CONTRE LA CARENCE EN PROTÉINES ET EN MATIÈRES GRASSES À CONDITION, BIEN SÛR, QUE L'ON N'Y VOIT PAS UNE PANACÉE MAIS QUE L'ON JOUE MIEUX CETTE CARTE QUE PAR LE PASSÉ.

M. EMILE CHONE DIRECTEUR DU CETIOM A DÉCRIT AVEC BEAUCOUP DE LUCIDITÉ ET DE PRÉCISION QUELLE ÉTAIT LA SITUATION DE LA FRANCE DANS LA PRODUCTION MONDIALE DE COLZA. ELLE TRADUIT UNE NETTE PROGRESSION DEPUIS QUELQUES ANNÉES PRINCIPALEMENT DANS CINQ DES GRANDES RÉGIONS PRODUCTRICES AVEC UN ACCROISSEMENT DE RENDEMENT MOYEN D'ENVIRON 35 %. NOTRE PAYS QUI A CONSACRÉ AU COURS

DE CES DEUX DERNIÈRES DÉCENNIES PRÈS DE 2,5 MILLIARDS DE FRANCS À LA RECHERCHE SUR LE COLZA ET QUI VIENT DE LANCER AVEC L'APPUI DU MINISTÈRE DE L'AGRICULTURE ET DE LA M.S.T. AU MINISTÈRE DE L'INDUSTRIE ET DE LA RECHERCHE UN NOUVEAU PROGRAMME OU PLAN D'ACTION SUR LA FILIÈRE DES OLÉAGINEUX, FONDE, COMME DE NOMBREUX AUTRES PAYS DU MONDE, UN TRÈS GRAND ESPOIR SUR LE COLZA.

QUANT AUX PAYS DE LA C.E.E., ON SAIT QU'ILS ONT CONNU EN 1981 UNE PROGRESSION DE LA PRODUCTION D'HUILE DE COLZA CORRESPONDANT À PRÈS DE 180.000 TONNES. DE MÊME LA TRITURATION DU COLZA EST DE TOUS LES OLÉAPROTÉAGINEUX LA SEULE QUI AIT ENREGISTRÉ UN ACCROISSEMENT RELATIF EN 1981 AVEC UNE PROGRESSION DE 26 %.

JE NE VEUX POINT VOUS INONDER DE CHIFFRES. ETANT PAR NATURE ET PAR FORMATION UN SCIENTIFIQUE ET DE SURCROIT UN BIOLOGISTE, JE SUIS CONVAINCU QUE LE COLZA QUI EN 1981 REPRÉSENTAIT 4,3 % DES BESOINS MONDIAUX EN HUILE ALIMENTAIRE (AVEC UNE PRODUCTION DE 11.400.000 TONNES) EST APPELÉ À UN GRAND AVENIR. CELA SERA DÛ POUR UNE LARGE PART AU FAIT QUE, DEPUIS PRÈS DE 30 ANS, LES CHERCHEURS DE TOUS LES PAYS DU MONDE ONT CONFRONTÉ LEURS DONNÉES ET SOUVENT JOINT LEURS EFFORTS. IL N'EST PAS DE PLUS NOBLE CAUSE QUE CELLE QUI VISE À LUTTER CONTRE LA FAIM DES HOMMES DANS LE MONDE.

DE NOMBREUSES PROPOSITIONS PRATIQUES PEUVENT DONC ÊTRE DÉGAGÉES À TRAVERS LES RÉSULTATS DU COLLOQUE ET NOTAMMENT, PUISQUE LES TRAVAUX ENTREPRIS POUR AMÉLIORER LA QUALITÉ DE L'HUILE DE COLZA PAR LA <u>SÉLECTION</u> ET PAR LA <u>TECHNOLOGIE D'EXTRACTION</u> (GRAINES DÉPELLICULÉES) DÉBOUCHENT SUR DES CONCLUSIONS EXTRÊMEMENT ENCOURAGEANTES ON EST LÉGITIMEMENT EN DROIT DE SE DEMANDER DANS QUELLE MESURE L'UTILISATION DE L'HUILE DE COLZA NE POURRAIT PAS ÊTRE FACILITÉE EN LA FAISANT ENTRER PAR EXEMPLE DANS DES <u>MÉLANGES</u> D'HUILES EN PROPORTIONS VARIABLES ET ADAPTÉES À CHAQUE UTILISATION ?

PARCE QU'IL Y A CONTRIBUÉ À SA MANIÈRE, CE CONGRÈS AURA ÉTÉ UNE BELLE RÉUSSITE INTERNATIONALE ET DE CELA, EN MA QUALITÉ DE CONSEILLER DU PREMIER MINISTRE MAIS PLUS MODESTEMENT, D'HOMME D'UN MONDE SOUVENT HEURTÉ PAR L'INCOMPRÉHENSION ET LA VIOLENCE ET QUI VOIT DANS LA RECHERCHE UN EFFORT DE SOLIDARITÉ HUMAINE VERS PLUS DE PAIX ET MOINS DE SOUFFRANCE, DE TOUT CELA, MESDAMES ET MESSIEURS, JE VOUS REMERCIE.

FRANÇOIS GROS

6ème CONGRES INTERNATIONAL SUR LE COLZA

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