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A METHOD OF BIOLOGICALLY DETOXIFYING RAPESEED MEAL

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Rapeseed meal is a good source of protein but it presents numerous inconveniences because it contains thioglucosides. Methods of eliminating these sulphur compounds have been developed (toasting, distillation, steaming, extraction by solvents, treating with salts of heavy metals). However, none of these techniques seems perfect.

During this project, we attempted to eliminate the toxic sulphur compounds through fermentation. We, therefore, tested some fifty very diverse micro-organisms (bacteria, yeasts, fungi). Among them, six gave satisfactory results.

For the research described in this report, we chose the Geotrichum candidum which, during the preliminary tests, appeared to us the most interesting technologically.

MORPHOLOGY AND BIOLOGY OF THE GEOTRICHUM CANDIDUM USED

Microscopically the mycelium is white, branched and with frequent divisions. Conidiophors are absent. The conidia are monocellular, without conceptacle, globular, cylindrical, short and straight; they are formed by segmentation of the mycelium (arthrospore conidia). Their dimensions are on an average 3-6 x 6 - 12 μ. The above characteristics permit the micro-organisms to be classified in the species Geotrichum candidum Link ex Persoon.

This fungus grows well on most of the usual media (Sabouraud, Czapek, potato nutrient, peptone), but it is on Sauton medium that its growth is more luxuriant. This strain is very polyphagous; it metabolizes all carbohydrates and all nitrogenous forms (nitric, ammoniacal, urea, amines, proteinaceous, pyrimidines, purines). However, it is the purine bases which give the best protein yields.

GROWTH MEDIUM AND CULTURE CONDITIONS

The strain is preserved on a solid Sauton medium.

The base for fermentation was obtained on a culture medium made up of:

a) a mineral solution at pH = 6.8 (KH_2PO_4 : 1g; Mg $SO_4.7H_2O$: 0.5g ; KCl : 0.2g ; $CaCl_2$: 0.2g ; Fe $SO_4 . 7H_2O$: 0.03g ; $ZnSO_4.7H_2O$: 0.01g; $CuSO_4.5H_2O$: 2mg; made up with distilled water to 1,000 ml);

b) glucose 35g/l,

c) uric acid 5g/l or urea 4g/l.

This medium is apportioned into 500 ml Erlenmeyer flasks in a proportion of 100 ml/ flask and then sterilized for 15 min. at 110°C.

The seeding is done under sterile conditions with an aqueous suspension of a 5-day Geotrichum candidum culture obtained on a Sauton medium.

The culture is prepared on a rotary agitator (130 rpm) at 30°C; its duration is 48 hours.

CONTROL METHODS

The release of VTO (5-vinylthiooxazolidine) into the fermentation juices and the residual content of this substance in the insoluble meal is checked as follows:

a) Obtaining an enzymatic preparation of myrosinase.

Since the oil-free white mustard meal gave us results comparable to the myrosinase solution, it is the one we are now using (See Figure I).

The trialba white mustard seeds are finely crushed, the oil is then extracted three times with five volumes of petroleum ether, and the seeds are dried at laboratory temperature and kept in a freezer at -20°C.

b) Enzymatic reaction.

Two grams of rapeseed meal to be analysed and 0.2 of oil-free mustard flour are weighed into a 500 ml Erlenmeyer flask, to which is added 100 ml of phosphate buffer pH = 7 ($Na_2HPO_4.12\ H_2O$ at 23.08g/l = 400 ml; KH_2PO_4 at 9.07g/l = 600 ml). It is incubated on the agitator at 30°C. for 2 hours.

c) Extraction of the VTO and proportioning.

When the enzymatic reaction is over, the solution is filtered through paper; 1 ml of solution is extracted twice with 10 ml of ether treated with sulfuric acid. The ether extracts are

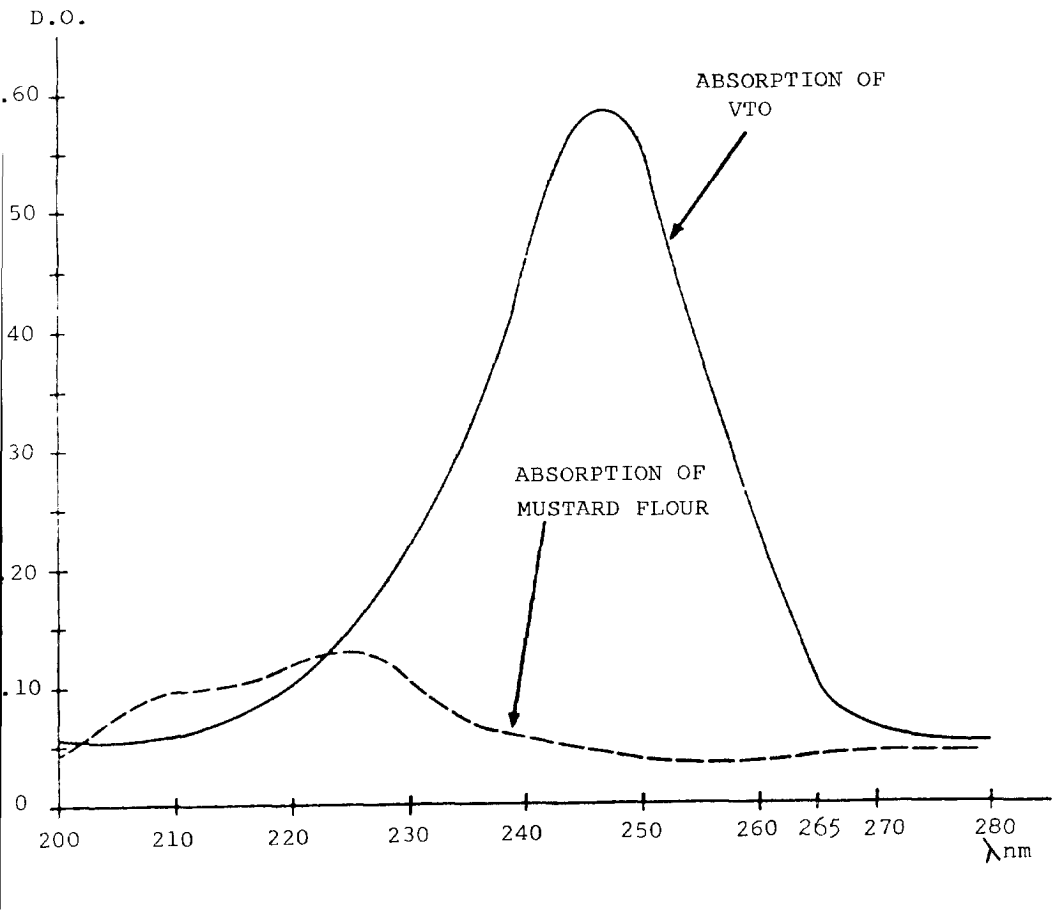

D.O.
.60
50
40
30
20
.10
0
ABSORPTION OF
VTO
ABSORPTION OF
MUSTARD FLOUR
200
210
220
230
240
250
260
265
270
280
λnm

FIGURE I

combined and made up to 25 ml, filtered through hydrophilic cotton. A sample is measured into a spectrophotometer (peak of VTO is at 248 nm).

The absorption is measured at 225, 248 and 265 nm and the corrected optical density is calculated by subtracting the average of the values at 225 and 265 from the value at 248 nm. The difference is plotted on the Wetter standard curve (Figure II) to obtain the VTO content in mcg/ml (X).

The VTO content in g per 100 g of meal is determined from the equation $\frac{0.25\ X}{M}$, where M is the weight of the test sample in g.

FERMENTATION METHOD USED AND RESULTS OBTAINED

The rapeseed meal was fermented in a 40 litre pilot fermenter according to the following procedure:

- Rapeseed meal . . . . . 6 kg
- Geotrichum candidum culture . . . 5 l
- Tap water . . . . . .19 l

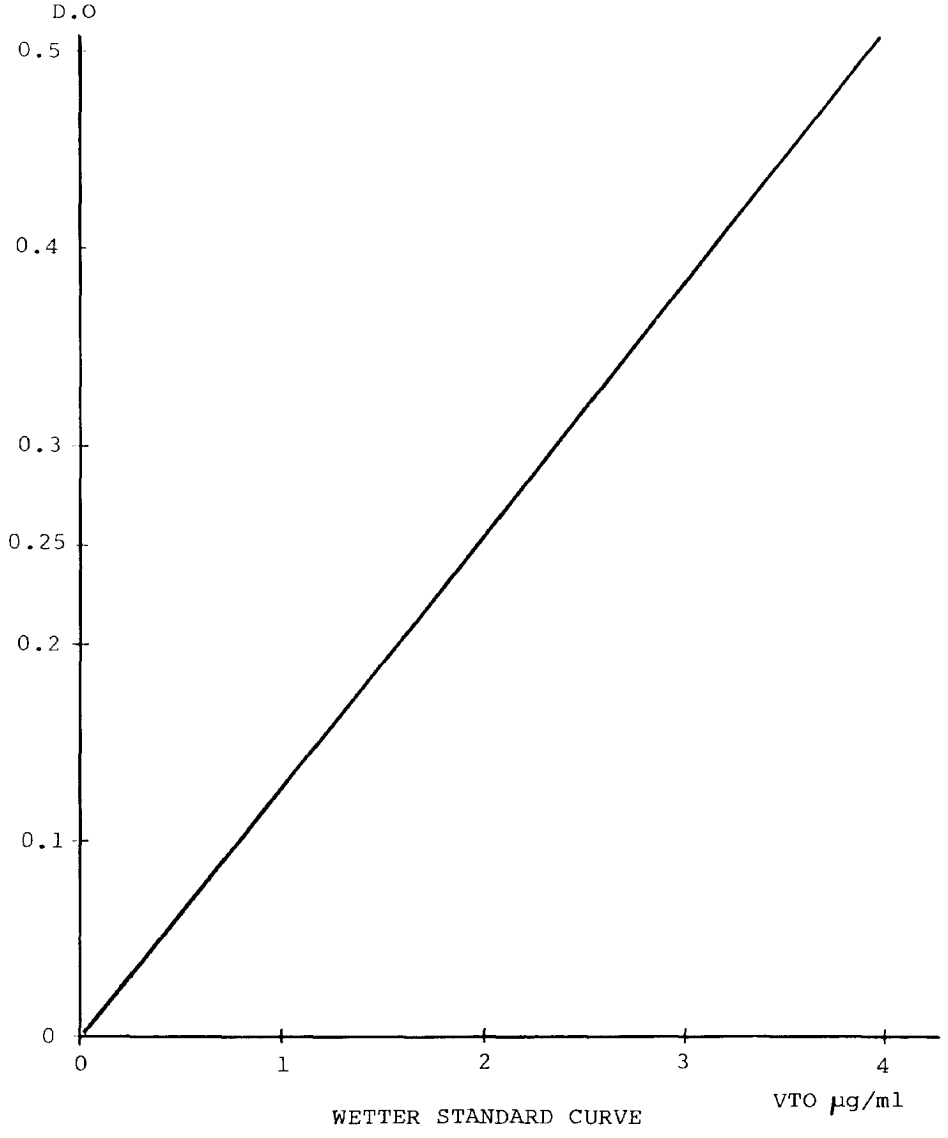
starting pH = 6.4; pH at extraction = 4. This drop in pH is due essentially to the release of proteins into the culture solution (Figure III).

The culture is obtained through slow agitation or through the stationary process for 30 to 60 hours at 30°C or 37°C, without prior sterilization. In fact, preliminary tests have shown that under the stated conditions, no contamination had ever taken place. On the other hand, agitation of the medium and aeration do not seem necessary when small volumes are being fermented.

Test samples make it possible to check the release of VTO into the fermentation solution and its progressive decomposition (Figure IV).

As shown in Figure IV, at 27°C the hydrolysis of the thioglucosides occurs first; it is only after the 35th hour of fermentation that the decomposition of the isothiocyanates commences, and their destruction is not complete until after 85 hours of fermentation. On the other hand, at 37°C the hydrolysis of the thioglucosides and the decomposition of the isothiocyanates that have appeared take place simultaneously.

During fermentation the Geotrichum candidum progressively renders the rapeseed meal proteins soluble. Thus, when the maceration is carried out from 60 to 80 hours at 37°C, 90 percent of the



WETTER STANDARD CURVE

FIGURE II

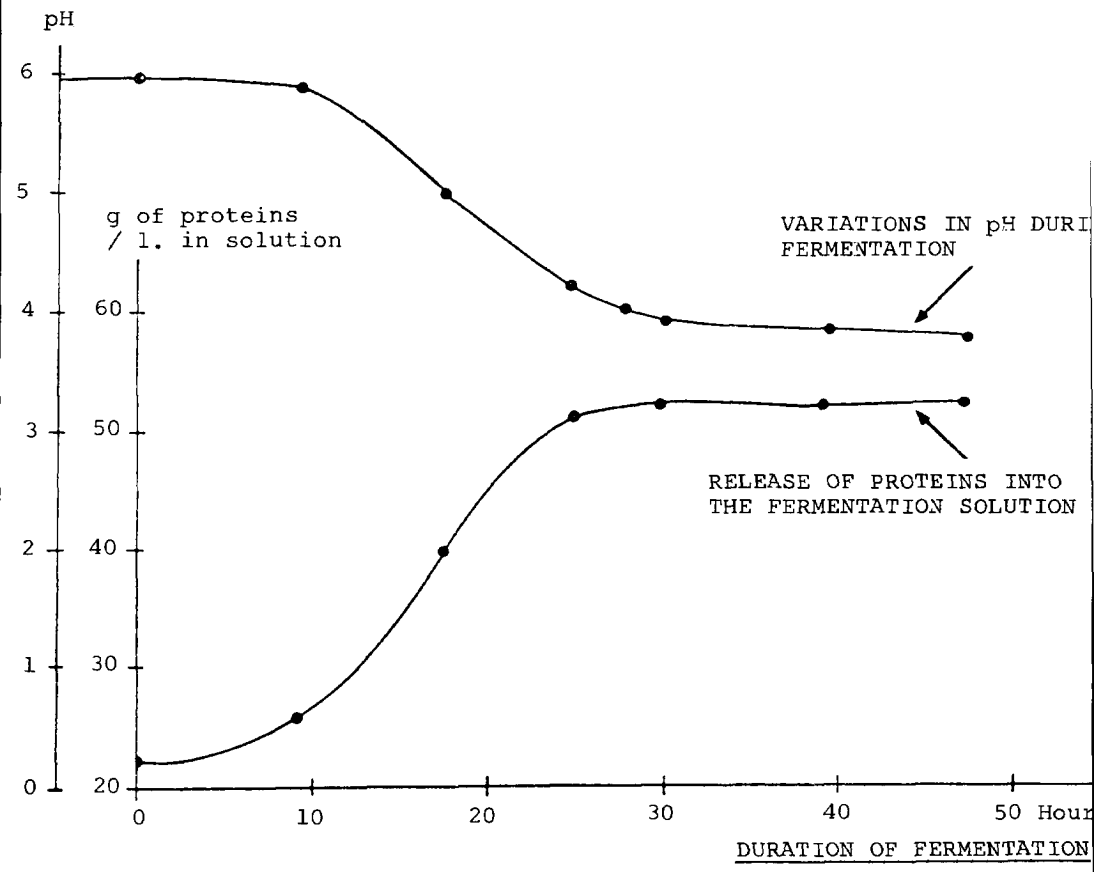

pH
6
5
4
3
2
1
0
g of proteins
/ l. in solution
60
50
40
30
20
VARIATIONS IN pH DURI
FERMENTATION
RELEASE OF PROTEINS INTO
THE FERMENTATION SOLUTION
0
10
20
30
40
50 Hour
DURATION OF FERMENTATION

FIGURE III

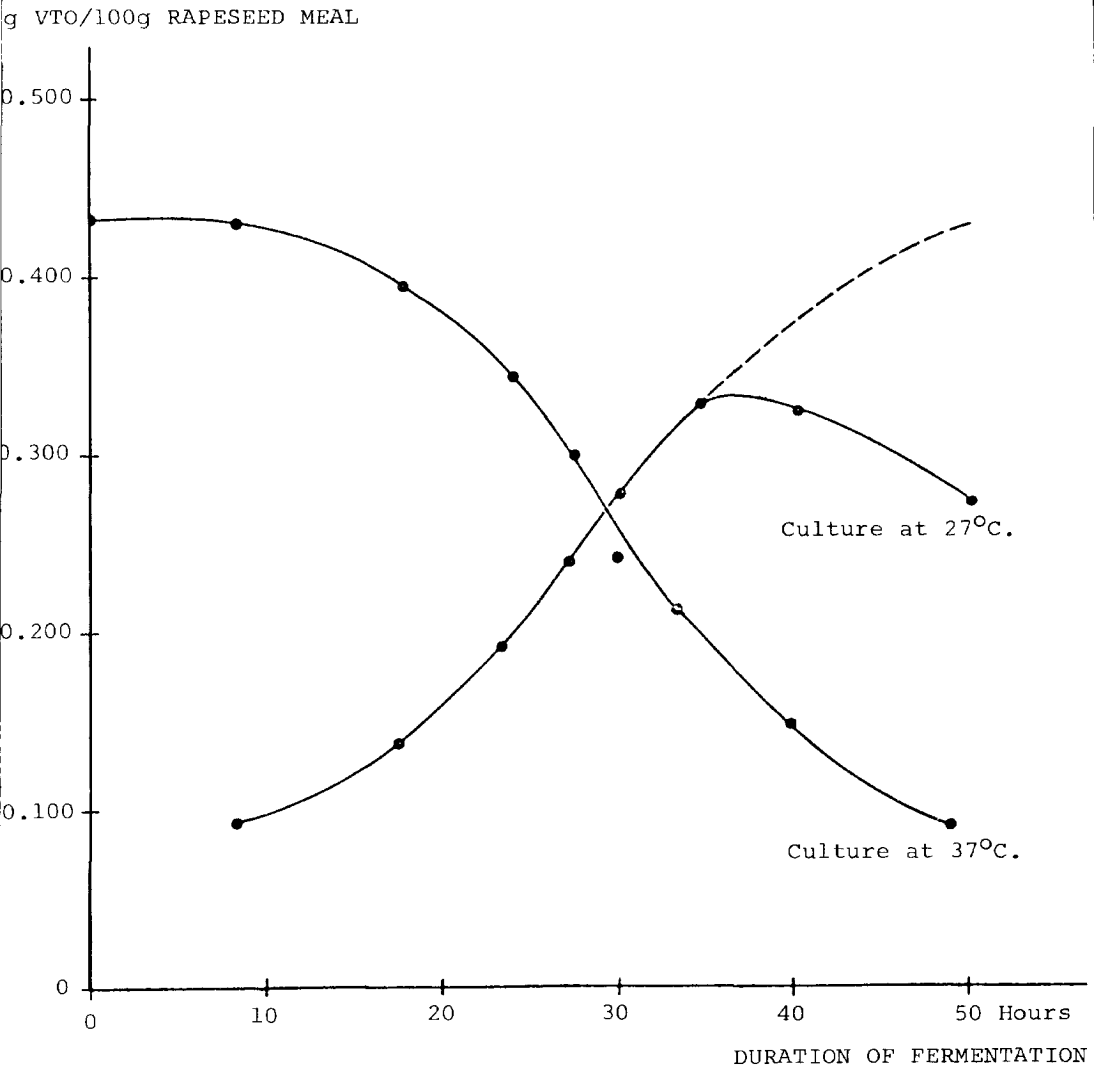


FIGURE IV

RELEASE AND DECOMPOSITION OF VTO BY GEOTRICHUM CANDIDUM

proteins pass into the solution. During the first 30 hours there are released: a heteroprotein which is toxic to mice (protein α) and a protein rich in glutamic acid, proline, lysine and sulphur amino acids (protein β). After the 35th hour, we observe the disappearance of protein α and the release of soluble portions whose amino acid composition is very close to that of whole rapeseed meal (See Table I).

The fractions thus obtained can be easily extracted by standard chemical methods and contain between 65 and 80 percent protein.

RECOVERY AND METHODS OF TREATING FERMENTED RAPESEED MEAL

The fermented rapeseed meal was treated experimentally in various ways:

a) Recovery of the total fermented amount and evaporation of water through spray drying.

b) Isolation of the insoluble portion through centrifuging and drying.

c) Separation of the soluble portion and drying.

d) Breaking up the soluble portion by $(NH_4)_2SO_4$.

Working under these conditions, we can distinguish the following fractions:

1) Total rapeseed meal fermented,
2) Fermented rapeseed meal, insoluble portion,
3) Fermented rapeseed meal, total soluble portion,
4) Protein α which precipitates at 20% (w/v) of $(NH_4)_2SO_4$.
5) Protein β which precipitates at 40% (w/v) of $(NH_4)_2SO_4$.

COMPARATIVE STUDY OF AMINO ACID COMPOSITION OF PROTEINS IN THE VARIOUS PORTIONS OBTAINED

The proteins are determined after decomposition according to the Kjeldahl method. The amino acids are analyzed with a Technicon autoanalyzer after hydrolysis of the proteins in a sealed tube in 6N HCl for 24 and 48 hours at 115°C; the tryptophan was determined after alkaline hydrolysis (Robin).

Table I shows that Geotrichum candidum does not damage the composition of proteins in rapeseed meal during fermentation; on the other hand, it progressively releases protein fractions whose amino acid composition is variable.

TABLE I

	Rapeseed Meal Control	Fermented Sprayed Rapeseed Meal Total	Fermented Rapeseed Meal Soluble Portion	Fermented Rapeseed Meal Total Soluble Portion	Protein β
Protein Content In Percent of Dry Weight	35.3	45.2	Variable	64	100
Aspartic Acid	7.0	6.9	7.0	7.0	2.4
Threonine	4.3	4.5	4.5	4.0	3.0
Serine	4.3	4.2	3.8	4.4	3.2
Glutamic Acid	18.3	18.7	17.0	19.2	26.0
Proline	7.4	7.1	7.3	7.2	9.2
Glycine	5.2	5.2	5.3	5.2	4.1
Alanine	4.2	4.9	5.0	5.2	3.8
Valine	4.7	4.9	5.1	4.8	3.9
Cystine	2.6	2.7	2.3	3.2	4.8
Methionine	1.9	2.0	1.6	2.1	2.4
Isoleucine	4.6	4.5	4.6	4.3	3.2
Leucine	7.0	6.9	7.1	7.3	6.1
Tyrosine	2.5	2.6	3.1	2.9	1.4
Phenylalanine	4.2	4.0	4.1	3.9	2.8
Ammonia	1.8	1.7	1.6	1.6	2.3
Lysine	5.3	5.6	5.1	6.1	6.8
Histidine	2.7	2.9	3.0	3.2	3.5
Arginine	6.5	6.6	6.8	6.6	5.6
Tryptophan	1.2	-	-	-	0.9
Cellulose	14%	17%	Variable	2%	0%

Protein β , which is rich in glutamic acid, proline, lysine, hystidine and sulphur amino acids represents 25 percent of the total proteins.

The cellulose content increases in the insoluble portion during fermentation because it is not attached, but nevertheless, it is easy to eliminate.

During fermentation, we recorded a weight loss of approximately 10%, which occurred mainly at the expense of the carbohydrates.

NUTRITIONAL EXPERIMENT ON MICE

The feed value of the fermented meal was checked on mice.

One hundred and eighty female mice were divided into 6 groups of 30 mice. I.N.R.A. acoustic physiology control feed was used. In the other five feeds tested, peanut and soya meals were replaced by rapeseed meal used in the experiment.

Table II shows the composition of the feeds used.

The experiment was conducted from June 16, to October 2, 1969. The mice were weighed twice a week. Figure V shows the growth curve for each group.

Since the mice in group 5 showed symptoms of capillary weakness (bleeding of the ears and nose), fifteen of them were destroyed on September 9, as well as ten test mice. The other fifteen mice of group 5 were fed feed 4 (group 5b) after that date; the symptoms mentioned above disappeared and the weight gain per mouse was 9.83g on October 3.

This nutritional experiment shows that the toxicity of the rapeseed meal is not apparent from the weight of the mouse.

Polish rapeseed meal produced good growth. With regard to the fermented feed (No. 5) in which protein ϙ and the free VTO were incorporated, its toxicity is very apparent and the experiment, therefore, had to be cut short. Feed 4 made from whole, sprayed rapeseed meal, and feed 6 with a base of fermented rapeseed meal, the total soluble portion gives a better growth than all the others.

The largest weight gains were obtained with feed 6. This result seems due to a better amino acid balance in the total soluble portion and to its low cellulose content (See Table I).

ANALYSES OF TISSUES AND ORGANS

The mice were killed on October 3, and the following tissues and organs were dissected: brain, heart, lungs, kidneys, spleen, liver, stomach, intestines, muscles, skin and fur, bones. For each of these samples, the dry weight, the free amino acid content, total protein content, and total phosphate content were determined. The results are given in Table III.

The analysis of the results in Table III reveals the following differences:

TABLE II

FEED COMPOSITION

Constituents	Feed Control No. 1	Feed No. 2	Feed No. 3	Feed No. 4	Feed No. 5	Feed No. 6
	%	%	%	%	%	%
Barley	10	8	9	9.5	9	11
Oats	15	12	13.4	14	14	16
Wheat	22	18	19.5	20.8	18.4	22
Corn	20	16.5	18	18.8	20.3	21.5
Peanut Meal	6.5	0	0	0	0	0
Soybean Meal	15	0	0	0	0	0
Rapeseed Meal	0	34	0	0	0	0
Polish Rapeseed Meal	0	0	28.6	0	0	0
Fermented, sprayed total rapeseed meal	0	0	0	25.4	0	0
Fermented rapeseed meal + protein % and free VTO reincorporated	0	0	0	0	26.8	0
Fermented rapeseed meal, total soluble portion	0	0	0	0	0	18
Norwegian Fishmeal	3	3	3	3	3	3
Yeast	0.5	0.5	0.5	0.5	0.5	0.5
Spray-dried milk powder	6	6	6	6	6	6
Mineral and vitamin additives	2	2	2	2	2	2
Percent of Total Proteins	21.83	21.20	21.04	21.31	21.25	21.53
Thioglucoside Content (expressed as VTO)	0	1.6mg/g	0	0	0	0
Free VTO Content	0	0	0	0	0.8mg/g	0
Weight Gain Per Mouse in g	10.2	10.14	11.04	12.8	-	14.2

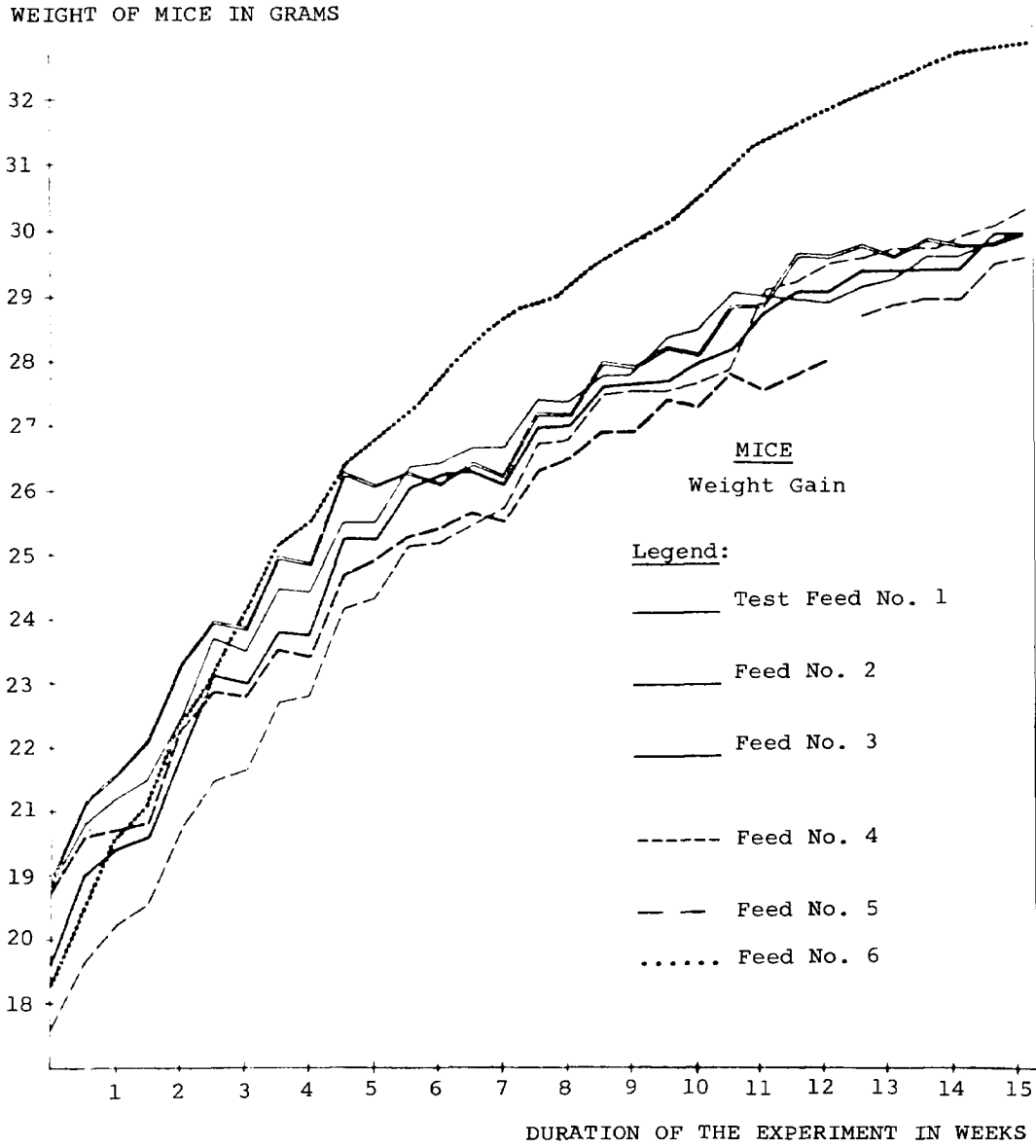

WEIGHT OF MICE IN GRAMS
32
31
30
29
28
27
26
25
24
23
22
21
19
20
18
MICE
Weight Gain
Legend:
Test Feed No. 1
Feed No. 2
Feed No. 3
Feed No. 4
Feed No. 5
Feed No. 6
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
DURATION OF THE EXPERIMENT IN WEEKS

FIGURE V

TABLE III

	Mice of Group 1 (Control) Killed on 9/9				Mice of Group 1 (Control) Killed on 3/10				Mice Receiving Feed 2 (Group 2)			
	Percent Dry Matter (D.M.)	Mg of Free Amino Acids per g of D.M.	Percent Protein D.M.	Percent Total Phosphorus D.M.	Percent Dry Matter (D.M.)	Mg of Free Amino Acids Per g of D.M.	Percent Protein D.M.	Percent Total Phosphorus D.M.	Percent Dry Matter (D.M.)	Mg of Free Amino Acids Per g of D.M.	Percent Protein D.M.	Percent Total Phosphorus D.M.
Liver	27.1	6.2	64	1.1	27.51	6.4	65	1.2	24.3	8.7	67.8	0.98
Spleen	22.2	5.8	73.8	1.9	22.25	5.7	72	1.75	21.3	6.3	79.5	1.65
Kidneys	25.5	5	57	0.9	26.2	4.5	59.5	0.82	23	5.9	65	0.88
Brain	19.6	7.3	54.5	1.29	19.6	7.15	55.5	1.37	18	8.5	59.8	1.34
Intestines	20.2	13.2	70.2	1.4	20.25	13.8	67.5	1.4	19	15	74.3	1.42
Skin & Fur	50.8	2.1	45.8	0.19	51	2.07	45.4	0.17	49.5	2.3	46.9	0.18
Muscles	27.2	4	60.7	0.75	27.5	3.8	60.6	0.73	29.4	3.6	56.5	0.70
Bones	42.2	2.2	45.7	8.3	43.5	2.1	48.6	8.15	42.4	2.1	46.5	6.6
Lungs	20	5.7	64.8	0.90	20	5.4	65	0.92	18.6	5.8	69	0.85
Heart	21.6	5.8	69.3	0.80	21.8	5.6	69.5	0.78	21.6	5.6	71.2	0.80
Stomach	19.1	11.1	65	1.	18.7	11.3	66	0.97	20.6	8.5	69	0.93
	MG OF GLUCOSE PER G OF D.M.											
Livers	38.2				38.2				52.6			

TABLE III (Continued)

	Mice Receiving Feed 3 (Group 3)				Mice Receiving Feed 4 (Group 4)				Mice Receiving Feed 5 (Group 5)			
	Percent Dry Matter (D.M.)	Mg of Free Amino Acids Per g of D.M.	Percent Protein D.M.	Percent Total Phosphorus D.M.	Percent Dry Matter (D.M.)	Mg of Free Amino Acids Per g of D.M.	Percent Protein D.M.	Percent Total Phosphorus D.M.	Percent Dry Matter (D.M.)	Mg of Free Amino Acids Per g of D.M.	Percent Protein D.M.	Percent Total Phosphorus D.M.
Liver	25.9	6.6	66.2	0.89	27.7	4.7	73.4	0.91	22.6	11.7	66.2	0.91
Spleen	22.8	5.8	75	1.51	22.1	6	80.6	1.66	21.8	6.6	80	1.44
Kidneys	23.7	6	58.6	0.81	26.2	4.6	61.6	1.07	22.2	5.6	79	0.51
Brain	17.5	10	56.3	1.3	19.8	7.6	55	1.37	17.4	10.4	60	1.03
Intestines	20.8	17.2	72.5	1.37	20.1	13.3	73.6	1.36	17.7	18.7	71.2	1.3
Skin & Fur	51.35	1.9	44.5	0.10	47.9	2.1	47.9	0.11	47.6	3	54.8	0.13
Muscles	29.85	3.3	56.3	0.63	29	3.2	62.4	0.89	29.65	4.6	55.6	0.50
Bones	40.2	2.3	43	7	44.6	2	45.8	7.6	36.9	2.5	51.2	6.4
Lungs	20.1	5.9	65.5	0.85	19.1	5.8	73	0.85	20.5	6.5	78	0.87
Heart	21.7	6	65.5	0.87	22.8	5.8	75	0.84	21.5	7.8	80	0.75
Stomach	20.25	9.2	67.5	0.90	21.5	8.6	71.5	0.91	21	11.6	76	0.95
	MG OF GLUCOSE PER G OF D.M.											
Livers	51.2				33.3				72			

TABLE III (Continued)

	Mice Receiving Feed (Group 5b) Until 9/9 Then Feed 4				Mice Receiving Feed 6 (Group 6)			
	Percent Dry Matter (D.M.)	Mg of Free Amino Acids Per g of D.M.	Percent Protein D.M.	Percent Total Phosphorus D.M.	Percent Dry Matter (D.M.)	Mg of Free Amino Acids Per g of D.M.	Percent Protein D.M.	Percent Total Phosphorus (D.M.)
Liver	24.5	9.7	70.6	0.81	27.3	5.9	69.9	1.4
Spleen	22.6	5.7	75.5	1.47	22	5.4	74	1.6
Kidneys	25	5.2	60.2	0.94	25.9	5.1	59.5	0.92
Brain	19.2	8.4	54.8	1.39	19.9	8.9	55.2	1.42
Intestines	19	15.8	70	1.25	20	13.6	71.2	1.6
Skin & Fur	44	2.5	51.6	0.12	48.7	2.1	47.1	0.20
Muscles	29.25	3	59.4	0.51	28.7	3.6	62.3	0.81
Bones	42.4	2.4	47	7.5	44	2.4	48.3	8.6
Lungs	19.1	5.2	64	0.87	19.8	5.5	68	0.94
Heart	21.2	5.8	69	0.8	22.3	5.3	72	0.8
Stomach	19.5	8.8	67	0.9	19.8	9.3	68.5	1
	MG OF GLUCOSE PER G OF D.M.							
Livers	49.5				38.4			

- The dry matter content remains practically constant for all organs and tissues in all six groups, except for the muscles in group 5 which are higher in fat.

- The free amino acids are found in larger quantity in all the organs of group 5; this reflects a disturbance in metabolism at this level.

- We observe an increase in proteins in all the organs of group 5, except in the muscle, where their content is lower. It is interesting to note that groups 2 and 3 occupy middle positions.

- The total phosphorus contents of the organs of group 5 are greatly lowered. The decrease in this element is also observed in groups 2 and 3, especially in the bones.

- The free glucose in the liver is greatly increased in the mice of groups 2, 3 and 5. This phenomenon might be caused by an inhibition of phosphorylations.

- The results of the analyses on the mice of group 5b show that the disturbances observed in group 5 are rapidly reversible when the diet is changed.

- The analyses of group 6 indicate that cellulose-free fermented rapeseed meal is an excellent feed for mice.

DISCUSSION AND CONCLUSIONS

Subjecting rapeseed meal to soaking in the presence a Geotrichum candidum strain improves the feed value of this meal.

The yeast acts in at least two ways: on the one hand, we observe a hydrolysis of the thioglucosides with decomposition of the isothiocyanates which appeared; on the other hand, there was a release, then destruction, of toxic heteroproteinaceous compounds. A fraction precipitating from a 20 percent $(NH_4)_2SO_4$ solution (protein α) has already been identified. This substance, administered orally to mice stopped their growth at a dosage level of 20mg/kg. More detailed toxicity tests are now underway.

By continuing the fermentation, we were able to make the rapeseed proteins completely soluble; they can be easily extracted by standard chemical methods. The fractions thus obtained total between 65 and 80 percent in proteins and lend themselves to texturization.

The overall amino acid composition of these fractions remains appreciably the same as in the original meal. However, it is possible to isolate a protein (protein β) which represents 25 percent of the total proteins and which has very special structure and properties.

The nutritional experiments on mice showed:

- that rapeseed meal does not have a toxicity that can be observed from the weight of the mouse.

Nevertheless, on the basis of the numerous analyses made on the organs, we discovered major disturbances with regard to the amino acids, free glucose, proteins and phosphates in the animals fed the rapeseed meal rich in thioglucosides, and in the Polish meal, which did not contain any.

If we admit that the digestive tract of a mouse does not have a flora capable of decomposing the thioglucosides, and that these are toxic only in the free isothiocyanate state (as in feed 5), numerous toxic signs observed remain unexplained. Hence, we actually observe a hypertrophic action on the thyroid together with a decrease in the amounts of fat in the organs, except in the muscles of group 5, where several substances seem to act together. These results agree with the observations of C. Calet, who observed a hypertrophy of the thyroid and a thinning effect on the carcass of a chicken receiving feed containing rapeseed meal. D.R. Clandinin also noted that isothiocyanates have no effect on a chicken's metabolizable energy rate for rapeseed meal.

Thus, the problems related to the feeding of rapeseed meal are not essentially due to sulphur compounds alone, but undoubtedly to several substances whose harmful effects accumulate and which still have to be identified.

Whatever the case may be, the Geotrichum candidum fermentation improves the feed value of rapeseed meal for mice.

This project was produced with the financial assistance of C.E.T.I.O.M., at its request.

We thank Mr. Chopin de Janvry, Mr. Y. Guilhaumaud and Mr. M. Chanet for the help and confidence they offered us.