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EFFECTS OF BIN-HEATING ON THE CHEMICAL COMPOSITION AND NUTRITIONAL VALUE OF RAPESEED FED TO MICE

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ABSTRACT

Undamaged and bin-heated rapeseed (*Brassica campestris*) were either: ground; ground and dry-cooked for 30 min at 110 C; ground and autoclaved for 30 min at 1.2 kg/cm^2, then dried at 100 C; ground, autoclaved, partially extracted with petroleum ether; or ground, autoclaved, extracted, steam-stripped, and dried. These meals were incorporated at 8, 12, 16, and 20% levels, seed basis, in approximately isocaloric, isonitrogenous mouse diets containing about 20% protein. Bin-heated rapeseed contained no glucosinolates, no myrosinase, and only a trace of hydroxynitriles, but had lost 65, 52, 28, 23, and 15% of its original tryptophan, lysine, histidine, arginine, and threonine, respectively. Mycotoxins were not detected. The odor of the meal derived from heated rapeseed was rated by a panel and found to be generally pleasant. Growth rates and feed utilization by mice fed bin-heated rapeseed were normal. Dietary protein levels were too high to allow the amino acid changes to be reflected in animal performance.

RESUME

Des grains de colza (*Brassica campestris*) non endommagés ou échauffes en entrepôt ont été soit moulus, moulus et cuits à sec pour 30 min à 110 C, moulus et étuvés pour 30 min à 1.2 kg/cm^2 et puis séchés à 100 C, moulus, étuvés et partiellement extraits avec de l'éther de pétrole, ou moulus, étuvés, extraits, dépouillés à la vapeur et séchés. Se servant du grain entier comme base, ces tourteaux ont été incorporés à des niveaux de 8, 12, 16, et 20% dans des régimes approximativement isoénergétiques et isoazotés contenant environs 20% de proteines. Le grain de colza échauffé en entrepôt ne contenait aucun glucosinolate, aucune myrosine et seulement un montant très faible de hydroxynitrile, mais il avait perdu, 65, 52, 28, 23, et 15% de la tryptophan, la lysine, l'histidine, l'arginine, et la thréonine originelle, respectivement. Des mycotoxins n'ont pas été détectés. L'odeur du tourteau provenant du colza échauffé a été évaluée par un groupe de juges et en general semble être plaisante. Les taux de croissance et l'indice de consommation par des souris nourries aux grains de colza échauffés en entrepôt ont été normales. Les teneurs en proteines des rations étaient trop élevés pour que les modifications chez les acides aminés influencent la performance des animaux.

INTRODUCTION

Rapeseed that is damaged because of unfavorable harvesting or storing conditions constitutes a serious economic problem to individual rapeseed producers. It also becomes a matter of considerable regional significance as rapeseed production expands and large quantities of off-grade rapeseed periodically may have to be utilized or disposed of in the most advantageous manner.

Although much research has been done on the use of rapeseed meal derived from good quality rapeseed, there is little if any direct evidence available on the nature or magnitude of problems inherent in the meal or oil obtained from bin-heated rapeseed. Therefore, the purpose of this study was to investigate the effects of bin-heating of rapeseed in terms of nutrient content, myrosinase activity, glucosinolate content, and occurrence of mycotoxins. In addition, feeding value was determined by tests with mice.

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MATERIALS AND METHODS

Design

A feeding experiment of 2 × 5 × 4 × 2 factorial design in four replicates was conducted. This involved two samples of rapeseed, each processed in five different ways and fed at four different levels in the diet to two strains of mice.

The two samples of rapeseed (*Brassica campestris* L., cult Echo) were derived from a rape field harvested with 9–10% moisture in the seed on a warm day (28 C) and immediately stored in a large plywood bin. The seed in the core of the bin subsequently heated severely although the damage was not detected until about 4 months after the seed was stored. The seed in the outer regions of the bin showed no evidence of damage and was used as the control sample.

Each sample of rapeseed was fed at 8, 12, 16, and 20% of the diet to provide the equivalent of up to about 10% rapeseed meal. Each kind of rapeseed, at each level, was subjected to five different treatments and incorporated in the diets as: (*i*) ground seed, G; (*ii*) ground seed dry-cooked at 110 C for 30 min, DC; (*iii*) ground seed autoclaved at 1.2 kg/cm^2 for 30 min, then dried at 100 C, A; (*iv*) ground seed, autoclaved as above, partially extracted with petroleum ether (35–58 C bp), then dried, AE; and (*v*) ground, autoclaved, extracted, steam-stripped (Reynolds and Youngs 1964) 30 min at 100 C and then dried, AES.

Animals, Management, and Diets

Two male weanling mice weighing 10–12 g initially were randomly allotted within replicates and within strains to the various diets. Mice of Carworth Farms No. 1 origin and crossbreds with Swiss white were used. They were housed in individual wire-bottom cages in a battery under conditions of controlled light and temperature. Fresh feed and water were provided daily ad libitum. Records were kept of weight gains, feed consumption, and liver weights for a 14-day growth period.

The rapeseed preparations were incorporated into the diets (Table 1) so as to maintain isocaloric and isonitrogenous conditions, based on analyzed protein

Table 1. Formulation of diets containing four levels of bin-heated and normal rapeseed meals (*B. campestris* cult Echo) processed† in five different ways

Ingredients	Level used in diet, seed basis, %			
	8	12	16	20
Basal mixture‡	80.0	80.0	80.0	80.0
Rapeseed	8.0 (4.8)	12.0 (7.2)	16.0 (9.6)	20.0 (12.0)
Soybean meal	5.82 (5.78)	3.88 (3.86)	1.94 (1.93)	
Starch	0.66 (0.60)	0.44 (0.40)	0.22 (0.20)	
Cellulose	0.72	0.48	0.24	
Corn oil	2.40 (4.05)	1.60 (4.03)	0.80 (4.015)	
Lard	2.40 (4.05)	1.60 (4.03)	0.80 (4.015)	

†Values represent quantities in unextracted rapeseed meal; those in parentheses represent the quantities in partially oil-extracted rapeseed meal.
‡Contains (%): oatmeal (100 mesh) 64.41, lard (ethoxyquin-stabilized) 3.39, alpha (soy) protein 1.0, casein 1.0, vitamin–mineral premix§ 5.0, cellulose (BW-100) 5.0, L-lysine 0.1, methionine (hydroxy analog) 0.1.
§Per kg of premix: 50 g $NaCl$; 651.54 g $CaHPO_4 \cdot 2H_2O$; 136.0 g $KHCO_3$; 18.0 g MgO; 3.0 g $MnSO_4 \cdot H_2O$; 5.17 $FeSO_4 \cdot 7H_2O$; 0.50 g $CuSO_4 \cdot 5H_2O$; 1.25 g ZnO; 0.30 g vitamin A (stabilized, 325,000 IU/g); 0.125 g vitamin D_3 (200,000 IU/g); 5.0 g vitamin E (275 IU/g); 3.0 g menadione; 0.1 g thiamine; 0.2 g riboflavin; 0.5 g calcium pantothenate; 0.15 g pyridoxine; 0.5 g niacin; 0.5 mg vitamin B_{12}; 20 mg biotin; 50 mg folacin; 40 mg inositol; 80 g choline chloride; 44.055 g cornstarch.

values and estimated digestible energy values. All diets were designed to be nutritionally adequate for growing mice (NRC 1962).

Analyses

The rapeseed samples were analyzed for protein, fat, and crude fiber by AOAC methods (1960), for heat of combustion (kcal/g) by oxygen bomb calorimetry, for fatty acids in the oil by gas–liquid chromatography, for amino acids by automated amino acid analyzer (Technicon TSM I), for sulfur in the oil by the method of Youngs (unpublished method based on reduction of sulfur to H_2S, by nacent hydrogen, and determination as PbS; developed by C. G. Youngs) and for glucosinolates according to Youngs and Wetter (1967). The two rapeseed samples were also examined for presence of mycotoxins (courtesy Dr. P. M. Scott, Food Division, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario).

The feeding trial data were subjected to analysis of variance and covariance, and Duncan's (1955) multiple range tests were used to assess significance of treatment differences.

RESULTS

Seed Characteristics and Composition

Bin-heating brought about distinct changes in the outward appearance of the rapeseed. The seed lost its shiny, reddish-brown color and became dark-greyish and dull and as well increased in kernel size. Weight/unit volume decreased by 13% (Table 2). The "spoiled" seed had a pronounced sour odor.

Processed, bin-heated rapeseed was evaluated for odor characteristics by an untrained panel of about 40 people. Most regarded the odor as pleasant, and although reactions varied, resemblance to the aromas of chocolate, coffee, tobacco, and molasses were frequently observed.

Glucosinolate analysis indicated typical values for the good quality seed but there was no evidence of glucosinolates or their hydrolytic products in the bin-heated rapeseed, although a trace of hydroxy-nitrile was found.

The moisture contents of both samples of rapeseed had declined somewhat from the values determined at time of harvesting. Protein and fat (oil) contents appeared to have been relatively unaffected by the heating during storage.

Amino acid analysis of the rapeseed samples revealed marked reductions in the essential amino acids arginine, histidine, threonine, tryptophan, and lysine in the protein of bin-heated rapeseed. The concentrations were 23, 28, 15, 65, and 52%, respectively, below those of the control rapeseed protein. There were reductions ranging from 9 to 18% below the control protein for aspartic acid, serine, glutamic acid, and proline. The sulfur-containing amino acids, methionine and cystine, seem to have been unaffected. About 11% less of the total nitrogen in the bin-heated rapeseed was accounted for in terms of the 18 amino acids measured.

The fatty acid composition of the oil showed several possible effects of bin-heating. Slight increases in the percentages of palmitic acid (16:0), oleic acid (18:1), and linoleic acid (18:2) and a reduction in erucic acid (22:1) were observed (Table 2). However, all the values reported here fall within the normal ranges for this species of rape.

Table 2. Density and chemical composition of normal and bin-heated rapeseed

Item	Quality of rapeseed	
	Normal	Bin-heated
Density		
Weight/bushel, lb	54	47
kg/hl	67	59
Rapeseed, ground		
Moisture, %	5.4	4.5
Protein, N × 6.25, %	24.7	24.7
Ether extract, %	42.7	42.2
Gross energy, kcal/g	6.3	6.5
Riboflavin, mg/kg	5.8	5.4
Niacin, mg/kg	105	100
Pantothenic acid, mg/kg	7.2	4.2
Rapeseed, ground, partially extracted, dry matter basis		
Protein, %	32.3	29.8
Ether extract, %	21.2	27.7
Crude fiber, %	8.5	18.0
Gross energy, kcal/g	5.62	6.06
Glucosinolates, aglycones, mg/g fat-free dry matter		
Allylisothiocyanate	0	0
Butenylisothiocyanate	4.7	0
Pentenylisothiocyanate	3.8	0
Oxazolidinethione	3.1	0
Hydroxy nitrile	0	.02
Amino acids, % in protein of whole seed		
Arginine	7.67	5.89
Histidine	3.41	2.46
Isoleucine	3.49	3.52
Leucine	6.60	6.61
Lysine	6.60	3.18
Methionine	1.75	1.69
Cystine	1.65	1.92
Phenylalanine	4.09	4.45
Valine	4.34	4.45
Threonine	4.34	3.68
Tryptophan†	1.13	0.40
Fatty acids, % of oil		
16:0	2.8	3.1
16:1	0.2	0.2
18:0	1.9	1.3
18:1	29.3	31.8
18:2	14.6	18.0
18:3	6.1	7.2
20:0	0.7	0.6
20:1	11.9	11.1
20:2	0.4	0.4
22:0	0.6	0.4
22:1	29.6	24.5
22:2	0.2	0.1
24:0	0.3	0.2
24:1	1.4	1.1
Sulfur in oil, mg/kg	0	55
Mycotoxins	0	0

†Seed protein hydrolyzed by triple enzyme (trypsin, pepsin, erepsin) and assayed by *Lactobacillus plantarum*. ATCC 8014.

Sulfur at 55 mg/kg of oil was found in oil extracted from bin-heated rapeseed, possibly reflecting breakdown of glucosinolates during the heating of the seed while in storage.

No mycotoxins were found in either of the two rapeseed samples although they were examined for aflatoxins, ochratoxin A, sterigmatocystin, diacetoxyscirpenol, and zearalenone.

Animal Responses

Weight gains were superior for both strains of mice fed the bin-heated rapeseed ($P < .01$; Table 3) and, among the methods of processing, the diets containing autoclaved seed (A, AE, and AES) promoted better growth. However, there was an interaction between rapeseed source and methods of processing that indicated that only the normal rapeseed responded to autoclaving. Thus, all animals fed either autoclaved normal rapeseed or damaged rapeseed regardless of processing grew normally; 14-day gains of 13–15 g being typical of well-nourished mice of these strains.

Dry cooking, partial extraction of oil, or steam-stripping failed to effect any improvement in the normal rapeseed, leaving autoclaving as the only effective treatment in this experiment.

The level of rapeseed incorporated into the diets had relatively little effect, although the 20% level resulted in significantly lower gains than the 8% level ($P < .01$).

Feed intake responses paralleled the growth rates, with mice fed bin-heated rapeseed consuming more than those fed the normal seed and with autoclaving of normal seed resulting in greater feed consumption (Table 3). Likewise, efficiency of feed conversion revealed superiority of bin-heated and of autoclaved normal seed. The level at which rapeseed was fed had no effect on feed intake.

Because of the correlation between weight gains and feed intakes ($r = 0.60$; $P < .01$) the observed gains were adjusted by regression to average feed intake (Table 3). This indicated that the superiority of bin-heated rapeseed diets was due mainly to the higher feed intakes, since adjusted gains for bin-heated and normal rapeseed diets were similar. However, methods of processing resulted in differences in growth responses not totally accounted for in terms of variations in feed intakes. Autoclaved normal seed remained superior to nonautoclaved normal seed independent of amounts consumed.

Trends in liver weights paralleled those of weight gains (Table 3) and contained similar treatment effects. The correlation coefficient for weight gains and liver weights was 0.80 ($P < .01$) and, as with weight gains adjusted for feed intake differences, liver weight differences largely reflected differences in weight gains or feed consumption. There may have been a tendency for the liver to be relatively larger for mice fed the ground or dry-cooked normal seed than for mice fed the autoclaved normal seed.

The strains of mice differed in their responses in some respects but displayed no basic difference in relation to the evaluation of bin-heated rapeseed (Table 3). The crossbred mice consumed more feed and grew more rapidly. They also ate more of the diets containing normal rapeseed than did the pure strain of mice.

Table 3. Growth and feed utilization responses

Treatment		Gain	Feed	Feed/gain ratio	Adjusted gain†	Liver wt	Adjusted liver wt‡
		g	g	g	g	g	g
Strain of mice							
Carworth		12.62	47.61	3.86	13.01	1.59	1.63
Carworth × Swiss		13.31	50.74	3.90	12.92	1.65	1.61
Seed quality							
Normal		12.34	47.13	3.96	12.84	1.56	1.63
Bin-heated		13.60	51.22	3.80	13.10	1.68	1.61
LSR§		0.56	0.52	0.15	0.44	0.08	0.05
Method of processing							
G		11.76	47.11	4.06	12.26	1.55	1.68
DC		11.60	45.67	4.09	12.46	1.52	1.67
A		13.58	50.89	3.80	13.16	1.62	1.56
AE		14.03	50.10	3.62	13.80	1.67	1.56
AES		13.88	52.13	3.84	13.15	1.74	1.64
LSR Min		0.88	2.16	0.26	0.93	0.12	0.08
Max		0.97	2.40	0.29	1.03	0.13	0.09
Level of meal or seed used, %							
8		13.57	49.57	3.71	13.50	1.68	1.62
12		13.09	49.78	3.84	12.94	1.64	1.62
16		12.91	49.29	3.96	12.88	1.64	1.64
20		12.30	48.17	4.01	12.55	1.52	1.59
LSR Min		0.78	1.94	0.23	0.63	0.10	0.07
Max		0.85	2.15	0.26	0.70	0.11	0.08
Strain × processing							
Carworth	G	11.65	46.46	4.02	12.32	1.58	1.72
	DC	12.15	45.95	3.93	12.94	1.50	1.59
	A	13.55	49.47	3.74	13.48	1.62	1.56
	AE	13.22	46.56	3.59	13.86	1.62	1.60
	AES	12.55	49.61	4.03	12.44	1.63	1.68
Carworth × Swiss	G	11.86	47.75	4.08	12.21	1.52	1.64
	DC	11.05	45.40	4.25	11.98	1.54	1.75
	A	13.60	52.31	3.86	12.83	1.62	1.55
	AE	14.83	53.63	3.64	13.74	1.72	1.51
	AES	15.21	54.64	3.65	13.87	1.85	1.60
LSR Min		1.24	3.06	0.37	0.99	0.17	0.11
Max		1.38	3.40	0.41	1.10	0.19	0.12
Seed quality × processing							
Normal	G	9.80	41.37	4.32	11.71	1.37	1.71
	DC	9.66	41.83	4.47	11.46	1.40	1.76
	A	14.29	51.04	3.61	13.83	1.69	1.54
	AE	14.24	49.37	3.51	14.19	1.64	1.50
	AES	13.69	52.05	3.89	12.99	1.72	1.64
Bin-heated	G	13.72	52.84	3.79	12.82	1.73	1.64
	DC	13.53	49.52	3.72	13.45	1.65	1.58
	A	12.86	50.73	4.00	12.48	1.56	1.57
	AE	13.82	50.83	3.72	13.41	1.70	1.61
	AES	14.06	52.20	3.80	13.32	1.76	1.64
LSR Min		1.11	2.74	0.33	0.89	0.15	0.10
Max		1.23	3.04	0.37	0.98	0.17	0.11
Error variance (80 df)		3.10	18.86	0.27	1.98	0.06	0.02

†Adjusted by regression to average feed intake.
‡Adjusted by regression to average weight gain.
§Least significant range, using Duncan's multiple range test, at which smallest or greatest differences between means are significant at $P < 0.05$.

The strains responded equally well to the diets containing nonautoclaved rapeseed but the crossbreds grew faster and ate more ($P < .01$) than the pure strain mice when fed diets containing autoclaved rapeseed.

DISCUSSION

Rapeseed that has been subjected to spontaneous heating during storage may undergo marked changes in its chemical, physical, and nutritional properties. Density, color, and odor were affected but perhaps the most important change was the apparent total disappearance of the glucosinolates. In view of their known goitrogenic properties (Bell and Belzile 1965) and probable effects on palatability, the loss of the glucosinolates can be regarded as beneficial from the standpoint of feeding value of the seed or meal derived from it. The excellent performance of the mice fed the bin-heated material confirmed the absence of glucosinolates and removed much of the concern over the possibility that the glucosinolates might have been converted to other deleterious substances.

The fact that livers were not enlarged and that no mycotoxins, possibly resulting from fungal action in the bin, were found provided additional confirmation of the nontoxicity of this sample of bin-heated rapeseed.

Another significant change associated with bin-heating was the substantial losses in five essential amino acids. Compared with the normal seed, tryptophan was reduced by 65%, lysine by 52%, histidine by 28%, arginine by 23%, and threonine by 15% (Table 2). The loss of lysine is probably the most serious because it involved extensive loss and is often the first limiting amino acid in cereal grains with which rapeseed or rapeseed meal might be fed. However, the losses in the other four amino acids are also potentially serious, along with lysine, in nonruminant diets. It is therefore probable that the occurrence of heat-damaged rapeseed in seed processed by crushers could lead to variations in the content of these amino acids and in nutritional value of the resulting meal without there being any measurable effects in terms of crude protein content.

The fate of the amino acids that were missing in the rapeseed after bin-heating was not determined. Possibly some were converted by enzymatic or microbial action to various forms of nonprotein nitrogen. It is also possible that the storage conditions during bin-heating resulted in amino acids becoming tightly bound to other constituents and therefore difficult to release by acid hydrolysis. In either event it is probable that the amino acids apparently lost would not be available to an animal in the normal course of digestion.

To the extent that this sample of bin-heated seed is representative of bin-heated rapeseed in general, it is probable that although such material may possibly be safe and palatable to swine and poultry, it would be a more appropriate ruminant feed, either as whole-fat seed or as extracted meal. This is because of the greater dependence of swine and poultry on the so-called essential amino acids.

The feeding trials were not designed to assess protein quality and since all diets contained about 18% protein, mostly from the basal portion of the diet, no indication of amino acid deficiencies could be expected to appear. However, further studies on protein quality and digestibility are in progress.

Any changes observed in the fatty acid composition of the oil from the bin-heated rapeseed were relatively minor. Further testing would be required to establish whether bin-heating significantly affects this aspect of oil quality. The appearance of sulfur in the oil from heated rapeseed is of interest to crushers and refiners because of its property of inhibiting hydrogenation reactions.

CONCLUSIONS

Analytical and feeding trial data involving mice revealed that extensive, spontaneous bin-heating of clean rapeseed (*Brassica campestris*) resulted in reduced density, a color change from reddish-brown to dark-grey, a sour odor, elimination of the glucosinolates, substantial losses in tryptophan, lysine, histidine, arginine, and threonine, and the appearance of sulfur in the extracted oil. There was no evidence of mycotoxins. The odor of processed bin-heated rapeseed was assessed by a panel and generally judged to be pleasant, somewhat resembling the aromas of coffee, tobacco, molasses, or chocolate.

Growth rates and feed utilization by mice fed bin-heated rapeseed at levels up to 20% of the diet were normal, but dietary protein levels were too high to permit the amino acid changes to affect growth responses.

These studies indicate that bin-heated rapeseed, or rapeseed meal derived therefrom, may differ markedly from material not damaged in storage. Extensive spontaneous heating, as occurred in the sample used in these tests, may result in total destruction of the glucosinolates and extensive losses of amino acids, presumably by conversion to simpler nitrogenous substances. The reduction in protein quality associated with the amino acid changes could be a substantial disadvantage in swine and poultry ration formulation. Such material would appear to be better suited for ruminant feeding.

Mixtures of ground and bin-heated rapeseed, as well as rapeseed less severely heat-damaged, need to be investigated. It is difficult to predict the effects of intermediate degrees of glucosinolate hydrolysis or degradation and of amino acid changes on nutritional value.

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