Part I.—Growth of Young Albino Rats as Influenced by Heat-treated Mustard Oil and Erucin Supplementation of Groundnut Oil in Fish Flour Diet

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A growth inhibitory factor present in mustard oil was investigated by feeding young albino rats with raw, boiled and steamed mustard oil and with groundnut oil supplemented with erucin. The results show a definite improvement of the growth due to heating of the mustard oil. Partial substitution of groundnut oil with erucin at 40% level did not produce any appreciable change in the growth rate calculated per 100 calories food intake. It appears that the lower growth rate as effected by mustard oil feeding is not due to high erucic acid content but may be attributed to the presence of some volatile compound which is removed during the ordinary process of cooking of the food-stuffs with this oil.

Introduction

The main fat component of the dietaries of the people of East Pakistan is mustard oil and its use for the last several centuries has not shown any evidence of mental or physical disorders amongst the population of this region. Nutritional studies of this oil, however, have been reported with divergent conclusions. Deuel *et al.*[1,2] reported its poor absorption and digestibility in rats, near about 77 % whereas experiment with human subjects[3] revealed about 99 %—a value which is almost the same as due to other vegetable oils. They attributed the low disgestibility to the presence of high quantity of erucic acid in this oil. The first report about the ill effect of erucic acid on the growth response in rats was communicated by Turpeinen,[4] but the acid was given in the form of ethyl eruciate, a compound which is not naturally present in the oil. Thomasson's[5,6] work showed that the lower growth of rats due to feeding of rape-seed oil was due to the presence of erucic acid. In the subsequent paper,[7] however, he reported after long-term feeding experiment on rats upto the death of the animals, that while the growth rate due to feeding of rape-seed oil was slower as compared to that due to winter butter fat, the life span of the rats fed on rape-seed oil was, however, more than those fed butter fat. In spite of the above difference, the total growth and the food consumption during the survival period of the rats were the same for both the rape-seed oil and the butter fat groups. Moreover, the rats fed on butter fat showed pathological changes like leg lesions and kidney degeneration of serious nature which were absent in the group fed on rapeseed oil.

Recently Naismith and Qureshi[8] have attempted to correlate the higher cholesterol content of the blood of the rats fed on the mustard oil with its high erucic acid content. But their results are not based on any feeding trial with erucin in support of the above conclusion. In the same experiment by feeding typical Pakistani diets they noted almost equal nitrogen balance due to ghee, hydrogenated cotton seed oil and mustard seed oil.[8a]

In summarising the above work it will appear that there is still a controversy about the nutritional character of mustard and rape-seed oil. Further, the mustard oil contains another important compound allylisothiocyanate, the effect of which on the growth rate and on the ultimate nutritive value of the oil has not been studied so far. This compound is volatile and so escapes easily with steam. As a matter of practice the mustard oil is consumed locally by boiling or steam-heating with vegetables, fish, meat, pulse etc. and not much in the raw state—a dietary condition on which the previous experiments were carried out. It is, therefore, possible that during boiling or steaming, the growth retardation effect of the oil is removed, because of the escape of allylisothiocyanate or any other steam-volatile compound with the ultimate result of normal growth of the rats. With the above possibilities in view the whole problem of mustard oil feeding as in vogue in this region has been reinvestigated so as to determine how far the erucic acid content and boiling and heat treatment of the oil may influence the ultimate nutritive value of the oil.

Experimental Series 1

Effect of Erucic Acid on Growth of Rats.—The composition of the diets used for this series is given in Table 1. In all the diets the Calorie percent from protein was kept constant by adjustment of the protein percent of the diets due to increase of Fat Calorie percents. Fish flour processed in this laboratory from a local species Boal fish, (*Wallaguatu*) which was found to be rich in available lysine, histidine, methionine, etc., and possessing high Relative Nutritive value, was used as the main protein source in the preparation of the diet.

TABLE 1.—SHOWING THE COMPOSITION OF DIET PREPARED BY SUBSTITUTION OF GROUNDNUT OIL WITH ERUCIN IN THE RATIO OF 60:40 AND WITH RAW, BOILED AND STEAM-HEATED MUSTARD OIL.

	Experimental Diet No.						
	24 Ground-nut oil	25 Ground-nut oil + erucin	26 Ground-nut oil	27 Ground-nut oil + erucin	32 Raw mustard oil	33 Boiled mustard oil	34 Steam heated mustard oil.
Starch	59·3	59·3	52·7	52·7	72·7	72·7	72·7
Fish Flour[a] ..	25·7	25·7	27·3	27·3	12·3	12·3	12·3
Salt Mixture[b] ..	4	4	4	4	4	4	4
Sugar with vitamin mixture[c] ..	4	4	4	4	4	4	4
Agar	2	2	2	2	2	2	2
Groundnut oil ..	5	3	10	6	—	—	—
Erucin	—	2	—	4	—	—	—
Raw Mustard Oil ..	—	—	—	—	5	—	—
Boiled Mustard Oil	—	—	—	—	—	5	—
Steam-Heated Mustard Oil ..	—	—	—	—	—	—	5
Total	100	100	100	100	100	100	100
Calculated calorie per g. of the diet ..	4.01	4.01	4.26	4.26	4.01	4.01	4.01
Protein percent of the diet	20.05	20.05	21.3	21.3	10.02	10.02	10.02
Protein-Calorie percent of the diet[d] ..	20	20	20	20	10	10	10
Fat percent of the diet	5	5	10	10	5	5	5
Fat-Calorie percent of the diet[e] ..	11.2	11.2	21.1	21.1	11.2	11.2	11.2

(a) Fish powder used in diets No. 24 to 27 contained 78% protein and that used in diets No. 32 to 34 contained 81% protein, (b) Hawk, Oser and Summerson, (c) Vitamin Mixture supplied: 0.4 mg. of Thiamine HCl. 5 mg. Niacin, 1 mg. Riboflavin, 2 mg. Calcium pantothenate, 2 mg. α—tocopherol acetate, 0.45 mg. pyridoxine HCl, 0.20 mg. Folic acid, 0.04 mg. Menadione, 0.02 mg. Biotin.150 mg. Choline C1, 10 mg. Inositol and 0.50 μg vitamin B_{12}. Vitamins A and D concentrate (Adexolin) were given in bi-weekly dose of 600 I.U. and 100 I.U. respectively per rat.

$$(d) = \frac{\text{Protein percent of the diet} \times 4 \times 100}{\text{Calorie per g. of the diet} \times 100} = \frac{\text{Protein percent of the diet} \times 4}{\text{Calorie per g. of the diet}}$$

$$(e) = \frac{\text{Fat percent of the diet} \times 9 \times 100}{\text{Calorie per g. of the diet} \times 100} = \frac{\text{Fat content of the diet} \times 9}{\text{Calorie percent the diet.}}$$

Preparation of Erucin.—The oil obtained from yellow variety of mustard seeds in cold solvent (Light Petroleum Ether) extraction method was saponified in the usual way. The washed and dried mixed fatty acids were fractionated and a fraction rich in erucic acid (I.V. 73-75) was separated by urea adduct method at 12-15°C. The purity of the acid was determined by its iodine value. The preparation of glycerides of erucic acid was effected by the use of sodium methoxide and heating the components for 7 hours continuously under reduced pressure. The end product-erucin was repeatedly washed and finally dried. The dried product showed the I.V. of 72-74.

Animal Feeding.—Thirty two male young albino rats of ages 25-35 days were divided into four groups of eight and each group of eight rats was fed with the experimental diets keeping them into two separate cages—each with four rats. The rats were obtained from our stock colony which is maintained by random breeding. The foundation stock was procured from the Basic Medical Science

Centre at Karachi. The feeding was continued for a consecutive period of four weeks after a preliminary period of 3 days to allow adjustment for the changeover to the experimental diets. Measurement of the body weight and collection of residual food was made at the end of each week. The weekly increase of the body weight and the food consumed was then determined and from these values the Calorie Consumption and the Calorie Efficiency Ratio (CER) *i.e.*, growth per 100 Calories consumption per rat was then calculated. All these values are shown in Table 2 (a).

Results

The results of feeding the Diets No. 24 and 26 show that weekly gain in body weight per rat due to feeding of groundnut oil at 5 and 10% level of dietary intakes *i.e.*, at 11 and 21% Fat Calorie levels, were found to be 17.5 and 19.7 g. respectively. The improvement in the gain in body weight due to increase of the level of the fat is due to sparing effect of fat on protein metabolism at the present intake level of 20% protein calorie. Such improvement in growth due to increase in the Fat component was previously reported by Elvehjem[9] and others.

Substitution of a part of the groundnut oil by erucin in the ratio of 60:40 caused a decrease of the weekly gain in body weight from 17.5 to 15.0 g. when the Fat-Calorie percent of the diet was 11 (Diet No. 25) and a decrease from 19.7 to 17.0 g. when the Fat Calorie percent was 21 (Diet No. 27). The important aspect whether this decrease in body weight is the direct inhibitory effect on the growth or due to the lower food consumption is clarified from the values of the Calorie Efficiency Ratio which almost remain unchanged due to partial substitution of the groundnut oil with erucin at both the Fat Calorie percent levels of 11 and 21. The above drop in the gain in body weight due to erucin substitution is, therefore, not due to direct inhibitory effect on growth but mainly due to less consumption of the foodstuff as is evident from the Table 2a. The drop in the consumption of foodstuff, on the other hand, appears to be due to less availability of the essential fatty acid from Groundnut Oil resulting from the proportionally lower intake of this oil when it is partly substituted by erucin in the ratio of 60:40.

Experimental Series 2.

Study on the Effect of Heat Treatment of Mustard Oil on the Growth of Rats.—(A) Preparation of Mustard Oil for Diets: In this part of Pakistan some food menu like meat, fish, vegetable and pulse cutlets are prepared by direct frying in the mustard oil. In the preparation of curries from the above products slight frying is followed by boiling in water. Only in the preparation of mashed potato and mashed cauliflower etc. raw mustard oil is used and only in small quantities. In order to equalise these conditions of dietary habitats in the consumption of mustard oil with various meal preparations, the following three batches of mustard oil were prepared.

TABLE 2 (a).—SHOWING THE GROWTH OF YOUNG ALBINO RATS DUE TO SUBSTITUTION OF GROUNDNUT OIL BY ERUCIN (60: 40—RATIO).

Diet No.	Nature of the fat used	Pcal value *	Fcal value **	Gain in body weight. (g.)		Consumption of food (g.)		Calorie consumption		Calorie Efficiency Ratio § (CER)
				Total for 4 weeks	Weekly average	Total for 4 weeks	Weekly average	Total for 4 weeks	Weekly average	
24	Groundnut Oil	20	11.2	70	17.5	200	50	802	200.5	8.72
25	Groundnut Oil + Erucin (60:40)	20	11.2	60	15.0	184	46	737.8	184.4	8.13
26	Groundnut Oil	20	21.1	79	19.7	204	51	869	217.2	9.08
27	Groundnut Oil + Erucin (60:40)	20	21.1	68	17	176	44	749.7	187.4	9.06

* Pcal value refers to Protein Calorie percent of the diet; ** Fcal value refers to Fat Calorie percent of the diet.

$$\S = \frac{\text{Gain in body weight per rat per week} \times 100}{\text{Calorie consumption per rat per week}}$$

Pure mustard oil was collected from a reputed oil supplier and it was then treated under three batches as below:

(1) One batch of the oil was kept as such without any heat treatment and this was marked as raw oil.

(2) Another batch was boiled with water under direct flame for 20 minutes, a period which is generally taken up in the preparation of meal with mustard oil. The water was separated out and the oil dried over desiccation. This treatment equalises the condition where the vegetables, meat and fish having 60 to 90% water are fried with a little mustard oil.

(3) To the third batch of the oil, steam was passed for the same period of 20 minutes. This was finally dried over desiccation under vacuum. This process is almost the same when fish, meat vegetable and pulse curry or soup are prepared with this oil by steaming.

(*B*) *Preparation of Diet.*—Three experimental diets (Diets No. 32, 33 and 34) of the composition as shown in Table 1 were used for this series. The protein supply was kept constant at the Protein Calorie percent level of 10. The oil was incorporated in the diet at 5 percent. level *i.e.*, at 11.2 percent Fat-Calorie level in each case.

Selection of the rats and procedure for growth study on these rats were the same as adopted in Experimental Series-I excepting that in this case a preliminary period of 1 week was allowed so as to get the rat accustomed to the new diet. It has been noted that at the first two days the rats showed aversion to the diet, specially to the raw mustard oil diet because of the pungent odour of the oil, afterwards they regained the appetite and consumed the usual quantity of the diet. For this reason one week was allowed as the preliminary period for adjustment to the new diets in this experiment.

The results of the study are presented in Table 2 (b). From the results it would appear that both ordinary boiled (Diet No. 33) and steam-heated mustard oil (Diet No. 34) produced higher weekly rate of growth of 17.25 and 18 g. per rat than that due to crude oil (Diet No. 32) showing a lower growth rate of 13.7 g. per rat. The average food intake also increased due to heat treatment of the oil and this is probably due to removal of the pungent odour of allylisothiocyanate during the process of frying and boiling.

Calorie Efficiency Ratio showed higher value of 5.64 in case of boiled oil. The value due to steam-heated oil was 5.23 though the food intake on this diet increased considerably. The Calorie Efficiency Ratio due to raw mustard oil is very poor (4.25). This low Calorie Efficiency Ratio of raw oil indicates that lower growth rate due to raw mustard oil is not mainly the result of decreased consumption of food, unlike the previous experiment on erucic acid. It appears that some factor other than erucic acid is present in the raw mustard oil which produces direct inhibitory effect on growth. This factor is volatile and is easily removed when the oil is heated.

Discussion

The results of Experimental Series 1 have given evidence against the previous claims that high erucic acid content in the rape-seed oil is the causa-

TABLE 2 (b).—SHOWING THE GROWTH OF ALBINO RATS DUE TO RAW, BOILED AND STEAM HEATED MUSTARD OIL.

Diet No.	Name of the fat used	Pcal value *	Fcal value **	Gain in body weight g.		Composition of food g.		Coloric consumption		Caloric efficiency ratio §
				Total for 4 weeks	weekly average	Total for 4 weeks	weekly average	Total for 4 weeks	weekly average	
32	Raw Mustard Oil	10	11.2	55	13.75	322.4	80.6	1292	323	4.25
33	Boiled Mustard Oil	10	11.2	69	17.25	304.8	76.2	1222	305.5	5.64
34	Steam-heated Mustard Oil	10	11.2	72	18	340.8	85.2	1374	343	5.23

* Pcal value refers to Protein Calorie percent of the diet; ** Fcal value refers to Fat Calorie percent of the diet.

$$\S = \frac{\text{Gain in body weight per rat per week} \times 100}{\text{Calorie consumption per rat per week.}}$$

tive factor in retarding the growth rate of the rats. The apparent decrease in body weight gain as observed here is mainly due to lower consumption of food and not related with the direct retardation effect on growth. Careful scrutiny of the results of other workers in this line also substantiate this fact. Thomasson in association with Boldingh[6] while reinvestigating the cause of lower growth rate of rapeseed oil as observed previously by him[6] has noted decrease in the gain in body weight due to substitution of groundnut oil or fatty acids by erucin or erucic acid in the ratio of 50:50 at the Fat-calorie level of 20%. These growth results were not supported by any food consumption data to judge in the decrease in gain in body weight due to erucic acid or erucin substitution was the result of direct inhibitory action on growth or of indirect effect through lower consumption of food. The observations submitted in the subsequent publication[7] that the rats fed on rape-seed oil survived longer, even with lower growth rate and with less food consumption than those fed on Summer butter fat with higher rate of growth and with more food consumption, led them to conclude that the lower growth rate due to rapeseed oil feeding is the result of lower consumption of food probably by the influence of erucin. Thus it is evident that erucin has no direct inhibitory effect on the growth process as viewed by the authors in the present investigations.

The results of second series of the experiment of the present investigations that the growth retardation of raw oil is mainly due to its direct effect on growth process rather than on the food consumption unlike the effect of erucic acid and that this effect is destroyed when the oil is boiled or steam-heated point to show that the growth retarding effect of mustard oil is associated with some volatile compound present in the raw oil. This volatile compound may be either the allyl-isothiocyanate or some other factors which are mostly removed during the ordinary process of cooking of the meals as practised in this region. It may be stated here that there is no possibility of development of any toxic effect in the body due to consumption of mustard oil in the proportion (Maximum of 20% Fat-calories) as used in the preparation of meals in this region. On the contrary, it will help in the prevention of obesity and increase of the life span as viewed by Thomasson[7] and Pennington[10] as compared to those fed on butter fat provided the protein and other essential ingredients in the rice eater's diet are safeguarded. If the oil is consumed as raw in all the dietary preparations, growth retardation and other toxic effects may develop. But occasionally this is consumed as raw and only in small quantity with meshed vegetables. The cumulative effect of consumption of such small quantity of raw mustard oil on the health of the consumers is not yet definitely known. Further work in this line is in progress and the results will be communicated in due course.

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