

## Chapter 3 (Study 2)

### ANIMAL EXPERIMENTS

#### EFFECT OF FENUGREEK (*TRIGONELLA FOENUM GRAECUM*) SEED BASED DIETS ON BIRTH OUTCOME AND LACTATIONAL PERFORMANCE IN ALBINO RATS.

##### INTRODUCTION

Methipak was believed to be a hot food and one that might cause abortions by several respondents in the habit survey (Chapter 2). Many of the women attributed this effect to fenugreek seeds. While methipak during pregnancy was avoided by some women, it was almost universally consumed during lactation. One of the properties ascribed to methipak was that it increased the secretion of milk.

Since, fenugreek seeds were to be incorporated into the food supplement, to be subsequently developed for pregnant and lactating women, it was considered necessary to establish the safety of its consumption during pregnancy and also to investigate if the belief that it increased milk secretion was well founded and could be scientifically proved.

Two separate studies were therefore planned and conducted to satisfy the above issues. The objectives, methods and findings of these two experiments are described in this chapter.

##### Experiment - I

##### *Effect of fenugreek seed based diets on the birth outcome in albino rats.*

##### Objectives

1. To determine the effect of fenugreek seed powder fed at a level of 5 and 20% on the birth outcome.
2. To investigate the effect of fenugreek seed powder incorporated into biscuits\* on the birth outcome (with or without casein).

##### MATERIAL AND METHODS

##### Selection of Animals

Forty five virgin female albino rats of the Charles Foster strain (taken from the stock colony of the Department) weighing atleast 170 g were selected for the experiment. The females were mated overnight with male rats of the same strain

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\*A biscuit product type was included for testing as it is in this form that the fenugreek seed based supplement was proposed to be offered to pregnant & lactating mothers.

and about the same size. First day of pregnancy was determined by the presence of sperm in the vaginal smears\*. After the pregnancy was confirmed, the dams were placed in individual cages and randomly assigned to experimental and control groups of 5 to 8 rats in each group.

#### Experimental groups and diets

The experiment consisted of the following groups :

| Group | n | Diet Fed                             |
|-------|---|--------------------------------------|
| E1    | 6 | 5% fenugreek diet.                   |
| E2    | 8 | 20% fenugreek diet.                  |
| C1    | 6 | 10.7% Casein protein diet (control)  |
| C2    | 8 | 13% Casein protein diet (control)    |
| FB    | 6 | 5% fenugreek biscuit without casein. |
| FBC   | 6 | 5% fenugreek biscuit with casein.    |
| PB    | 5 | Plain biscuit (without fenugreek).   |

Fenugreek seed powder was either given in the raw form (groups E1 and E2) or incorporated into biscuits (groups FB and FBC). The level of fenugreek in groups E1, FB and FBC was 5% and that in group E2 was 20%. A 5% level was selected because in the proposed food supplement, the level of fenugreek would not exceed 5%. A 20% level was selected to represent a high intake of fenugreek seed powder. There were three control groups C1, C2 and PB, which were fed diets without fenugreek. Group C1 was fed 10.7% casein protein diet; group C2 was fed 13.1% casein protein diet and group PB was fed plain biscuit (crushed) diet without casein (Two control groups C1 and C2 were included because addition of fenugreek seed powder specially at a level of 20% increased the protein content of the diet). Groups C1, C2 and PB were the corresponding controls for the groups E1, E2 and FB. Tables 22 and 23 show the composition of the experimental diets. Tables 24, 25 and 26 show the composition of mineral mix, water soluble vitamin mix and fat soluble vitamin mix respectively. The diets and water were offered ad libitum for period of 21 days.

#### Autopsy Procedure

On day 22, the dams were mildly anaesthetized by ether and laparatomized. The uterus was examined intact and the number of implantations was determined by

\*With the help of a dropper, a drop or two of normal saline was introduced into the vagina and the fluid was sucked back in the dropper. A drop was placed on a slide and observed under the microscope for the presence of sperm. The test was carried out in the morning, between 6 and 8 am.

Table 22 : Composition of the control and experimental diets

| Group | Diet                                   | Casein<br>g | Fenugreek<br>g | Groundnut<br>Oil g | Sago<br>g | Biscuits<br>(crushed)<br>g | Nutrients<br>Protein g<br>Cal |
|-------|--|-------------|----------------|--------------------|-----------|----------------------------|-------------------------------|
| E1    | 5% fenugreek                           | 10.9        | 5.0            | 5.0                | 73.1      | -                          | 11.31 358                     |
| C1    | 10.7% casein<br>protein control        | 11.7        | -              | 5.0                | 77.2      | -                          | 10.77 360                     |
| E2    | 20% fenugreek                          | 10.9        | 20.0           | 5.0                | 58.1      | -                          | 15.24 356                     |
| C2    | 13% Casein<br>protein control          | 14.3        | -              | 5.0                | 74.6      | -                          | 13.13 360                     |
| FB    | 5% Fenugreek<br>Biscuit                | -           | -              | -                  | -         | 97.0                       | 6.26 430                      |
| FBC   | 5% Fenugreek<br>biscuit with<br>casein | 8.0         | -              | -                  | -         | 89.0                       | 5.43 428                      |
| PB    | Plain Biscuit                          | -           | -              | -                  | -         | 97.0                       | 5.43 424                      |

Diets E1, C1, E2 and C2 contained 4% (W/W) Hawk-Oser mineral mix. (Table 24 ), and 2% vitamin Mix (Table 25.) Fat soluble vitamin mix (Table 26 ) was added to all diets at a level of 1 g per kg of the diet.

**Table 23 :** *Composition of fenugreek & plain biscuits for the experiment*

| <b>Ingredient</b>     | <b>Amount (g)</b>             |                           |
|-----------------------|-------------------------------|---------------------------|
|                       | <b>Fenugreek<br/>Biscuits</b> | <b>Plain<br/>Biscuits</b> |
| Whole wheat flour     | 39.4                          | 45.0                      |
| Jaggery               | 40.0                          | 40.0                      |
| Hydrogenated fat      | 15.0                          | 15.0                      |
| Fenugreek seed powder | 5.6                           | -                         |
| <b>Total</b>          | <b>100.0</b>                  | <b>100.00</b>             |

Table : 24                      Composition of the mineral mix. (Oser 1976)

| S.No. | Compound                              | Amount (g) |         |
|-------|---------------------------------------|------------|---------|
| 1.    | Calcium citrate, $4H_2O$              | 308.2      |         |
| 2.    | $Ca(H_2PO_4)_2 \cdot 2H_2O$           | 112.8      |         |
| 3.    | $K_2HPO_4$                            | 218.7      |         |
| 4.    | KCl                                   | 124.7      |         |
| 5.    | NaCl                                  | 77.0       |         |
| 6.    | $CaCO_3$                              | 68.5       |         |
| 7.    | $3 MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$ | 35.1       |         |
| 8.    | $Mg SO_4$ (anhydrous)                 | 38.3       |         |
| 9.    | $FeNH_4$ Citrate                      | 91.36      | }       |
| 10.   | $CuSO_4 \cdot 5H_2O$                  | 5.97       |         |
| 11.   | NaF                                   | 0.76       |         |
| 12.   | $MnSO_4 \cdot 2H_2O$                  | 1.07       |         |
| 13.   | $KAl (SO_4)_2 \cdot 12H_2O$           | 0.54       |         |
| 14.   | KI                                    | 0.24       |         |
| 15.   | $ZnSO_4 \cdot H_2O$                   | 0.06       |         |
|       |                                       | 100.00     | 1000.00 |

Table : 25      *Composition of the Water soluble Vitamin Mix for 500 g mix.*  
( Rajlakshmi et al 1969, 1974 )

| S.No. | Compound                 | Amount                |
|-------|--------------------------|-----------------------|
| 1.    | Thiamine hydrochloride   | 40 mg                 |
| 2.    | Riboflavin               | 60 mg                 |
| 3.    | Pyridoxine hydrochloride | 60 mg                 |
| 4.    | Calcium pantothenate     | 100 mg                |
| 5.    | Nicotinic acid           | 500 mg                |
| 6.    | Folic acid               | 8 mg                  |
| 7.    | Biotin                   | 1 mg                  |
| 8.    | Vitamin B12              | 0.75mg                |
| 9.    | Ascorbic Acid            | 1.0 g                 |
| 10.   | Choline Chloride         | 5 g                   |
| 11.   | Inositol                 | 10 g                  |
| 12.   | Para-amino-benzoic acid  | 10 g                  |
| 13.   | Dextrin                  | to make<br>upto 500 g |

**Table 26 :**      **Composition of the fat soluble vitamin Mix. (for 100 g mix.)**

|    | <b>Compound</b>          | <b>Amount</b>       |
|----|--------------------------|---------------------|
| 1. | Vitamin A Acetate        | 3.3 g               |
| 2. | DL - $\alpha$ Tocopherol | 3.5 g               |
| 3. | Calciferol (Vitamin D)   | 9.0 mg              |
| 4. | Oil (groundnut)          | to make upto 100 g. |

1. Vitamin A Acetate 1.5 million IU/g  
Fluka AG. Buchs SG. Switzerland
2. DL  $\alpha$  Tocopherol  $C_{29}H_{50}O_2$   
E. : Merk Darmstadt
3. Calciferol (Vitamin  $D_2$ )  $C_{28}H_{44}O$ .  
BDH Chemical Ltd. Poole England.

counting the metrial nodes. The number of resorptions, if any, was also recorded. The uterus was then cut open and the fetuses and their placentas were removed, cleared of adhering tissue and weighed individually.

#### **Parameters**

1. Food consumption (every alternate day).
2. Weight change (every alternate day).
3. Number of implantations.
4. Number of resorptions.
5. Placental weight.
6. Fetal weight.

#### **Statistical Analysis**

Means and standard errors were calculated and students 't' test was used to determine if the differences between the means were significant.

### **RESULTS AND DISCUSSION**

#### **Food intake and fenugreek intake**

Table 27 shows the food intake and fenugreek intake in different groups. In spite of the bitter taste of fenugreek seed powder, the consumption of food in the experimental or fenugreek fed groups (14.5 to 18.2 g per day) was comparable to that of control groups (14-15 g per day). There was no difference in the consumption of food by groups E1 and E2, indicating that increasing the amount of fenugreek had no effect on the food intake. Food intake of group PB was less than that of group FB but it was not significantly different. Subbulakshmi (1967) and Sharma (1983) also reported that there was no difference in the food consumption of groups which were fed diets containing 5 to 60% fenugreek seed powder.



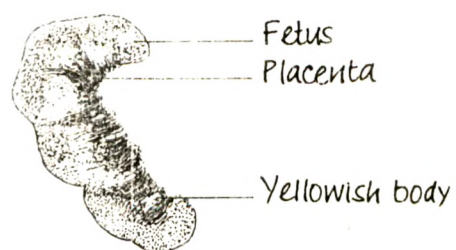
The intake of fenugreek seed powder on an average was 0.75 to 0.9 per day in groups fed 5% fenugreek diets (E1, FB and FBC) and 2.9 g per day in the group fed 20% fenugreek diet (E2).

### **Body weight changes**

The dams in all the groups (except groups FB and PB) gained on an average 95 to 110 g of weight during the period of gestation (Table 28, 29). There was no significant difference in the weight gain in groups fed fenugreek or control diets (E1 Vs C1, E2 Vs. C2). Increasing the level of fenugreek from 5 to 20% also had no effect on the weight gain (E1 Vs E2). Groups FB and PB (groups fed biscuit diets) lost some weight initially and gained little weight by the end of gestation. The poor weight gain in these groups could be attributed to the inadequate protein content of the diets i.e. 5.43 g% for PB and 6.26 g% for FB diet. However, when the fenugreek biscuit diet was supplemented with casein (group FBC), the gain in weight was comparable to that in control groups C1 and C2 which were fed 10.7 and 13.1 g% casein protein diets. Sharma (1983) has reported lower body weights of animals fed 60% fenugreek seed diet compared to control groups or groups fed 15 and 30% fenugreek diets. However, the weight gain in experimental groups fed 15 and 30% fenugreek diets was not found to be significantly different from the weight gain in the control group. Subbulakshmi (1967), observed higher body weights in groups fed 10% fenugreek diets compared to control or 5% fenugreek fed groups. Thus, inclusion of fenugreek seeds (either in raw form or biscuit form) in the diets does not seem to adversely affect the weight gain.

### **Reproductive Performance**

The average number of implantations in the control groups C1, C2 and PB was 11.3, 10.6 and 12.4 respectively. The corresponding figures for experimental groups E1, E2, FB and FBC were 11.3, 10.8, and 12.3 respectively (Table 30). There was no significant difference between any of these values. Similarly, the number of resorptions which ranged from 0.25 to 0.6 was not significantly different in control and experimental groups fed low and high levels of fenugreek (E1 Vs E2). The litter size (range 10.3 to 12.0, fetal weights (3.3 to 4.0 g) and placental weights (0.43 to 0.5 g) were also comparable in control and experimental groups, except in groups FB and PB in which the fetal and placental weights were low because of inadequate protein in the diets. However, it is to be noted that inspite of the low protein intake of these groups (PB & FB), there was no increase in the resorption rate or a decrease in the number of implantations. The average litter size in different groups was comparable to the figures reported by the National Research Council,



**Fig. 2 :** The laparatomized rat. The black area is the placenta surrounded by the fetus enclosed in amniotic bags. The yellowish area on the placenta is the metrial node. In case of resorption, only the metrial node shows.

**Table 27 :** Food intake and fenugreek intake by the pregnant dams in fenugreek fed and control groups

| Group | Food Intake / day<br>(g) |   |      | Fenugreek intake<br>per day (g) |   |       |
|-------|--------------------------|---|------|---------------------------------|---|-------|
| E1    | 17.7                     | ± | 0.65 | 0.89                            | ± | 0.028 |
| C1    | 15.3                     | ± | 2.32 | -                               |   |       |
| E2    | 14.6                     | ± | 0.77 | 2.93                            | ± | 0.155 |
| C2    | 14.0                     | ± | 0.73 | -                               |   |       |
| FB    | 15.1                     | ± | 1.55 | 0.75                            | ± | 0.076 |
| FBC   | 18.2                     | ± | 1.73 | 0.90                            | ± | 0.087 |
| PB    | 11.7                     | ± | 0.43 | -                               |   |       |

**Table 28 :** Body weights of pregnant dams in fenugreek fed and control groups on day 1, 7, 14 and 21 of pregnancy

| Group | Day 1         | Body<br>Day 7 | Weights (g)<br>Day 14 | Day 21        |
|-------|---------------|---------------|-----------------------|---------------|
| E1    | 201.8 ± 5.83  | 223.1 ± 5.14  | 245.3 ± 5.49          | 313.8 ± 1.84  |
| C1    | 195.6 ± 7.47  | 215.6 ± 10.22 | 238.5 ± 9.41          | 298.0 ± 15.60 |
| E2    | 242.1 ± 20.02 | 260.8 ± 21.91 | 280.1 ± 21.87         | 341.6 ± 25.77 |
| C2    | 219.5 ± 11.62 | 240.3 ± 13.23 | 261.2 ± 14.63         | 317.2 ± 19.16 |
| FB    | 194.8 ± 7.80  | 195.4 ± 9.23  | 195.8 ± 8.33          | 216.0 ± 8.70  |
| FBC   | 207.8 ± 9.71  | 227.1 ± 10.54 | 250.3 ± 9.94          | 327.5 ± 13.29 |
| PB    | 201.0 ± 9.91  | 199.0 ± 11.78 | 197.4 ± 9.39          | 209.2 ± 11.62 |

**Table 29 :**      *Body weight changes (g) of the pregnant dams in fenugreek fed and control groups on day 7, 14, & 21 of pregnancy*

| Group | Day 7        | Day 14       | Day 21        |
|-------|--------------|--------------|---------------|
| E1    | 21.3 ± 2.23  | 43.5 ± 5.13  | 112.0 ± 7.04  |
| C1    | 19.0 ± 4.74  | 42.8 ± 7.76  | 102.0 ± 14.61 |
| E2    | 18.7 ± 3.82  | 44.0 ± 5.31  | 97.8 ± 9.62   |
| C2    | 20.8 ± 2.81  | 41.7 ± 5.31  | 97.7 ± 9.42   |
| FB    | -0.6 ± 1.20* | 1.0 ± 0.93*  | 21.1 ± 4.59*  |
| FBC   | 19.3 ± 3.15  | 42.5 ± 4.15  | 119.6 ± 5.59  |
| PB    | -4.7 ± 1.65* | -3.6 ± 2.31* | 8.2 ± 8.70*   |

\*Significantly different from all other groups.

Table 30 : Reproduction performance of dams in fenugreek fed and control groups.

| Groups | No | Implantation    |  | No | Resorption      |  | Litter size     |  | Placental wt*. (g)            |  | Fetal wt*. (g)               |  |
|--------|----|-----------------|--|----|-----------------|--|-----------------|--|-------------------------------|--|------------------------------|--|
|        |    | Mean $\pm$ SE   |  |    | Mean $\pm$ SE   |  | Mean $\pm$ SE   |  | Mean $\pm$ SE                 |  | Mean $\pm$ SE                |  |
| E1     | 68 | 11.3 $\pm$ 1.10 |  | 2  | 0.33 $\pm$ 0.21 |  | 11.0 $\pm$ 0.85 |  | 0.43 <sup>a</sup> $\pm$ 0.01  |  | 3.5 $\pm$ 0.12               |  |
| C1     | 68 | 11.3 $\pm$ 0.76 |  | 2  | 0.33 $\pm$ 0.21 |  | 11.0 $\pm$ 0.68 |  | 0.43 <sup>b</sup> $\pm$ 0.02  |  | 4.0 <sup>ab</sup> $\pm$ 0.31 |  |
| E2     | 91 | 11.3 $\pm$ 1.17 |  | 5  | 0.60 $\pm$ 0.18 |  | 10.7 $\pm$ 1.09 |  | 0.43 $\pm$ 0.01               |  | 3.5 $\pm$ 0.83               |  |
| C2     | 85 | 10.6 $\pm$ 0.86 |  | 2  | 0.25 $\pm$ 0.16 |  | 10.3 $\pm$ 0.82 |  | 0.43 $\pm$ 0.01               |  | 3.3 $\pm$ 0.09               |  |
| FB     | 65 | 10.8 $\pm$ 1.04 |  | 2  | 0.33 $\pm$ 0.21 |  | 10.5 $\pm$ 1.05 |  | 0.37 $\pm$ 0.03               |  | 2.6 <sup>a</sup> $\pm$ 0.15  |  |
| FBC    | 74 | 12.3 $\pm$ 1.17 |  | 3  | 0.50 $\pm$ 0.34 |  | 11.8 $\pm$ 1.30 |  | 0.50 <sup>ab</sup> $\pm$ 0.01 |  | 3.3 $\pm$ 0.07               |  |
| PB     | 62 | 12.4 $\pm$ 0.39 |  | 2  | 0.40 $\pm$ 0.24 |  | 12.0 $\pm$ 0.44 |  | 0.30 <sup>b</sup> $\pm$ 0.31  |  | 2.3 <sup>b</sup> $\pm$ 0.13  |  |

a, b Values sharing a common superscript within the same column are significantly different from each other ( $P < .05$ ). For 't' test please see Table 30 a.

\*Mean of means of litter.

Table 30 a : Statistical analysis (values of 't' test) for table 30

| Comparison | Implantation | Resorption | Litter Size | Placental wt. | Fetal wt. |
|------------|--------------|------------|-------------|---------------|-----------|
| E1 Vs C1   | 0            | 0          | 0           | 0             | 1.51      |
| E2 Vs C2   | 0.48         | 1.45       | 0.29        | 0             | 0.24      |
| FB Vs PB   | 1.30         | 0.22       | 1.32        | 2.33*         | 1.51      |
| FB Vs C1   | 0.39         | 0          | 0.4         | 2.00          | 4.11*     |
| FBC Vs C1  | 0.71         | 0.43       | 0.54        | 3.5*          | 2.25*     |

\* P &lt; .05

Washington (1981) and the National Institute of Nutrition, India (1981), which are 11 (average) and 7 to 14 (range) for rats respectively. In a concurrent experiment conducted to determine the effect of similar fenugreek diets (5 & 20% fenugreek) on lactational performance, (please see page 89 of this chapter), we found that when the dams were allowed to deliver, the litter size was not different in experimental and control groups.

Thus, we failed to see any adverse effects of fenugreek seed based diets on either the weight gain during pregnancy or on the reproductive performance. These findings were different from those reported by Khare et al (1983), who observed mild antifertility effects of feeding an ethereal extract of fenugreek seeds at a dose of 25 mg per 100 g body weight. In their study, 2 rats (out of 13) showed complete absence of fetal implants in the control group, the average number of implants being 5.57. In contrast, in the test group, seven rats (out of 12) showed complete absence of implants; the average number of implants being 3.2.

On calculating the equivalent of the ethereal extract in terms of whole fenugreek seeds, based on the reported value of 7% ethereal extract (Shankaracharya and Natarajan, 1972), it was found that 0.25 mg of ethereal extract was equivalent to 0.375 g of fenugreek seeds. Thus a rat weighing about 200 g in the study of Khare et al (1983) would have received about 0.72 g of fenugreek seeds, which is less than the amount of fenugreek seeds fed in our experiments which was 2.9 g per day in group E2. It is postulated that perhaps the ethereal extract is a concentrated source of steroidal substance **diosgenin** which is used as a starting material in the synthesis of sex hormones and oral contraceptives (Shankaracharya and Natarajan, 1972), and feeding of this in a single dose could have resulted in the antifertility effects seen by Khare et al (1983).

## Experiment II

### Effect of fenugreek seed based diets on the lactational performance in albino rats.

#### Objectives

To determine the effect of feeding 5% and 20% fenugreek seed diets on lactational performance (growth of the pups).

#### MATERIAL AND METHODS

##### Selection of Animals

The experiment was conducted on 30 virgin female albino rats of the Charles Foster strain, weighing at least 170 g. The females were mated overnight with male rats of the same strain and about the same size. First day of pregnancy was determined by the presence of sperm in the vaginal smears. After confirming pregnancy, the dams were placed in individual cages and randomly assigned to six groups of 5 rats in each group.

##### Experimental groups and diets

The experimental diets consisted 5 and 20% fenugreek seed powder (E1 and E2). The control diets were C1 (10.7% casein protein) and C2 (13.1% casein protein). The composition of these diets has been described previously (see page 76 of this chapter). The experimental groups were as follows :

| Group | n | Diet given during pregnancy | Diet given during lactation |
|-------|---|-----------------------------|-----------------------------|
| E1 E1 | 5 | E1                          | E1                          |
| E2 E2 | 5 | E2                          | E2                          |
| C1 C1 | 5 | C1                          | C1                          |
| C2 C2 | 5 | C2                          | C2                          |
| C1 E1 | 5 | C1                          | E1                          |
| C2 E2 | 5 | C2                          | E2                          |

The diets and water were fed ad libitum during gestation and lactation (upto 21 days). In groups C1 E1 and C2 E2, the diets were changed within 12 h of parturition.

##### Parameters

Food consumption and weights of the dams were recorded every alternate day. Pups were weighed within 12 h of the birth and subsequently every alternate day. The litter size was adjusted to eight pups for all the dams. The weight of the pups at weaning (22nd day of lactation) was considered as the indicator of the lactational performance of the dams.



### **Statistical Analysis**

Means and standard errors were calculated and student's 't' test and analysis of variance were used to determine if the differences in the means of the various groups were significant.

## **RESULTS AND DISCUSSION**

### **Food Consumption**

Table 31 shows the food intake in fenugreek fed and control groups during pregnancy and lactation. The average food intake during pregnancy was 15 to 18 g per day and during lactation 25 to 32 g per day. The results of analysis of variance (ANOVA) showed that there was no significant difference in the food intake in different groups. In the previous experiment (page 81 of this chapter), in which rats were given similar experimental and control diets, the food consumption during gestation also ranged from 14 to 18 g per day.

### **Fenugreek consumption**

As shown in Table 31 intake of fenugreek seed powder during pregnancy was on an average 0.78 g per day in group E1E1 (5% fenugreek diet) and 2.9 g per day in group E2E2 (20% fenugreek diet). These diets were fed throughout gestation (21-22 days) and lactation (upto 21 days). The fenugreek intakes during lactation in groups E1E1 and E2E2 were 1.5 g and 6.0 g per day respectively. The fenugreek intakes in groups C1E1 and C2E2 were 1.2 g and 5.5 g per day respectively (only during lactation).

**Body weight changes of dams during pregnancy and lactation :** : Table 32 & 33 and Fig. 3 show the weight changes during pregnancy and lactation in different groups. During pregnancy, the dams gained on an average 95 to 132 g of weight. The gain in weight in experimental groups E1E1 and E2E2 was 95 g and 99 g and that in the control groups C1C1 and C2C2 was 108. g and 119 g respectively. The difference although large was not found to be statistically significant. The gain in weight during pregnancy was similar to that observed in the previous experiment. At the time of parturition the dams lost on an average 55 to 64 g of weight. Throughout lactation, there was not much change in the weights of the dam (Fig.3).

### **Litter size, birth weight and growth of the pups**

The litter size ranged from 10.2 to 12.8 in control and experimental groups and was not significantly different in the various groups. These results support the previous study wherein the effect of fenugreek diets on the birth outcome was

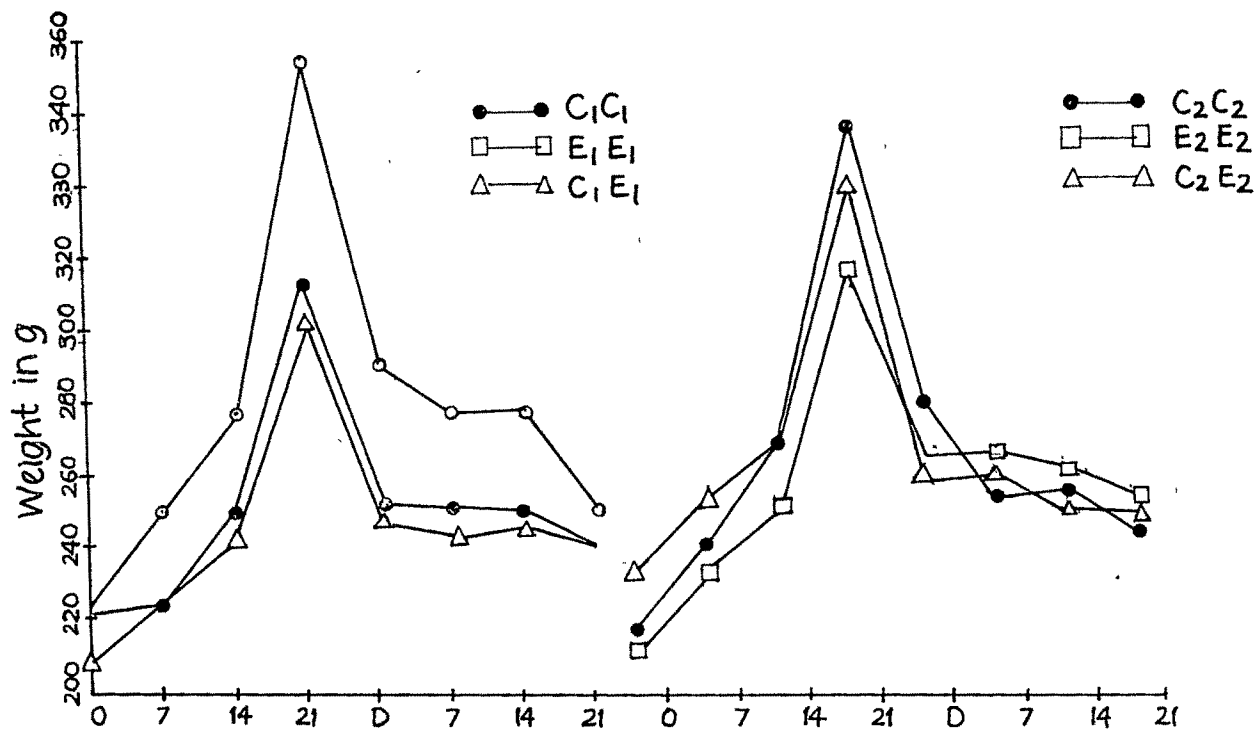


Fig.3 Weight changes of dams Fed Fenugreek and control diets during pregnancy (P), parturition (D) and lactation (L)

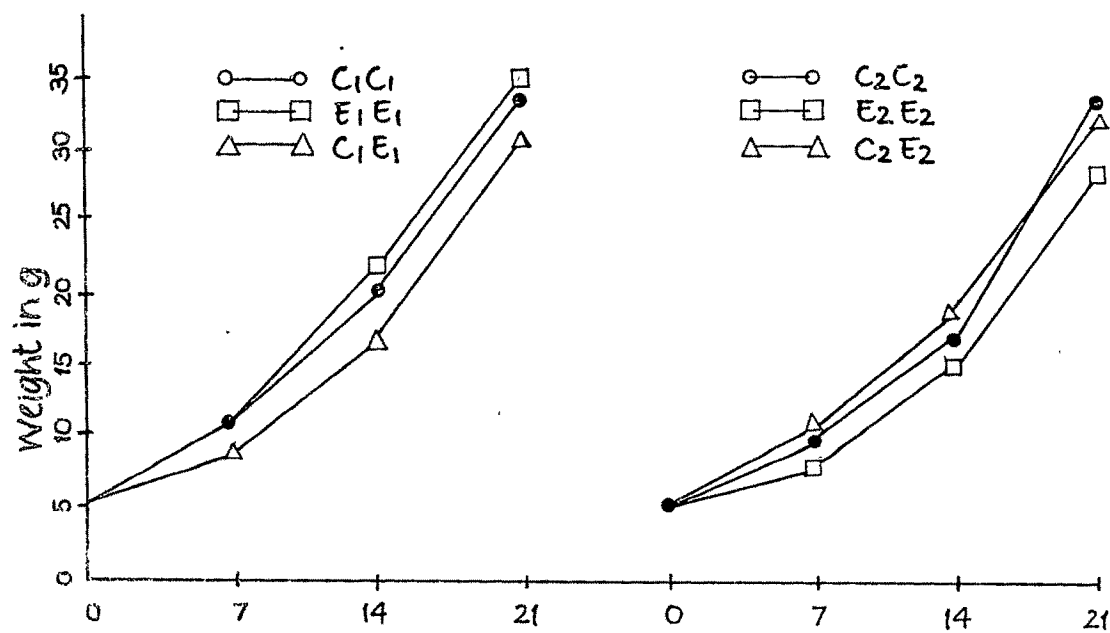


Fig.4 Growth of pups in Fenugreek fed and control groups.

**Table 31 :** Food consumption by dams during pregnancy and lactation in fenugreek fed and control groups

| Group              | Food intake ( g day) |                    | Fenugreek intake (g day) |            |
|--------------------|----------------------|--------------------|--------------------------|------------|
|                    | During Pregnancy     | During Lactation   | Pregnancy                | Lactation  |
| E1E1               | 15.7 ± 0.46          | 30.1 ± 1.49        | 0.78 ± 0.02              | 1.5 ± 0.07 |
| C1C1               | 16.2 ± 1.15          | 32.3 ± 3.02        | -                        | -          |
| C1E1               | 17.7 ± 1.37          | 25.2 ± 2.13        | -                        | 1.2 ± 0.16 |
| E2E2               | 15.0 ± 2.00          | 30.2 ± 4.07        | 2.9 ± 0.40               | 6.0 ± 0.81 |
| C2C2               | 17.3 ± 1.03          | 27.7 ± 2.27        | -                        | 5.5 ± 0.45 |
| F value<br>(ANOVA) | 0.70 <sup>NS</sup>   | 0.97 <sup>NS</sup> | -                        | -          |

NS - Not significantly different.

Table 32 : Body weights (g) of dams on day 1, 7, 14, 21 of pregnancy and at parturition

| Group | Pregnancy Weights (g) |            |            |            | Weight at Parturition |
|-------|-----------------------|------------|------------|------------|-----------------------|
|       | Day 1                 | Day 7      | Day 14     | Day 21     |                       |
| E1E1  | 209 ± 10.9            | 225 ± 9.8  | 243 ± 8.6  | 304 ± 13.8 | 249 ± 9.4             |
| C1C1  | 222 ± 13.0            | 225 ± 11.6 | 251 ± 12.2 | 315 ± 14.5 | 253 ± 7.7             |
| C1C1  | 224 ± 22.1            | 250 ± 23.3 | 278 ± 24.4 | 356 ± 20.5 | 292 ± 23.1            |
| E2E2  | 234 ± 12.9            | 255 ± 19.9 | 271 ± 18.7 | 333 ± 23.3 | 262 ± 18.7            |
| C2C2  | 219 ± 7.2             | 242 ± 8.8  | 271 ± 12.0 | 338 ± 15.2 | 272 ± 15.0            |
| C2E2  | 211 ± 6.4             | 234 ± 3.8  | 253 ± 7.3  | 320 ± 17.8 | 263 ± 9.27            |

Table 33 : Body weight changes (g) in dams during pregnancy and parturition in fenugreek fed and control groups

| Groups             | Diet given | Body weight gain (g) |                    |                    | Wt loss<br>At parturition |
|--------------------|------------|----------------------|--------------------|--------------------|---------------------------|
|                    |            | Day 7                | Day 14             | Day 21             |                           |
| E1E1               | E1         | 16.8 ± 2.22          | 34.4 ± 2.80        | 95.0 ± 5.81        | - 55.0 ± 5.46             |
| C1C1               | C1         | 18.0 ± 3.99          | 43.6 ± 5.63        | 108.6 ± 13.53      | -62.0 ± 7.37              |
| C1E1               | C1         | 25.4 ± 6.02          | 53.6 ± 10.87       | 132.0 ± 9.61       | -64.0 ± 4.78              |
| E2E2               | E2         | 19.0 ± 6.05          | 36.2 ± 8.43        | 99.0 ± 15.45       | -71.0 ± 6.49              |
| C2C2               | C2         | 22.4 ± 4.80          | 51.2 ± 6.02        | 119.0 ± 8.70       | -66.0 ± 4.45              |
| C2E2               | C2         | 22.7 ± 3.83          | 48.7 ± 8.57        | 120.0 ± 18.26      | -57.0 ± 9.66              |
| F Value<br>(ANOVA) |            | 0.62 <sup>NS</sup>   | 1.10 <sup>NS</sup> | 1.22 <sup>NS</sup> |                           |

NS - Not Significantly different.

Table 34 G Litter size, birth weight and growth of pups in fenugreek fed and control groups

| Groups          | Litter size        | Weight (g)         |                    |                    |                    |
|-----------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                 |                    | At birth           | Day 7              | Day 14             | Day 21             |
| E1E1            | 10.2 ± 0.73        | 5.3 ± 0.09         | 9.2 ± 0.91         | 16.6 ± 1.25        | 30.7 ± 1.60        |
| C1C1            | 10.6 ± 1.39        | 5.3 ± 0.10         | 10.9 ± 1.22        | 20.3 ± 1.83        | 33.8 ± 1.49        |
| C1E1            | 10.8 ± 0.86        | 5.8 ± 0.23         | 11.3 ± 0.91        | 21.6 ± 1.10        | 35.4 ± 1.92        |
| E2E2            | 12.7 ± 0.47        | 5.4 ± 0.12         | 10.2 ± 0.52        | 19.1 ± 1.50        | 32.9 ± 2.76        |
| C2C2            | 12.8 ± 0.96        | 5.2 ± 0.22         | 9.6 ± 0.50         | 17.5 ± 0.46        | 33.9 ± 1.78        |
| C2E2            | 10.7 ± 0.47        | 5.5 ± 0.14         | 8.4 ± 0.79         | 15.5 ± 1.54        | 27.8 ± 1.58        |
| F Value (ANOVA) | 1.13 <sup>NS</sup> | 1.66 <sup>NS</sup> | 1.35 <sup>NS</sup> | 2.95 <sup>NS</sup> | 2.70 <sup>NS</sup> |

NS - Not significantly different  
 Figures are mean ± S.E.



*Fig. 5 : Weanling pups in group C1E1*



*Fig. 6 : Weanling pups in group C2C2*

investigated. The birth weight of the pups ranged from 5.2 g to 5.8 in different groups. The results of ANOVA showed that the difference was not significant between the control and experimental groups. Thus, feeding of 5% or 20% fenugreek seed powder diets during pregnancy had no adverse effect on the birth weight of the pups. The subsequent growth of the pups followed more or less a similar pattern (Fig. 4. Table 34). At day 21, the weights of the weanling pups were 28 g to 31.4 g in the experimental groups and about 34 g in the control groups. These values were not significantly different from each other. Thus, inspite of a lower weight gain during pregnancy, the birth weight and growth of the pups in fenugreek fed groups was comparable to that in the control group.

The results of the present study do not indicate any beneficial effect of whole fenugreek seed powder on the growth of the pups, over and above that seen in the casein control groups. These findings do not support the studies carried out earlier (El Reidi et al 1944; El Reidi et al 1954) in which the oil extracted from the seed was found to promote lactation in rats. Perhaps, the galactogenic effect of the oil extract is more pronounced.

#### CONSLUSION

In the rat model, the strongly held belief that fenugreek seeds are abortive if consumed during pregnancy was not supported by the present study. Likewise, the strong belief that 'it increases milk secretion' was equally not substained. Since fenugreek seeds had no deleterious effect on either the birth outcome or growth of the pups, and foods containing fenugreek seeds are popular in Western India, it was considerd safe to incorporate it at levels of 50% into the maternal food supplement for pregnant and lactating women beneficiaries of the existing nutrition programs (as a means to reduce its sharing among other members of the family).