CHAPTER 3

Use of Mahuda flowers as an energy yielding component in the diet

(Experiment II and III)

Objective 2, To explore the effect of feeding pressure cooked for 10 or 20 minutes, Mahuda flowers as the source of carbohydrate on growth and biochemical status of weanling rats.

Objective 3, To investigate the safe level of Mahuda flowers in the diet that would support growth in weanling rats when used as carbohydrate source.

Highlights of the results

The degree of adverse effect on growth rate of rats was related to the levels of Mahuda flowers in the diet(25 or 50 g/100 g diets). The findings suggested that during the period of growth, if Mahuda flowers are to be consumed due to shortage of food, they should be pressure cooked for not less than 20 minutes and should be consumed not more than 25 g per 100 g diet.

Introduction

Literature regarding consumption of Mahuda flowers as an energy yielding component is scanty. Studies conducted on the use of Mahuda flowers as a feed for live stock indicated that cattle fed for more than 30 days on a feed mixture containing 10% Mahuda flowers exhibited no adverse effect on yield and quality of their milk(Wealth of India, 1962). Earlier Chandra and Joshi(1952) had investigated protein digestibility of a diet comprising of Mahuda flowers and wheat bhoosa in the ratio of 1.5:1. The digestibility co-efficient of crude protein was found to be 60%. The investigators reported that bulls fed on Mahuda flower containing diet maintained a positive balance for nitrogen and calcium.

Later Lahar et al (1959) fed bullocks for 27 days. Mahuda flowers and wheat bhoosa in equal amounts and observed that the animals maintained health, appearance throughout the experimental period. On an average there was an increase in the body weight of 5 lbs per head. The average digestibility co-efficient for crude protein of the diet was found to be 46%.

Vyas (1967) examined the effect of feeding chicks for 28 days isonitrogenous diet containing Mahuda flowers at 0, 4, 8, 12 and 16% levels. The author observed superior weight gain and maximum feed utilization in chicks fed diet containing 8% Mahuda flowers but at 12 and 16% level the growth rate tended to decrease.

Mahuda flowers are utilized by tribals whenever there is a scarcity of foods. Gopaldas et al (1981b) observed that tribals of Chhotaudepur district, Gujarat, consumed Mahuda flower admixed with cereals such as Maize, Bajra, Jowari and Rice. Often the flowers were

roasted or crushed and incorporated into dal(cereal soup) or into chapatis(unleavened flat bread) dough. Recently, Mane(1984) conducted dietary survey among Gond tribe of Maharashtra and reported that Mahuda flowers were mixed with other cereals to prepare Khakri(thick chappati) and Vada(small round balls, fried in oil).

As discussed earlier Mahuda flower contain 72% carbohydrates, mainly in the form of sugars. Therefore studies were conducted in this department to investigate the feasibility of Mahuda flower sugar, concentrated water extract of Mahuda flowers(Mahuda syrup), to replace carbohydrate in rat diet. Weanling rats fed ad libitum for 28 days, diet containing Mahuda syrup exhibited decreased food intake and consequently lower weight gain. However, the decrease in food intake was attributed to stickiness of the diet caused by Mahuda sugar fed in the form of syrup (Rajgor et al 1996). It was therefore considered necessary to avoid stickiness of the diet. Thus Mahuda flowers were incorporated instead of Mahuda sugar syrup as the carbohydrate source into rat diet.

Since Mahuda seeds contain saponin (Dhull and Gandhi 1977) and since heat treatment to foods is known to destroy anti-nutritional factors such as trypsin inhibitor (Kumar et al 1980), saponin (Liu et al 1980), haemagglutinins (Pender 1978), cyanogenetic glycosides and goitrogens (Delange et al 1982), polyphenolic compounds and phytic acid (Deosthale 1984) present in various plant products it was considered essential to subject the flowers to heat treatment prior to their incorporation into rat diet. The present investigation was therefore, planned with the main objective of using pressure cooked Mahuda flowers as an energy yielding component in the diet of

weanling rats.

The two specific objectives of the experiment II and III were

a) To explore the effect of feeding pressure cooked for 10 or 20 minutes, Mahuda flowers as the source of carbohydrate, on growth and biochemical status of weanling rats(objective two).

b) To investigate the safe level of Mahuda flowers in the diet that would support growth in weanling rats when used as a carbohydrate source(objective three).

The effects of feeding diet containing pressure cooked Mahuda flowers as the source of carbohydrate were evaluated against those of feeding diet containing sago as the carbohydrate source.

To fulfill the above objectives the parameters used or tests performed were :

1. Growth rate in terms of weight gain
2. Food intake
3. Organ weights(Liver, Heart, Kidney, Intestines, Spleen)
4. Moisture content of the organs
5. Hepatic lipid content
6. Hepatic glycogen content
7. Blood sugar levels
8. Haemoglobin concentration
9. Serum protein levels and A/G ratio

Materials and Methods

This investigation was conducted in two separate experiments(experiments II and III). Experiment II was designed to determine the acceptability and growth promoting quality of diet

containing pressure cooked Mahuda flowers. While experiment III determined (a) the optimal time for pressure cooking Mahuda flower which would increase growth promoting quality of Mahuda flower diets and (b) the level of Mahuda flowers safe for consumption.

In experiment II, 12 albino weanling male rats weighing between 30 to 40 g were randomly divided into two groups of 6 rats each and were fed ad libitum for 28 days, control or pressure cooked mahuda flowers diet.

Heat treatment to Mahuda flowers : Mahuda flowers were washed and pressure cooked for 10 minutes without addition of water. The cooked flowers were churned in a mixer to get a smooth pulp. The pulp was air dried and coarsely powdered. Mahuda sugars being hygroscopic, the powder tended to become lumpy. One g of powder equalled one g of sun dried flowers.

Experimental diets

The composition of control and Mahuda flower diets is presented in Table 3.1. The control diet (sago-bengal gram diet) provided 11.2 g protein and 69 g of carbohydrate. Of the total carbohydrate in this diet 33 g was provided by bengal gram and 36 g by sago. In Mahuda diet, to obtain 36 g of carbohydrate, 50 g of Mahuda powder (calculation based on 72% carbohydrate content) was incorporated per 100 g diet. As discussed earlier, the tribals of Ghatsila-Dhalbhumgarh district consume Mahuda flowers admixed with cereals and pulses. Therefore to simulate tribal diet the prepared Mahuda powder was mixed with bengal gram flour which also served as the protein source. The carbohydrate content of the diets supplied by Mahuda flower or sago (control diet) was maintained at comparable levels.

Table 3.1 Composition of control and pressure cooked Mahuda flower diets.

Ingredients	Casein diet	Sago-Bengal gram diet	Pressure Cooked Mahuda flowers to provide 24 g of CHO	Pressure Cooked Mahuda flowers to provide 19 g of CHO
			g/100 g	
1. Bengal gram flour [1]	-	54.00	41.00	48.00
2. Casein	12.00	-	-	-
3. Oil [1]	06.00	07.50	03.50	07.50
4. Sago [1]	76.00	41.00	-	23.00
5. Mahuda pulse powder	-	-	50.00	20.00
6. Vitamin mix [2]	02.00	00.50	00.50	00.50
7. Mineral mix [3]	04.00	01.00	01.00	01.00
Total	100.00	100.00	100.00	100.00
Protein (g)	11.04	11.2	11.2	11.1
Calories (K Cal)	388	375	372	369
Carbohydrate (g)	68.00	68.00	62.00	66.00
Fat (g)	8.0	7.0	4.0	7.0

* [1] Local Market

* [2] See table 3.2

* [3] See table 3.3

The diets were isocaloric and isonitrogenous. The water soluble vitamin mixture (Table 3.2) and the mineral mixture (Table 3.3) were prepared as recommended by National Academy of Science National Research Council (1978) and Osei (1979) respectively. Both vitamin and mineral mixtures were prepared in sufficient quantities to last for the entire experiment. The water soluble vitamin and mineral mixtures were added directly into the diet while the fat soluble vitamins were mixed in oil and then added to the diet to provide 1500 mcg of vitamin A, 90 mcg of vitamin D, and 25 mg of vitamin E per kg of diet (NAS – NRC 1978).

For experiment III, forty weanling albino male rats weighing between 30 to 40 g were randomly divided in 5 groups of 8 rats each. The rats were fed for 28 days, diets containing either 50 or 25 g (to provide 16 or 18 g of carbohydrate) of Mahuda powder prepared from Mahuda flowers pressure cooked for 10 or 20 minutes or sago as the carbohydrate source (Table 3.1). The protein was provided by either Casein or bengal gram flour. The allotment of the diets to the different groups and symbols used are outlined below.

Allotment of the control and Mahuda powder diets to the rat

Group 1 Casein diet (6 diet/group)

Group 2 Sago-bengal gram diet (SB diet/group)

Group 3 10 minutes cooked Mahuda flower diet at 25% level (25M10 diet/group)

Group 4 20 minutes cooked Mahuda flower diet at 25% level (25M20 diet/group)

Group 5 20 minutes cooked Mahuda flower diet at 50% level (50M20 diet/group)

Table 3.2 Composition of water soluble vitamin mixture

Vitamin	Allowance/rat/day	Allowance/1000 doses
Thiamine HCl[1]	40 mcg	40 mg
Riboflavin[2]	60 mcg	60 mg
Pyridoxin[3]	40 mcg	40 mg
Calcium pantothenate[4]	100 mcg	100 mg
Nicotine acid[4]	500 mcg	500 mg
Folic acid[3]	8 mcg	8 mg
Biotin[5]	1 mcg	100 mg
Vitamin B12[1]	.75 mcg	750 mg
Ascorbic acid[6]	1 mcg	1 mg
Choline chloride[6]	5 mcg	5 g
Inositol[7]	10 mg	10 g
Para amino benzoic acid[3]	10 mg	10 g
Dextrin[8]		to make up to 500 g

* [1]British Drug House Pvt. Ltd. , Bombay, India
* [2]Sigma Chemicals Company, Missouri, U.S.A.
* [3]Loba Chemie, Bombay, India
* [4]E Merck A.G. Darmastadt, Germany
* [5]Roche P Hoffman I Roche Inc. U.S.A.
* [6]Sarabhai M. Chemicals, Baroda, India
* [7]SDS Fine Chem Pvt. Ltd. , Biosar, India
* [8]Wellfield Ltd. , Bombay, India

Table 3.3 Composition of salt mixture

Salts		Amounts / g / kg
Calcium Citrate. 4 H_2O[1]		309.2
$Ca(H_2PO_4)_2 . H_2O$[2]		112.8
K_2HPO_4[2]		218.7
KCl[2]		124.7
NaCl[2]		77.0
Ca Co- [3]		63.5
$MgCO_3 . Mg(OH)_2 . H_2O$[4]		35.1
Mg SO_4(anh.drous)[2]		38.3
Fe NH_4 Citrate[3]	g 91.35)	
$CuSO_4 . 5H_2O$[2]	5.97)	
NaF[2]	0.78)	
$MnSO_4 . 2H_2O$[4]	1.07)	16.7
$KAl(SO_4)_2 . 12H_2O$[3]	0.54)	
KI[2]	0.24)	
$ZnSO_4 . H_2O$[2]	0.05)	
	100.00	1000.00

* [1]Thomas Baker and Co., London.

* [2]Sarabhai M. Chemicals, Baroda, India.

* [3]British Drug House Pvt. Ltd, Bombay, India.

* [4]Loba Chemeic, Bombay, India.

The rats were housed in individual galvanised iron cages. Food and water were offered ad libitum. Every alternate day the rats were weighed and food bowls were changed. A piece of paper was kept under each cage to hold spilled food and fecal material. The papers were removed when food bowls were changed. Fecal material was carefully removed and the left over food along with the paper was allowed to air dry overnight. On the following day, the left over food was weighed and the food consumed was calculated as follows

Weight of fresh food - Weight of left over food = food consumed.

Autopsy procedure

On the morning of the 29th day of the experiment the rats were weighed and their food intake was recorded. The rats were lightly ether anaesthetized and the blood was collected from jugular vein for serum protein estimation. At the same time, 0.02 ml of blood was measured and immediately expelled into 5 ml of Drabkin's solution for haemoglobin estimation. Blood was also collected in oxalated bulb for blood sugar estimation. Midline and crosswise incisions were made in the abdominal and thoracic regions. The liver was quickly removed and immediately placed on ice. The non hepatic tissues were trimmed, the liver was blotted on filter paper and weighed. Immediately appropriate amount of the liver tissue was placed in 30% potassium hydroxide for estimation of liver glycogen. Liver was also sampled for total hepatic lipid content. The sampled tissue was stored frozen until analysed. Heart, kidney, spleen and intestines were removed and weighed.

Analytical procedures

1. Moisture content of the organs

Moisture content of the organs was determined by the method of AOAC (1975). Approximately 0.5 to 0.6 g of organ tissue (previously sampled and kept frozen) was dried in an oven at 100 to 105° C and cooled in a desiccator. The process of heating and cooling was repeated till a constant weight was achieved. The percent moisture content was calculated using following formula :

$$\text{Moisture \%} = \frac{\text{Initial weight} - \text{final weight}}{\text{weight of the sample}} \times 100$$

2. Haemoglobin

Haemoglobin was determined by Cyanomethemoglobin method as described by Oser (1976).

Principle of the procedure : A sample of blood is mixed with a solution containing potassium cyanide (KCN) and potassium ferricyanide ($K_3Fe(CN)_6$). Ferricyanide oxidises the haemoglobin to methemoglobin which then reacts with cyanide ion to form cyanomethemoglobin. The cyanide methemoglobin formed, absorbs light at 540 nm and is stable.

Procedure : In duplicate, 0.02 ml of blood was pipetted into 5 ml of the Drabkins solution and mixed well. After 10 minutes, reading was taken in Klett Summerson using 540 nm filter (Green filter) against reagent blank.

The standard solution of cyanomethemoglobin corresponding to 14.5% haemoglobin was obtained from V.P. chest Institute.

Drabkin. The optical density (O.D.) of the standard solution was measured and the haemoglobin content of the sample was calculated as follows :

$$\frac{\text{O.D. of the sample}}{\text{O.D. of the standard}} \times \text{Concentration of the standard.}$$

The results were expressed in g haemoglobin per dl blood

2. Serum protein

Serum protein was estimated using Biuret method as modified by Varley (1967).

Principle of the procedure : The biuret test is given by those substances whose molecules contain 2 carbomyl (- $CONH_2$) groups, joined either directly together or through a single atom of nitrogen or carbon. Proteins respond positively since these are pairs of -CONH groups in the molecule (User, 1979).

Procedure : In a test tube 2.5 ml of sulphate sulphite solution was taken and 0.5 ml of serum was added. The contents were mixed by inversion.

For the total protein estimation, 2 ml of the above mixture was transferred into another test tube to which 5 ml of the biuret solution was added.

For determination of albumin, 1 ml of ether was added to the remaining mixture. The tubes were stoppered and were shaken 40 times in 20 seconds by inversion. The contents were centrifuged for 10 minutes at 200 rpm. Then 2 ml of the clear bottom solution without disturbing the precipitate, was transferred into a test tube to which 5 ml of biuret reagent was added.

A standared solution was prepared by dissolving 0.4 g of bovine

albumin in 100 ml of 0.2N NAOH. Different aliquots of the solution were made upto 2 ml with 0.2N NAOH and were treated in a similar manner as the sample. For the blank, 5 ml of biuret reagent was added to 2 ml of 0.2N NAOH.

All the tubes with biuret solution were placed in the water bath at 37° C for 10 minutes. for colour development. The colour intensity was read at 540 nm (green filter) in Klett summerson. Serum proteins were expressed as g/dl.

4. **Hepatic lipids**

Hepatic lipids were extracted according to the method of Folch et al (1957) and estimated gravimetrically.

Procedure : Approximately, 0.5 g of liver was homogenised in a pestle and mortar with 2 to 3 ml of chloroform : methanol (2 : 1) mixture. The homogenate was filtered through whatman No. 1 filter paper. The filterate and the residue suspended into 2 to 3 ml of chloroform : Methanol mixture. were kept over night in the refrigerator. On the following day. the residue was subjected to second extraction. The two filterates were combined and the volume made up to 5 ml with chloroform : methanol mixture.

In duplicate. 1 ml aliquot was pipetted into preweighed beakers. They were kept in an oven at 80° C for 15.5 hours. The beakers were then cooled in a desiccator for 1 hours and weighed. The differences between the two weights, i.e. weight of the empty beaker and that of the beaker containing dried lipids, gave the weight of the lipids.

Hepatic lipids were expressed as g/100 g fresh liver.

5. Hepatic glycogen

Hepatic glycogen was estimated using method described by Seifter et al (1950).

Principle of the procedure : Glycogen is hydrolysed to glucose and the glucose thus formed is estimated by using Anthrone reagent.

Procedure : Approximately 0.2 g of liver tissue was dropped in a sugar tube containing 3 ml of 30% potassium hydroxide solution. The tissue was digested over a boiling water bath for 30 minutes. The contents were cooled and diluted with water to 10 ml. Then 2 ml of the solution was pipetted in a sugar tube and diluted to 5 ml with water. The tubes were placed in cold water and 10 ml anthrone reagent was added and the contents were mixed well. The tubes were covered with foils and kept in boiling water bath for 10 minutes. They were removed and immediately cooled under the tap. The colour intensity was measured at 620 nm in spectronic 20 against reagent blank.

The percent glycogen content of the sample was calculated as follows :

$$\frac{\text{Reading of unknown} \times \text{dilution factor} \times 100 \times 1.11}{\text{Reading of std} \times \text{g tissue taken} \times 10000}$$

1.11 is the factor for conversion of glucose to glycogen.

6. Blood sugar

Blood sugar was analysed by the method of Nelson (1944) and Somogyi (1945).

Principle of the procedure : Blood is deproteinized by a zinc sulphate - barium hydroxide procedure which gives a filtrate containing practically no reducing substances other than

glucose. The zinc - barium filtrate is heated with an alkaline copper reagent and then treated with a special arsenomolybdate colour reagent. The colour developed is compared with that obtained from a known amount of glucose.

Procedure : 0.1 ml of blood was placed in a centrifuge tube containg 3.5 ml of distilled water. To this, 0.2 ml of 0.3 N barium hydroxide and 0.2 ml of 0.5% zinc sulphate were added. The contents were shaken well,centrifuged and filtered. One ml of the filterate, 1 ml water for blank and 1 ml of standard were pipetted separately into three test tubes. To all the three test tubes, 1 ml of water and 2 ml of alkaline copper solution were added. The contents were heated in a boiling water bath for 10 minutes. They were cooled quickly for 1 minute and 1 ml of the arsenomolybdate reagent was added. The volume was made upto 10 ml with distilled water. The colour intensity was read at 540 nm (green filter) in Klett Summerson. The blood sugar content (mg/dl) of the sample was calculated as follows :

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times \text{conc. of standard} \times \frac{100}{0.025\text{ml blood}}$$

Statistical Analysis

Means and standard errors were calculated for each parameters. The data on food intake, gain in body weight, percent organ weights, moisture content of the organs, haemoglobin, serum protein, A/G ratio, hepatic lipids, glycogen, and blood sugar levels were subjected to analysis of variance. Where F value was significant, the group means were tested for significance of differences by using the students-t-test. All tests were considered significant at

p = 0.05 level (Snedecor and Cochran 1967).

Results and discussion

The present study was conducted in two separate experiments (experiment II and III). Experiment II, determined acceptability of the diet containing cooked Mahuda flowers as indicated by food intake as well as the growth promoting quality in terms of growth rate and g food needed per g weight gain. While experiment III, determined the optimal cooking time of Mahuda flowers to maximise its growth promoting quality and attempted to find out the level of Mahuda flowers safe for consumption along with other dietary components.

Twelve weanling rats were divided into two groups to be fed for 28 days either sago-bengalgram (SB diet) or mahuda flower diet (50M10 diet). The SB diet provided 68g of carbohydrate/100g diet of which 32g came from bengalgram and 36g from sago. In 50M10 diet, the amount of carbohydrate provided by sago (36g) in SB diet was replaced by Mahuda flowers. Considering 72% carbohydrate content of Mahuda flowers (chapter two), 50 g flowers were incorporated into 100g diet at the cost of sago, to obtain 36g carbohydrate. The flowers were pressure cooked for 10 minutes and air dried prior to their incorporation into the diet.

It was observed that rats fed 50M10 diet apparently ate as much as those fed SB diet indicating that the former diet was as acceptable as the latter diet (Table 3.4). But it is possible that owing to the relatively higher moisture content of the 50M10 diet the actual food intake of the Mahuda fed rats was lower than the observed value. However no attempts were made to determine moisture content

Table 3.4 Food intake, body weight gain and haemoglobin concentration of rats fed sago-bengal gram and pressure cooked Mahuda flower diets for 28 days.

Variables	DIETS	
	Sago-bengal gram (SB)	Pressure cooked Mahuda flowers (SBMHD)
	MEAN ± SE	
Food intake (g)	102.08 ±17.85	192.08 ±27.71
Body weight gain (g)	71.20 ± 2.29	24.80* ± 1.45
g Food needed per g weight gain	4.76	10.97
Haemoglobin (g/dl)	17.80 ± 0.78	12.91 ± 0.59

* Values significantly different from the control values.

of the SBM10 diet.

The weight gain of the rats fed diet containing cooked Mahuda flowers was significantly lower than those fed SB diet (74.8 Vs 91.2g). Consequently, the efficiency to utilize food for growth in SBM10 diet fed rats was significantly lower than that of those fed SB diet. The Mahuda diet fed rats required about 2.7 times more food to gain one g of weight as compared to the SB diet fed rats. However, no significant difference was noted in their haemoglobin concentration between both the groups (Table 3.4).

Table 3.5 presents value of fresh organ weights expressed as percentage of body weight. Except for liver weight, the mean value for weight of intestine, heart, brain and kidney of Mahuda fed rats were significantly higher and that of spleen was significantly lower than those fed SB diet indicating that feeding of Mahuda flowers at 50% level exerted injurious effects on vital organs.

These findings are consistent with those of Kalkad et al (1956). The authors had fed weanling rats for 28 days cereal-pulse diet containing either 25 or 50g/100g of biscuits prepared by using Mahuda extract as the sweetening agent. It was observed that the kidneys, intestine and heart of Mahuda fed rats were heavier than those of the controls. Between the two Mahuda biscuit fed groups, those fed 50% Mahuda biscuits had heavier kidney, intestine and heart than that of those fed 75% Mahuda biscuits. The authors attributed these effects to some toxic factor present in Mahuda sugar.

The findings of the present experiment 1) suggested that SBM10 diet was nutritionally inferior to SB diet, perhaps due to the presence

Table 3.5 Fresh organ weight as percent body weight of rats fed sago-bengalgram and pressure cooked Mahuda flowers diets for 28 days.

Organs	DIETS: Sago-bengal gram (SB)	Pressure cooked Mahuda flowers (50M10)
	MEAN ± SE	
Liver	4.61 ±0.22	4.46 ±0.20
Intestine	4.22 ±0.19	5.83* ±0.50
Heart	0.3[illegible] ±0.01	0.45* ±0.01*
Spleen	0.[illegible]4 ±0.02	0.2[illegible]* ±0.02
Brain	1.16 ±0.02	1.02* ±0.02
Kidney	0.81 ±0.04	1.08* ±0.66

- *Values significantly different from the control values.

of some growth depressor which was either not heat labile or that the Mahuda flowers were not cooked for long enough time to destroy the so called, antinutritional factor/s. Hence, it was considered worthwhile to explore whether increasing cooking time and/or reducing Mahuda content of the diet would improve nutritional quality of the Mahuda diet. Thus, it was decided to increase the cooking time of flowers from 10 to 20 minutes and at the same time to reduce the level of Mahuda flowers from 50g to 25g/100g diet. Therefore experiment III was set up to investigate the effects of feeding diet containing 25 or 50% Mahuda flowers pressure cooked for either 10 or 20 minutes, on growth and biochemical status of weanling rats.

Fort, weanling rats were randomly divided into five groups to be fed for 28 days either casein diet (C diet) or Sago-bengalgram diet (SB diet) or diet containing Mahuda flowers cooked for 10 minuts at 25% level (25M10 diet) or diet containing Mahuda flowers cooked for 20 minutes at 25% level (25M20 diet) or diet containing Mahuda flowers cooked for 20 minutes at 50% level (50M20 diet). The air dried pressure cooked Mahuda flowers provided 5g or 10g of carbohydrate at 5B or 25% level respectively. In this experiment, moisture content of all the diets was determined to calculated actual food intake. Since the moisture content of all the Mahuda diets was higher than that of the SB and C diets (17.89% of 25M10 diet or 22.53% of 25M20 diet or 29.48% of 50M20 diet Vs 10.1% of SB and C diets),the food intake data of the Mahuda diets fed rats was adjusted for the excess moisture content (i.e. 17.89-10.1% = 7.7B excess moisture) of the diets.

Food intake

The adjusted means for food intake are presented in Table 3.6. The F ratio of 7.1 was significant at P = 0.05 level indicating that various dietary treatments exerted significant effects on their food intake (Table 3.6). The individual comparisons between the two means revealed that the food intake of rats fed Mahuda diets was significantly lower than those fed SB diet. It might be that Mahuda diets fed rats began to eat less consequently started to grow at a slower rate, thenceforth they continued to eat less because of their decreased requirements owing to their small body size. It could also be that Mahuda diet contained some toxic factor which acted as appetite depressor and/or growth depressor. The hypothesis that Mahuda diet contain antinutritional/toxic factor seems to be more probable as the food intake varied with the Mahuda content of the diets. The rats fed 25M20 diet ate significantly more than those fed 50M20 diet indicating that keeping the cooking time constant, reducing the content of Mahuda flowers from 50 to 25g/100g diet increased food intake by 21% . No such effects however, were observed when the content of Mahuda flower in the diet was held constant and cooking time was varied although the 25M20 diet fed rats ate 20.4g more food than the 25M10 diet fed rats during the entire experimental periods (284.2 Vs 263.8g).

Many investingations have reported that feeding of polyphenolic gossypol pigments that are present in certain plants have led to loss of appetite (Bailey 1948, Eagle and Bialel 1950, Couch et al 1955). Likewise, saponins present to the extent of 2 to 3% in alfalfa (lucerne) have been held responsible for a depressant effect on feed consumption in chicks (Draper 1948, Cooney et al 1949.

Table 3.6 Food intake, body weight gain and FER of rats fed control and Mahuda flower diets for 28 days.

Variables	DIETS					F-ratio
	C	SR	25M10	25M20	50M20	
	MEAN ± SE					
Food intake[1] (g)	290.25 ±10.22	231.25 ±13.77	260.88 ±19.[illegible]	284.19 ± 7.37	235.62 ±13.08	7.2*
Bod, weight gain (g)	71.00 ± 7.67	90.12 ± 4.81	52.38 ± 7.41	60.12 ± 1.86	41.75 ± 2.90	202.98*
g Food needed per g weight gain	4.11 ± 0.42	2.68 ± 0.12	5.04 ± 0.50	4.57 ± 0.11	5.29 ± 0.32	

- [1]Values of Mahuda diets adjusted for the moisture content of the diet.

- *Significance at P = 0.05 level.

Lepkovsky et al 1950, Hewang 1957). Recently, Joshi et al (1984) have reported that unprocessed Mahuda seed cake containing 4.5% crude saponin and 6.0% tannins incorporated in to buffalo diet at 75% level, depressed digestibility of nutrients (crude protein and fiber). The authors attributed this depressed digestibility of nutrient to the presence of saponin or tannins in unprocessed Mahuda seed cake.

Among the diets tested in the present experiment it appeared that the 25M20 diet was relatively superior in terms of food intake to 25M10 and 50M20 diets.

The pattern of week-wise food intake of rats fed various diets (Figure 3.1.A) indicated that in relation to SB diet fed rats, those fed casein or Mahuda diets ate significantly less throughout the experimental period. The gap in food intake widened between the SB and 25M20 diet fed group from the second week and continued to remain wide throughout the experimental period. Likewise the rats fed 25M10 or 50M20 diets started to eat less than those fed 25M20 diet from the 1st week of the experimental period and continued to eat less throughout the experiment (Table 3.7). The gap in food intake between 25M20 and 50M20 became wider after 2nd week of the experiment. The pattern of food intake points to the fact that it is the amount of Mahuda flower ingestion and not the cooking time that is responsible for loss of appetite.

Body weight gain

The pattern of weight gain of rats fed various diets was the mirror image of their food intake (Table 3.6). The 'F' ratio of 202.98 was found significant at P = 0.05 level which suggested that the

Figure: 3-1-A Weekwise food intake in rats fed various diets for 28 days.

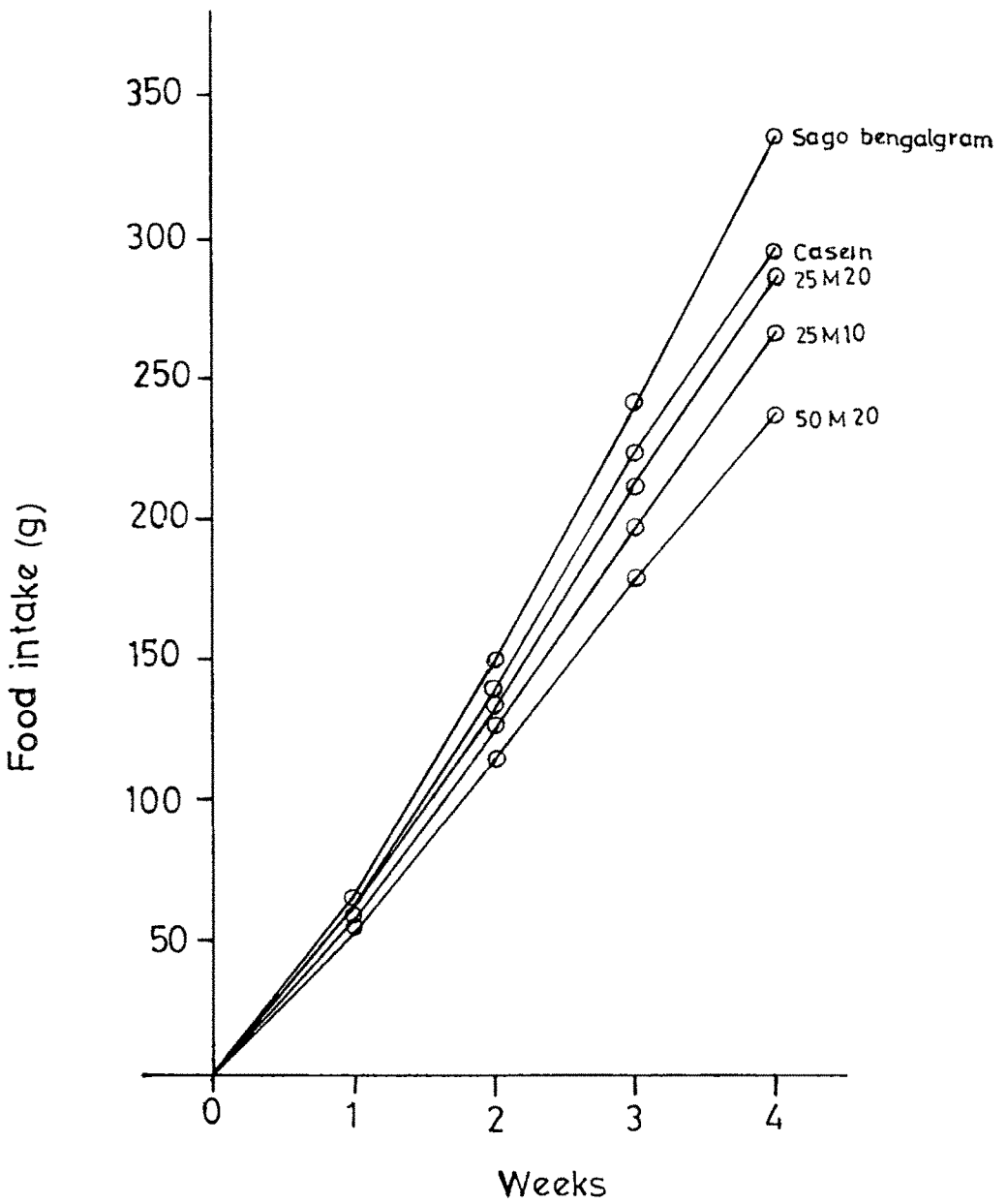


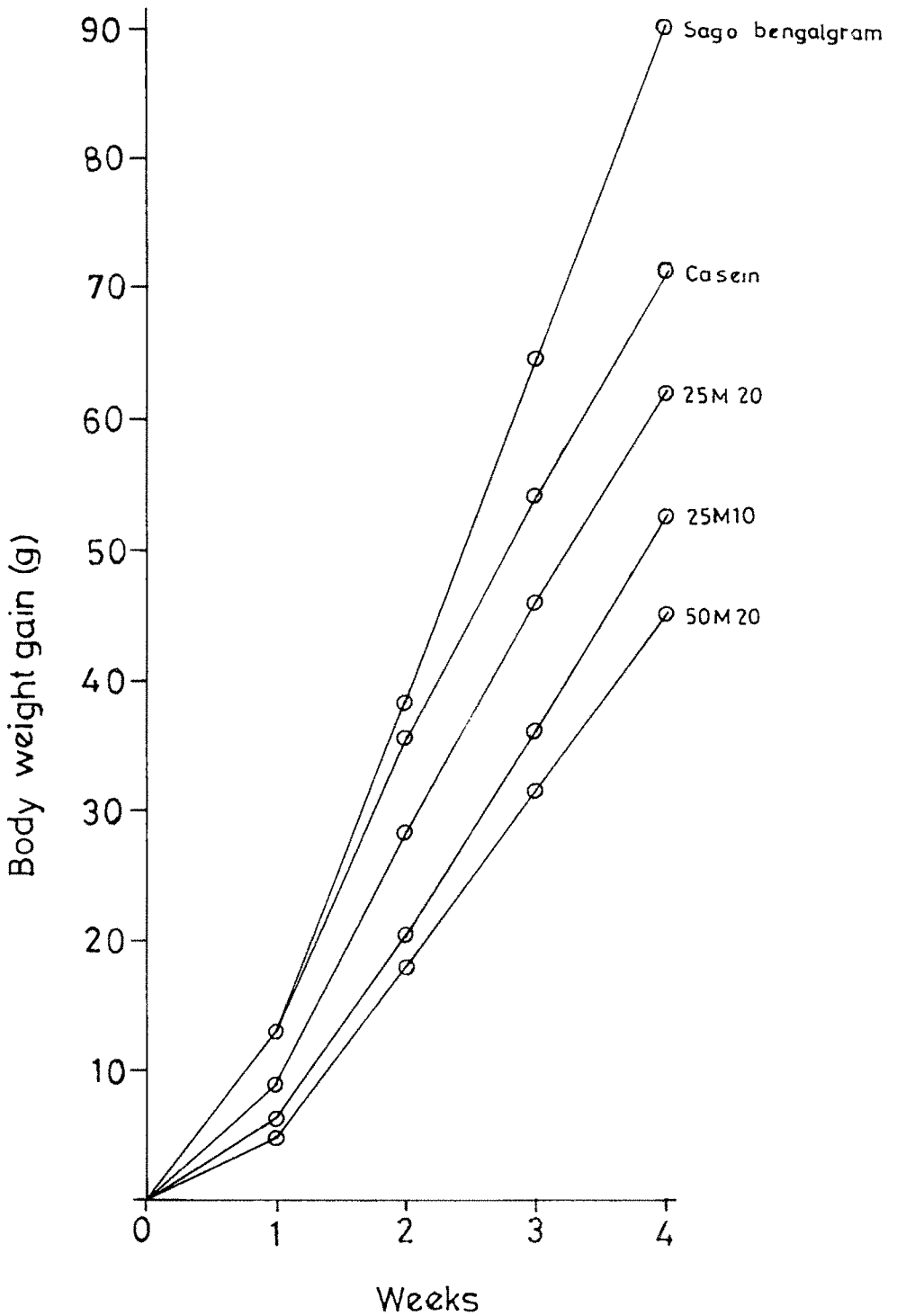
Table 3.7 Weekwise food intake and body weight gain of rats fed control and Mahuda flowers diets for 28 days.

	1st week	2nd week	3rd week	4th week
			MEAN ± SE	
Food intake (g)				
C	60.75 ±4.01	74.5 ±2.51	80.80 ±2.78	74.38 ±6.85
GB	60.75 ±2.41	80.5 ±6.54	94.5 ±4.82	94.25 ±6.58
25M10	55.90 ±4.40	66.16 ±4.31	70.54 ±6.12	66.74 ±7.47
25M20	58.14 ±2.20	71.71 ±1.29	77.40 ±4.37	76.92 ±2.72
50M20	52.90 ±2.28	58.14 ±1.92	63.28 ±4.32	58.65 ±5.24
Body weight gain (g)				
C	12.12 ±1.14	21.68 ±1.52	18.50 ±1.42	17.5 ±7.14
GB	12.75 ±1.77	25.62 ±2.97	24.38 ±1.34	27.12 ±2.08
25M10	06.28 ±2.24	13.38 ±1.25	16.12 ±2.22	16.50 ±2.13
25M20	09.25 ±0.45	18.75 ±1.79	17.75 ±1.90	16.28 ±1.67
50M20	04.75 ±0.41	17.38 ±1.42	13.60 ±1.92	12.25 ±1.47

various dietary treatments had exerted significant effects on weight gain of the rats (Table 3.6). The individual comparisons between the two means indicated that the rats fed SB diet gained significantly more than those fed casein or Mahuda diets. No significant differences in weight gain was observed between the rats fed casein and 25M20 diets (71 Vs 62.1g). Keeping the cooking time constant, increasing the Mahuda flowers into the diet from 25 to 50% led to growth arrest. The weight gain in rats fed 50M20 diet was 70% of those fed 25M20 (43.75 Vs 62.13g) and 49% of those fed SB diet (43.75 Vs 90.13g). At the same level of Mahuda flower intake no significant difference in weight gain was observed when the cooking time was varied although the 25M20 diet fed rats gained 9.7g more weight than the 25M10 diet fed rats (62.1 Vs 52.4g). These data suggested that Mahuda flowers contain some growth depressor whose adverse effects corresponded to the amount that was ingested by the rats. These findings are supported by those of Vyas (1967) who had earlier demonstrated a depressed growth rate in chicks fed diet containing 12 to 16% Mahuda flowers residue. The author reported that the adverse effects on growth of chicks disappeared when the diets contained 8% Mahuda flowers residue instead of 12 to 16%.

The pattern of weekly body weight gain of rats fed various diets (Figure 3.1.2 and Table 3.7) was a supportive pattern to that of weekly food intake. The rats fed casein diet were found to trail behind those fed SB diet and the rats fed 25M20 followed casein diet fed rats. Likewise, the rats fed 25M10 diet trailed behind those fed 25M20 diet and the rats fed 50M20 were behind 25M10 diet fed rats. As was observed in case of food intake, the gap in weight gain became wider between 25M20 and 50M20 as the experiment progressed. It was also observed that between 25M20 and SB diet fed rats, the

Figure: 3-1-B Weekwise body weight gain in rats fed various diets for 28 days.



gap in weight gain was wider than that observed in food intake indicating decreased efficiency of utilization of food for growth.

Table 3.5 also includes the values for gram food needed per gram weight gain. It appeared that the rats fed casein diet needed 0.45g more food per gram weight gain than those fed SB diet (4.1 Vs 3.65g). The difference between the values were however, not statistically significant. The amount of food required per gram weight gain significantly differed among SB and 25M10 or 25M20 or 50M20 fed rats (3.68 Vs 5.04 or 4.57 or 5.38g). All the Mahuda diets fed rats were less efficient in utilization of food for growth than those fed SB diet. However the efficiency of food utilization increased by decreasing the amount of Mahuda flowers in the diet. The rats fed 25M20 diet needed 0.81g or less food per gram of weight gain than that needed by those fed 50M20 diet (4.57 Vs 5.38g). These data supported the hypothesis that Mahuda flowers contain some growth depressor which perhaps reduced the growth promoting quality of the diet.

Earlier, Molly and Gandhi (1977) had demonstrated that Mahuda seed meal containing 7% saponin was toxic when administered parenterally or orally. The authors observed destruction and sloughing of the superficial layers of the intestinal mucous membrane followed by intense inflammation when the saponin was orally administered to mice. This might explain the decrease in food intake and consequently, lower body weight gain observed in Mahuda diets fed rats as Mahuda flowers like Mahuda seed meal do contain saponin although in a smaller amount as discussed subsequently.

The experiments of Heywang (1950) showed that both dehydrated and sun cured alfalfa meals contain factor/s that retard the growth of

young chicks when included in the diet at levels as low as 10 percent. However, Peterson (1950 a,b) demonstrated that the growth inhibiting effect of quillaja saponin was similar to that of dehydrated alfalfa meal and opined that growth inhibiting effect of alfalfa meal might have been due to saponins. Later, Heywang and Bird (1953) fed diets containing 0.2 and 0.4% saponin extracted from alfalfa meal, to chicks untill they were six weeks old and observed that the saponin inhibited their growth, reduced their diet consumption and decreased efficiency of diet utilization. The authors observed greater effect on growth inhibition when diet contained 0.4% versus 0.2% saponin.

Since no report as per author's knowledge, has been published stating the presence of saponin in Mahuda flowers and in the present investigation Mahuna flowers were found to contain about 7% of soluble crude saponin it can only be hypothesised at this point that the ill effects observed on weight gain, food intake and efficiency of food utilization were perhaps due to the presence of water soluble saponin in the flowers. It may as well be due to some other toxic components such as polyphenolic comopunds, like tannins which are known to react with proteins reducing their solubility (Hough et al 1977). Tannins (Gandhi et al 1975), trypsin inhibitor and haemagglutinin (Ramamani and Subramaniam 1981) have also been labeled as growth depressors. Gandhi et al (1975) have reported that sal seed meal contains 8-10% tannins which inhibit growth and cause other toxic effects in rats when added at 30% level into the diet.

The authors observed that the deleterious effects observed in rats were dependent on the amount of sal meal and quantity of protein in the diet. It appears that some of the agricultural and wild edible forest products contain antinutritional toxic factor/s, otherwise they could be utilized for human and animal feeding for obvious economic reasons.

Organ weights

Table 3.8 exhibits mean values for weight of fresh organs expressed as percent body weight, of rats fed control and Mahuda flowers diets for 28 days. Except for liver weight, the F ratios for the weight of intestine (7.24), heart (5.53), spleen (5.18), brain (7.12) and kidney (9.08) were found significant at P = 0.05 level indicating that organ weights were significantly altered in response of various dietary treatments (Table 3.0). The comparisions between the two means revealed that the mean weight for intestines of Mahuda fed diets was significantly higher than those fed casein diet but no such significant differences were observed between intestinal weight of rats fed SB or any of Mahuda diets. Except for SB and 25M10 diets fed rats, no significant differences were observed in mean weight of heart between C and SB or 25M30 or 50M20 diets fed rats. Likewise, no significant differences were observed in mean values for liver, intestine, heart, spleen and kidney weights between the rats fed SB and 25M20 diets indicating no injurious effects of feeding 25M20 diet on the vital organs.

It may be recalled here that the adverse effects of feeding 50M20 diet on food intake and body weight gain were more pronounced than that of feeding 25M20 diet. The data on organ weights along with those of food intake and body weight gain suggested that it would

Table 3.8 Fresh organ weight as percent body weight of rats fed control and Mahuda flower diets for 28 days.

Organs (g)		DIETS					F-ratio
		C	SB	25M10	25M20	50M20	
		MEAN ± SE					
Liver	A	4.60 ±0.40	5.90 ±0.39	4.41 ±0.48	4.36 ±0.16	5.04 ±0.40	
	B	4.26 ±0.26	4.37 ±0.16	4.84 ±0.15	4.49 ±0.14	4.56 ±0.11	1.15
Intestine	A	5.92 ±0.20	5.70 ±0.22	4.18 ±0.70	4.81 ±0.23	5.96 ±0.26	
	B	5.66 ±0.08	4.56 ±0.10	4.72 ±0.24	4.90 ±0.23	5.01 ±0.28	7.21*
Heart	A	0.42 ±0.01	0.46 ±0.02	0.36 ±0.02	0.38 ±0.01	0.40 ±0.02	
	B	0.39 ±0.002	0.47 ±0.02	0.40 ±0.02	0.38 ±0.008	0.39 ±0.01	0.51*
Spleen	A	0.47 ±0.07	0.45 ±0.07	0.34 ±0.05	0.35 ±0.02	0.42 ±0.02	
	B	0.41 ±0.06	0.36 ±0.06	0.32 ±0.01	0.34 ±0.01	0.42 ±0.02	5.18*
Brain	A	1.59 ±0.04	1.41 ±0.04	1.49 ±0.04	1.37 ±0.02	1.51 ±0.04	
	B	1.32 ±0.04	1.13 ±0.04	1.62 ±0.14	1.40 ±0.04	1.65 ±0.08	7.72*
Kidney	A	0.82 ±0.04	0.88 ±0.04	0.82 ±0.06	0.79 ±0.02	0.74 ±0.04	
	B	0.70 ±0.04	0.71 ±0.02	0.72 ±0.04	0.80 ±0.02	0.94 ±0.01	7.02*

A = Weight of fresh organ

B = As percent body weight

*Significance at P = 0.05 level

not be unsafe to incorporate into diet. Mahuda flowers cooked for 20 minutes at 25% level.

The higher weight of various organs in relation to body weight in response to feeding diet containing Sal meal providing 3% tannins has been reported earlier (Gandhi et al 1975). Such effects on organ weight have been considered as a physiological response of the rats to decreased food intake (Scharer 1977).

In the present experiment since 25M10 and 50M20 diets fed rats ate respectively 20% and 29% less food than the SB diet fed controls, it is possible that the higher organ weights expressed as percent body weight, were actually due to decrease in food intake and consequently body weight.

Moisture content of the organs

To examine further whether the relatively higher weight of organs of Mahuda diets fed rats were intact due to increased moisture content, the moisture content of organs were determined. The percent moisture content of the fresh organs of rats fed C or SB or 25M10 or 25M20 or 50M20 diet is displayed in Table 3.7. Except for the 'F' ratio calculated for moisture content of intestine, the ratio for liver, heart, spleen, brain, and kidneys indicated that various dietary treatments had not altered moisture content of these organs (Table 3.7). The comparisons between the group means revealed that the moisture content of intestines of rats fed 25M10 diet was significantly lower than that of rats fed SB diet. However, there were no significant differences observed in moisture content of the intestine among the rats fed C or SB or 25M20 or 50M20 diets. These results indicated that feeding of diet containing Mahuda flowers

Table 3.9 Percent moisture content of the organs of rats fed control and Mahuda flower diets for 28 days.

Moisture content of organs (g/100g)	DIETS					
	C	SE	25M10	25M20	50M20	F-ratio
	MEAN ± SE					
Liver	72.40 ±1.75	77.70 ±2.24	67.80 ±3.18	76.60 ±1.42	72.10 ±3.20	1.87
Intestine	80.00 ±0.76	81.60 ±1.97	78.50 ±1.44	80.10 ±1.60	81.70 ±1.56	1.07*
Heart	78.60 ±0.35	79.10 ±0.32	87.30 ±2.41	77.70 ±3.08	74.50 ±3.07	1.60
Spleen	78.10 ±1.04	79.70 ±0.80	80.20 ±1.92	79.70 ±1.66	81.30 ±1.58	0.61
Brain	78.80 ±1.07	80.60 ±0.75	80.30 ±1.81	80.00 ±2.46	75.60 ±2.01	1.08
Kidney	82.50 ±2.68	78.10 ±1.24	78.10 ±2.71	79.10 ±1.55	75.10 ±2.49	2.36

*Significance at P = 0.05 level.

produced no ill effect on the moisture content of the organs. These findings support the speculation made earlier that in the present experiment, higher organ weights in proportion to body weight observed in Mahuda fed rats, in contrast to those of the controls, was indeed due to decreased body weight.

Hepatic lipids

Since elevations in hepatic fat content as a consequence of the presence of some toxic substances in animal feeds have been reported (Sharma et al 1981, Bullman et al 1982), in the present experiment the hepatic lipid content was determined to ascertain whether feeding of Mahuda flowers would bring about similar changes. Table 3.10 presents the mean values for hepatic lipids of rats fed control or Mahuda diets. The 'F' ratio of 10.86 significant at P = 0.05 level suggested that the various dietary treatments had exerted significant effect on hepatic lipid contents. The group mean comparisons indicated that hepatic lipid content of 25M10 and 50M20 were elevated as compared to C or SB or 25M20 diets. But the value of hepatic lipids of rats fed C or SB diet did not significantly vary from each other (3.02 Vs 3.06g/100g wet tissue). Likewise, no significant difference in hepatic lipid content was observed between rats fed 25M20 diet and SB or C diets (3.94 Vs 3.06 or 3.02 g/100g wet tissue). The livers of rats fed 50M20 diet contained significantly more fat than those fed 25M20 diet indicating that increasing the content of Mahuda flowers from 25 to 50g /100g diet increased hepatic fat content. The feeding of diet containing Mahuda flowers cooled for 10 minutes (25M10 diet) led to increased accumulation of fat into hepatic tissue as compared to feeding of 25M20 diet.

Table 3.10 Hepatic lipids and glycogen, blood sugar and heamoglobin levels of rats fed control and Mahuda flower diets for 29 days.

Variables	C	5B	25M10	25M20	50M20	F-ratio
			DIETS			
			MEAN ± SE			
Hepatic lipids (g/100g wet tissue)	3.02 ±0.07	3.02 ±0.04	3.48 ±0.09	3.94 ±0.04	3.41 ±0.10	30.85*
Hepatic glycogen (g/100g wet tissue)	5.39 ±0.51	5.08 ±0.49	4.54 ±0.26	5.00 ±0.17	4.42 ±0.22	2.72*
Blood sugar (mg/dl)	80.87 ±5.00	87.20 ±4.64	77.71 ±7.45	80.24 ±5.20	71.79 ±7.69	1.08
Haemoglobin (g/dl)	12.00 ±0.11	12.47 ±0.22	11.52 ±0.42	12.52 ±0.46	11.74 ±0.28	2.41

*Significance at P = 0.05 level.

Sharma et al (1981) had observed that lipid content of liver tissue increased in response to oral administration of Lantana camara leaf in guinea pigs. Later Sulaiman et al (1982) reported that total lipids were higher in the liver, kidneys and heart of Cassia occidentalis poisoned goats. The authors attributed these findings to some toxic substances present in Lantana camara and Cassia occidentalis.

Recently Pallad et al (1986) have demonstrated that inclusion of Mahuda biscuits made out of Mahuda sugar, into cereal-pulse diet at 25 or 50% level, led to a fatty liver in rats within 28 days. The hepatic lipids content of rats fed diet containing 25 and 50% Mahuda biscuits was 2.5 and 5 times respectively, higher than that of the control diet fed rats. The authors reported that the toxic factor present in Mahuda sugar perhaps had caused elevations in hepatic lipids contents.

Hepatic glycogen content

The mean values for hepatic glycogen levels are presented in Table 3.10. The F ratio of 2.72 was found significant at P = 0.05 level which indicated that the hepatic glycogen content differed in response to various dietary treatments (Table 3.10). By comparing the group means, the hepatic glycogen content was found to be significantly lower in the rats fed 25M10 or 50M50 diet than that of SB diet fed rats (4.04 or 4.42 Vs 5.89g/100g wet tissue). The hepatic glycogen content of rats fed C or SP diet did not significantly vary from each other (5.39 Vs 5.88g/100g wet tissue). Similarly, no significant difference in hepatic glycogen content was observed between the rats fed 75M50 and SB or C diet (5.00 Vs 5.88 or 5.39g/100g wet tissue). Among the Mahuda diets fed rats, the

hepatic glycogen content of those fed 25M20 diet was higher than those fed 25M10 and 50M20 diets, however, these values were not found significantly different from each other.

Earlier Hornbrook (1970) had related the lower glycogen content in the hepatic tissue to alterations in the control of glycogen synthatase activity to store small amount of glycogen while Garfield and Cardell (1979) attributed the decreases observed in hepatic glycogen content of diabetic rats to increases in hepatic glucose 6 phosphatase activity which was related to the alterations in hepatocytes.

The association of saponins with the lipid and protein metabolisms have also been reported. Sirtori et al (1977) have related the beneficial effects of soybean preparation in lowering the serum cholesterol to the ability of saponins present in soybean which formed nonabsorbable complexes with cholesterol. Later in 1979, Sirtori et al observed that saponins present in lupines seeds of legumes interfered with the full utilization of seed protein.

In the present study, it is plausible that saponin present in Mahuda flowers might have produced alterations in carbohydrate metabolism. The decreased hepatic glycogen contents observed in rats fed 25M10 or 50M20 diet perhaps were due to an impairment in carbohydrate absorption. Earlier Mully and Gandhi (1977) had demonstrated that oral ingestion of saponin, damaged intestinal tissue causing impairment in carbohydrate absorption. However, consumption of 25M20 diet by rats did not exert alteration in glycogen storage by the hepatic tissues.

Blood sugar levels

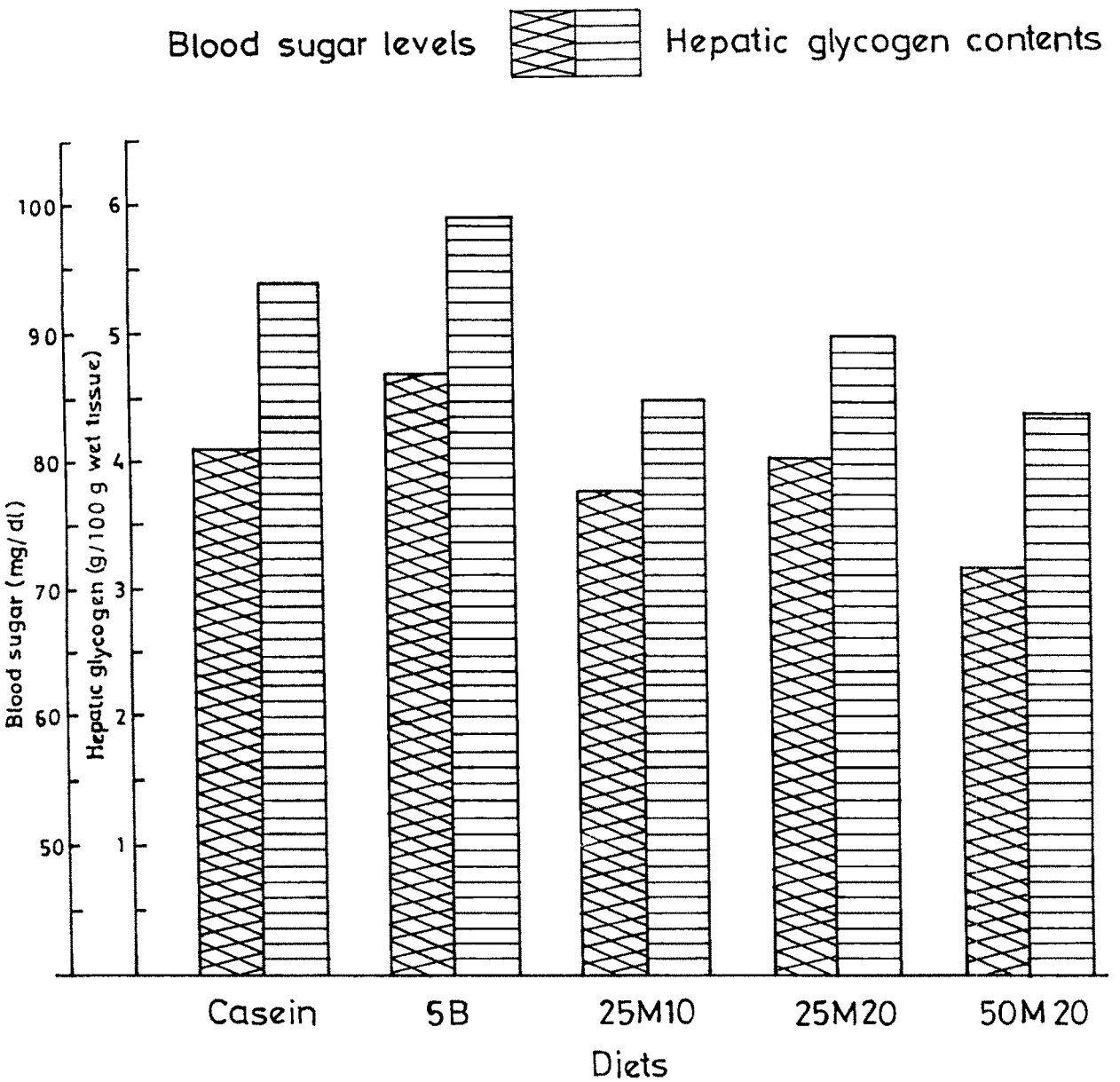
The average blood sugar levels of rats fed control or Mahuda diets are exhibited in Table 5.10. The calculated F ratio was not found significant at P = 0.05 level. The mean values for blood sugar ranged between 71.77 to 87.20 mg/100 ml blood. The hepatic glycogen and blood sugar levels followed the complementary pattern (Figure 5.2). Both the values were lowest in 50M20 diet fed rat. and highest in those fed 50 diet. The blood sugar level as well as hepatic glycogen content of 25M20 or 5B diets fed rats were comparable.

The characteristic sugar of blood and of tissue fluids is glucose. The liver has chief responsibility to regulate blood sugar concentration since it possesses a supply of glycogen directly convertable to glucose (West et al 1966). Sorkin et al (1976) have demonstrated that rise in blood glucose levels decreased the output of glucose by the liver whereas fall in glucose resulted in an increased output of sugar by the liver. However at any given time the blood sugar levels represents the balance between the processes adding the glucose to the blood and glucose uptake by the hepatic tissue for storage (West et al 1966). In the present experiment these findings suggested that the blood sugar levels were maintained at the cost of glycogen storage (Figure 5.2)

Haemoglobin concentration

Table 5.10 also displays the mean haemoglobin levels of rats fed various diets. The F ratio was not significant at P = 0.05 which suggested that various dietary treatments produced no ill effects on haematological status of the rats (Table 5.10). The haemoglobin levels of C and 5B diets fed rats was found comparable (12.9 vs

Figure:3-2 Blood sugar levels and hepatic glycogen contents of rats fed various diets for 28 days.



12.72g/dl). Likewise, no difference was observed in their haemoglobin levels between the rats fed SB or 25M20 diets (12.7% Vs 12.51g/dl).

The haemoglobin values did not vary among the Mahuda diets fed rats. These results are in line with those of Jaiprad et al (1986). They had observed no marked unfavourable effects on haemoglobin status when weaning rats were fed Mahuda biscuits into the diet of wheat : bangalgram mixture (1 : 1, at 25 or 50% level).

Generally, toxic components of food are associated with hemolysis of red cells and anaemia. Bailey (1948), Eagle and Bialel (1950) and Couch (1955) reported that gossypol pigment (constituent of cotton flower) toxicity led to decreases in haemoglobin level and number of red blood cells in rats. Later George (1965) reported that saponins possess a powerful hemolytic property but they are needed in larger doses to produce hemolysis.

The data of the present experiment (1) indicated that Mahuda flowers cooled for 10 or 20 minutes and incorporated into the diet at 25 or 50% level exerted no adverse effects on the haematological status of rats. It may be that the amount of toxic substance ingested through Mahuda flower diets by rats was not sufficient to produce haemolytic effects.

Serum proteins

Table 7.11 highlights the serum protein status of various diets fed rats for 28 days. The serum of rats fed SB diet contained significantly less proteins than C diet fed rats (7.06 Vs 7.74 g/dl). The decrease in total protein seemed to be due to decrease in globulin levels because globulins of the SB diet fed rats were

Table 3.11 Serum protein and A/G ratio of rats fed control and Mahuda flower diets for 28 days.

Variables	DIETS				
	C	SE	25M10	25M20	50M20
	MEAN ± SE				
Serum total proteins (g/dl)	7.74 ±0.01	7.06 ±0.18	6.95 ±0.17	6.94 ±0.22	7.00 ±0.25
Albumins (g/dl)	3.69 ±0.14	3.73 ±0.14	3.21 ±0.21	3.07 ±0.12	3.22 ±0.15
Globulins (g/dl)	4.07 ±0.26	3.34 ±0.10	3.74 ±0.14	3.87 ±0.24	3.77 ±0.16
A/G ratio	0.94 ±0.09	1.16 ±0.12	0.88 ±0.08	0.82 ±0.06	0.85 ±0.05

significantly lower than those of casein fed rats (5.14 Vs 4.00 g/dl). The albumins in serum of SB diet fed rats as compared to C diet fed rats showed an upward trend (3.73 Vs 3.69g/dl). Consequently the A/G ratio of the former group of rats was not significantly different than that of the latter group of rats (1.15 Vs 0.94g/dl). However, lower total proteins observed in SB diet fed rats as compared to those fed C diet could be a consequence of feeding a diet containing protein of low biological value.

The serum total proteins observed in rats fed diets containing Mahuda flowers did not significantly differ as compared to serum protein of SB diet fed rats (Table 3.11). Also, serum total proteins did not vary among any of the groups fed Mahuda diets. The serum albumin levels were found lower in Mahuda diets fed rats than those fed SB diet. But no such differences in serum globulin levels were observed between SB or Mahuda diets fed rats. However, the A/G ratio of the former group did not differ significantly from that of the latter groups indicating no ill effects on protein status in response to feeding Mahuda flowers. Also, all the Mahuda diets fed rats exhibited comparable pattern of serum total protein, albumin and globulin levels which suggested that level of feeding or time of pressure cooking Mahuda flowers had no adverse effects on serum proteins.

The decreases in serum proteins observed in response to feeding Mahuda diets as compared to C diet fed rats were due to decrease in albumin levels but the A/G ratio did not differ significantly among C, 25M10, 25M20 and 50M20 diets fed rats. Earlier, adverse effects of Mahuda biscuits containing diets for 28 days on the serum proteins of rats were observed when compared with that of the casein

diet fed rats (Prasad et al 1986).

Since no significant effects in total serum proteins were observed between SD and Mahuda diets fed rats, it appeared from the data of the present experiment that inclusion of Mahuda flowers into the diet exerted no ill effects on the total serum proteins of the rats. However, decreases found in albumin levels in rats fed diets containing Mahuda flowers than that of C or SD diet fed rats were perhaps due to unavailability of the dietary protein for synthesis of albumins and/or the target organ mainly, liver was not functioning effectively.

The salient features of feeding weanling rats for 28 days, Mahuda flowers cooled for 10 or 20 minutes, at 25 or 50% level were :

(i) decreases in food intake and body weight gain of 50M20 versus 25M10 or 25M20 diet fed rats ;

(ii) higher organ weights expressed as percent body weights of 25M10 or 50M20 versus 25M20 diet fed rats ;

(iii) elevation in hepatic lipids of rats fed 25M10 or 50M20 diet versus those fed 25M20 and SD diets ;

(iv) decrease in hepatic glycogen, and moderate decrease in blood sugar levels in 25M10 or 50M20 versus 25M20 diet fed rats ;

(v) no adverse effect on the haematological status of rats fed Mahuda diets ;

(vi) moderate alteration in serum albumins but no significant effects on total serum proteins of rats fed diets containing Mahuda flowers as compared to those fed SD diet.

The results of experiments II and III suggested that cooked Mahuda flowers incorporated at 25 or 50% into sago-bengalgram diet as a carbohydrate source, adversely affected food intake, growth rate, organ weights and biochemical status of the weanling rats. However, the degree of adverse effect was related to the levels of Mahuda flowers in the diets. Also, favourable effects on growth and biochemical status were observed when cooking time was increased from 10 to 20 minutes, perhaps due to inactivation and/or destruction of part of the toxic substance present in Mahuda flowers. The data on food intake, growth rate, organ weights and biochemical status indicated that rats fed 25M20 diet fared better than those fed 25M10 or 50M20 diet. It appeared from these data that it would not be unsafe to consume if necessary, Mahuda flowers cooked for 20 minutes at the level of not more than 25g per 100g diets as a carbohydrate source. Since the 25M20 diet was found nutritionally superior to other alternative Mahuda diets, in all further experiments only 25M20 diet was used.