M.Sc. NUTRITION & DIETETICS
LAB MANUAL
3rd Semester

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PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for PG Nutrition & Dietetics Third Semester. Hope this edition will help you during practical. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers , and non-teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

Laboratory Practice Safety Rules

1. Use safety glass when dealing with fire and chemical.
2. Should use front cover clothes during biochemistry practical.
3. Always use hand wash after dissection and any type of chemical use.
4. Carefully handle needles , forceps, microscope and any other dissecting instrument.

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NUD – 396 **Nutritional Survey and Advanced Food & Nutrient Analysis**

Unit – 35 Nutritional Survey

1. Determination of socio-economic status:

Socio economic status (SES) refers to an individual's position within a hierarchical social structure, which is one of the important determinants of health status. Composite scales are generally used to measure the SES, which has a combination of social and economic variables. There is no direct measure of the social status of an individual; therefore, an attempt had been made by many eminent researchers and social scientists in the past to formulate a composite index to measure it. Several methods or scales have been proposed for classifying different populations by socioeconomic status. Most commonly used and acceptable method is Kuppuswamy scale. 1976 and modified on 2017.

Table 1: Modified Kuppuswamy Scale, 2017

Detailed information	Score
Education of head of the family	
Profession or honours	7
Graduate or postgraduate	6
Intermediate or post high school diploma	5
High school certificate	4
Middle school certificate	3
Primary school certificate	2
Literate	1
Occupation of head of family	
Profession	10
Semi Profession	6
Clerical, Shop-owner	5
Skilled worker	4
Semi-skilled worker	3
Unskilled worker	2
Unemployed	1
Monthly income of family (January 2017)	
>41430	12
20715-41429	10
15536-20714	6
10357-15535	4
6214-10356	3
2092-6213	2
<2091	1
Socio economic class	**Total Score**
I Upper	26-29
II Upper middle	16-25
III Lower middle	11-15
IV Upper lower	5-10
V Lower	<5

Table 2: Explanation and example of classification of education of head of the family

Education category	New name for the category	Salient point	Examples
Profession or honours	Post graduate or professional degree	Any Post graduation, Any high grade professional education (which may directly be after class XII)	M.A, M.Sc, Ph. D, M Ed, MBBS, BE
Graduate or Post graduate	Graduate degree	Any graduation degree (other than high grade professional education)	BA, B Sc, B Ed.
Intermediate or High post school diploma	High secondary school certificate	Class XII pass	Class XII pass, Class XII pass with vocational diploma
School certificate	Madhyamik school certificate	Class X pass	Class X pass, XI pass (do not complete XII)
Middle school certificate	Middle school certificate	Class VIII pass	Class VIII pass, class IX pass.
Primary school or illiterate	Literate, less than middle school certificate	Literate would be as per the definition followed by census of India that is person aged ≥7 years who can read and write with understanding in any language.	Any level of literacy below class VIII (that is did not get class VIII pass certificate
Illiterate	Illiterate	Person aged ≥7 years who can not read and write with understanding in any language	Person who can only understand a language

Table 3: Explanation and examples of classification of occupation of head of the family

Occupation category	Salient points	Examples
Professionals	Decision making, formulating policies and execution of policies job that need creative work. Jobs involved high organizational ability and control of large number of humans or jobs that involve dealing with large amounts of money. Most requires very high general or professional education but this is not compulsory.	Doctors, advocates, engineers, directors, managers, architect, senior administrator, readers and professors, newspaper editors, college principles, bank managers
Semi professionals	Jobs requiring post high school education but routine wise jobs.	High school teachers, college lecturer, junior administrator, junior medical practitioners
Clerical, Shop owner	Jobs that require some training in arithmetic and probably also reading and writing. But jobs that are basically repetitive in nature. Arithmetic skill in context of higher arithmetic skills requiring for job.	Clerk, accountant, typist, elementary school teacher, farm owner, shopkeeper, salesman, insurance agent, news journalist
Skilled worker	Long training in complicated work.	Driver, telephone operator, carpenter, mechanical worker
Semi-skilled worker	Jobs that require some training.	Factory labour, car cleaner, petty shopkeeper
Unskilled worker	No education or training required.	Domestic servant, peon, watchman
Unemployed	Irrespective of education level.	Self explanatory

Observation and analysis of data:

Place of study: Saratpally, Midnapore, Paschim medinipur, West Bengal, India

Date: 12.09.2019

Name of Head of the family: Subrata Jana

Total family member: Four

Number of earning: Two

Total Income of the family: Rs. 110000.00/ Month

Education of head of the family: Engineer

Table 4: Socio economic status according to Kuppuswami scale, 2017

Detailed information	**Score**
Education of head of the family	
Profession or honours √	7
Graduate or postgraduate	
Intermediate or post high school diploma	
High school certificate	
Middle school certificate	
Primary school certificate	
Literate	
Occupation of head of family	
Profession	10
Semi Profession	
Clerical, Shop-owner	
Skilled worker	
Semi-skilled worker	
Unskilled worker	
Unemployed	
Monthly income of family (January 2017)	
>41430	12
20715-41429	
15536-20714	
10357-15535	
6214-10356	
2092-6213	
<2091	
Socio economic class	**Total Score**
I Upper	29
II Upper middle	
III Lower middle	
IV Upper lower	
V Lower	

Interpretation:

The data is collected from a family belongs to very high Socio economic status as the total score is 29 that is consider to be an upper class.

2 & 3. Determination of energy requirement of sedentary persons moderate and heavy workers.

Energy requirement of sedentary moderate and heavy workers can be determined by different methods like EER method, according to RDA and using PAL value.

Formula of EER

BOY AND GIRL - INFANTS AND TODDLERS
0-3 months EER (kcal/d) = (89 x Wt [kg] - 100) + 175 4-6 months EER(kcal/d) = (89 x Wt [kg] - 100) + 56 7-12 months EER (kcal/d) = (89 x Wt [kg] - 100) + 22 13-36 months EER (kcal/d) = (89 x Wt [kg] - 100) + 20
BOYS 3-8 YEARS OLD
EER (kcal/d) = 88.5 - 61.9 x Age [y] + PA x (26.7 x Wt [kg] + 903 x Ht [m]) +20
GIRLS 3-8 YEARS OLD
EER = 135.3 - (30.8 x age [y]) + PAx { (10.0 x weight [kg]) + (934 x height [m]) } + 20
BOYS 9-18 YEARS OLD
EER (kcal/d) = 88.5 - 61.9 x Age [y] + PA x (26.7 x Wt [kg] + 903 x Ht [m]) +25
GIRLS 9-18 YEARS OLD
EER = 135.3 - (30.8 x age [y]) + PA x { (10.0 x weight [kg]) + (934 x height [m]) } + 25
ADULTS 19 YEARS AND OLDER - MEN
EER = 662 - (9.53 x age [y]) + PA x { (15.91 x weight [kg]) + (539.6 x height [m]) }
ADULTS 19 YEARS AND OLDER - WOMEN
EER = 354 - (6.91 x age [y]) + PA x { (9.36 x weight [kg]) + (726 x height [m]) }
PREGNANCY (14-50 YEARS OLD)
1st trimester EER = Non-pregnant EER + 0 2nd trimester EER = Non-pregnant EER + 340 3rd trimester EER = Non-pregnant EER + 452
LACTATION
0-6 months postpartum EER = Non-pregnant EER + 330 7-12 months postpartum EER = Non-pregnant EER + 400

PA= Physical activity

Category	PA value
PA for boys 3-18 years	
Sedentary	1.0
Low active	1.13
Active	1.26
Very active	1.42
PA for girls 3-18 years	
Sedentary	10
Low active	1.4
Active	1.6
Very active	1.9
PA for girls adult men	
Sedentary	1.0
Low active	1.11
Active	1.25
Very active	1.48
PA for girls adult woman	
Sedentary	1.0
Low active	1.12
Active	1.27
Very active	1.45

4. Determination of nutritional consumption by questionnaire method:

A survey will be done in a community for analysis of nutritional status of a family by oral questionnaire method of diet survey.

Following format are used for the diet survey.

SCHEDULE FOR DIET SURVEY-1

General Information of the family

Name of Head of Family:	Address:
Age:	Sex:
No. of Family Members:	
Age group of Family Members:	
Total income of the family:	
Total earning member of the family:	
Socio-economic status of the family:	
Dietary pattern of the family:	Veg ( ) Non- Veg ( )
Any type of food allergy is present:	
Mother's nutritional status:	
Timing of Initiation of Breastfeeding:	
Seasonal availability of the foodstuffs:	
Timing of meal intake:	Regular ( ) Irregular ( )
Any type of kitchen garden is present:	
Supplementary Food Received from an *Anganwadi* Centre (AWC) by Children Under Six Years	
Mid –Day Meal Received from School by Children	

Information about sanitation & hygiene

Sources of drinking water:

Cooking facilities:

Kitchen sanitary condition:

Storage facilities:

By whom food is prepared: Family member ( )

Worker ( )

Food bring from canteen ( )

Waste disposal:

Toilet facilities:

Cooking utensils are washed by: Only water ( )

water with soap or detergent ( ) Hot water ( )

SCHEDULE FOR DIET SURVEY-2

(NUTRITIONAL REQUIREMENT OF FAMILY BY ICMR)

Name of the investigator:

Place:

Head of the family: House hold Number:

Total ACU:

Sl No.	Name	Sex	Age (Yrs)	Body Weight (Kg)	Energy (Kcal)	Carbohydrate (gm)	Protein (gm)	Fat (gm)	Vit A (mcg)	Vit C (mcg)	Iron (mg)	Ca (mg)

SCHEDULE FOR DIET SURVEY-3

(Food consumption schedule by family by icmr method)

Name of the Town / Village: District:

Head of the Family: House hold number:

Name of the respondent: Type of family:

Foodstuffs	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	Total	Avg

SCHEDULE FOR DIET SURVEY-4

(NUTRIENT ANALYSIS SCHEDULE OF FAMILY BY ICMR METHOD)

Name of the investigator: Place:

Head of the family:

Sl No	Foodstuffs	Amount (gm)	Energy (Kcal)	Carbohydrate (gm)	Protein (gm)	Fat (gm)	Vit A (mcg)	Vit C (mcg)	Iron (mg)	Ca (mg)

SCHEDULE FOR DIET SURVEY- 5

Nutrients	Required amount	Amount consumption	Deficiency/ excess
Total energy (kcal)			
Total carbohydrate (g)			
Total protein (g)			
Total fat (g)			
Total iron (mg)			
Total calcium (mg)			

5. Study on nutritional status of the beneficiaries under National nutritional Programme:

A survey will be done in an ICDS center or a primary school for analysis of nutritional status of school going or preschool children in a community

NUD – 396 **Nutritional Survey and Advanced Food & Nutrient Analysis**

Unit – 36 Advanced Food and Nutrient Analysis

1. Estimation of calcium in milk by using EDTA by titrimetric method

Principle:

In acid base titration the end point is marked by sudden change in pH and it can be detected by an indication. Here the determination of calcium in milk is based on a complexometric titration of calcium with an aqueous solution of EDTA bind to free calcium ion in the solution and the solution become calcium free. In presence of calcium in a particular pH, the calcon shows pink or purple colour. In absence of calcium calcon shows blue colour.

Requirements:

i. Alcoholic calcon solution

ii. NaOH – 2 Molar (2.05 g of NaOH is added to 250ml of distilled water

iii. EDTA – 0.01 Molar (Dissolve 3.723 g of EDTA in 1 litre of distilled water in a volumetric flask.

iv. Burette with stand

v. Conical flask

vi. 5 ml pipette

vii. 25ml measuring cylinder

viii. Funnel

ix. Milk sample

Procedure:

i. A 50ml conical flask was weighed and 1 ml supplied milk was taken. Then the conical is again weighed to get the weight of the supplied milk.

ii. 25ml of distilled water and 4 ml of 2M NaOH was added to it.

iii. Then 2-3 drops of calcon solution was added and mixed well. The solution will become purple colour.

iv. Titrate the solution against 0.01M EDTA solution in a burette.

v. When purple colour is turned into blue colour that time will be the end point of the titration and the volume of EDTA used was noted in the burette.

Observation:

No of observation	Burette reading (ml)		Difference	Average
	Initial	Final		
1	15.5	19.5	4	4.15
2		23.19.58	4.3	

Result:

Volume of EDTA required for titration is- 4.15 ml= 0.0041 litre

Weight of milk- 0.98g

So,

$$\text{Calcium (mg\%)} = \frac{\text{Molarity of EDTA X volume of EDTA(lit) X Molecular weight of calcium X 100}}{\text{Weight of milk (g)}}$$

$$= \frac{0.01 \text{ X } 0.004 \text{ X } 40.078}{0.98} \text{ X } 100$$

$$= 0.0017 \text{ X } 100$$

$$= 0.172 \text{ g}$$

$$= 172\text{mg}/100\text{g}$$

Interpretation:

Calcium level of Amul milk is 102mg/100ml. Supplied sample contain 183.5mg% calcium. So, supplied sample contain 172mg% calcium. So, this value is higher than the normal value.

2. Estimation of iron in food

Iron is an important element in our diet. It is used in the manufacture of the oxygen carrying proteins, hemoglobin and myoglobin. Although considered a trace mineral (one that is needed in relatively small quantities, diets lacking iron can contribute to the deficiency condition known as anemia.

In this analysis the iron present in a food sample is extracted to form a solution containing Fe 3+ (Ferric ions. To make the presence of these ions in solution visible, thiocyanate ions are added. These react with the Fe3+ ions to form a blood red colour complex.

$Fe^{3+} + SCN^{-} \longrightarrow (FeSCN)^{2+}$

By comparing the intensity of the colour of this solution with the colours of a series of standard solutions, with known Fe3+ concentrations, the concentration of iron in food sample may be determined. This technique is called colorimetry.

Equipment:

- Filter paper
- Funnel
- Beaker
- Test tubes
- Ring stand
- Burner
- Spectrophotometer/ Colorimeter

Materials:

- Distilled water
- Fecl3 solution- 0.001 M
- KSCN
- HCL

Procedure:

Preparation of standard solution:

- Three stock solutions were made ready before the experiment and were stored in 500ml standard flask.
- Firstly, 0.001 M $Fecl_3$ stock solution was prepared by adding approximately 0.162g Fecl3 in 500ml of distilled water followed by the addition of 5ml concentrated HCL. The contents were diluted to 1 liter and were mixed well before being transferred to a standard flask.
- Secondly, 1.5 M KSCN solution was prepared by adding approximately 36.375g of KSCN in 500ml ditilled water. The contents were mixed well before being transferred to standard flask. This solution was made on the basis of colorimetry involved in the analysis and used till the end of the experiment.

Preparation of sample:

- 1-15 g of the edible portion of food was weighed. (Here 1g of food was taken)
- They were finely chopped for the purpose of ashing.
- Then heated in a stainless steel over a hot induction plate at 200-240^{0}c.
- The heating time varied depending on the amount of sample and the rate at which the sample is burned to ash.
- The sample were heated till a greyish ash were observed and then they were powdered using a grinder.
- After that the sample were cooled, they were transferred to a small beaker of 100ml capacity and the iron in the ash was dissolved in 10-30 ml of 2 M HCL. The ash solution was stirred using a glass stirring rod for about 15 minutes and then filtered. Then 5 ml of 1.5 M KSCN was added to it (10ml was used in this method).
- The absorbance was measured at 480nm.

Calibration curve:

Seven standard solutions were prepared each having a molarity of 0.5x10-4 M, 1x10-4 M, 1.5x10-4 M, 2x10-4 M, 2.5x10-4 M, 3x10-4 M and 4x10-4 M. The first solution was prepared by diluting 0.5 mL of 0.001 M FeCl3 solution with 9.5 mL of 2 M HCl solution. Similarly, the corresponding solutions are made by diluting 1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL and 4 mL of 0.001 M FeCl3 solution to 10 mL by 2 M HCl solution. After this, 5 mL of 1.5 M KSCN was added to each of the solution and mixed by swirling the test tubes. This step diluted the 10 mL solution to 15 mL causing the concentration to decrease by 2/3rd of its original molarity value. Thus, the values read by the spectrophotometer were for two-thirds of the actual concentration. After adding KSCN, the absorbance was measured immediately because absorbance value can be affected as the colour of the solution fades within 15-20 minutes. 2M HCl was used as the blank. Using these solutions, the concentration vs absorbance curve was plotted.

Flow chart:

- First solution was prepared by diluting 0.5 ml of 0.001 M Fecl3 with 9.5 ml of 2 M HCl solution. So, it is 0.5x10-4 M
- Similarly, 1ml of Fecl3 with 9ml of HCl was added (1 $x10^{-4}$ M).
- 1.5ml of Fecl3 with 8.5 ml of HCl was added (1.5 $x10^{-4}$ M).
- 2ml Fecl3 with 8ml HCl was added (2 x10-4 M).
- 2.5ml Fecl3 with 7.5ml of HCl was added (2.5 $x10^{-4}$ M).
- 3ml fecl3 with 7ml HCl was added (3 x10-4 M).
- 3.5 ml Fecl3 with 6.5 ml HCl was added (3.5 $x10^{-4}$ M).
- 4ml Fecl3 with 6ml HCl was added (4 $x10^{-4}$ M).
- After this 5ml of 1.5 m KSCN was added to each solution and mixed well.
- 2 M HCl was used as blank
- Absorption was taken at 480nm.

A. Concentartion of Fe on standard solution

Fecl3 (ml)	Absorbance	Concemtration of Iron (mg)
0.5	0.34	0.162
1.0	0.74	0.324
1.5	1.05	0.486
2.0	1.39	0.648
2.5	1.69	0.81
3.0	2.0	0.972
3.5	2.32	1.134

B. Sample and its iron concentration

Name of Sample	Absorbance	Concentration of Iron from plotted graph	Iron (In 100g)
Cowpea seed	0.10	0.05mg	5mg
Jackfruit seed	0.11	0.06mg	6mg
Tamarind seed	0.12	0.07mg	7mg
Turmaric powder	0.49	0.22mg	22mg
Cauliflower green	0.68	0.3mg	30mg

Interpretation:

According to colorimetric method of iron estimation highest iron containing food stuff is cauliflower greens.

3. Estimation of Ascorbic acid in lemon

Vitamin C or ascorbic acid standard in metaphosphoric acid is titrated against 2,6 dichlorophenol indophenol salt. This salt is blue in colour in alkaline medium and became pink in acidic medium. Pink colour indicates the complete oxidation of ascorbic acid. The dye in this titration is coloured in the oxidised form and colourless in reduced form. Ascorbic acid is a strong reducing agent because of which it reduces the dye and converted to dehydro ascorbic acid (Oxidised form).

Requirements:

i. 2,6, dichlorophenol indophenol was taken and added with 150 ml of distilled water. Then 42 mg of NaHCO3 was added to it. Then 50 ml of distilled water was added with it or volume upto 200 ml with distilled water.

ii. 6% meta phosphoric acid

iii. 50mg of ascorbic acid was added with 100 ml of 6% meta phosphoric acid and the concentration is 50mg%.

iv. 50ml conical flask

v. 10 ml pipette, burette and beaker

vi. Measuring cylinder

vii. Lemon juice

Procedure:

i. 1 ml of lemon juice was taken in a 50ml conical flask. Similarly, 1ml of standard Vitamin C was taken in a conical flask

ii. 9 ml of 6% metaphosphoric acid was added to each conical flask

iii. Both standard vitamin C and sample was titrated against the 2, 6, dichlorophenol indophenol in a burette

iv. Volume of the dye was recorded when a light pink colour persists for 30 seconds.

v. The total procedure of titration was repeated for 3 times.

Observation of the sample:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**0**	**3.3**	**3.3**	**3.15**
2	**3.3**	**6.3**	**3**	

Observation of the standard vitamin C:

No of observation	Burette reading (ml)		Difference(ml)	Average (ml)
	Initial	Final		
1	**0**	**2.5**	**1.5**	**1.5**
2	**2.5**	**5**	**1.5**	

Result:

Titration value of standard vitamin C is 2.5 ml.

Titration value of lemon juice sample is 3.1

1 ml standard vitamin C solution contain 0.5mg vitamin C

So, 2.5 ml dye reduces 0.5 mg of vitamin C

1ml dye reduces 0.5/2.5 mg vitamin C

3.15 ml dye reduces 0.5/2.5 X 3.15

=0.63 mg

As 1 ml of lemon juice was taken so 1 ml lemon juice contain 0.63 mg vitamin C,

So, 100ml lemon juice contain 0.63 X 100 =63.0 mg

Interpretation:

The supplied lemon juice contains 63mg% Vitamin C. As per the standard value available at the book of C Gopalan, the vitamin C content in lemon juice is 40mg%. So the supplied lemon juice contain a high amount of vitamin C which may be due to the variety or the change in climate for cultivation of vitamin C.

4. Estimation of food Composition

Estimation of food composition can be done by estimation of any type of nutrients in foods like the other methods. As for example total carbohydrate content in foods, lactose in milk etc may be used.