






APPENDICES

Appendix I

Institutional Ethical Clearance Letter

INSTITUTIONAL HUMAN ETHICS COMMITTEE	
 <p>Avinashilingam Institute for Home Science and Higher Education for Women University (Estd. u/s 3 of UGC Act 1956)</p>	
<p>Chairman Dr. S. Ramalingam Principal, PSG Institute of Medical Sciences & Research, Coimbatore</p> <p>Member Secretary Dr. P. R. Padma Professor, Department of Biochemistry, Biotechnology and Bioinformatics</p> <p>Members Dr. P. Santhanakrishnan Mr. C. G. Kumar (Legal Expert) Dr. S. Premakumari Dr. A. Saraswathy Mrs. S. Radha Devi Dr. N.S. Rohini Mrs. Judith Justin Dr. S. Kowsalya Dr. Subhashini K. Sripathi</p>	<p style="text-align: right;">24th December 2014</p> <p>To Ms. Pavithra Krishna Department of Food Science and Nutrition Avinashilingam Institute for Home Science and Higher Education for Women Coimbatore – 641 043</p> <p>Dear Madam,</p> <p>Ref : Our letter dt. 21st February 2014 in response to your proposal No. AUW.IHEC.2013:107 entitled "Effect of intervention on vitamin A and iron nutritional status among primitive tribal children in Nilgiris Dt."</p> <p>With reference to the above letter, in continuation with the documents submitted by you in support of your proposal, as per the suggestions made by the IHEC, the Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No.AUW.IHEC.2013:107 entitled "Effect of intervention on vitamin A and iron nutritional status among primitive tribal children in Nilgiris Dt.". The Approval number for the same is AUW/IHEC-13-14/FHP-20.</p> <p>We wish you all the best in your research endeavours.</p> <p style="text-align: right;">Regards,  Dr.P.R.Padma Member Secretary</p> <p style="text-align: right;"></p>

Appendix II

Clinical Examination Schedule

Name :

Sex:

Age:

Religion:

Caste:

Address:

S.No.	Site	Sign
1	General appearance	Loss of subcutaneous fat Sunken or hollow cheeks
2	Hair	Easily plucked hair, alopecia Dry, brittle hair Corkscrew hairs
3	Nails	Spooning Transverse depigmentation
4	Skin	Dry and scaly flaky paint Nasolabialseborrhoea Psoriasiform rash Pallor Follicular haemorrhage Easy bruising Hyperpigmentation
5	Eyes	Night blindness Photophobia, xerosis Conjunctival inflammation Retinal field defect
6	Mouth	Glossitis Bleeding gums Angular stomatitis Cheilosis Decreased taste or smell Tongue fissuring Tongue atrophy Loss of tooth enamel
7	Neck	Goitre Cretinism Parotid enlargement
8	Extremities	Edema Bone tenderness Bone/ joint pain muscle pain joint swelling
9	Neurological	Nystagmus Wide based gait
10	Any other Symptoms	

Srilakshmi, 2006

Appendix III

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN,
COIMBATORE- 641 043

Effect of Interventions on Vitamin A and Iron Nutritional Status among
Primitive Tribal Children in Nilgiris District

INTERVIEW SCHEDULE

I. SOCIO ECONOMIC BACKGROUND

1. Name of the Interviewer :
2. Name of the interviewee :
3. Age :
4. Sex :
5. Name of Father :
6. Address:
7. Family type : Joint Nuclear
8. Religion :
9. Caste :
10. Family Status

S.No.	Name of the Family Members	Relation to the Child	Age	Sex	Education	Occupation	Monthly Income

11. Other sources of Income

- I, Rent House :
- li, Agriculture :
- lii, Handicrafts :
- lv,Others :

12. Total Family Income :

13. Monthly Expenditure pattern

S.No.	Details	Amount Spent	Percentage of Amount spent from the income
1	Food		
2	Clothing		
3	House Rent/Tax for House/ Home Loan		
4	Education		
5	Medicine		
6	Electricity/ Fuel		
7	Pan/ Betel nut/ Alcoholic drinks		
8	Household things/ Repairs		
9	Transport/ Bus charge/ fuel for vehicles		
10	Savings		
11	Recreation		
12	Debts		

II. DIETARY INTAKE

14.Type of diet

- A, Vegetarian :
 B, Non- Vegetarian :
 C, Ova- Vegetarian

15.Type of Meal pattern

- A, Two Meal :
 B, Three Meal :
 C, Four Meal :

16.Food Frequency

S.No.	Food	Quantity	Frequency					
			Daily	Weekly	Monthly	Fortnight	Occasionally	Not at all
1.	Cereals:							
	Bajra							
	Jowar							
	Maize							
	Panivaragu							
	Ragi							
	Rice							
	-Parboiled							
	-Hand pounded							
	-Raw							
	-Flakes							
	-Puffed							
	Samai							
	Varagu							
	Wheat flour							
	-Whole							
	-Refined							
Semolina								
Vermicelli								
Bread								
-Brown								
-white								
2.	Pulses:							
	Bengal Gram							
	-Whole							
	-Dhal							
	-Roasted							

SNo.	Food	Quantity	Frequency						
			Daily	Weekly	Monthly	Fortnight	Occasionally	Not at all	
3.	Black Gram								
	-Dhal								
	Cow pea								
	Green gram								
	-Whole								
	-Dhal								
	Horse gram								
	-Whole								
	Peas								
	-Green								
	-dry								
	-Roasted								
	Red gram								
	-Dhal								
Soya bean									
4.	Roots & tubers								
	Beet root								
	Carrot								
	Colocasia								
	Onion								
	Big								
	Small								
	Potato								
	Radish								
	Sweet potato								
	Yam								
	Turnip								
	Tapioca								
	Others (specify)								
5.	Spices:								
	Ginger								
	Garlic								
	Coriander								
	Cinnamon								
	Cardamom								
	Cloves								
	Turmeric								
	6.	Greens:							
		Agathi							
		Amaranth							
		Cabbage							
		Coriander leaves							
		Curry leaves							
Drumstick leaves									
Fenugreek									
leaves									
Manathakkali									
leaves									
Mint leaves									
Paruppukeerai									
Ponnangani									
Spinach									
7.	Other vegetables								
	Ash gourd								
	Beans								
	Bitter gourd								
	Bottle gourd								
	Brinjal								
	Cauliflower								
	Cluster beans								
	Cucumber								
	Drumstick								
	Ladies finger								
	Pumpkin								
	Tomato (green)								
	Fruits								
Amla									
Apple									
Banana									

SNo.	Food	Quantity	Frequency					
			Daily	Weekly	Monthly	Fortnight	Occasionally	Not at all
8.	Dates							
	Grapes							
	Guava							
	Jack fruit							
	Lemon							
	Mango							
	Papaya							
	Pineapple							
	Pomegranate							
	Sapota							
	Tomato							
	Water melon							
	Non- vegetarian foods							
9.	Chicken							
	Egg							
	Fish							
	Liver							
	Mutton							
10.	Prawn							
	Milk & milk products							
10.	Buffalo's milk							
	Butter milk							
	Cheese							
	Cow's milk							
	Curd							
11.	Skimmed milk							
	Nuts							
	Cashew nut							
	Coconut							
	Ground nut							
12.	Walnut							
	Fats & oils							
	Butter							
	Coconut oil							
	Ghee							
	Gingelly oil							
	Refined oil							
	Sunflower oil							
	Vanaspathi							
	Sugar							
Honey								
Jiggery								
sugar								

17. Type of beverage consumed

Beverage	Quantity	Frequency
Black tea		
Tea		
Coffee		
Aerated drinks		
Natural drinks		
Natural fruit juices		
Alcohol		
Others		

18. Cooking methods

Foods	Boiling	Steaming	Pressure cooking	Roasting	Deep fat frying	Shallow fat frying
Cereals						
Pulses						
Vegetables						
Egg						
Fish						
Greens						
Meat						
Others (specify)						

III. NUTRITIONAL ANTHROPOMETRY

Height (cm) :
 Weight (kg) :
 Body mass index :
 MUAC (cm) :
 Chest circumference (cm) :
 Head circumference (cm) :

Appendix IV

Description of Procedures for Anthropometric Measurements

Standing height

The subject should stand erect looking straight on a levelled surface, without shoes, with heels together and toes apart. The anthropometer rod should be placed behind the subject in the centre of the heels perpendicular to the ground. The investigator standing on the left side of the subject should firmly hold the chin of the subject with his/her left hand and the occiput of the subject with his right little finger in the Frankfurt horizontal plane (an imaginary line joining the tragus of the ear and infra orbital margin of the eye). The moving head piece of the anthropometer should be placed in the sagittal plane over the head of the subject applying a slight pressure to reduce the thickness of hair. The reading should be taken when the anthropometer rod is still in position. An average of three measurements is taken as the final measurement.

Measure the height up to 200cm/78inch with memo paper

For Measuring Both at Standing and Sitting Positions Wall Type, Measuring Bar :- Vertical, Easily Moved and Fixed Horizontal Rod, Maximal Height of Measuring :- 2 M, Measurement Resolution :- 0.5 cm, Material : Plastic

Body weight

The weight was taken using digital weighing balance. The subjects were asked to remove footwear, wearing minimal clothing and stand on the platform with one foot on either side of the scale without holding on to anything and they were erect. The weight was recorded to the nearest of 0.1 kg. Zero error was corrected every time before actual weighing.

The weighing balance should be tested periodically for its accuracy with known standard weights.

The following precautions should be taken to measure body weight:

1. The Zero error of the weighing scale should be ensured before taking the weight and corrected as and when required.
2. The individual should wear minimum clothing, and be without foot wear.
3. The individual should not lean against or hold anything, while the weight is recorded.
4. The measurements should preferably be taken under basal conditions in early mornings.

The Salter 9000 electronic bathroom scale is suitable for use on carpets as well as hard floor surfaces. It has an easy to read 1" LCD screen. These scales use 2 lithium CR2032 batteries

(included) Size; 27 x 28.5 x 4cm approx. Large platform for generous foot room .Max 150kg ÷ 100g, 23st 8lb ÷ 0.2/1lb

Mid Upper Arm Circumference

The Mid Upper Arm Circumference is taken on the left hand. The mid-point between the tip of the acromion of scapula and the tip of the olecranon process of the (fore- arm bone) ulna, with the arm flexed at the elbow, is marked with a marker pen. Now, the arm should hang freely and the fiber glass tape is gently, but firmly placed embracing the arm without exerting too much pressure on the soft tissues. The reading is taken to the nearest millimetre, with the tape still in position.

Head and Chest Circumference

Head size relates to the size of brain which increases quite rapidly during infancy. The chest in a normally nourished child grows faster than head during the second and third year of life. As a result, the chest circumference overtakes head circumference overtakes head circumference by about one year age. The head and chest circumference are measured with a flexible fibre glass tape used for measuring arm circumference. The chest circumference is taken at the nipple level preferably in mid inspiration. The head circumference is measured passing the tape around the head over the supra-orbital ridges (just above the eyes) of the frontal bone in front, and the most protruding point of the occiput on the back of the head. Slight pressure should be exerted to allow for the thickness of the hair.

Appendix V

Procedure for Biochemical Analysis

Blood Hemoglobin was determined using Sahlis method (Mukherjee, 2004)

The blood sample was drawn from the fingertip of the children and the hemoglobin concentration (g/dl) was estimated by Sahli's method by using haemoglobinometer before and after interventions. Graduated tube was filled with N/10 HCl up to mark 2. The finger of the subject and the needle was sterilized with alcohol, then prick the finger with needle and drawn the blood with the help of the pipette up to 0.2 ml. Care should be taken not to allow any air bubbles inside the pipette while sucking the blood. If the blood goes beyond the mark it was drawn out by touching the tip of the pipette with cotton. The blood was transferred into the graduated tube by blowing gently and the test tube was allowed to stand for 3-4 minutes. The liquid was diluted with distilled water till the colour matches with the standard colour prism and hemoglobin level was recorded.

Serum Retinol was estimated using HPLC method (Raghuramulu, 2003).

Principle

The vitamin A is extracted with a suitable organic solvent and an aliquot of the organic phase is injected onto a normal or reversed phase HPLC column, followed by an eluting solvent of suitable polarity. Retinol, which is eluted as a sharp peak within 1-6 min is detected by a sensitive UV detector set at 325-328nm. Retinol is quantitated by use of peak height ratios or peak area ratios relative to an internal standard (retinyl acetate or other appropriate analogues).

Reagents

Solvents of HPLC graph must be free of particles.

Standards: a stock standard solution of retinyl acetate in ethanol (50µg retinol/ ml) is prepared by dissolving about 1 mg of retinyl acetate in 10 ml of ethanol, determining the concentration in a 1/30 dilution of an aliquot in ethanol by use of the $E_{1CM}^{1\%}$ at 325 nm as 1795, and then diluting the stock standard appropriately with ethanol.

A typical calculation is as follows:

A 1:30 dilution of a solution of all-trans retinyl acetate, prepared and assayed as indicated above, gave an absorbency reading at 328 nm of 0.58 in ethanol. The concentration of vitamin A expressed as retinol in this solution is:

$$\frac{0.58 \times 30}{0.1795} \text{ or } 96.9 \mu\text{g/ml}$$

Then 10ml of this solution is diluted to 19.4 ml to yield the stock standard with 50µg retinol/ ml.

Procedure

1. Normal Phase

Transfer 100µl of serum, 15 µl of internal standard solution (4 µg/ml of retinyl acetate or propionate) and 100µl of methanol to a conical centrifuge tube. Mix the contents of the tube with a vortex mixer. Add 200µl of extraction solvent (petroleum ether 80, dichloromethane 19.3, isopropanol 0.7 by volume) and cap the tube. Extract by interrupted mixing on the vortex mixer for 60s. After centrifugation (3000 rpm, 2 min) inject 100 µl of the supernatant into the column by the use of a Hamilton syringe. Elute with the same solvent as used for extraction.

Chromatographic conditions:

Column	15×0.2cm i.d. Micro Pak Si-10
Mobile phase	petroleum ether: dichloromethane :isopropanol (80:19.3:0.7)
Flow rate	0.5ml/min
Pressure	10kg/cm ²
Detector wavelength	328nm
Detection sensitivity	0.04 AUFS* on recorder
Temperature	Ambient
Recorder speed	Not specified
Retention time (min)	
Retinol	5.0
Retinyl acetate	6.2

*AUFS- Absorbance unit at full scale

Calculation

By use of an internal standard, losses due to incomplete extraction, inaccurate aliquots, oxidation etc. are automatically corrected. The internal standard should have physical and chemical properties sufficiently similar to retinol, is suitably separated from retinol on HPLC, should not coincide with other 325nm absorbing materials in serum, and is not converted to retinol under the assay conditions. A precisely known amount of the internal standard is added to the aliquot of plasma to be analysed. By determining the relative extraction efficiency and detector response of retinol and the internal standard, a standard curve is fashioned in which the ratio of peak heights (or areas) is plotted against the retinol concentration in plasma. In experimental samples, the peak height (or area) ratio is determined and the appropriate plasma retinol concentration determined from the standard curve.

A standard curve is prepared by adding varying amounts of retinol (i.e.10-120 ng) to a fixed amount (i.e.50 ng) of internal standard in a final volume of 100µl of eluting solvent, injecting the solution on HPLC under assay conditions, measuring the peak heights, and calculating the peak height ratio. The peak height ratio is then plotted as the abscissa with the standard retinol concentration (for a 100µl plasma aliquot) as the ordinate.

Appendix VI

24 Hour Recall Method

Date:

Early morning	Breakfast	Lunch	Tea time	Dinner

Appendix VII

Formulation and Preparation of the Spirulina Incorporated Food Supplement (SIFS)

To prepare 1 kg of Spirulina Incorporated Food Supplement, the following amount of ingredients were used

Ingredients

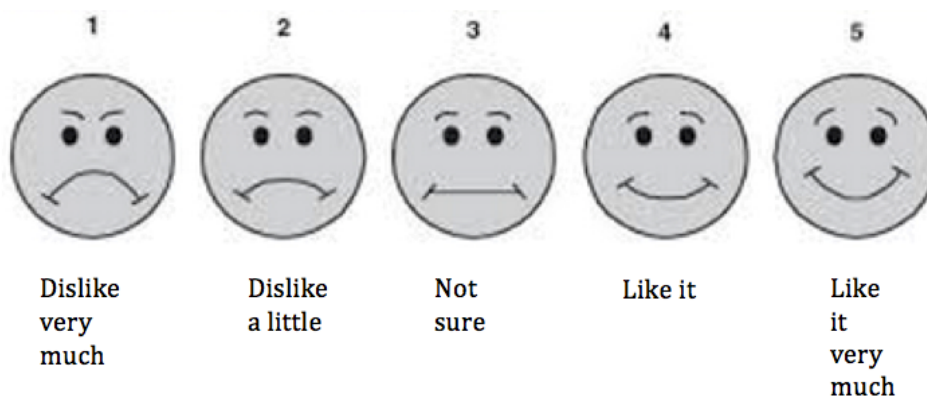
Wheat	- 180 g
Maize	-160 g
Jowar	- 160 g
Roasted Bengal gram	- 120 kg
Ragi	- 50 g
Jaggery	- 300 g
Spirulina powder	- 30 g

Preparation

Spirulina powder was purchased from Antenna Nutritech Foundation, Madurai. Wheat, Maize, jowar, Roasted Bengal gram, ragi and jaggery were purchased from the local grocery shops. It was ensured that all ingredients were of good quality. They were cleaned well, dry roasted separately, one by one and powdered in the mill, sieved and stored in air-tight containers. These ingredients were included at 30g, based on the formulation of the ICDS health mix. On the before day of supplementation, all the ingredients were mixed well in a vessel, weighed and packed as 30 g for each children in zip lock covers.

Appendix VIII

A Child Friendly Five Point Scale



Appendix IX

Estimation of total microbial count

Protocol

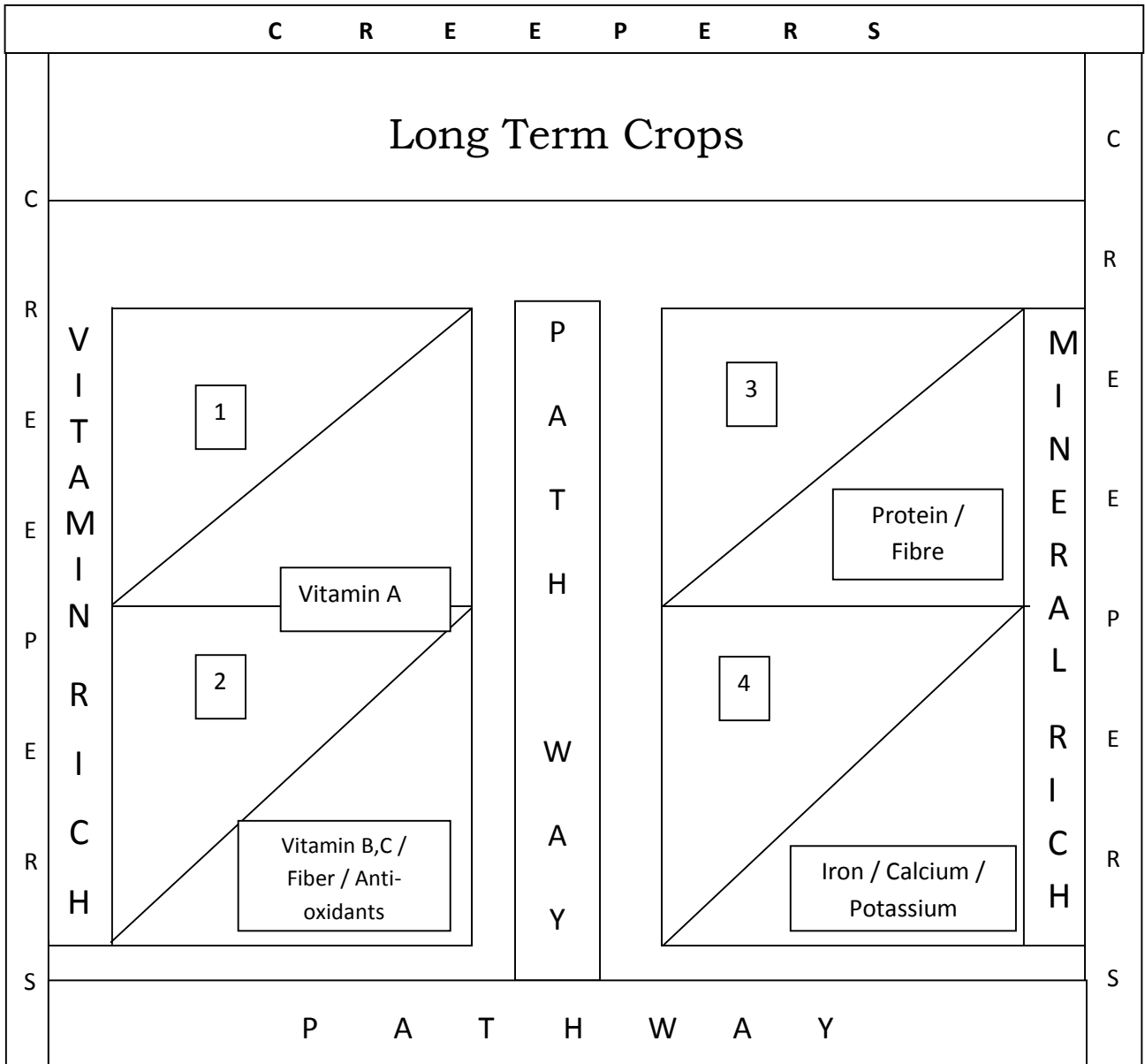
Isolation and identification of bacteria / yeast and molds from food sample

Sample preparation: 10 g of sample was dissolved in 90ml of distilled water, mixed well and serially diluted up to 10^{-4} . The serially diluted samples (0.1ml) were plated on Nutrient agar medium (20ml) and mix by rotating clockwise and anticlockwise. After solidification the plates were incubated at 37°C for 24 hrs for bacteria.

For Yeast and mould Malt agar medium was prepared and 0.1ml sample was added to the plate. After solidification the plate was incubated at room temperature for 4 to 5 days.

Appendix X

The Kitchen Garden Model



(Source : Resource book, KVK, Gandhigram University, Horticulture Department, The Nilgiris)

Appendix XI

KAP Schedule

KNOWLEDGE

- 1, Among the following which is rich in fats
A, Fruits B, Vegetables C, Oil D, Cereals
- 2, Low intake of food throughout life
A, Impairs growth and development B, Improve body size
C, Improve working efficiency D, Do not know
- 3, Which among the following builds your body
A, Pulses B, Ghee C, Rice D, Do not know
- 4, Which nutrient is present in Green Leafy vegetables
A, Protein B, Carbohydrate C, Vitamins & Minerals D, Fat
- 5, Which among the foods are protein rich
A, Meat, Fish and egg B, Rice, ragi and wheat C, leafy vegetables & fruit D, Do not know
- 6, Which type of oil is good for health
A, Gingely oil B, coconut oil C, castor oil D, Do not know
- 7, Three foods required for health
A, pulses, animal foods, nuts and oil seeds B, Fruits, cereals, milk
C, cereals, fats and vegetables D, Do not know
- 8, What does vegetables contain
A, vitamins and minerals B, fat C, Protein D, Do not know
- 9, Rich source of protein
A, vegetables B, milk C, potato D, oils
- 10, Is it essential to consume vegetables daily
A, yes B, no
- 11, Which foods can prevent bleeding gums
A, amla & guava B, meat & fish C, dry grains D, Do not know
- 12, Which food is required for good eye sight
A, carrot, papaya, greens B, rice, wheat, ragi C, sugars, nuts and oilseeds D, Do not know
- 13, Which among the following foods is rich in iron
A, rice flakes B, coconut C, vegetables D, Do not know
- 14, Anaemia is caused due to the deficiency of
A, iron B, protein C, vitamin A D, Do not know
- 15, Which among the following is a symptom of anaemia
A, tiredness B, bleeding gums C, fever D, Do not know
- 16, Which among the following is rich in fibre
A, whole grains & greens B, sugar and jaggery C, sweets & biscuits D, Do not know
- 17, Which of the following will result, if the diet is deficit of fibre
A, constipation B, diarrhea C, vomiting D, Do not know
- 18, Ragi is rich in
A, calcium and energy B, vitamin C C, vitamin A D, Do not know
- 19, Foods for treatment of diarrhoea
A, salt, sugar solution B, coffee/tea C, ragi, kool D, Do not know
- 20, Usual weight gain during pregnancy is
A, 10-12 kg B, 8-10 kg C, 6-8 kg D, Do not know

ATTITUDE

- 1, Do you consume sprouted pulses
A, yes B, no
- If yes, how do you consume
A, raw B, boiled C, fried D, steamed
- 2, Sprouted gram is a good source of
A, protein B, fat C, vitamin C D, calcium
- 3, Which among the following food should a pregnant women consume
A, green leafy vegetables B, rice C, ash D, do not know
- 4, Why additional food is required during pregnancy and lactation
A, to prevent maternal mortality B, to decrease birth weight
C, to increase maternal mortality D, do not know
- 5, Breast feeding the new born must be started
A, immediately after birth B, 2 days after birth C, 1 week after birth D, do not know
- 6, Breast feeding can be till
A, 18-24 months B, 12-18 months C, 6-12 months D, do not know
- 7, Advantages of breast feeding
A, rich in nutrients B, commercial food C, increases risk of infection D, do not know
- 8, Breast fed infants require weaning foods
A, yes B, no C, do not know
- 9, Colostrums is
A, rich in nutrients B, impure C, toxic D, do not know
- 10, Immunization is essential for new borns because
A, to fight against infection B, to grow fast C, do not know
- 11, One of the reasons for maternal death is
A, severe anemia B, late marriage and pregnancy C, lack of exercise D, do not know
- 12, Which type of rice is good for health
A, raw hand pounded B, parboiled C, raw milled D, do not know
- 13, Banana helps in
A, relieving constipation B, causing diarrhea C, causing fever D, do not know
- 14, Which among the following foods could be given to a ulcer patient
A, milk B, coffee/tea C, soda D, do not know
- 15, Which of the following foods can be given during fever
A, rice kanji B, ragi roti C, egg D, do not know
- 16, Diet deficit of green leafy vegetables and yellow fruits and vegetables leads to
A, night blindness B, jaundice C, constipation D, do not know
- 17, Chewing beetle leaves causes
A, mouth cancer B, obesity C, lung disorder D, do not know
- 18, Who should consume nutritious food in the family
A, women B, men C, adolescents D, old age
- 19, Stagnation of water
A, breeds mosquitoes B, acts as a good manure C, increases hygiene D, do not know
- 20, Fried foods should be avoided during
A, jaundice B, underweight C, vitamin A deficiency D, do not know

PRACTICE

- 1, When do you wash the vegetables
A, before cutting B, after cutting C, both D, need not wash at all
- 2, How many times do you wash rice
A, 1 time B, 2 times C, 3 times D, > 3 times
- 3, By which method do you cook rice
A, pressure cooking B, boiling C, both
- 4, What do you do with the excess of water after cooking
A, drained off B, used in the meal preparation C, used for animal feeds
- 5, Do you have a kitchen garden in your house
A, yes b, no
- 6, Soaking the cut vegetables in water for long periods
A, enhancing nutrient loss B, prevents nutrient loss C, neither gain nor loss D, do not know
- 7, How often you include greens in your diet
A, daily B, once a week C, occasionally D, never
- 8, How often you drink milk
A, once a day B, twice a day C, thrice a day D, never
- 9, How often you include fruits and vegetables in your diet
A, daily B, twice a week C, thrice a week D, once a week
- 10, How do you consume vegetables
A, raw B, cooked C, both
- 11, Which among the following foods do you give among diarrhoea
A, dehydration therapy B, fluids C, soda D, regular diet
- 12, Which among the following foods do you adapt in storing agricultural commodities
A, tin with tight lid B, tin with loose lid C, gunny bags D, any other
- 13, Do you use cooking soda
A, yes b, no
- 14, Which among the following method do you practice to prevent nutrient loss
A, steaming B, boiling C, frying D, roasting
- 15, Which among the following foods do you consume to relieve cough
A, pepper B, garlic C, cumin seeds D, rice flakes
- 16, How often you take bath
A, daily B, alternate days C, once in 2 days
- 17, Where do you go to seek medical help during illness
A, hospitals b, home remedies c, taking to priests
- 18, Which you eat often for breakfast
A, poori B, idly C, noodles D, dosai
- 19, Do you take any special foods during disease conditions
A, yes B, no
- 20, Which among the following foods do you give for lactating women to increase milk secretion
A, garlic B, vegetables C, sweets D, castor oil