Stage 1: Theoretical development of indigenous enteral formulae
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1: Abstract

The main objectives of nutrition intervention are to improve the nutritional status of patients and decrease the number of hospital days. Enteral nutrition is one of the numerous modalities of nutrition support. This mode of therapy is only considered when the patient is unable to consume regular food.

In order to meet the nutritional needs of a wide variety of patients, a number of commercial formulae are available in developed countries. However, developing countries have to rely on an imported products. This is usually not feasible because of their erratic supply and high cost. Hence, there is an obvious need for an indigenous enteral product.

With this view, an enteral formula was developed on the basis that it will be easy to prepare, relatively inexpensive and nutritionally equivalent to commercial formulae. The procedure for developing the enteral formulation involved a number of steps:

- Identification of food items
- Determining the nutritional composition of food items
- Selecting Recommended Dietary Allowance (RDA) table
- Enteral diet formulation

A computer aided master sheet was developed which contained information on selected food items, their nutritional composition, fibre, moisture content, essential amino acid profile, nitrogen and cost of the formula.

Initially various combinations and food ratio were tried selecting food items from the Master Sheet and were analysed by using What if cell, constraint cell and Target cell which are the main features of “What If Problem Solver®”, an attachment of Lotus spread sheet.
The main advantage of using this program was the instantaneous formulation of diets and prompt information on their nutritional composition. Further it proved that theoretical formulation of an enteral diet is possible. However, in order to determine the efficiency of these diets scientific testing is very much required.
Chapter 1.1: Introduction

An adequate diet is necessary for good health. Only recently, there has been an increased emphasis on nutrition support as an integral part of medical health care. The main objectives of support are to improve the nutritional status of patients, reduce morbidity and mortality and decrease the number of days spent in hospital and the cost of the health care system. Numerous modalities of nutrition support are available; enteral feeding is one of them.

**Definition** Enteral feeding is defined as the provision of nutrition via the gastrointestinal tract, either by mouth or through a tube or catheter that delivers nutrients distal to the oral cavity (1.1).

**Indications and rationale** Enteral feeding should be considered for the patients who are unable to eat but have an intact and functioning gastrointestinal tract (1.2). This route of alimentation is more physiological and less costly than the parenteral route (1.3-1.4).

**Statement of the problem**

A wide variety of commercial enteral formulae are available in developed countries. Developing countries have to rely on imported products. The high import cost, erratic supply and short shelf life of such products are serious limiting factors.

The single commercial formula available in Pakistan offers very little flexibility for varying energy, macronutrient and electrolytes according to individual requirements. For patients unable to afford commercial formula, only clear soups, squashes and soft drinks are available which do not supply adequate nutrition. Thus, there is clearly a need for a hospital made formula feed.

**Aim**

The purpose of this study is to develop a procedure for preparing an
enteral formula with Pakistani indigenous food items using simple technology at affordable cost. The formulation should furnish 1 kcal* / mL and provide an energy ratio of carbohydrate: fat: protein of 50 : 30 : 20.

**Hypothesis**

The computer aided formulation of low cost indigenous enteral formula is possible.

*The reason for using kcal unit for energy value is because it is commonly used in Pakistan.*
Chapter 1.2: Literature Review

History

Enteral feeding originated in ancient times with the Egyptians who used nutritional enemas for preservation of general health. Greek physicians used an enema containing wine, whey, milk and barley broth for the treatment of diarrhoea and for the provision of nutrients. In the nineteenth century European physicians used the patient’s rectum for instillation of various foods and liquids such as beef extract, milk and whisky. Rectal feeding was widely used until the beginning of the 20th century. A major development in the provision of nutrition occurred at the end of the eighteen century when John Hunter, a famous surgeon proposed that a nasogastric tube be made from eel skin to feed a patient suffering from neurogenic dysphagia. The use of nasogastric tube both for feeding and for emptying the stomach became wide spread in the nineteenth century.

In 1918, Anderson introduced the concept of early post operative enteral feeding by starting jejunal feeding in a patient following a gastrojejunostomy (1.5). With the increasing popularity of this practice, regular food stuffs were mixed and ground or blenderised into a fine solution which was instilled into the stomach through a tube.

In the 1930s, specialised enteral feeding formulas appeared with the introduction of casein hydrolysate for use by both enteral and parenteral route. The first commercial formula introduced to the market in 1942 was Nutramigen. It was especially designed for children with intestinal disease and allergies.

Barron in 1959 reported on enteral feeding through a polyethylene tube passed through the nose of the post operative patient. Interestingly, beside using juices and food broken into solution, he also infused G.I secretions collected during drainage of biliary, pancreatic, gastric and intestinal fistulas (1.6). Major achievements in knowledge and utilisation of chemically defined formula were gained through studies sponsored by the National Aeronautics and Space Administration (1.7-1.8).
Indications for enteral feeding

Enteral feeding is a method of providing nutrients in the gastrointestinal (G.I) tract orally or through a tube. It is for patients who cannot ingest a sufficient amount of solid food. There are four categories of such patients (1.9).

1. *Patients with severely impaired gastrointestinal function* such as those with high output intestinal fistulae, subacute intestinal obstruction, prolonged ileus, severe malabsorption and complicated acute pancreatitis.

2. *Patients with inadequate intake* such as those with extensive burns, major trauma, severe and prolonged sepsis and severe and extensive inflammatory disease.

3. *Patients undergoing major surgery* who have poor nutritional status. It has been shown in several studies that there is a close association between the pre-operative nutritional status and the outcome of surgical patients (1.10 -1.11).

4. *Cancer patients*

Rationale for prescribing enteral feeding

The advantage of enteral nutrition over parenteral feeding are as follows:

- *Maintenance of the integrity of gut structure and function* is confirmed on the basis of theoretical and scientific evidence that the enteral route is superior to parenteral with regard to maintaining gut structure and function (1.4-1.12).

- *Fewer infectious and metabolic complications* are reported with enteral nutrition support. Parenteral nutrition is associated with complications such as pneumothorax, hydrothorax, arterial puncture, catheter embolus and sepsis (1.13).
• **Ease of administration** as compared with complex techniques required for parenteral feeding (*1.14*).

• **Cost effective** as opposed to commercial enteral product and intravenous nutrition (Table 1.2).

### Table 1.2.1. Comparative cost of parenteral and enteral nutrition

<table>
<thead>
<tr>
<th>S.no</th>
<th>Products</th>
<th>Cost/ 2400 kcal (Rs / Aus $)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hospital prepared formulae</td>
<td>Rs 90/-</td>
</tr>
<tr>
<td>2</td>
<td>Commercial enteral product</td>
<td>Rs 325/-</td>
</tr>
<tr>
<td>3</td>
<td>Intravenous product:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Dextrose (25%)</td>
<td>Rs 102/-</td>
</tr>
<tr>
<td></td>
<td>b. Aminovil (5%)</td>
<td>Rs 2880/-</td>
</tr>
<tr>
<td></td>
<td>c. Intralipos (10%)</td>
<td>Rs 2050/-</td>
</tr>
</tbody>
</table>

*Aus $ 1.00 = Pak Rs 22.00 (1/3/95)*

Intravenous nutrition regimes are very expensive as compared with indigenous enteral formulae (*1.15 -1.16*).

**Enteral feeding solutions**

Twenty years ago, enteral products consisted of natural food stuff, milk shakes and baby formulae. Only recently the use of enteral nutrient formulae has broadened and range from the home blenderised whole food diets to highly refined "predigested" nutrients in combination or in isolated form. Since 1989 the term "medical food" has been used to define enteral nutrition products by the United States Food and Drug Administration (FDA) (*1.17*):

"Medical Foods (MF) are distinguished from other foods for especial dietary purposes or foods which make health claims (eg., fibre in relation to cancer) by the requirement that they (MF) be used under medical supervision. In addition single ingredient nutrient products that are promoted for the treatment of specific disease states will continue to be regulated under existing drug law (eg., zinc sulfate for the treatment of acrodermatitis enteropathica, as well as all injectable nutrient formulations. In general, in order to be considered a MF a product must, at a minimum, meet the following criteria:

• The product is a food for oral or tube feeding.
- The product is labelled for the dietary management of medical disorder, disease or condition.

- The product is labelled to be used under medical supervision".

Solutions for enteral feeding can be classified in different ways. Shike's (1.18) classification of enteral formulae is based on the clinical indication for the solution:

**Natural blenderised foods** are available commercially and can be prepared at home. Commercially available blenderised diets are mainly constituted of milk, beef, fruits, vegetables and fibres. Although they may have the advantage of being natural foods, nutritional completeness is not assured. Currently the use of a blenderised product is limited.

**Polymeric formulae** refers to those formulae that contain macronutrients in the form of isolates of intact protein, triglycerides and carbohydrate polymers. Polymeric solutions contain whole protein isolated from casein, lactalbumin, whey, egg white or a combination of these. The carbohydrates are usually glucose polymers in the form of starch and its hydrolysates. The fats are of vegetable source, such as corn oil, safflower oil, sunflower oil and others. In most of these solutions, the energy ratio of macronutrients is as follows: protein constitutes 12 to 18%, carbohydrate 40 to 60% and fat 30 to 40% of total calories. In the standard formula the ratio of non-protein calories to nitrogen is approximately 150:1. Vitamins and essential trace elements are present in adequate quantities so that a daily intake of 1500 to 2000 kcal furnishes the recommended daily allowance (RDA) of these nutrients, except sodium and potassium which may vary considerably among the various polymeric solution in order to cater to individual patients. The osmolality varies between 300 - 400 mosm/kg in a solution that contains fibre up to 6-14 g/1000 kcal. The caloric density is between 1-2 kcal / mL.

Polymeric solution can be infused through a tube in patients with a functioning upper gastrointestinal tract. Patients being fed through a nasogastric tube can tolerate up to 300-500 mL in 3 to 5 bolus feedings daily (1.19). Patients fed through a jejunostomy can take up to 250 mL/hour/day through continuous drip.
Monomeric formulae require less digestion than a regular food or polymeric formula. In monomeric solutions protein is in the form of peptides and/or free amino acids which are hydrolysates of casein, whey and other proteins. The study of Grimble, et al (1.20), shows that absorption of dipeptides and tripeptides is more rapid and efficient than the absorption of an equivalent amount of amino acids.

In monomeric solutions carbohydrates are in the form of partially hydrolysed starch such as maltodextrins and glucose oligosaccharides. Fat is a mixture of long chain triglycerides (LCT) and medium chain triglycerides (MCT) of vegetable origin. Monomeric formula are lactose free and fibre free. The caloric ratio of macronutrient in monomeric formula is quite different from polymeric solutions because 80% of calories are present as carbohydrate, protein contribute 12 to 20%, and only 1 to 5% are in the form of fat. The caloric density of monomeric solutions is between 1 and 1.5 kcal/mL. The osmolality of monomeric solutions is high and ranges between 400 to 700 mosm/kg, which can induce rapid transit and diarrhoea. Keeping in view the characteristics of monomeric formula, these solutions might be considered as suitable for patients with impaired fat digestion, such as those with pancreatic insufficiency or short bowel syndrome, but such an advantage has yet to be demonstrated through scientific clinical trials (1.18).

Modular formulae consist of a single nutrient or multiple nutrients which when combined produce a nutritionally complete formula or enhance an existing fixed ratio formula (1.21-1.22).

The major types of modules available for commercial use are carbohydrate, fat and protein. The protein module is available in the form of intact protein, hydrolysed protein and crystalline amino acid; carbohydrate is available in the form of glucose polymers. Butter fat, vegetable (corn, safflower and sunflower) oils and fat emulsions are the major source of lipid module (1.23). According to Shike (1.18), the use of these solutions to modify commercial solution or to prepare a special solution is limited because no clear cut specification exists for their use.
For example fat emulsions require an emulsifier to keep them dispersed in solution after they have been mixed with other solutions. Glucose polymer added to a solution can increase the osmolality and induce diarrhoea. Protein powder is difficult to mix and tends to clump, thereby clogging the feeding tube when administered.

**Hydration formulae** are especially designed to provide fluid and minerals to children and adults with acute diarrhoea in order to prevent dehydration. These solutions can be given through tubes to patients with excessive fluid and mineral requirements such as patients with certain types of bowel syndrome (1.18).

**Formulae for specific metabolic needs** are almost complete nutritional solutions in which specific nutrients are added or removed to meet the special metabolic requirement (1.17). These solutions are divided into two categories.

1. Solutions designed for patients with an inborn error of metabolism such as formula low in or devoid of specific nutrients, eg. Phenylalanine or other amino acid that can not be metabolised due to enzymatic defects or deficiencies.
2. Solutions developed for patients with a specific medical condition, such as renal failure, liver failure or with multiple organ failure.

**Foods and their nutritive value**

Each food may supply some energy and a combination of nutrients, where as the nutritional value of any meal or diet depends on the individual foods used and how it is prepared (1.24).

The availability and the use of food varies from place to place. However, in order to examine the nutritive value of foods for this study they are grouped under the following headings:
Food of plant origin

Cereals and grains have approximately the same nutritive value. All cereals, when raw and dried contain 7 to 14 % protein, up to 75 % carbohydrate and apart from oats, quinoa and maize, negligible amounts of fat. The protein quality varies among different foods (ie. different proteins have different amino acid patterns). Among cereals and other grains, protein quality of oats is superior to rice but rice is more available. Most commonly used milled rice contain little fibre and therefore can be digested and absorbed easily. In fact the use of rice has proved to be effective in dietary management of persistent diarrhoea (1.25-1.26). However, the percentage of protein in rice is low (7 %) and lysine is present in even smaller proportion to the reference pattern. Rice has an amino acid score of approximately 65 which is considered to be of marginal quality, but if consumed with other food items such as legume or from animal origins, the quality of the protein becomes much better. It is noted in FAO Nutritional Studies # 1 (1.27) that supplementation of rice with milk powder results in better utilisation of rice protein. The fat soluble vitamins A and D are in negligible quantities but vitamin E content of whole rice is considerable (1.28). In its mineral contents, rice like other cereals is poor in iron and calcium. Also the calcium to phosphorous ratio is unfavourable ie 1:10 (1.29). Wheat appears to vary more widely in nutrient content than any other cereal. The percentage of protein may vary from 8-15 % and the limiting amino acid is lysine. Wheat is poor in riboflavin but relatively high in thiamine and niacin content (1.30). Overall, the nutrient content of cereal is affected by the way in which it has been processed.

Tuber, roots and starchy fruits are lower in protein content when compared with cereal because of high water content but unlike cereals, they do provide useful amounts of vitamin C. These foods are very bulky and nutritionally less nutrient dense.

Legumes (dried beans and peas) varieties are eaten world wide and most of them have a similar nutrient value. Although moderate in protein
quality, they are considered valuable in supplementing other food protein such as cereals. However appropriate processing for legumes is more important to improve palatability and nutrient availability (1.31). In order to aid digestibility and palatability, legumes must be soaked, skinned and well cooked. Nearly all legumes contain some form of toxin or anti-nutrient which must be removed by careful preparation.

Mung bean (dhal) is considered in India/Pakistan to be a light food and easily digestible. This traditional belief is confirmed by trials that show a low tendency to produce flatulence. The beans contain a high level of protein but are limiting in sulphur amino acids, especially methionine and tryptophan but are high in lysine content. It has highly digestible carbohydrate (oligosaccharide is the principal constituent of the beans) and a low level of free sugar (1.32-1.33). On the whole, legumes are rich in dietary fibre and like cereals, absorb water during cooking which increases their bulk and at the same time reduces the concentration of their nutrients.

**Oilseeds and nuts** Oilseeds and nuts contain more and better quality protein than most of the legumes. They are used less frequently and in smaller amount than legumes. Their main contribution to the diet is to add a particular flavour and texture and increase energy concentration. In central America and India, mixes of flour from soybeans, cotton seed, sesame, sunflower seeds are used as a supplement on their own or mixed with some cereal flour (1.24). The protein contents of different oil seeds (1.34) are as follow:

<table>
<thead>
<tr>
<th>Soybeans</th>
<th>Peanuts</th>
<th>Sunflower seed</th>
<th>Sesame seed</th>
<th>Almonds</th>
<th>Cashew nut</th>
<th>Coconut (dried)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.1 %</td>
<td>26.3 %</td>
<td>24 %</td>
<td>18.6 %</td>
<td>18.6 %</td>
<td>17.3</td>
<td>7.2 %</td>
</tr>
</tbody>
</table>

Adopted from CRC Handbook of nutritional supplement. CRC Press 1983.

Soy proteins are good sources of all essential amino acid except methionine. Protein from peanut, safflower seed and almond are deficient in lysine, methionine and threonine, while sesame seed is only deficient in lysine. The protein efficiency ratio of nuts and oil seeds range from 1.5 to 2.3 depending on the kind of nuts or oil seeds. However, significant
increase has been shown in protein efficiency ratio (PER) as a result of fortification with lysine \((1.35)\). In India, protein food formulation based on blends of sesame, soybeans, chickpea and peanuts flour fortified with vitamins and minerals have been developed \((1.36)\). A study by Doraiswamy, et al revealed \((1.37)\) that a daily supplement of 50 g of a protein food based on a blend of sesame seed, chickpea and peanut flours, over a six month period brought highly significant increase in height and weight of children aged 5 to 11 years when compared with an isocaloric control group.

The fat content of soybean is 17.7 % with other oils seed ranging from 34 to 54%. Among all of these, sunflower, soybean, safflower and sesame seeds oils are rich in polyunsaturated fatty acids and peanuts and almonds have a moderate quantity of them.

Carbohydrate contents such as starch, sugar, cellulose, hemicellulose vary among oilseeds and nuts. Oil seed and nuts are generally a good source of all minerals except calcium and sodium. Sesame seed contain vitamin E, an antioxidant which helps to prevent other fats from going rancid. Due to this quality, in Sierra Leone ground sesame seed have been added to home made weaning mixture to prevent rancidity \((1.24)\).

*Vegetables* are higher in water content and low in energy and protein contents. Fresh vegetables are an important source of carotenoids and C and folate. The darker the colour of the vegetables, the higher is the content of Beta-carotene. All vegetables contain some fibre but they are important not only for their nutritive value but also for their additional flavour and texture.

*Fruits* are similar in nutritional content to vegetables but because they contain easily digestible sugar and are sweet in taste, they are generally eaten raw. Many fresh fruits are an excellent source of vitamin C and yellow and orange fruits contain vitamin A. Dried fruits have a higher concentration of energy and nutrients than the fresh varieties.
Food of animal origin (1.24)

Food of animal origin contain a good proportion of high quality protein, which makes them an excellent supplement for cereals and other staple foods. Although they are relatively expensive and short in supply, only a small amount is required if legumes are used in the same meal. They are readily digested and are almost completely absorbed by the body. Only food from animal origin sources provide adequate amount of vitamin B 12.

Milk from many species is available but the most commonly used is cow’s milk. All milks provide protein of high quality, fat and lactose in varying amount. Lactose is most concentrated in human milk. The amount of fat is high in buffalo and sheep’s milk. All milk fat also contains a little vitamin D and some vitamin A and is a good source of vitamin B particularly riboflavin. Milk is a rich source of calcium but the concentration of iron is low. Since milk is a highly perishable food item, great care should be taken to keep it safe for consumption. Milk and milk products have potential use to supplement food to improve nutritional values. However care should be taken before supplementing food with dairy products, particularly with regard to the type of consumer to be served, their physiological state, age and food allergies. Commercially milk is preserved in several ways which include:

- Removing the water
- Lowering the pH using a lactobacillus culture
- Canning
- Treating it with heat.

Most products retain much of their nutrient value but this depends on the method of processing.

Meats and sea foods when fresh are an excellent source of good quality protein, iron and B vitamins particularly niacin. Meats from young animals contain less fat than from more mature animals, but the proportion of protein is the same (about 16%).
Poultry has a nutritive value similar to meat. Poultry usually have less fat, although duck and other water birds contain more fat. In nutritive value, fish is similar to meat but has less iron. Mussels, shrimps and prawn have abundant iron. The liver and fish of oily sea fish (herring and sardines) are rich in fat and vitamin A and D.

Eggs are an excellent source of good quality protein, as well as fat, vitamin A, thiamine and riboflavin. Also, they contain some vitamin D but they are not a good source of iron because it is not bioavailable.

**Concentrated energy sources**

*Fats and oils* are energy dense and improve palatability and consistency of food and add to energy value of a meal without increasing bulk. Care should be taken that they do not supply more than about 25 - 30 % of the total energy intake. Oils from plant sources contain more unsaturated fatty acid than does animal fats. However, coconut has a high proportion of saturated fatty acid, a low proportion of polyunsaturated fatty acids and little of the essential fatty acids.

*Sugar* in its refined white form is a source of energy only. It has no protein, no vitamins and no minerals. The energy concentration of sugar is less than half of that of oil but compared with other foods, it is a concentrated source of energy. Since sugar does not increase the bulk or volume, it can be a useful ingredient for fluid or convalescent diet especially when appetite is small.

*Herbs, spices and condiments* lack particular nutritive value, but add flavour when taken in food or beverages.

**Nutritional composition of enteral formulae**

*Protein* content is the most critical component of enteral formulae because it is required for the maintenance of the body cell mass and for the major functions of the body (1.38). The kind of protein present in formula
has an impact on osmolality, palatability and digestibility. Protein in enteral formulae is divided into three categories (1.39) depending on the degree of digestion required:

1. Intact protein.
2. Hydrolysed protein.
3. Pure amino acids.

*Intact proteins* are present in enteral formulae in their complete and original forms as found in whole foods. These proteins require complete digestion to small peptides and amino acids in order to be absorbed in the gut. Intact proteins have very little effect on the osmolality of the formulae. Examples of intact proteins are protein isolates such as lactalbumin and casein salts (1.38).

*Hydrolysed proteins* have been enzymatically hydrolysed to peptides and to free amino acids. As the protein molecules become smaller, they tend to increase the osmotic load. According to MacBurney (1.38), protein hydrolysates often require the addition of free amino acids (methionine, tyrosine, tryptophan) to enhance the protein quality of a formula.

*Crystalline amino acids* require no further digestion in the gastrointestinal tract and are absorbed directly via active transport. These particles due to their small size, affect the osmolality and taste of the formula (1.1).

Whether or not the administration of peptides or amino acids as a part of enteral formulae has an advantage over the intact or hydrolysed protein is questionable. Numerous studies have addressed this issue by comparing the use of peptides and amino acids solution versus intact protein on normal subjects and on patients with a compromised digestive tract.

Several studies found no nutritional evidence in favour of using expensive enteral diets containing peptides or amino acid over whole protein for patients with normal gastrointestinal tract (1.40 -1.43). A study with head injured unconscious patients indicated that nitrogen balance was achieved
more readily with intact protein than with the elemental diet (1.44). Moriarty, et al (1.45) evaluated the relative merits of different nitrogen sources in the maintenance of nitrogen balance using healthy volunteers. He concluded that whole protein is as effective as amino acids and protein hydrolysate in maintaining nitrogen balance and that no logic exists for prescribing elemental feeds to subjects with normal gastrointestinal function.

Studies on animals with normal G.I tract showed significantly less weight gain on elemental diets when compared to polymeric diet (1.46). Trocki, et al (1.47) compared the nutritional effect of diet containing intact protein with corresponding free amino acids in a burn guinea pig model with normal G.I tract and observed a significant benefit in using intact protein for maintaining body weight and providing better nitrogen retention (p<0.05).

While discussing the application of different kinds of protein, the question arises as to whether a peptide based formula offers any advantage in patients with a compromised absorption and digestive capacity. According to Rees and Hare (1.48) no indication exists for prescribing an enteral diet with a predigested nitrogen source for patients with moderately impaired gastrointestinal function. However, studies indicate the use of peptide based formula to be extremely useful in critically ill patients with impaired gastrointestinal absorption associated with hypoalbuminemia (1.49 - 1.51). Intestinal perfusion studies have consistently shown more efficient absorption of peptides over amino acid mixture (1.52 - 1.54). The result of a study by Smith, et al (1.55) indicated that the non elemental diet is better tolerated in most patients with a moderate degree of small bowel abnormality. Di and tripeptides (small peptides) have been found beneficial for patients with a severely reduced intestinal function (1.52, 1.56 - 1.57).

The use of peptide based enteral diets in trauma patients is associated with better hepatic protein response and less diarrhoea when compared to intact protein (1.58). Shike (1.18) quoted studies which have shown that feeding
a peptide based formula (Reabilan) resulted in a higher level of serum transferrin and prealbumin than those attained by feeding a solution with intact protein.

The use of an elemental diet is indicated for patients who are critically ill (1.59), patients with severe exocrine pancreatic insufficiency and those with short bowel syndrome (1.60). In patients with a severe bowel abnormality, the elemental diet may be better tolerated, but a non elemental diet should still be the initial formula.

There is enough supporting evidence to maintain that the use of a polymeric diet is equal or superior to a peptide and amino acid diet for patients with an intact digestive tract. Its use is recommended for patients with intact G.I tract who require nutritional support to maintain energy intake (1.48). There are studies which have acknowledged the superior absorption and other benefits of peptides over elemental and intact protein diet. However, controlled clinical trials in various disease states are required to compare their efficiency and tolerance against elemental diets as well as whole protein formula.

**Protein quality** of a particular formula, regardless of whether it has an intact, peptide or amino acid based composition, must be critically analysed. Protein quality depends upon the profile of amino acids in a particular formula (1.38). Imbalance of amino acids can result in growth failure or negative nitrogen balance. In study by Marable and associates (1.61) in which the chemical score was used to assess the protein quality of supplements and meal replacements it was observed that predigested products had a low chemical score and failed to meet the suggested adult requirements for some essential amino acids.

**Nitrogen: energy ratio** of 1: 300 (1 g of nitrogen per 300 kcal) may be considered adequate for a healthy subject (1.62). But hospitalised patients are thought to have a higher protein requirement which is estimated to be from 1:100 to 1:80 (1.63). In another study (1.64), nitrogen balance was achieved in a group of malnourished patients, when a formula with a
calorie to nitrogen ratio of 1:150 was infused. Subsequently, a study conducted by Peter and Fischer (1.63) revealed a nitrogen to calorie ratio of 1:163 as most efficient for nitrogen equilibrium in non septic individuals. Cerra, et al (1.65) demonstrated that patients who were fed low non-protein calories to nitrogen ratio had significantly greater nitrogen retention (p< .05), increased plasma transferrin level (p< .05) and a lower respiratory quotient (RQ) (p< .05). This suggests that formula composition is also an important determinant for optimum nutritional outcome.

*Carbohydrate and fat allowances* to meet the requirements of the body under various condition varies. In order to minimise the risk of heart disease and cancer, the current recommendations are 50 to 60 % calories from carbohydrate and 30 to 35 % calories from fat sources (1.66). The same ratio is used for this experiment.

*Carbohydrate* In enteral formulae, the percentage of calories from carbohydrate ranges from 40 to 90 (1.67). With the exception of lactose, carbohydrate is an easily digested and absorbed component of most commercial formulae. Carbohydrate is present in different forms and concentrations, such as starch in the form of tapioca starch or modified food starch and glucose prepared by the partial hydrolysis of corn starch as well as monosaccharides, disaccharides and polysaccharides (1.38). The type of carbohydrate present in the formulae in turn affects the osmolality, sweetness and digestibility with shorter carbohydrate molecules being hyperosmolar, sweet and requiring less digestion than the longer ones (1.38).

*Starch* is composed of glucose molecules, which are either present in straight glucose chain (amylose) or in a form of branch chain (amylopectin). These chains vary in length from 400 to many thousand glucose molecules (1.67). Starch in the lumen is hydrolysed by the pancreatic enzyme, alpha amylase to dextrin, maltose and isomaltose. The types of starch source used in commercial formula and blenderised diets are cereal solids, food starch and to some extent fruits and vegetables (1.38). Starch is digestible and readily absorbed by the patients when it is
cooked (1.67). In addition to providing equivalent calories, it does not affect the osmolality of the formulae due to its high molecular weight (1.68).

**Polysaccharides and oligosaccharides** are glucose polymers which are the hydrolysates of starch (1.38). Polysaccharides are glucose polymers of more than 10 units length and oligosaccharides are glucose polymers from 2 to 10 units. The more the starch is hydrolysed, the higher its solubility. One advantage of using glucose polymers in enteral formulae is that they are more soluble than starch. Secondly, glucose polymers rapidly hydrolyse in the intestine and contribute less to the osmotic load of a formulae in comparison to glucose (1.38). As cited by MacBurney, Jones and associates (1.93) did a comparative study on the absorption of glucose, using three solutions containing 1). starch hydrolysate with 95% of its glucose as polymers greater than 10 glucose molecules (> G10), 2). an amylase hydrolysate of the > G10 solution and 3). free glucose. Data revealed that > G10 solution was efficiently absorbed in the jejunum in the absence of luminal amylase. Moreover, the > G10 hydrolysate was absorbed even faster than the glucose polymer or free glucose. The same investigator carried out another study to compare the jejunal absorption of three solutions, 1). maltotriose, 2). glucose oligomer (containing maltotriose, tetrose, pentose and heptose), and 3). free glucose. The glucose absorption from maltotriose and glucose oligomer solution was significantly better than that from free glucose (1.69). Intolerance to glucose polymers is rare but the final hydrolysis of starch is dependent upon the enzyme activity of the brush border and the small bowel function (1.38). These studies suggest that energy content may be increased without actually increasing the osmolality of the formulae for patients with normal G.I tract.

**Disaccharides** found in enteral formulae are lactose, sucrose and the byproduct of starch digestion, maltose. The presence of disaccharides in the formulae increases the osmotic concentration when compared to starch. In the healthy intestine maltose and sucrose are hydrolysed rapidly and lactose is hydrolysed at 50% of the rate of maltose (1.38). Several
jejunal perfusion studies have shown that the absorption of sugar may be enhanced with the addition of the disaccharide, sucrose. Sucrose is hydrolysed by sucrase to glucose and fructose. Fructose absorption is mediated by a carrier which is different from that required for glucose uptake (1.70-1.71). Low lactase activity is common in most racial and ethnic groups such as Blacks, Orientals, Indians and Jews. Several investigators have carried out studies on lactose absorption with subjects with confirmed lactose malabsorption. They concluded that it is the load (concentration) of lactose which is administered to patients at a given time which affects the malabsorption. If the load of lactose administered per unit time is low, symptoms will not occur (1.51, 1.71-1.72). However, symptoms will develop if feed is administered by the bolus technique (1.94).

Monosaccharides in enteral formulae containing glucose as a sole source of carbohydrate are very sweet and hypertonic (1.38). The early elemental diets contained glucose but due to its hypertonicity it was replaced in part by sucrose and later by glucose polymer mixtures (1.73-1.74). Rapid infusion of hypertonic formulae in large volume into the stomach or jejunum should be avoided in patients with gastrectomy and intestinal dysfunction because this infusion can induce rapid transit, glucose malabsorption, abdominal discomfort and diarrhoea (1.18).

Lipids' major role in enteral formulae is to provide a concentrated source of energy. The amount of lipid present in enteral formulae varies from less than 2% to 45% of the total calories (1.18). Like other nutrients, the efficiency of lipid absorption depends on the rate of infusion, the concentration of nutrients and the digestive and absorptive capacity of the gastrointestinal tract. The major sources of fat in standard formulae are butterfat from milk in lactose based formulae and corn, soy, safflower or sunflower oils, the medium chain triglycerides (MCT), lecithin, monoglycerides and diglycerides. Fat enhances the flavour and palatability of the formulae but does not affect its osmolality (1.38).
**Essential fatty acids** are usually provided in vegetable oils. Deficiency of essential fatty acid is unlikely in patients on formulae containing vegetable oil \((1.75)\). Essential fatty acid deficiency has been recorded in patients on parenteral nutrition, patients on a long term low fat diet, on a fat free elemental diet or on a diet containing MCT as a sole source \((1.76-1.77)\). The estimated requirement for essential fatty acids for adult is approximately 1 to 2% of the total calories \((1.78)\).

**Medium chain triglycerides** are used as an alternative to long chain triglycerides (LCT) in enteral formulae and provide 8.2 to 8.4 kcal/gram \((1.38)\). "Medium chain triglycerides (MCT) are made up of a mixture of C 6:0 (1-2%), C 8:0 (65-75%), C 10:0 (25-35%) and C 12:0 (1-2%) medium chain fatty acids (MCFAs) obtained by the hydrolysis of coconut oil followed by the fractionation of the fatty acids" \((1.79)\). They do not contain linoleic acid or \(\approx\) linolenic which are the essential fatty acid. Hence, long term use of medium chain triglycerides (MCTs) as the only source of lipid can lead to an essential fatty acid deficiency. However, its use in enteral formulae is advantageous because medium chain triglycerides are more water soluble and intraluminal hydrolysis is more efficient when compared to long chain triglycerides. Also, MCTs do not require pancreatic or bile salt for absorption and are transported directly into the blood via the portal system \((1.38)\). The use of MCTs are beneficial in fat malabsorption, pancreatic insufficiency, post gastrectomy small bowel resection and healing of fistulas \((1.80-1.81)\), but are not found superior in ketosis or acidosis because they are rapidly oxidised to provide ketone bodies \((1.80)\). Medium chain triglyceride oil should be gradually introduced in order to avoid side effects such as nausea, diarrhoea and abdominal distension because they may be rapidly hydrolysed intraluminally and increase osmotic load \((1.38)\). Currently in commercial formulae, MCTs are used in combination with long chain fatty acids due to their easy digestibility and absorbability \((1.38)\). Although medium chain triglycerides and long chain triglycerides compete for absorption, but if fed together, the absorption of both increases as compared to when one or the other is administered alone \((1.80-1.83)\).
Vitamins and minerals Complete enteral formulae contain a range of vitamins and minerals and are designed to provide adequate quantities for 1600-2000 kcal. A major concern exists for the patient on a low energy intake or on a diluted formulae, especially when very little data is available for vitamin requirements for sick patients. Current recommendations on therapeutic doses are that they do not exceed two to ten times the Recommended Daily Allowance (RDA) depending on the vitamin (1.84).
Chapter 1.3: Methodology

A methodology was developed for the theoretical development of indigenous enteral formulae, which involved the following steps:

1. Identification of food items

The food suitable for a population varies from place to place depending on the availability, cost, culture, food preference and religious beliefs. In order to provide a healthy diet, it is important that food items are in the right proportion and the diet is nutritionally balanced. Initially, 15 food items were chosen for the development of enteral formulae including rice, rice flour, wheat flour, sago, dried milk, whole milk, skimmed milk, yoghurt, egg whole, egg white, dried egg, peanuts, sesame seeds, honey and sugar (Table 1.3.1). Selection of these Pakistani food items was based on the consideration that they are:

- Available throughout the year
- Relatively inexpensive
- Easy to prepare using the local method of cooking
- Acceptable in taste and odour when prepared.

Table 1.3.1. Selected food items for indigenous enteral formulae

<table>
<thead>
<tr>
<th>Cereals</th>
<th>Legumes</th>
<th>Milk</th>
<th>Eggs</th>
<th>Nuts</th>
<th>Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>Mung beans</td>
<td>Whl. drd milk</td>
<td>Whl. drd. egg</td>
<td>Sesame seed</td>
<td>Plain sugar</td>
</tr>
<tr>
<td>Rice flour</td>
<td></td>
<td>Skm. milk</td>
<td>Whole egg</td>
<td>Peanuts</td>
<td>Honey</td>
</tr>
<tr>
<td>Sago</td>
<td></td>
<td>Yogurt</td>
<td>Egg white</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semolina</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2. Determining the nutritional composition of food items

The FAO Food and Nutrition paper # 26: Food Composition Table for the Near East 1982 (1.85) was used to determine the nutritive value and amino acid contents of the food items. The reason for using this Food Composition Table was to best represent the nutritive value of the Pakistani indigenous food items. Food Composition Table for Pakistan and Bowes and Church's Food Values of Portion Commonly Used, were also referred to for the food items not listed in the FAO paper (1.86 - 1.87).

3. Selecting Recommended Dietary Allowance (RDA) table:

The Recommended Dietary Allowance (RDA) for the Pakistani population lacks information on some vitamins and minerals. Therefore FAO Recommended Dietary Allowance for all nutrients (Table 1.3.2) and for essential amino acid (Table 1.3.3) was selected (1.88-1.90). The information was entered into a computer program Lotus® spread sheet to analyse nutrients furnished through 2000 mL of indigenous enteral formulae feed.

**Calories**  Recommended Allowance of food energy for a 70 kg, 177 cm male is 2000 kcal per day at 75 years of age and 2800 kcal per day for 21 years of age. A mean value of 2400 kcal was used as an energy requirement for this project (Table 1.3.2).

**Protein**  FAO Recommended Allowance for protein is .83 g per kg of body weight which comes to 58 g per day for the reference 70 kg male (Table 1.3.2). FAO recommendation for essential amino acids for the reference male are also listed in Table 1.3.3.

**Vitamins and minerals**

The FAO allowance for vitamins and minerals (1.89 -1.90) recommended for 70 kg reference man has been used in this project (Table 1.3.2).
Table 1.3.2. FAO Recommended Dietary Allowance for reference adult (1.88-1.90)

<table>
<thead>
<tr>
<th>kcal / kJ</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>2400 / 10032</td>
<td>58 g</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamins</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Ascorbic acid</td>
</tr>
<tr>
<td>650 RE</td>
<td>30 mg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minerals</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>Potassium</td>
<td>Calcium</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>500 mg</td>
</tr>
</tbody>
</table>

Table 1.3.3. FAO Recommended Essential Amino Acids Allowance (mg) for reference adult (1.88)

<table>
<thead>
<tr>
<th>Try</th>
<th>Thr</th>
<th>Iso</th>
<th>Leu</th>
<th>Lys</th>
<th>Meth / Cys</th>
<th>Phe / Tyr</th>
<th>Val / Arg</th>
<th>His</th>
</tr>
</thead>
<tbody>
<tr>
<td>245</td>
<td>490</td>
<td>700</td>
<td>980</td>
<td>840</td>
<td>910</td>
<td>980</td>
<td>700</td>
<td>840</td>
</tr>
</tbody>
</table>

4. Enteral diet formulation

A master sheet was developed which contained information on selected food items, their nutritional composition, fibre, moisture content and essential amino acid profile. Using the master sheet, a computer program was designed on Lotus spread sheet using its attachment “What If Problem Solver®” to achieve the best food combination, which satisfied the following specifications:

- Calories ratio from Carbohydrate: 50, Fat: 30, Protein: 20.
- Non-protein calorie to nitrogen ratio 100:1 - 150:1.
- Amino acid profile of the diet meeting the recommended allowance for the healthy adult population.
Additional equations were introduced into the programme to obtain information on the nitrogen content of diets, nitrogen conversion factor, and cost of the formula.

**Calorie ratio of macronutrients**

In order to calculate the calorie ratio of macronutrients, equations were fed into the program as indicated in Figure 1.3.1.

**Figure 1.3.1. Calories ratio of macronutrients**

1. **Calories from protein:**

\[
\% \text{ Protein} = \frac{\text{protein (g) per 100 g diet} \times 4}{\text{kcals per 100 g diet}}
\]

2. **Calories from carbohydrate:**

\[
\% \text{ CHO} = \frac{\text{Carbohydrate (g) per 100 g diet} \times 4}{\text{kcals per 100 g diet}}
\]

3. **Calories from fat:**

\[
\% \text{ Fat} = \frac{\text{Fat (g) per 100 g diet} \times 9}{\text{kcals per 100 g diet}}
\]

**Nitrogen conversion factor**

For this study, a calculated nitrogen conversion factor was used for each individual diet. The calculation of nitrogen factor was based on the proportion of food items present in each formulae. The nitrogen conversion factor of 6.25 was used for milk and egg, and 5.8 was used for cereals and seeds. These factors were than used to calculate the nitrogen factor for each enteral formula as illustrated in Figure 1.3.2.
Figure 1.3.2. Method for calculating the nitrogen conversion factor

\[
\begin{align*}
(A \text{ food} + B \text{ food} + C \text{ food} + D \text{ food} + E \text{ food}) \\
\text{Rice} + \text{ Milk} + \text{ S.seed} + \text{ Eggs} + \text{ Sugar}
\end{align*}
\]

\[
\frac{\text{Quantity per 100 g}}{100 \text{ g of food}} \times 5.8 = X
\]

\[
\frac{\text{Quantity per 100 g}}{100 \text{ g of food}} \times 6.25 = Y
\]

\[
X + Y = Z \times \text{NCF*}
\]

* Nitrogen conversion factor

**Nitrogen content of enteral diet**

Nitrogen content of indigenous enteral formulae was determined using the calculation below:

\[
\text{Nitrogen content} = \frac{\text{Protein in g (Formula X)}}{\text{Nitrogen conversion factor (Formula X)}}
\]

**Nitrogen : Calories ratio**

A formula used to calculate the amount of calories per gram of nitrogen present in a particular indigenous enteral formula is as follow:

\[
\text{Calories:Nitrogen ratio} = \frac{\text{Total nitrogen in g (Formula X)}}{\text{Total calories in enteral (Formula X)}}
\]

**Non protein calorie to nitrogen ratio**

The amount of non protein calories to each gram of nitrogen is calculated using the given below formulae:
Non protein calorie : Nitrogen = \( \frac{(\text{Total carbohydrate} \times \text{Formula X}) \times 4 + (\text{Total fat} \times \text{Formula X}) \times 9}{\text{Total nitrogen in g (Formula X)}} \)

The reason for determining the calories to nitrogen and non-protein calories to nitrogen ratio was to match the amount of substrate present in the indigenous enteral diet with the estimated substrate requirement. For this study, the estimated substrate requirement suggested for level 2 stress (low) (1.91) was used as a base for the development of indigenous enteral diets.

**Costing of indigenous enteral formulae**

The cost of each food item per 100 g was listed in the master sheet, which was carried forward to individual diet sheets with additional information. Each formula’s costing was based on the ratio of food items present in the diet and was determined in the following manner:

\[
\text{Cost per food item} = \frac{\text{Cost per 100 g}}{100} \times \text{Proportion of food}
\]

*Cost of food item: Pak Rs 18.00 = Aus $ 1.00 (1-8-91)*

Each food item was calculated separately as explained and added to determine the cost of formulae per 100 g.

**Computer aided diet formulation**

Initially, food items from each group (Table 1.3.1) were chosen to work on various combinations and ratios. These combinations and ratios were analysed by using "What if cell, Constraint cell and Target cell", in the computer which are the main features of the program (Figure 1.3.3).
Figure 1.3.3. Flow process for diet formulation

The amount of food items in 100 g was specified using *What if cell*,. Initially, the fixed values for protein, carbohydrate and fat were specified using *constraint cell* but the desired combinations could not be achieved. Hence, the approach was changed in which the range of values was tried using the same cell to allow flexibility. Using the *constraint cell*, calorie ratio for macronutrients, non protein calorie to nitrogen ratio and nitrogen content of enteral formulae were also specified. The total amount of food was restricted to 100 g per diet using *Target cell*. (Figure 1.3.4).

Figure 1.3.4. Use of ‘What If Problem Solver’ for diet formulation
Combinations chosen were then refined by adjusting the weight of food or substituting with another food item until a desired specification for enteral formula was met.

More than 100 combinations were formulated using this program. In this process, the best multimix were obtained, although sometimes no combination was found, in which case the computer was programmed to find the closest combination to the target range. Some multimix that were nutritionally within the target range were rejected due to inappropriate combination of food items in term of ratio, for example the amount of cereals was too low or egg quantity was too high in a particular formulation. In other cases, the food combination was perfect but the energy ratio was not desirable, such as the high fat content or low protein content in a particular diet.

**Diet screening**

Out of one hundred indigenous enteral formulations, six diets were short listed to assess the protein quality of indigenous diet formulae. An adjusted protein efficiency ratio (PER) assay as per AOAC 1990 specifications was used (1.92).

Two diets were also short listed for a pilot study to determine the efficacy of diet in improving the nutritional status of adult patients.
Chapter 1.4: Result

Master sheet

The nutritional composition of each food and its cost in Pakistani Rupees is given in Appendix 1.4.1.

Formulation of indigenous enteral formulae

Using different combinations and ratio of food items listed in the master sheet, theoretical formulation of more than 100 combinations were made possible. Some of these combinations are shown in Table 1.4.1.

Table 1.4.1. Indigenous enteral formulation process

<table>
<thead>
<tr>
<th>Food items</th>
<th>Diet # 4</th>
<th>Diet # 7</th>
<th>Diet # 11</th>
<th>Diet # 13</th>
<th>Diet # 22</th>
<th>Diet # 26</th>
<th>Diet # 31</th>
<th>Diet # 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice flour</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>Sago</td>
<td></td>
<td>20</td>
<td></td>
<td></td>
<td>15</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Mung beans</td>
<td></td>
<td></td>
<td>15</td>
<td></td>
<td>40</td>
<td></td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Egg whole</td>
<td></td>
<td>25</td>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Egg white</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whl. drd. egg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Whl. drd. milk</td>
<td>26</td>
<td>25</td>
<td>30</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skm. milk</td>
<td></td>
<td></td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yogurt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sesame seed</td>
<td>6</td>
<td>15</td>
<td>10</td>
<td>21</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Sugar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Honey</td>
<td>13</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>28</td>
<td>20</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>
Food composition of indigenous enteral formulae

Out of the various food combinations tried, the most appropriate combinations in term of food ratio and nutritional content were selected. Combinations short listed were mainly composed of food items shown in Table 1.4.2.

Table 1.4.2. Composition of enteral formulae

| Fresh whole egg |
| Whole dried milk |
| Rice flour |
| Sago |
| Sesame seed |
| Sugar |

Nutritional profile of the selected indigenous enteral formulae:

A nutritional profile of the indigenous enteral formulae are mainly comprised from information on the nutritional profile, non-protein calorie to nitrogen ratio and calorie to nitrogen ratio (Appendix 1.4.1). For the purpose of explanation as to how these diets were computed, six diets, short listed for an animal study are being discussed here. Since these diets were especially designed to conduct protein efficiency ratio (PER) assay to determine the protein quality, the nutritional composition of the diets are tailored to meet AOAC specifications (1.92) and not to the nutritional requirements for humans.

Nitrogen conversion factor and nitrogen content of indigenous enteral formulae

Since the calculation of a nitrogen factor was based on the proportion of food item in each formulae, all six formulations have their own nitrogen conversion factor. (Figure 1.4.2). Using the nitrogen conversion factor of
each enteral formulae, the nitrogen content of the selected diets are shown in Figure 1.4.1.

Figure 1.4.1. Nitrogen conversion factor and nitrogen content of indigenous enteral formulae

<table>
<thead>
<tr>
<th>Diet #</th>
<th>Nitrogen Conversion Factor</th>
<th>Nitrogen Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>5.89</td>
<td>1.89</td>
</tr>
<tr>
<td>18</td>
<td>5.81</td>
<td>1.90</td>
</tr>
<tr>
<td>19</td>
<td>5.81</td>
<td>1.89</td>
</tr>
<tr>
<td>23</td>
<td>5.81</td>
<td>1.90</td>
</tr>
<tr>
<td>21</td>
<td>5.89</td>
<td>1.88</td>
</tr>
<tr>
<td>26</td>
<td>5.96</td>
<td>1.89</td>
</tr>
</tbody>
</table>

Energy and nitrogen profile of the selected enteral formulae

Figure 1.4.2. shows the energy and nitrogen profile of the selected indigenous enteral formulae indicating the number of calories per gram of nitrogen. Further it gives the ratio between non-protein calories to nitrogen of each selected indigenous enteral formulae.

Figure 1.4.2. Nitrogen profile of the selected enteral formulae
Chapter 1.5: Discussion

This section particularly dealt with the theoretical development of indigenous enteral formulae whereby the combinations of indigenous enteral formulations were made.

To test whether theoretical formulation of enteral formulae is possible or not, a master sheet was designed in which the identified food items, their nutritional profile and cost were specified. The purpose of developing the master sheet was to try different combinations in order to choose the best combination for enteral formulae.

With the help of this master sheet, 100 combinations were developed. Working manually on these combinations would have been a very time consuming exercise. Hence a computer aided program was specifically designed to work on combinations to produce enteral formulation and to reveal information on the nutritional profile, calorie ratio, nitrogen conversion factor, nitrogen ratio and nitrogen content of the diet.

Initially, the computer was programmed to formulate enteral diet by feeding the specific quality of food items, using what if cell but this approach was found to be very time consuming. Therefore, the programme was refined to feed a quantity of each food item in ranges to allow flexibility in diet formulation.

Constraint cell was used to specify the desired calorie ratio for macronutrients, non protein calories to nitrogen ratio and nitrogen content of enteral formulae. Protein was the main constraint nutrient because it had the direct impact on the ratio of essential amino acid in the diet. In this exercise, often constraints were achieved but the ratio of food items was out of proportion. To overcome this problem, the food quantity was specified in ranges as mentioned in the What if cell.
*Target cell* was particularly helpful in a rapid determination of the diets. The total amount of food was restricted to 100 g/diet using *Target cell*. Formulations not meeting the amount of target cell were out listed.

This exercise proved that theoretical formulation of indigenous enteral diet was possible. However, the efficacy of these formulations required scientific testing. Thus, to assess the protein quality of indigenous enteral formulae, PER assay is being carried out on six diets. These six diets are selected out of 100 combinations and are specifically designed to conduct the assay. Nutritional profile of these diets and details of the study will be discussed in Stage 2.
Chapter 1.6: References


1.86 *Food Composition Table for Pakistan*. Dept of Agricultural Chemistry and Human Nutrition, NWFP. Agriculture University, Peshawar. 1985.


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### Appendix 1.A.1

#### Reference

- [Appendix 1.A.1](#)

#### Table

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<td>2.4.2.</td>
<td>Protein efficiency ratio (PER) of experimental and control diets</td>
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2: Abstract

The main objective of this experiment was to demonstrate the nutritional efficacy of the formulated diets because the ultimate use of these diets is meant for seriously ill patients. Though theoretically these diets appeared to be nutritionally adequate but required some scientific proof to show their effectiveness. Therefore, protein efficiency ratio was carried out on six selected diets.

Methodology involved processing and formulation of test diet as per AOAC (1990) specification. However, for the purpose of this study PER was modified with all the diets designed to be isonitrogenous but with slightly different carbohydrate and fat content.

The experiment was carried out in 3 batches and in each batch, 2 experimental diets and one control diet were used. In each batch 10 Sprague Dawley male rats aged 21-28 days old were randomly allocated. The animals were weighed at the initiation of the study, at the end of the adaptation period and thereafter every 7 days. Total weight gain was calculated at the end of each week. Food intake was recorded after every 3 days. On 28th day of the study period, the PER was calculated for each group from the total weight gain and protein consumption. The Tukey-HSD test of significance was used with significance level of 0.05.

Results showed that when compared to the control group, the food intake and weight gain of experimental group were high. Although the protein content of all experimental and control diet was similar, the PER of experimental diets were higher. Protein digestibility corrected amino acid Score (PDCAAS) was also calculated. Result indicated higher digestibility value for experimental diets # 1.1, 3.1 and 3.2 and low for diets # 1.2, 2.1 and 2.2. The results of PER and PDCAAS indicated that the indigenous enteral formulae can be developed using the multi-mix approach to yield a good quality protein formulae.
Chapter 2.1: Introduction

Statement of the problem

Theoretically the formulation of the selected indigenous enteral diets appeared to provide adequate nutrition. However, in order to demonstrate nutritional efficacy it is necessary to assess the quality of the protein in the six selected indigenous enteral formulae using protein efficiency ratio (PER) and protein digestibility corrected amino acid score (PDCAAS) as the parameters.

Aim

To assess the adequacy of selected indigenous enteral formulae in terms of:
- protein efficiency ratio and to compare the protein quality against a reference casein diet.
- protein digestibility corrected amino acid score and calculate the digestibility value.

Hypothesis

The protein quality of the selected indigenous enteral formulae is equivalent to or better than that of the reference casein diet and maintains the high digestibility value.
Chapter 2.2: Literature Review

Whether commercial or home made, the enteral formula must meet the patient’s nutritional requirements for normal maintenance and any increase demand related to the disease state and repair and repletion.

Protein content of the formula is most important. ‘Protein is the basis of essential body tissues and in a state of malnutrition, injury and infection depletion of body protein occurs’ Jeejeebhoy (2.1). The capacity of a protein to meet the nitrogen requirement of an organism is dependent on the amino acid composition and digestibility of the protein. The disease process may alter digestion and absorption. Age and associated chronic illness may also modify utilisation of dietary protein (2.2).

Amino acid structure:

Atoms of Carbon, Oxygen, Hydrogen and Nitrogen bond with each other to form amino acids which join together to form proteins by means of the peptide links (2.3). Conversely protein on hydrolysis yields amino acids. Hence protein nutrition is essentially amino acid nutrition (2.4).

All amino acids have the same basic structure, a carbon with three groups of atoms attached to it; an amino group (NH2), an acid group (COOH) and a hydrogen atom (H) and a fourth attachment, a group of atoms known as the side group or side chain that make each amino acid distinctive (2.3). Most naturally occurring amino acids are of the L configuration. The pattern of essential amino acid which is found in milk and egg is considered most suitable for building human body protein (2.5). This pattern may be taken as reference against which other proteins are measured.
Classification of amino acids

The question of which amino acids are indispensable for human is clearly important for maintaining good nutrition in healthy adults, for normal growth and development of infants and in disease state. Absence or deficiency of an indispensable amino acid from the diet can lead to negative nitrogen balance, weight loss and impaired growth in young children and clinical symptoms. Over 30 years ago, Rose (2.6) identified eight amino acid as nutritionally indispensable which are as follows:

- Isoleucine
- Leucine
- Lysine
- Methionine
- Phenylalanine
- Threonine
- Tryptophan
- Valine

More recently histidine has been added to this list of indispensable amino acid for both children and adults. In the past few years, several questions have raised concerning the indispensability of dispensable amino acids for specific populations such as pre-term and term infants, sick patients and individuals who require nutritional support. Jackson (2.7), Laidlaw and Kopple (2.8) have classified amino acids according to their functional role indicating that amino acids that are normally dispensable may become indispensable under certain physiological conditions. The classification is given below:

- **Totally indispensable amino acids** (lysine and threonine) Omission from diet can lead to serious nutritional and metabolic effects.

- **Carbon skeleton indispensable amino acids** (histidine, isoleucine, leucine, methionine, phenylalanine, tryptophan and valine) Through
transamination, the ketoacid or hydroxyacid of each amino acid can be converted into its respective amino acid

- **Conditionally indispensable amino acids** (cysteine, tyrosine) Can reduce the requirements for the indispensable amino acids methionine and phenyalanine respectively. Under certain conditions cysteine and tyrosine may become indispensable themselves.

- **Acquired indispensable amino acids** Cysteine and tyrosine become indispensable during immaturity of synthetic processes in the premature infants or new born. Cysteine, tyrosine, arginine and citrulline in genetic disorders or acquired disease state. Cysteine, tyrosine, arginine, possibly citrulline and taurine due to extended periods of large intake of amino acids eg. TPN.

- **Completely indispensable amino acids** (alanine, glutamic acid, aspartic acid, glycine, proline, glutamine, asparagine). These amino acids are extensively synthesised in the body and are not essential component of the diet.

**Importance of protein in the diet:**

Dietary protein is the source of essential amino acid and provides nitrogen for the synthesis of non essential amino acid (2.9). In the body, proteins are the structural constituents of the cell and function as enzymes, antibodies and hormones. An adequate diet whether from normal food or a specially formulated medical product must contain appropriate levels of protein including essential amino acids in addition to calories, vitamins and minerals to ensure maintenance and growth of the body and in the case of illness to restore tissues that are depleted (2.9).

**Protein quality:**

The quality of a protein depends upon its amino acids profile and "its capacity to meet the amino acid and nitrogen requirements of an organism"
(2.2, 2.10)". As indicated by Whitney and Rolfes (2.3) ‘An essential amino acid supplied in less than the amount needed to support protein synthesis is called a limiting amino acid’. The protein quality of a food is considered to be poor if any single essential amino acid is found short in supply relative to the amount needed for protein synthesis in the body.

Table 2.2.1. shows that for a normal adult 15% of the total amino acids intake should be from the essential amino acids. At least 40 percent of the total amino acids intake should be supplied from the essential amino acid to traumatised, catabolic or seriously undernourished patients to promote tissue restitution, a requirement which is similar to that of a growing child (2.11).

Table 2.2.1. Essential amino acid and protein requirements for human infants and adults*

<table>
<thead>
<tr>
<th>Amino acids (mg/kg body weight)</th>
<th>Infants</th>
<th>Adults</th>
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<tbody>
<tr>
<td>Isoleucine</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td>Leucine</td>
<td>161</td>
<td>14</td>
</tr>
<tr>
<td>Lysine</td>
<td>103</td>
<td>12</td>
</tr>
<tr>
<td>Methionine/Cystine</td>
<td>58</td>
<td>13</td>
</tr>
<tr>
<td>Phenylalanine/tyrosine</td>
<td>125</td>
<td>14</td>
</tr>
<tr>
<td>Threonine</td>
<td>87</td>
<td>7</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>17</td>
<td>3.5</td>
</tr>
<tr>
<td>Valine</td>
<td>93</td>
<td>10</td>
</tr>
<tr>
<td>Histidine</td>
<td>28</td>
<td>8-12</td>
</tr>
<tr>
<td>Total essential amino acids</td>
<td>742</td>
<td>91.5</td>
</tr>
<tr>
<td>Tot. amino acid requirements</td>
<td>1700</td>
<td>570</td>
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</table>

Essential amino acid as % of total* 43% 16%


Types of Food Proteins

- **Complete protein** foods are the ones, which contain all the nine
essential amino acids in sufficient quantity and ratio to meet the body’s need (2.12).

- **Incomplete protein** foods are deficient in one or more of the nine essential amino acids. Grains, legumes, nuts, seeds, vegetables and fruits are called incomplete protein food (2.12).

- **Complementary protein** foods may complement each other to provide adequate amounts of essential amino acids (2.3). For example wheat protein contains very little lysine and beans are high in lysine. If wheat and beans are eaten together, the amino acids will complement each other to provide a complete protein diet (2.5).

**Assessment of protein quality**

The quality component of the dietary protein not only depends upon the amount and the amino acid make-up of the protein but also upon its digestibility and bioavailability.

*Digestibility* is ‘a measure of the amount of amino acids absorbed from a given protein intake’ (2.3). The most complete protein is worthless to the body if it is not digestible. Protein digestibility not only depends on its configuration but on other factors such as other food items on the diet and the reactions that influence the release of amino acids (2.3).

*Bioavailability* is defined as the utilisation of nutrient compound after digestion and absorption (2.13). It is a long known as an important factor in determining the protein quality and is defined by Kies (2.14) as the degree to which amino acids or small peptides from a test protein consumed by a living organism ultimately are transported across the intestinal membrane and into the body.

*Bioavailability with reference to protein digestion* There are a number of factors in protein digestion which have an impact on bioavailability, such as protein configuration whereby fibrous protein tends to be insoluble and
resistant to digestion as compared to globular protein which is soluble and has a relatively high digestibility. Another factor is the stomach pH which has an effect on the efficiency of protein digestion. Moreover absolute or apparent lack of digestive enzymes can result in decreased digestibility of protein and in turn lower bioavailability (2.14).

**Bioavailability with reference to protein absorption** When talking about absorption, one may assume that the greater the degree of digestibility, the greater the degree of bioavailability. This is not alway the case. The timing of intake of an individual amino acid or a combination of amino acids has an influence on absorption due to a shared transport mechanism. Amino acid absorption mechanisms involve competition among amino acids for absorption sites. For efficient absorption, release of an ideal mixture of amino acids at the right time is stressed. The other factor which has an impact on absorption is whether the protein is available in the form of peptides or amino acids in the diet (2.15). Matthews and Adibi (2.16), Silk (2.17) and Selisinger, et al (2.18) indicated that the peptide based products are better utilised than the amino acid based products.

**Other factors affecting bioavailability**

1. **Species difference** Different animal species have different digestive systems due to physiological and biochemical differences within their gastrointestinal system, resulting in differing ability to digest cereal protein.

2. **Stress factor** Bioavailability of a protein can also vary considerably within an individual due to day to day physiological and psychological stress which can cause profound alteration in gastrointestinal activity.

3. **Constituents of food products** Protein contained in the bran fraction of cereal is not available to the human. A study by Kies and Fox (2.19) indicated that fibre intake adversely affected nitrogen balance and decreased apparent protein bioavailability at the low protein intake level.
Methods for evaluation of protein quality

To evaluate protein quality, several measures have been devised including amino acid scoring, biological value, net protein utilisation and the protein efficiency ratio.

*Amino acid score* is a method for determining the protein quality of food, approved by the FAO/WHO (2.20). The current method relates the amino acid profile of a food or a meal to that of a reference pattern based on the amino acid needs of a 2-5 years old child (2.20).

<table>
<thead>
<tr>
<th>Amino acid score</th>
<th>mg of amino acid in 1 g of test protein</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>mg of amino acid in 1 g of reference protein</td>
</tr>
</tbody>
</table>

Advantages of amino acid scoring are its convenience, scientific soundness and its ability to identify the limiting amino acid (2.21) and major weakness is that it does not predict the digestibility of a protein (2.3). In order to overcome the weakness, amino acid score value is then corrected for digestibility yielding what is now called a protein digestibility corrected amino acid score (PDCAAS). It is calculated by multiplying the lowest amino acid ratio X true protein digestibility (2.20).

*Biological Value* of a protein measures its efficiency in supporting the body needs (2.3). It is expressed as a percentage of nitrogen absorbed that is retained. Biological value is determined by nitrogen balance and is calculated as:

\[
BV = \frac{\text{Food } N - (\text{FN} - F0) - (\text{UN} - U0)}{\text{Food } N - (\text{FN} - F0)} \times 100
\]
Food N: nitrogen intake., U: urinary nitrogen., F: fecal nitrogen and Uo and Fo are urinary and fecal nitrogen excreted when subjects are maintained on nearly nitrogen free diet. It can be simply expressed:

\[
BV = \frac{N_{\text{retained}}}{N_{\text{absorbed}}} \times 100
\]

A protein with a biological value of 70 or greater can support human growth as long as energy intake is adequate, et al. (2.22) indicated that the lower the biological value, the greater the amount of a protein required to achieve nitrogen equilibrium. Table 2.2.2 shows the biological value (BV) of proteins commonly found in commercial formulae.

**Table 2.2.2. Biologic value of proteins found in commercial formulae**

<table>
<thead>
<tr>
<th>Protein source</th>
<th>Biologic value</th>
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<tr>
<td>Lactalbumin w/ methionine</td>
<td>130</td>
</tr>
<tr>
<td>Egg whole</td>
<td>100</td>
</tr>
<tr>
<td>Milk, cow</td>
<td>90</td>
</tr>
<tr>
<td>Lactalbumin</td>
<td>84</td>
</tr>
<tr>
<td>Soybeans</td>
<td>75</td>
</tr>
<tr>
<td>Casein</td>
<td>72</td>
</tr>
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</table>

*Adopted from Oser B.L.: JADA 27: 396 - 402, 1951 (2.4)

**Net protein utilisation** determines the proportion of nitrogen that is retained from a given amount of nitrogen consumed (2.3). Like the biological value, the intake of nitrogen and urinary and fecal nitrogen must be determined (2.23). The formula for calculating NPU is as follow:

\[
NPU = \frac{\text{Food } N (U - Uo) + (F - Fo)}{\text{Food } N} \times 100
\]
It is more simply expressed as:

\[
NPU = \frac{N_{\text{retained}}}{N_{\text{intake}}} \times 100
\]

**Protein efficiency ratio** was originally proposed by Osborne, et al (2.24) using gram gain per gram of protein eaten as a measure of nutritive value of protein for growing rats. Osborne proposed that the optimum protein is determined not only by the absolute amount furnished but also its quality. The protein efficiency ratio (PER) is expressed as:

\[
\text{Protein efficiency ratio} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}
\]

According to Geraldine, et al (2.25) the protein efficiency ratio (PER) method is the official method used in the United States and Canada. Pike and Brown (2.26) in support of the protein efficiency ratio assay (PER) mentioned that it is the simplest method for evaluating protein quality because it only requires accurate measure of food intake and weight gain. Although the PER assay is a simple and extensively used method for determining protein quality, it has some limitations. Its major limitation is that it does not differentiate between the protein utilised for maintenance and for growth (2.27 - 2.29).

**Factors affecting PER**

**Study period** According to AOAC (2.30) specifications, there should be a 28 days study period and 10 animals should be assigned to each treatment group (2.31). Geraldine, et al (2.25) have shown that the precision of PER values for animal protein sources was improved when the PER assay period was extended.
Age Chapman, et al (2.32) demonstrated that the PER value of casein is influenced by the age of the animal at the time of initiation of the study. It was noted that the rat's age was inversely related to PER. Weanling rats 21-28 days are usually used.

Sex Morrison and Campbell (2.33) have indicated that the PER value of a test material should be corrected for the sex of the animal used. The AOAC (1990) (2.29) specifications uses male animals.

Protein content According to Hurt, et al (2.34), the protein level of the test diet should be closely standardised. Canadian regulations propose a content between 9.7 and 10.3% (2.35). Mclaughlan, et al (2.36) reports that egg and casein proteins show a maximum PER at 8% protein level.

Fat content The official AOAC (2.30) procedure recommends a minimum fat level of 8% in the form of cotton seed oil. However moderate adjustment in the lipid level may be made without affecting the assay.

Mineral content Mineral composition has little if any effect on the biological evaluation of protein quality (2.34).

Fibre Content AOAC (2.27) specifications suggest a crude fibre level of 1% whereas Chapman., et al (2.32) propose a 5% minimum level. Hurt, et al (2.34) are of the view that the level of fibre is of relatively little importance in determining the protein quality provided that the fibre ratio of the test diet is adjusted to the control diet.

Net protein ratio devised by Bender and Doell (2.37) is calculated using the following formula:

\[
NPR = \frac{Wt\ gain\ of\ test\ group\ (g) + Wt\ loss\ of\ non\ protein\ group\ (g)}{Protein\ consumed\ (g)}
\]
This calculation is based on the assumption that the protein required to prevent weight loss of the rats fed the non-protein diet is the protein needed for maintenance. However, the NPR assay overestimates the value of poor quality protein and PER underestimates the value of low quality protein.
Chapter 2.3: Methodology

Processing of test diets

Experimental diets were prepared using food items which were purchased from a local Indian/ Pakistani grocery store in Australia. Preference was given to available Pakistani food items. A processing protocol was developed for preparing experimental diets as shown in Figure 2.3.1.

Figure 2.3.1. Processing Protocol

```
  Purchasing
    ↓
  Cleaning
    ↓
  Grinding
    ↓
  Mixing
    ↓
  as per diet specification
    ↓
  Storing
    ↓
(5°C - 10°C)
```

The food items which needed to be ground were rice, sesame seed, sago and mung beans. After grinding into flour, they were mixed as per diet specifications and stored in plastic containers between 5°C - 10°C for later use.

Formulation of experimental diets

Six experimental diets were formulated using different food items in varying ratios but bearing approximately equivalent amounts of protein, i.e.
10.56 g (1.82 g of nitrogen) per 100 g of diet. The six diets were therefore isonitrogenous.

The amino acid profile of the experimental diets was calculated using the analytical information on the food items received from CSIRO, Australia. The information on the amino acid composition of controlled diet was gathered by SIGMA ALDRICH PTY. LTD. Australia. Amino acid composition of mixtures and complete diets, indirectly reflects the protein quality and show how well a particular diet meets the essential amino acids need (2.38).

The maximum difference in calories among these diets was 28 kcal per 100 grams. Table 2.3.1. shows the formula compositions of the six experimental diets. Detailed nutritional composition of these diets is specified in Appendix 2.3.1.

**Table 2.3.1. Ingredients components (g/100 g) of experimental diets**

<table>
<thead>
<tr>
<th>Components</th>
<th>Batch # 1</th>
<th></th>
<th>Batch # 2</th>
<th></th>
<th>Batch # 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet # 1.1</td>
<td>Diet # 1.2</td>
<td>Diet # 2.1</td>
<td>Diet # 2.2</td>
<td>Diet # 3.1</td>
<td>Diet # 3.2</td>
</tr>
<tr>
<td>Rice flour</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>-</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Sago flour</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Mung beans</td>
<td>-</td>
<td>18.6</td>
<td>17.0</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Egg whole dried</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.7</td>
<td>-</td>
</tr>
<tr>
<td>Egg whole fresh</td>
<td>1.4</td>
<td>-</td>
<td>1.9</td>
<td>-</td>
<td>-</td>
<td>1.6</td>
</tr>
<tr>
<td>Milk whole dried</td>
<td>18.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13.3</td>
<td>34.7</td>
</tr>
<tr>
<td>Yogurt</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sesame seed</td>
<td>24.6</td>
<td>28.6</td>
<td>29.5</td>
<td>31.8</td>
<td>12.6</td>
<td>-</td>
</tr>
<tr>
<td>Honey</td>
<td>35.5</td>
<td>31.3</td>
<td>31.6</td>
<td>26.2</td>
<td>46.3</td>
<td>23.7</td>
</tr>
</tbody>
</table>

**Formulation of control diet**

A standard control diet for all three batches was formulated according to AOAC specification (2.30). Table 2.3.2. shows the composition of the control diet.
Table 2.3.2. Composition of a 100 g of the control diet

<table>
<thead>
<tr>
<th>Components</th>
<th>g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>11.74</td>
</tr>
<tr>
<td>Sucrose</td>
<td>55.86</td>
</tr>
<tr>
<td>Cotton seed oil</td>
<td>15</td>
</tr>
<tr>
<td>Ash</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin mix (Rat and mouse pre-mix)</td>
<td>1.4</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>1</td>
</tr>
<tr>
<td>Water</td>
<td>5</td>
</tr>
</tbody>
</table>

Addition of rat and mouse pre-mix to experimental diets

Table 2.3.3. shows the composition of rat and mouse pre-mix purchased from Fielder Agriculture Product, 53 Belmore Street, Tamworth, NSW 2340 (A Division of Barastoc Stock Feed PTY, Limited). Approximately 73% of this pre-mix consisted of vitamins and the remaining 27% of minerals.

The natural vitamin composition of the experimental diet was not equivalent to the control diet. Therefore for the purpose of this experiment, the equivalent amount of Fielders' Rat and Mouse pre-mix was added to all the experimental diets. Table 2.3.3. also indicates minerals that were present in the rat mix. However minerals which were lacking in the rat and mouse pre-mix were added as per AOAC nutrient content specification. The amount of minerals required to be added in per kg feed is indicated in Table 2.3.4.

The mineral content of the experimental diets was found to be approximately 50% that of the control diet. Thus, a mineral mix was added to the experimental diets to make up the 5 g of mineral mix present in the control diet.
## Table 2.3.3. Composition of Fielders’ Rat and Mouse pre-mix / kg feed

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>per kg</th>
<th>Minerals</th>
<th>per kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>12000 L.U</td>
<td>Copper</td>
<td>35 mg</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>2000 L.U</td>
<td>Iron</td>
<td>200 mg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>80 L.U</td>
<td>Magnesium</td>
<td>1500 mg</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>10 mg</td>
<td>Manganese</td>
<td>100 mg</td>
</tr>
<tr>
<td>Thiamine</td>
<td>20 mg</td>
<td>Selenium</td>
<td>0.1 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>10 mg</td>
<td>Iodine</td>
<td>4 mg</td>
</tr>
<tr>
<td>Pantothenate</td>
<td>20 mg</td>
<td>Zinc</td>
<td>50 mg</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>50 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>10 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td>6 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td>1 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>15 ug</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline</td>
<td>500 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BHT Antioxidant</td>
<td>25 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inositol*</td>
<td>100 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraminobenzoic acid*</td>
<td>100 mg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: *Tablets were ground to powder form to add in the mixture.

## Table 2.3.4. Composition of mineral mix (g/ kg feed)

<table>
<thead>
<tr>
<th>Components</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>.0093</td>
</tr>
<tr>
<td>Iodide</td>
<td>.0218</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>6.9733</td>
</tr>
<tr>
<td>Potassium Dihydrogen Phosphate</td>
<td>19.4733</td>
</tr>
<tr>
<td>Cobalt Chloride</td>
<td>.0012</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>19.0930</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.4074</td>
</tr>
</tbody>
</table>
Moisture content of the control and experimental diets

Moisture content of six experimental and a controlled diet was measured using a vacuum oven (2.39). Samples of each diet in duplicate were weighed and left overnight in a vacuum oven (approx 16 hrs). Next morning, these samples were taken out from the oven and were kept in the desiccator for cooling. After cooling, the samples were weighed again and the moisture content of the diet was measured using the following formula:

\[
\frac{\text{Loss in weight of sample}}{\text{Weight of sample}} \times 100 = \% \text{ Moisture}
\]

Selection, housing, adaptation and feeding of animals

Weanling Sprague-Dawley male rats aged 21 to 28 days at the start of experiment were obtained from SPF Unit at the University of NSW, Biological Facility, 1408 ANZAC Parade, Little Bay. The experiment was carried out in 3 batches and in each batch 2 experimental diets and one control diet were used to compare the protein efficiency ratio (PER). A batch consisting of 36 rats were obtained at the initiation of each experiment. For each batch the same procedure was followed. Rats were housed individually in plastic cages with wired screen lids on the top and fed a rat chow ad libitum for 3 days allowing for adaptation. At the end of the adaptation period, the rats were weighed and 3 groups of 10 animals in each group were allocated to experimental or control diets (Table 2.3.5). The average weight variation between the groups at the time of initiation of the study was less than 5 grams. Six rats in each batch with extremely low or high weights were discarded.

Determination of the nitrogen content of the diets

The nitrogen content of the control and experimental diets was measured using Kjeldhal method. Duplicate samples were analysed twice. Hence four readings for each diet were obtained. The three closest readings were
Table 2.3.5. Experimental design of animal study

<table>
<thead>
<tr>
<th>Adaptation time, days</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adaptation diet</td>
<td>Rat chow</td>
</tr>
<tr>
<td>Animal No/group</td>
<td>10</td>
</tr>
<tr>
<td>Test protein level</td>
<td>10.56 %</td>
</tr>
<tr>
<td>Test days</td>
<td>28 days</td>
</tr>
<tr>
<td>Data collection</td>
<td>Rat weight on arrival</td>
</tr>
<tr>
<td></td>
<td>Rat weight on 0 day of test</td>
</tr>
<tr>
<td></td>
<td>Rat weight gain after every 7 days</td>
</tr>
<tr>
<td></td>
<td>Food consumption after every 3 days</td>
</tr>
</tbody>
</table>

chosen to calculate the mean nitrogen content of each diet and the reading which had the largest variation (highest or lowest) was discarded. As explained in Stage I in figure 1.4.1, the nitrogen factor for each diet was calculated on the basis of the ratio of food items present in a particular diet.

**Food intake and weight gain**

The rats were weighed at the initiation of the study, at the end of the adaptation period and thereafter every 7 days. Total body weight gain (growth) was calculated at the end of each week. Food intake was recorded after every 3 days. The amount of spilled and leftover food was subtracted from the total amount provided in order to calculate the food intake of each individual rat.

**Determination of protein quality of the diets**

Protein quality was evaluated by determining the protein efficiency ratio. The PER was calculated for each group from total weight gain and protein consumption (2.40). The corrected PER was calculated using a factor of 2.5 for the control (casein) diet. The formulae used to determine PER and corrected PER are as follow:
\[ \text{PER} = \frac{\text{Weight gain of test animals (gms)}}{\text{protein consumed by test animals (gms)}} \]

Corrected \( \text{PER} = 2.5 / \text{PER of control diet} \times \text{PER of experimental diet} \)

**Statistical analysis**

The data were analysed by analysis of variance (ANOVA) using the statistical analytical system computer package (SPSS) to determine the differences in PER means among treatment groups. Differences between PER mean were considered statistically significant at \( P < 0.05 \) (or 95\% level of confidence). The Tukey - HSD test of significance was used with significance level of .05. Calculation of this test is as follow:

\[
(Mean (J) - Mean (I)) > = 0.1956 \times \text{Range} \times \sqrt{\frac{1}{N (J)} + \frac{1}{N (I)}}
\]

(with the following values \( s \) for range : 3.50)

**Amino acid score**

Amino acid ratios for 9 essential amino acid were calculated by using FAO/WHO/UNU suggested pattern of amino acid requirements for preschool children (2-5 yrs) as the reference protein. The formula used for calculating amino acid score is as follow:

\[
\frac{\text{mg of an essential amino acid in 1 g of test protein}}{\text{mg of same amino acid in 1 g of reference protein}} \times 100
\]

**Protein digestibility corrected amino acid score**

Protein digestibility of all six experimental diets was then calculated by the following equation:
True protein digestibility \( \times \) The lowest amino acid ratio

Digestibility factors of each food item in the diets were used from table 8 of reference 2.20.
Chapter 2.4: Results

Proximate nutrient composition of diets

The proximate composition of one control and six experimental diets is shown in Table 2.4.1. The restriction imposed on experimental diets was that they should be isonitrogenous even though different protein sources were used. In practice the Kjeldhal digestion method showed a minor difference of protein content which is indicated in Table 2.4.1. The fat content of diet # 3.2 in batch 3 was the lowest whereas the carbohydrate content was the highest of the experimental diets. This is most likely the result of the exclusion of sesame seed and the increase of milk whole dried in this particular diet. There were minor differences in the quantities of carbohydrate and fat in other experimental diets.

Table 2.4.1. Proximate composition of experimental and control diets per 100 g

<table>
<thead>
<tr>
<th>Composition*</th>
<th>Batch #1</th>
<th>Batch #2</th>
<th>Batch #3</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet #1.1</td>
<td>Diet #1.2</td>
<td>Diet #2.1</td>
<td>Diet #2.2</td>
</tr>
<tr>
<td>kcal</td>
<td>427</td>
<td>411</td>
<td>414</td>
<td>418</td>
</tr>
<tr>
<td>kJ</td>
<td>1793</td>
<td>1720</td>
<td>1732</td>
<td>1746</td>
</tr>
<tr>
<td>(Kjeldhal)</td>
<td>10.96</td>
<td>10.23</td>
<td>10.28</td>
<td>11.10</td>
</tr>
<tr>
<td>Carbohydrate %</td>
<td>55.7</td>
<td>58.4</td>
<td>57.9</td>
<td>56.9</td>
</tr>
<tr>
<td>Fat %</td>
<td>18.0</td>
<td>15.3</td>
<td>16.0</td>
<td>16.8</td>
</tr>
<tr>
<td>Moisture %</td>
<td>9.6</td>
<td>9.6</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Minerals (g/100 g)</td>
<td>2.2</td>
<td>1.8</td>
<td>1.8</td>
<td>2</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>2.8</td>
<td>3.2</td>
<td>3.2</td>
<td>3</td>
</tr>
<tr>
<td>Rat pre-mix</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>(g/100 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Nutrient composition is based on g/100 g mixture, which was calculated after adding Rat pre-mix and mineral mix.
Moisture content of the experimental and control diets

Figure 2.4.1. shows the moisture content of the experimental and control diets. In theory the percentage of moisture in all the experimental diets was 9.6% and in the control diet 10%. However in practice the moisture content of the experimental diets ranged from 12.68 - 14.33% and was 10.32% in control diet.

Amino acid profile of experimental and control diets (mg/100 g of feed)

The detailed composition of essential amino acid of experimental and control diets is indicated in Table 2.4.2. In all the experimental and control diets, the ratio of essential amino acid to total amino acid was greater than the 40% required for growth.
Table 2.4.2. Amino acid composition of experimental diets (mg/100 g of feed)

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Batch # 1</th>
<th>Batch # 2</th>
<th>Batch # 3</th>
<th>Controlled diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet #1.1</td>
<td>Diet #1.2</td>
<td>Diet #2.1</td>
<td>Diet #2.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>113</td>
<td>107</td>
<td>106</td>
<td>99</td>
</tr>
<tr>
<td>Threonine</td>
<td>370</td>
<td>373</td>
<td>375</td>
<td>366</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>400</td>
<td>434</td>
<td>433</td>
<td>422</td>
</tr>
<tr>
<td>Leucine</td>
<td>759</td>
<td>774</td>
<td>774</td>
<td>739</td>
</tr>
<tr>
<td>Lysine</td>
<td>391</td>
<td>473</td>
<td>463</td>
<td>476</td>
</tr>
<tr>
<td>Methionine</td>
<td>272</td>
<td>281</td>
<td>270</td>
<td>260</td>
</tr>
<tr>
<td>Cysteine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>820</td>
<td>869</td>
<td>870</td>
<td>841</td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>1319</td>
<td>1617</td>
<td>1629</td>
<td>1609</td>
</tr>
<tr>
<td>Arginine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>319</td>
<td>321</td>
<td>322</td>
<td>317</td>
</tr>
</tbody>
</table>

Nitrogen conversion factors

The nitrogen conversion factors for the experimental diets (Table 2.4.3) fell in the range of 5.81-5.99. For details see Appendix 2.4.1. For the control diet the nitrogen conversion factor was 6.25 (Table 2.4.3) as per AOAC specification (2.30).

Table 2.4.3. Nitrogen conversion factors for experimental and control diets

<table>
<thead>
<tr>
<th>Control diet</th>
<th>Batch # 1</th>
<th>Batch # 2</th>
<th>Batch # 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet #1.1</td>
<td>Diet #1.2</td>
<td>Diet #2.1</td>
</tr>
<tr>
<td>6.25</td>
<td>5.99</td>
<td>5.81</td>
<td>5.81</td>
</tr>
</tbody>
</table>

Protein efficiency ratio (PER)

Figure 2.4.2 shows that the protein efficiency ratio (PER) of experimental diets was higher than that of control diet in all three batches. The PER mean of all diets were also calculated to show the corrected PER mean. The data were further analysed by analysis of variance (ANOVA) to determine the difference with significance level .050 in PER means among
Figure 2.4.2. Protein efficiency ratio of experimental and control diets

![Graph showing protein efficiency ratio for different diets]

treatment groups. The statistical analysis showed that the PER means of the experimental diets were significantly higher than that of control diet in their respective batches (Table 2.4.4).

Table 2.4.4. Protein efficiency ratio (PER) means of experimental and control diets

<table>
<thead>
<tr>
<th>Batch # 1</th>
<th>Batch # 2</th>
<th>Batch # 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Diet # 1 1</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Diet # 1.2</td>
<td></td>
</tr>
<tr>
<td>1.984</td>
<td>3.040**</td>
<td>1.407</td>
</tr>
</tbody>
</table>

(*) Indicate significant difference

These diets were also short listed for the preparation of indigenous enteral formulae. The reasons for selecting the diets were as follow:

- High PER ratio as compared to the control diet.
• The food items and their ratio indicated ease in preparation, acceptability and palatability.

Growth performance of weanling rats fed the control and experimental diets

In this experiment the weight gain, protein intake and feed utilisation were observed for each rat in order to determine the protein efficiency ratio of the experimental diets. The first two variables may be measured independently and the third is interdependent.

*Weight gain* is a relatively sensitive indicator of the adequacy of amino acid supply (2.28). The average weight gain ± SD of rats fed the experimental diets over a 28 days period is shown in comparison to the control diet in Table 2.4.5. The weight gain with experimental diets in all three batches was higher than with the control diet. Diets 1.1 of batch # 1 and 3.1 of batch # 3 showed two fold growth.

**Table 2.4.5. Average weight gain of rats fed experimental and control diets**

<table>
<thead>
<tr>
<th>Batch # 1</th>
<th></th>
<th>Diet # 1.1</th>
<th>Diet # 1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>83 ± 5.64</td>
<td>81.5 ± 5.67</td>
</tr>
<tr>
<td>Average initial weight (g)</td>
<td></td>
<td>83 ± 5.64</td>
<td>81.5 ± 5.67</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>149.4 ± 12.25</td>
<td>212.9 ± 11.12</td>
<td>187.2 ± 10.03</td>
</tr>
<tr>
<td>Average weight gain in 28 days (g)</td>
<td>66.4 ± 9.42</td>
<td>131.4 ± 11.59</td>
<td>103.4 ± 10.80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Batch # 2</th>
<th></th>
<th>Diet # 2.1</th>
<th>Diet # 2.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>111.4 ± 11.17</td>
<td>108.8 ± 9.78</td>
</tr>
<tr>
<td>Average initial weight (g)</td>
<td></td>
<td>111.4 ± 11.17</td>
<td>108.8 ± 9.78</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>163.1 ± 10.20</td>
<td>232.3 ± 22.81</td>
<td>235.2 ± 19.64</td>
</tr>
<tr>
<td>Average weight gain in 28 days (g)</td>
<td>51.7 ± 7.48</td>
<td>123.5 ± 19.38</td>
<td>122.4 ± 11.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Batch # 3</th>
<th></th>
<th>Diet # 3.1</th>
<th>Diet # 3.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>87 ± 5.85</td>
<td>85.8 ± 7.35</td>
</tr>
<tr>
<td>Average initial weight (g)</td>
<td></td>
<td>87 ± 5.85</td>
<td>85.8 ± 7.35</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>149.5 ± 9.74</td>
<td>242.9 ± 17.12</td>
<td>195.4 ± 12.79</td>
</tr>
<tr>
<td>Average weight gain in 28 days (g)</td>
<td>64.7 ± 7.30</td>
<td>155.9 ± 12.64</td>
<td>109.6 ± 9.96</td>
</tr>
</tbody>
</table>

*Food intake* Table 2.4.6. shows that the average consumption ± SD of
control diet was less than that of the experimental diets. The experimental diets # 2.1 and # 3.1 provided the highest protein intake.

Table 2.4.6. Average feed intake of rats fed experimental and control diets

<table>
<thead>
<tr>
<th>Batch # 1</th>
<th>Control</th>
<th>Diet # 1.1</th>
<th>Diet # 1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. feed intake (g / 28 days)</td>
<td>304.4 ± 29.75</td>
<td>396.1 ± 28.29</td>
<td>404.2 ± 44.86</td>
</tr>
<tr>
<td>Av. protein intake (g / 28 days)</td>
<td>33.7 ± 3.29</td>
<td>43.4 ± 3.10</td>
<td>41.4 ± 4.60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Batch # 2</th>
<th>Control</th>
<th>Diet # 2.1</th>
<th>Diet # 2.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. feed intake (g / 28 days)</td>
<td>333.1 ± 43.55</td>
<td>499.3 ± 39.97</td>
<td>456.4 ± 31.92</td>
</tr>
<tr>
<td>Av. protein intake (g / 28 days)</td>
<td>36.3 ± 4.81</td>
<td>51.6 ± 3.98</td>
<td>50.7 ± 3.54</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Batch # 3</th>
<th>Control</th>
<th>Diet # 3.1</th>
<th>Diet # 3.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. feed intake (g / 28 days)</td>
<td>339 ± 33.94</td>
<td>479.8 ± 52.42</td>
<td>396.1 ± 31.29</td>
</tr>
<tr>
<td>Av. protein intake (g / 28 days)</td>
<td>38.1 ± 3.82</td>
<td>53.7 ± 5.87</td>
<td>42.3 ± 3.34</td>
</tr>
</tbody>
</table>

**Feed utilisation** Emphasis is also put on the feed utilisation whereby weight gain per gram of the feed is calculated as well as calculating the weight gain per gram of protein. Although the diets were all isonitrogenous, the experimental diets showed greater growth promoting power as compared with the control diet. Comparison between

Table 2.4.7. Average feed utilisation of rats fed experimental and control diets

<table>
<thead>
<tr>
<th>Batch # 1</th>
<th>Control</th>
<th>Diet # 1.1</th>
<th>Diet # 1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed utilisation (gain / g of food)</td>
<td>.22 ± .033</td>
<td>.33 ± .020</td>
<td>.26 ± .032</td>
</tr>
<tr>
<td>Feed utilisation (gain / g of protein)</td>
<td>1.97 ± .292</td>
<td>3.03 ± .196</td>
<td>2.50 ± .325</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Batch # 2</th>
<th>Control</th>
<th>Diet # 2.1</th>
<th>Diet # 2.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed utilisation (gain / g of food)</td>
<td>.16 ± .014</td>
<td>.25 ± .036</td>
<td>.27 ± .023</td>
</tr>
<tr>
<td>Feed utilisation (gain / g of protein)</td>
<td>1.40 ± .123</td>
<td>2.39 ± .351</td>
<td>2.42 ± .187</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Batch # 3</th>
<th>Control</th>
<th>Diet # 3.1</th>
<th>Diet # 3.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed utilisation (gain / g of food)</td>
<td>.19 ± .018</td>
<td>.32 ± .028</td>
<td>.28 ± .021</td>
</tr>
<tr>
<td>Feed utilisation (gain / g of protein)</td>
<td>1.70 ± .162</td>
<td>2.90 ± .257</td>
<td>2.59 ± .175</td>
</tr>
</tbody>
</table>
experimental diets showed that weight gain had no direct relationship with protein intake, rather it was the quality of protein and amino acid composition that made the difference in weight gain. Table 2.4.7. indicates that diet # 1.1 of batch # 1 caused relatively more gain in weight/ per gram of protein in comparison to diets # 2.1 and 3.1 of batch # 2 and 3 respectively whose intake was much higher.

**Amino acid score** Table 2.4.8 shows the amino acid score of experimental diets. Lysine found to be the first limiting amino acid that is .90, .81, .79, .79 in diets # 1.1, 1.2, 2.1 and 2.2 respectively. Diets # 3.1 and 3.2 did not have any limiting amino acids and score of 1 (100%).

**Table 2.4.8. Amino acid score and protein digestibility corrected amino acid score of experimental diets**

<table>
<thead>
<tr>
<th>Diets #</th>
<th>Amino acid score</th>
<th>Protein digestibility corrected amino acid score</th>
<th>The first limiting amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>.90 (90%)</td>
<td>.83 (83%)</td>
<td>Lysine</td>
</tr>
<tr>
<td>1.2</td>
<td>.81 (81%)</td>
<td>.69 (69%)</td>
<td>Lysine</td>
</tr>
<tr>
<td>2.1</td>
<td>.79 (79%)</td>
<td>.68 (68%)</td>
<td>Lysine</td>
</tr>
<tr>
<td>2.2</td>
<td>.79 (79%)</td>
<td>.68 (68%)</td>
<td>Lysine</td>
</tr>
<tr>
<td>3.1</td>
<td>1 (100%)</td>
<td>.94 (94%)</td>
<td>-</td>
</tr>
<tr>
<td>3.2</td>
<td>1 (100%)</td>
<td>.95 (94%)</td>
<td>-</td>
</tr>
</tbody>
</table>

**Protein digestibility corrected amino acid score** of six experimental diets was calculated which is shown in table 2.4.8. Diets # 3.1 and 3.2 had high PDCAAS of .94 (94%) and .95 (95%) respectively. Diet # 1.1 had score of .83 (83%) whereas diets # 1.2, 2.1 and 2.2 had low PDCAAS of .69 (69%), .68 (68%) and .68 (68%) respectively due to deficiency of lysine.
Chapter 2.5. Discussion

The protein efficiency ratio (PER) remains the method of choice for evaluating the protein quality of a wide variety of food stuff (2.36) in the absence of detailed information on the amino acid requirement for human despite of its shortcomings such as lack of accuracy, poor reproducibility and high cost (2.41 -2.42). However, very recently a committee of joint FAO/WHO consultation on protein quality evaluation has concluded that the casein/rat growth assay procedure do not accurately judge a food protein for human diets and the use of amino acid score related to human requirement would provide a realistic basis for defining the value of food protein based on human needs rather than the need of growing rats (2.20). Therefore in order to assess the protein quality of experimental diets both these method were used: protein efficiency ratio (PER) and protein digestibility corrected amino acid score (PDCAAS).

In this study PER was modified with all the diets designed to be isonitrogenous but with slightly different carbohydrate and fat contents. This variation was due to the use of selected food items and their contribution in 100 g of food mix to achieve the level of protein content as specified by AOAC (1990) (2.30). All other components were equivalent to control diet (Table 2.4.1).

A comparison of the control and six experimental diets in Table 2.4.1. shows that in theory the protein content of all diets was similar but when determined through Kjeldhal, there was slight variation. The reason may be the under or over estimation of food items transcribed from the references. However the difference is too small to impact on the PER.

A comparison of essential amino acid profile in control and experimental diets (Table 2.4.2) shows negligible difference. Moreover, the ratio of essential amino acids to total amino acids in the control diet is in excess to the minimum requirement (ie 40%) for establishing human growth (2.40).
Despite an adequate amount of essential amino acids present in the control diet, the PER was quite low when compared to experimental diets. There is no obvious reason for such low PER of control diet except the low intake of diet by control groups as compared with their respective experimental groups or other reason could be that casein furnishes only 70 - 87% of the sulfur amino acid required by growing rats (2.20). Since casein powder was not supplemented with the sulfur amino acid, it might have affected the growth rate of control group. This is further supported by the feed utilisation data which indicated less weight gain per gram of protein of control group. In any event, the experimental diets clearly out performed the control diet.

When compared within experimental diets protein efficiency ratio (PER) of diet # 1.1 of group 1 and diets # 3.1 and 3.2 of group 3 showed higher PER when compared with other experimental diets. A combination of cereal and animal protein in these diets may have resulted in a superior protein quality. These finding are in accordance with the others previously reported by and Pellet and Mitchell, et al (2.43 - 2.44). They showed that PER value of food mixes was comparable to casein and lactalbumin based diets and rice based diets gave better growth than wheat and millet (2.44). This fact was also observed by Hernandez and associates (2.45) that milk-rice and milk-corn mixtures had a protein quality similar to that of milk alone with similar PER and digestibilities.

Since protein efficiency ratio does not take into consideration the factors of digestibility, protein digestibility corrected amino acid score (PDCAAS) was determined. Initially amino acid score determined the first limiting amino acid which was lysine for the experimental diets # 1.1 and 1.2 of group 1 and 2.1 and 2.2 of group 2 where as diets # 3.1 and 3.2 had no limiting amino acid.

Protein digestibility corrected amino acid score data supported the PER results for experimental diets whereby diets # 1.1 of group 1 and 3.1 and 3.2 of group 3 had relatively higher PDCAAS as compared with diets # 1.2 of group 1 and 2.1 and 2.2 of group 2. The higher digestibility score of
these experimental diets could be due to appropriate ratio of vegetable and animal protein sources. Studies of protein digestibility on human indicated high true protein digestibility value of 93 - 100% for animal food and food products and low protein digestibility value of 70 - 85% for different beans and lentils (4.46 - 4.47). The low protein quality of vegetable protein source can be improved by the addition of animal protein sources which is apparent from protein digestibility corrected amino acid value of experimental diets # 1.1, 3.1 and 3.2, which had egg and milk in sufficient quantity to increase the digestibility value. Whereas diets # 1.2, 2.1 and 2.2 had low protein digestibility value ranging from 68 - 69% due to low ratio of animal protein source.

In summary, the main purpose of conducting the PER and PDCAAS were to assess the protein quality of food mixes at the initial stage of development. The ultimate use of these formulations are for the development of indigenous enteral formulae for seriously ill patients whom nutritional requirements cannot be met because of insufficient food intake or no oral intake at all. Moreover, not only was the protein quality of the experimental diets determined, but also the most appropriate formulation for short listing further experiments was ascertained. Additionally, this study strengthened the idea that an acceptable protein quality enteral diet may be developed from an indigenous source.
Chapter 2.6: References


2.45 Henanadez, M., Montalvo, I., Sousa, V., Sotelo, A.: The protein
efficiency ratio of 30:70 mixtures of animal:vegetable protein are similar or higher than those of the animal foods alone. *J. Nutr.* 1996; 126 (2) 574-581


| 1.60 | 1.50 | 1.40 | 1.30 | 1.20 | 1.10 | 1.00 | 0.90 | 0.80 |
| 1.60 | 1.50 | 1.40 | 1.30 | 1.20 | 1.10 | 1.00 | 0.90 | 0.80 |
| 1.60 | 1.50 | 1.40 | 1.30 | 1.20 | 1.10 | 1.00 | 0.90 | 0.80 |
| 1.60 | 1.50 | 1.40 | 1.30 | 1.20 | 1.10 | 1.00 | 0.90 | 0.80 |
| 1.60 | 1.50 | 1.40 | 1.30 | 1.20 | 1.10 | 1.00 | 0.90 | 0.80 |
| 1.60 | 1.50 | 1.40 | 1.30 | 1.20 | 1.10 | 1.00 | 0.90 | 0.80 |
| 1.60 | 1.50 | 1.40 | 1.30 | 1.20 | 1.10 | 1.00 | 0.90 | 0.80 |
| 1.60 | 1.50 | 1.40 | 1.30 | 1.20 | 1.10 | 1.00 | 0.90 | 0.80 |
| 1.60 | 1.50 | 1.40 | 1.30 | 1.20 | 1.10 | 1.00 | 0.90 | 0.80 |

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<td>Protocol for the preparation of partially digested diets</td>
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<td>3.4.5</td>
<td>Cooking method # 5 for the development of partially digested diets</td>
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<td>Nutritional profile of selected diets</td>
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3: Abstract

This part of the study deals with the preparation trial for the development of an indigenous enteral formula, furnishing one calorie / ml at full strength and maintaining a macronutrient energy ratio of 50: 30: 20 carbohydrate, fat and protein. Moreover, to select the ones which are easy to prepare, safe to administer and cost effective.

The methodology used for the development of these enteral formulae involved:

- Theoretical diet formulation using varied food combinations.
- Preparation methods for the development of polymeric and partially digested diets.
- Physical and chemical analysis of indigenous enteral formulae.
- Microbiological screening of diets.

Twenty nine diets out of 100 were selected for the preparation of polymeric and 13 out of 29 were chosen for the preparation of partially digested diets. Two techniques: incubation and cooking were used and under each technique various preparation methods were tried. Incubation technique did not give favorable results. Cooking methodologies # 13 for polymeric and # 5 for the partially digested diets gave the desired results.

Both methodologies are easy to prepare, less time consuming and cost effective as compare to other preparation methods. These methodologies were later tried on various diets and diet # 4a and 8a were ultimately shortlisted for pilot study. Both of these diets meet the desired energy ratio and nitrogen to calorie ratio. They are isocaloric, homogenous and easy to administer.

Bacteriological screening indicated a need for an aseptic technique for the preparation of these feeds to minimize contamination.
Chapter 3.1: Introduction

The advantage of enteral nutrition over parenteral nutrition is that gut integrity and function are maintained and administration is safe and cost effective. Enteral nutrition has advanced significantly from blenderised food to highly refined predigested foods which are commercially available in developed countries (3.1). In developing countries such food have limited application due to their high cost, limited shelf life and erratic supply. Under these circumstances, it would be useful to develop an affordable enteral product prepared with local food items. As patients cannot afford long stays in the hospital, it is important that such formulae should be easy to prepare and administer to patients at home also.

Statement of the problem

This stage of the study deals with the preparation trials for the development of an indigenous enteral formula, and to select one which is simple to prepare, safe and cost effective. Further, an effort will be made to provide one calorie per mL, maintaining a macronutrient energy ratio of 50:30:20 for carbohydrate, fat and protein and a nonprotein calories : nitrogen ratio in the range of 100:1 - 150:1. The feed will be isotonic and capable of being administered orally or through a tube.

Aim

The purpose of this section is to identify a simple and cost effective preparation method for developing an indigenous enteral formula.

Objectives

1. To try various preparation methodologies for an indigenous enteral formulae.
2. To shortlist a preparation technique which is simple, safe and cost effective.
3. To prepare an isotonic indigenous enteral feed furnishing 1 calorie per mL.
4. To develop an indigenous enteral formulae for patients with an intact digestive system as well as for the compromised digestive tract.

**Hypothesis**

An indigenous enteral formula is simple, safe, cost effective and meets the specified macronutrients energy ratio and non protein calories to nitrogen ratio.
Chapter 3.2: Literature Review

There has been an increased growth of commercial enteral products over the past decade which has stemmed from an increased awareness by health care providers of patients’ nutritional need (3.1). Enteral food may be used as a complete diet furnishing the total nutritional requirement of a patient or as a food supplement. In preparing an enteral formulae the patient’s energy, protein, mineral, vitamin, electrolyte and water requirements should be taken into consideration as well as the disease process which may influence the ability to digest and absorb food (3.1).

Non-commercial liquid food for tube feeding has the advantage over commercial formulae in that the carbohydrate, protein, fat and fluid content can be tailored to an individual patient’s needs and is relatively less costly (3.1). However there are problems associated with its preparation and delivery such as viscosity and bacterial contamination.

General considerations

Since this project specifically deals with the preparation techniques for the development of an indigenous enteral formula, emphasis will be placed on the food components which are used for the formulation, their nutritional composition and desirable mix and methodology to ensure minimum contamination. “In food, the purpose of supplementation is to bring about a meaningful interrelationship among constituents in such a fashion that obvious benefits are obtained in the resulting composite products”(3.2). In this project, specific benefits resulting from supplementation are related to the nutritional value of the product and its affordibility.

Physical characteristic of enteral formulae

Physical characteristics of enteral formulae may affect the patient’s tolerance, by causing gastric retention, diarrhoea and constipation. These symptoms may be the result of hyperosmolality nutrient density, caloric density, microbial contamination of feed, inappropriate administration technique or drug interaction. (3.1).
**Osmolality**

Electrolytes have a major effect on the osmolality of enteral formulae. Large molecular weight carbohydrates such as starch and glucose polymers exhibit less osmotic pressure in a solution than smaller units such as sucrose or glucose. Therefore juices and drinks provided in clear liquid and full liquid diets range in osmolality between 565 - 836 mOsm/kg (3.3) and should be diluted to less than 400 mOsm/kg.

Food increases the osmolality of the gastric juice but a normally functioning stomach empties isotonic solution into the duodenum. In disease states however, or after prolonged fasting, the stomach may function less effectively and either empty less rapidly leading to distension or empty more rapidly causing hyperperistalsis and diarrhoea. (3.4 - 3.5). Zarling, et al (3.6) found no significant difference in symptoms in patients, given isotonic or hyperosmolar solutions. Other studies (3.7 - 3.8) have shown that nutrient absorption is relatively more efficient with moderately hyperosmolar solutions. Gastric emptying is slowed by solutions with osmotic concentration higher or lower than 200 mosm, the higher the osmolality, the greater the inhibitory effect (3.9). Hypertonic formulae fed into the stomach especially in bolus form, may cause gastric retention and vomiting (3.9). However, Keohane, et al (3.10) have suggested that poor tolerance is more often the result of antibiotic treatment rather than a hypertonic diet.

Proteins when intact are very large molecules and exert little or no osmotic effect in solution. The more the protein is hydrolysed, the higher is the osmolality of the product. Fat does not have a profound effect on osmolality due to the way it is digested and transported across the cell membrane. However, medium chain triglycerides (MCT) are more osmotically active than other types of fats (3.1).

The major contributing constituents of formulae are: urea (breakdown product of protein) and the electrolytes: sodium, potassium and chloride. Collectively they make up the renal “solute load”. Enteral formulations that produce a large renal solute load may lead to clinical dehydration. The kidneys are only able to concentrate urine to a certain extent (about 1300 mosm/kg of urine) in the normal healthy adult and there is an obligatory
loss of water with each unit of solute (3.11). It is estimated that the minimal amount of water required to excrete 1 g of nitrogen is 40 to 60 mL (3.12). Dehydration and hyperosmolarity associated with high protein tube feeding have been reported in several studies (3.11, 3.13 - 3.16). In most of these studies, the dehydration or negative water balance was due to the increased renal solute load secondary to a protein intake of 150 - 200 g/day in 2000 to 3000 mL of fluid. The renal solute load was increased further due to liberal amount of sodium and potassium present in the formula. In some situations, this condition is further aggravated due to negative nitrogen balance seen in some critically ill patients or the specific dynamic action of a high protein intake (3.14, 3.17 - 3.18). The steps to prevent hyperosmolarity associated with high protein tube feeding include lowering the protein to water ratio in enteral formula and monitoring the patients’ fluid status (3.11).

**Palatability**

Although palatability is a crucial factor, it is highly subjective and highly variable. Generally formulae with fibre are less palatable than those without fibre. Formulae of intact protein are more palatable as compared to elemental formulae. Moreover, liquid formulae taste better than powdered formulae reconstituted with water (3.19).

**Rheology**

Rheology is defined as a science concerned with the law of deformation of various materials. Deformation is the process of changing the relative position of a part of a material. Viscosity is the principal parameter that characterises the flow properties of fluids (3.20). It can be divided into two general types:

1. Reversible deformation called elasticity.
2. Irreversible deformation called flow.

**Types of flow**

Products which are being evaluated for viscosity are classified as Newtonian or non-Newtonian.
**A Newtonian** product is characterised by a viscosity which is independent of the shear rate at which it is measured. For example water, mineral oil, liquid lecithin and various syrups.

**A Non-Newtonian** product is dependent on the shear rate at which it is measured. For example emulsions, dispersions, cake frosting and beaten egg white.

In medical foods, generally the non-commercial blenderised products are more viscous as compared to commercial enteral products.

**Bacterial contamination**

Regardless of where it is prepared, food can become microbiologically contaminated in many ways (3.21), examples are as follows:

1. During slaughtering
2. Through mishandling of food
3. Stored at ambient temperature after cooking
4. Poor facility for storing food.

Food contamination can lead to mortality and morbidity. It is widely recognised that enteral feed do become contaminated during preparation and administration (3.22 - 3.24). Studies have shown that gross contamination of both commercial and home made feed in the hospital may occur with bacterial counts up to $10^9$ organism per mL (3.25 - 3.27).

Although, no universal definition of unacceptable contamination exists and it is not known at what bacterial count contamination will cause infectious complications. However, based on Milk Standards and the Center for Disease Control Standards for food borne disease, unacceptable contamination may be defined as bacterial counts $\geq 10^5$ CFU/mL (3.28-3.30). Kohn (3.31) in her study defined bacterial count of $\geq 10^5$/mL in enteral formula unacceptable. Further, she defined unacceptable contamination of enteral feed with bacterial count exceeding 100 cfu/mL or with any one of the following organism; E.coli, Clostridium, Salmonella, Staphylococcus aureas, Bacillus cereus or Klebsiella.
Enteral formulations provide a rich source of nutrients for contaminating organisms. Once contaminated, rapid growth may occur. There are many potential source of contamination which are listed in Figure 3.2.1.

**Figure 3.2.1. Potential sources of enteral feed contamination***

<table>
<thead>
<tr>
<th>Potential sources of feed contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exogenous</strong></td>
</tr>
<tr>
<td>Kitchen environment</td>
</tr>
<tr>
<td>Feed component</td>
</tr>
<tr>
<td>Preparation utensils</td>
</tr>
<tr>
<td>Reservoir container</td>
</tr>
<tr>
<td>Sub-optimal storage condition</td>
</tr>
<tr>
<td>Handlers (dietetic, nursing, medical)</td>
</tr>
<tr>
<td><strong>Endogenous</strong></td>
</tr>
<tr>
<td>Patients</td>
</tr>
<tr>
<td><strong>Ward environment</strong></td>
</tr>
<tr>
<td>Air and dust</td>
</tr>
<tr>
<td>Administration technique</td>
</tr>
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</table>

*Intensive Care 1991: p 135-139 (3.46)

*Kitchen environment* Casewell (3.32) and Cooke (3.33) have shown that the main source of Klebsiella in food is the hospital kitchen where liquidisers, ice-cream utensils, dish cloths, water taps, sinks and work surfaces provided a reservoir for the organism. The organism was also isolated from a cutting knife, a scrapping machine and a chopping board. Several studies (3.32, 3.34-3.35) have shown contamination of feed during mixing and during the diluting stage as a result of contamination of the mixers and blenderisers used in preparation of hospital food.

Nasogastric feeds left at room temperature for several hours or delay in transport to the ward provide a further opportunity for bacterial multiplication (3.36). Diet reconstituted with unsterile water may rapidly become contaminated, but even sterile water does not guarantee that a diet will remain uncolonised (3.36). Moreover suboptimal storage of nutrient solution before administration to the patient may result in bacterial contamination.
**Ward environment** In a study by Kresky (3.37) "sterile water", hexachlorophene soap dispensers and sink taps have yielded Klebsiella species, and other gram negative bacilli. Hospital flower vases have been shown to contain gram negative bacilli with counts ranging from $10^4$ to $10^6$ organism per mL (3.38).

**Air and dust** Several studies (3.39 - 3.40) have indicated that air in the hospital ward may contain 1-5 microgram per mL so the possibility of feed contamination from the air would appear to be quite low. However, Miller and Logan (3.41) indicated that feeds which were kept open became contaminated with a variety of gram negative rods and yeast, where as no contamination was detected in closed bags. Hansen and Helper (3.42) concluded that the closed system gave significantly better protection against microbial contamination.

**Nasogastric feed ingredients** Regardless of whether feeds are commercially made or prepared in hospitals, using dried whole milk, raw eggs, sugar and water, failure to follow sanitation procedures in preparation and handling can lead to bacterial growth.

**Handling** Studies have shown that the level of contamination is proportional to the extent of handling required in setting up the system (3.43- 3.44). Anderton and Aidoo (3.45) have shown that the use of sterile gloves in setting up enteral feed delivery systems prevents initial contamination. Administration sets should be changed at least every 24 hours especially in a warm climate or in a ward setting (3.46).

**Duration of feeding** Continuous feeding at 125 mL/ hour as opposed to bolus feeding predisposes toward bacterial contamination (3.47).

**Temperature** A temperature ranging 20-25°C, commonly found in hospitals provides a suitable environment for bacterial growth (3.23). Patients receiving tube feeding are generally in a poor state of health and their immunity is compromised. Administration of contaminated feeds to these patients may result in colonisation, infection and or food poisoning. In a situation where the use of commercial formulae is not possible, care should be taken to prepare home based feed under aseptic condition, avoid delays between preparation and administration, strict hygiene precaution
during preparation and handling of feeds and regular monitoring of the microbial quality (3.23).

Cost of enteral feed

Commercial products are preferred for their safety. In developing countries where availability of these products is a major issue, it is necessary to look at the cost effective indigenous options for nutritional support. Even in developed countries escalated medical care cost has forced the health providers to look for economical options.

Role of enzyme in enteral formulation (3.48)

A compromised digestive system may require hydrolysed starch or hydrolysed protein for maximum absorption. In such circumstances, enzymes are useful.

More than 80% of all industrial enzymes are hydrolytic in action and are used for the depolymerisation of natural substances. Sixty percent of them are proteolytic, 3% are amylases and the remainder are lipases.

Sources of enzymes

Enzymes are available from plant, animal and microbial sources. Dry products have a longer shelf life than liquids and cool conditions extend enzymatic time further (3.49). Enzymes may be used to:

1. Reduce viscosity
2. Improve extraction
3. Make bioconversion
4. Cause separation
5. Change functionality
6. Synthesise chemicals
7. Improve cleaning

Selection of enzymes

Many factors are involved in the selection of enzymes such as specificity,
pH, temperature, availability and cost.

**Types of enzyme (3.48)**

**Protease** enzymes are those which hydrolyses peptide bonds in proteins. Those attaching at the C terminus are called carboxypeptidases and those at the N terminus are called aminopeptidases. Protease enzymes are categorised into two on the basis of their actions, which are indicated below:

*Exoprotease* systematically cleave amino acids from either N or C terminus of the protein.

*Endoprotease* cleave interior of peptide bonds. The hydrolysed products are usually smaller polypeptides and peptides.

The degree of hydrolysis or the number of peptide bonds hydrolysed depend on the specificity of the enzyme and on the condition of the substrate. Not every "susceptible" bond is accessible to the enzyme. For example, protease will make fewer cuts on a globular protein in its native state than on the one which has been denatured and opened up.

**Amylase** accelerates the hydrolysis of starch. Starch in its native state is a polymer made up of glucose linked together to form either linear polymers called amylase or branched polymers called amylopectin.

Starch is gelatinised to promote hydrolysis. When this happens, viscosity rises to a maximum and the starch becomes more susceptible to hydrolysing enzyme. Two kinds of amylase of commercial importance are available:

*α - Amylase* which randomly cleaves interior bonds of starch to yield dextrin.

*β - Amylase* which cleaves off maltose units from ends of the starch chain.

**Lipases and phospholipases** are enzymes that hydrolyse fats and oils and
attack only the ester bonds (a combination of a carboxylic acid and an alcohol) in these compounds.
Chapter: 3.3. Methodology

Theoretical diet formulations using varied food combinations

The 100 formulae combinations (stage 1) were initially manipulated using a procedure, which is indicated in Figure 3.3.1.

**Figure 3.3.1. Protocol used for enteral formulation**

![Diagram]

*For details see Appendix 3.3.1

These 100 combinations were investigated for formulation of enteral diets and only 29 (Appendix 3.3.2) were selected for preparation trials using selection criteria.

**Selection criteria:**

1. Appropriate food ratio especially 20% cereal content.
2. Macronutrient energy ratio approximately Carbohydrate: Protein: Fat; 50:20:30 respectively.
3. Formulation furnishing 1 kcal / 1 mL at full strength.
4. Nitrogen : Nonprotein calorie ratio within the range of 1:100 to 1 :150
5. Cost effective.

The other 71 combinations were rejected because they did not meet one or more specifications of the criteria.
Preparation methods for the development of indigenous enteral formulae

This exercise involved preparation of polymeric diets for patients with a functionally intact digestive system and partially digested diets for patients with a compromised digestive system. Initially polymeric enteral diets were developed involving various preparation trials and the selection of the most appropriate procedure. At the initial stage, experiments were carried out to yield 300 mL of enteral feed furnishing 1 kcal per mL at full strength. Subsequent experiments were carried out to yield 600 mL, 1200 mL of full strength and half strength polymeric diets. The whole process of standardising the preparation methodology involved optimising the quantity of water, emulsifier, thickener, preparation and cooking time.

Polymeric diet

Preparation methods for polymeric diet

Initially rice water was prepared using a ground rice and water ratio of 20 : 80. This ratio is being used to prepare rice ORS (oral rehydration salt) for anti-diarrhoeal treatment at the Aga Khan University Hospital, Karachi, Pakistan. Sesame seed was processed in a blender and strained through muslin cloth before adding to the mixture. Two techniques used for the preparation of polymeric diets are mentioned below:

Incubation technique

In this technique feeds were incubated at 65°C. To prevent the mixture from getting viscous due to gelatinisation of starch, malted barley was added at different stages of preparation which is shown in Table 3.3.1.

Cooking technique

Rice water was prepared as before and 20% malted barley was added at different stages of cooking to observe any change in the feeds’ viscosity. Other ingredients were added and various cooking methods were tried, as described in Table 3.3.2.
Table 3.3.1. Incubation technique for preparation of polymeric diet

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method 1.</strong></td>
<td>Twenty percent malted barley was added in the prepared rice water. It was then incubated at 64°C for 20 minutes. Feed was strained through muslin cloth after mixing with other ingredients.</td>
</tr>
<tr>
<td><strong>Method 2.</strong></td>
<td>All the ingredients were mixed. After adding 20% malted barley, feed was incubated for 20 minutes at 64°C. Feed was not strained.</td>
</tr>
<tr>
<td><strong>Method 3.</strong></td>
<td>All the ingredients were mixed together except egg. After adding malted barley, it was incubated for 20 minutes at 64°C. Strained and then egg added.</td>
</tr>
<tr>
<td><strong>Method 4.</strong></td>
<td>Feed was prepared without adding malted barley. Rice water was prepared, other ingredients were added and then the mixture was strained.</td>
</tr>
</tbody>
</table>

Table 3.3.2. Cooking techniques for the preparation of polymeric diets

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method 1.</strong></td>
<td>Rice water was cooked without malted barley. Other ingredients were added and the feed was strained through muslin cloth.</td>
</tr>
<tr>
<td><strong>Method 2.</strong></td>
<td>All ingredients were mixed and was cooked without malted barley. The feed was not strained.</td>
</tr>
<tr>
<td><strong>Method 3.</strong></td>
<td>Rice water was cooked using malted barley. Other ingredients were mixed and the feed was strained through muslin cloth.</td>
</tr>
<tr>
<td><strong>Method 4.</strong></td>
<td>All ingredients were mixed and cooked with 20% malted barley for 10 minutes. After cooling, the feed was strained through muslin cloth.</td>
</tr>
<tr>
<td><strong>Method 5.</strong></td>
<td>Rice water was cooked and poured into a blender after cooling. Other ingredients were weighed and put into a blender for 2 minutes. Feed was strained after blending.</td>
</tr>
<tr>
<td><strong>Method 6.</strong></td>
<td>Same as method # 5. The feed was not strained after blending.</td>
</tr>
<tr>
<td><strong>Method 7.</strong></td>
<td>In order to overcome the dispersion problem, an emulsifier lecithin was added using the same method as #5. In this method feed was not strained.</td>
</tr>
<tr>
<td><strong>Method 8.</strong></td>
<td>Same as method # 7. The feed was strained.</td>
</tr>
<tr>
<td><strong>Method 9.</strong></td>
<td>All ingredients were weighed and mixed in 300 mL of water except egg. The mixture was cooked till it gelatinized. It was cooled down to 30 - 40°C. Egg and 4 g of lecithin were added and blenderized for one and a half minutes on high speed. The feed was strained using a muslin cloth.</td>
</tr>
<tr>
<td><strong>Method 10.</strong></td>
<td>Same as method # 9, but standardised the water quantity to yield 300 mL furnishing 1 calorie per mL at full strength.</td>
</tr>
<tr>
<td><strong>Method 11.</strong></td>
<td>Same as method # 9, but standardised the lecithin quantity to yield 300 mL furnishing 1 calorie per mL at full strength.</td>
</tr>
<tr>
<td><strong>Method 12.</strong></td>
<td>Same as method # 9, but standardised the blending time to yield homogenous feed.</td>
</tr>
<tr>
<td><strong>Method 13.</strong></td>
<td>All ingredients were weighed and put into a saucepan except egg. The mixture was cooked till it gelatinized (approx 10 -12 minutes at 90 - 100°C). It was cooled down to 30 - 40°C. Two gram of lecithin were added and blended at high speed for 1 minute. Egg white was added and blended for half minute at medium speed and strained through a strainer.</td>
</tr>
</tbody>
</table>
Use of thickener

For half strength polymeric diets, different thickeners as listed below were used:

* Psyllum husk
* Corn flour
* Vegetable gelatin
* Guar gum

Use of emulsifier

Originally an emulsifier was not added to the feed, but after observing the settling down of food particles, an emulsifier (lecithin) was added to obtain a homogenised feed. The quantities used were as follows:

* 0.4 g of lecithin per 300 mL yield.
* 0.2 g of lecithin per 300 mL yield.
* 0.1 g of lecithin per 300 mL yield.
* 1.0 g of lecithin per 300 mL yield.
* 2.0 g of lecithin per 300 mL yield.
* 4.0 g of lecithin per 300 mL yield.

Partially digested diets

* Preparation methods for partially digested diets

In the preliminary stages, the diets used for the development of polymeric diets, were as well tried for the preparation of partially digested diets. In a series of experiments different preparation methodologies and different enzymes for the hydrolysis of protein and carbohydrate and were tried. Initially, experiments were carried out to yield 300 mL of partially digested diets furnishing 1 kcal/mL. Later experiments were conducted to yield 600 mL and 1200 mL full strength and half strength partially digested diets. Two techniques were used to prepare partially digested diets as mentioned below:
**Incubation technique**

In the incubation technique, four different sets of experiments were carried out, whereby different enzyme for starch liquification and protein hydrolysis were used. In these experiments, incubation period was varied to determine the incubation time required for optimum enzyme activity. Details are shown in Table 3.3.3.

**Cooking techniques**

Partially digested diets were prepared using the cooking methodology. This technique involved series of experiments, whereby termamyl was used for starch liquification and alcalase for protein hydrolysis at different ratio. In this series of experiment, the cooking time required for optimum enzyme activity was also determined. Details are stated in Table 3.3.4.

**Use of thickener**

Partially digested diets demonstrated reduced viscosity and a very fast flow rate. Hence it became imperative to use a thickener to increase the viscosity in order to control the flow rate. Below mentioned thickeners were used in different ratio and the most suitable thickener was shortlisted for the preparation of partially digested diets.

* Psyllum husk
* Guar gum
* Vegetable gelatin
* Corn flour

**Use of emulsifier**

For partially digested diets, an emulsifier in varying quantity was used to obtain homogenous feed. The details are as follow:

**Treatments**

* 0.4 g of lecithin per 300 mL yield.
* 0.2 g of lecithin per 300 mL yield.
**Table 3.3.3. Incubation technique for the preparation of partially digested diets**

<table>
<thead>
<tr>
<th>Preparation methodologies</th>
</tr>
</thead>
</table>

**Method 1.** All ingredients were mixed and a blend of malted barley (for starch liquification) and neutrase enzyme (protein hydrolysis) in different quantities were used per 100 g of solid. The mixtures were incubated for 30 minutes, boiled for 1 minute to inactivate the enzyme activity and strained through muslin cloth. The same methodology was used for all five treatments.

**Treatments**
- 2 g malted barley, .5 mL neutrase, .2 g lecithin.
- 2 g malted barley, 1 mL neutrase, .2 g lecithin.
- 2 g malted barley, 2 mL neutrase, .2 g lecithin.
- 5 g malted barley, .5 mL neutrase, .2 g lecithin.
- 2 g malted barley, 2 mL neutrase, .2 g lecithin.

**Method 2.** All ingredients were mixed. Two mL of neutrase was added. Incubated as specified at 64°C. Boiled for 1 minute. Blenderised and strained through muslin cloth.

**Treatments**
- 30 minutes incubation period.
- 60 minutes incubation period.
- 90 minutes incubation period.

**Method 3.** All ingredients were mixed. Two mL of alcalase (protein hydrolysis) was added and incubated as specified. Boiled to inactivate the enzyme. Two mL of termamyl (starch liquification) was added and the diets were cooked on stove for 5 minutes at 100°C. Cooled to 30 - 40°C, 0.2 gm of lecithin was added, blenderised and strained through muslin cloth.

**Treatments**
- 30 minutes
- 60 minutes
- 90 minutes

**Method 4** Rice was mixed with water and boiled to gelatinized. Two mL of termamyl was added and the preparation were cooked for 15 minutes. The rest of the ingredients were added along with 2 mL of alcalase and incubated for the time listed. The preparations were boiled for 1 minute to inactivate the enzyme activity and 0.2 g of lecithin was added. The diets were blenderised and strained through muslin cloth.

**Treatments**
- 30 minutes
- 60 minutes
- 90 minutes
Table 3.3.4. Cooking technique for the preparation of partially digested diets

<table>
<thead>
<tr>
<th>Methods of preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method 1.</strong> Rice flour mixed with 350 mL of water and cooked to boil. One mL of termamyl was added and cooked at 80 °C for the time specified. Cooled to 45 - 55 °C, mixed other ingredients and 1 mL of alcalase and incubated for the time specified below. Boiled to inactivate the enzyme, cooled to 30 - 40 °C; 0.2 g of lecithin was added and blenderised for 1 minute at high speed and strained through muslin cloth.</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>- 15 minutes cooking with termamyl + 60 minutes incubation after alcalase addition.</td>
</tr>
<tr>
<td>- 30 minutes cooking with termamyl + 90 minutes incubation after alcalase addition.</td>
</tr>
<tr>
<td>- 60 minutes cooking with termamyl + 120 minutes incubation after alcalase addition.</td>
</tr>
<tr>
<td><strong>Method 2.</strong> Ingredients except egg were mixed in 400 mL of water; 3 mL of termamyl was added and the diet was cooked for 15 minutes at 90 °C, then cooled to 30 - 40°C. Egg and 3 mL of alcalase were added and incubated for 60 minutes; boiled to inactivate the enzyme and cooled to 30 - 40°C. 0.2 g of lecithin was added, blenderised for a minute and strained through muslin cloth.</td>
</tr>
<tr>
<td><strong>Method 3.</strong> Diet # 4a and 8a were chosen to conduct this experiment. All ingredients were mixed in 400 mL water to yield 300 mL of feed,. The diets were cooked to boil, than 1 mL of termamyl was added and cooked for another 15 minutes at 95 - 100°C. The preparations were cooled to 55 - 65°C and egg white and 2 mL of alcalase were added and kept in water bath for half an hour at temperature 55 - 65 °C, The solution was then boiled to inactivate the enzyme, cooled to 40°C 0.5 mL of lactozym and .2 g of lecithin were added and blended for 2 minutes and strained. Using the same preparation methodology, alcalase and termamyl in different quantities were tried for 600 mL and 1200 mL yield.</td>
</tr>
<tr>
<td><strong>Method 4.</strong> In this series of experiment, different amount of enzymes were tested on diet # 4a to yield 600 mL. All ingredients were mixed in 800 mL of water, 3 mL of termamyl was added and was cooked for 15 minutes at 90°C. The mixture was cooled to inactivate enzyme, then gg was added and alcalase as specified below:</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>- Add 2 mL of alcalase and keep in water bath for 30 minutes at temperature ranging between 55 - 65°C. Add .2 g lecithin and blenderize for 3 minutes.</td>
</tr>
<tr>
<td>- Add 3 mL of alcalase and follow the treatment # 1.</td>
</tr>
<tr>
<td>- Add 1 mL of termamyl and cook for 10 minutes. Add 2 mL of alcalase and follow the treatment # 1.</td>
</tr>
<tr>
<td>- Add 2 mL of termamyl and cook for 10 minutes. Add 1 mL of alcalase and follow treatment # 1.</td>
</tr>
<tr>
<td><strong>Method 5.</strong> All ingredients except egg were mixed and boiled. Four mL of termamyl was added and cooked for 10 minutes at temperature 90 - 100°C. Cooled to 60°C and 2 mL of alcalase and egg was added and left in hot water sink maintaining the temperature between 55- 65°C for 30 minutes. Boil to inactivate the enzyme. Cooled to 40°C. Add .2 g of lecithin and .2 mL of lactase and blenderised for 2 minutes and strained.</td>
</tr>
</tbody>
</table>
* 0.1 g of lecithin per 300 mL yield.
* 1.0 g of lecithin per 300 mL yield.
* 2.0 g of lecithin per 300 mL yield.
* 4.0 g of lecithin per 300 mL yield.

**Physical and Chemical analysis of indigenous enteral formulae**

Physical and chemical properties of indigenous enteral formulae were analysed to ensure that they were similar to commercial formulae and safe to administer.

**Viscosity measurement**

Viscosity is the measure of the internal friction of a fluid. This friction becomes apparent when a layer of fluid is made to move in relation to another layer. The greater the friction, the more the force required to cause this movement. This is called “shear”. Highly viscous fluids, therefore require more force to move than less viscous materials. Viscosity of indigenous formulae was measured to make sure that formulae were of liquid consistency to pass through 8 -12 size French feeding tube. Viscosity of the diets was measured at varying spindle size and speeds, using the Brookfield Synchroelectric Dial-reading Viscometer (Appendix 3.3.3).

**Flow rate assessment**

Enteral feeding pumps are not used in Pakistan. For tube feeding, 8 French and 12 French calibre tubes are used, because they are commonly available in Pakistan. The flow rate of polymeric and partially digested diets was assessed to ensure smooth flow through different size feeding tubes. The flow rate was determined using a gravity clamp controlled Kangaroo feeding set (Sherwood Medical) of 500 mL capacity with 8 or 12 French calibre tubing (Appendix 3.3.4).

**Moisture content**

Moisture content was determined to make sure that the moisture content of indigenous enteral formulae was equivalent to commercial enteral
products. Moisture content of the diets was determined using a hot air oven (3.50). This method involved the measurement of weight loss due to evaporation of water (Appendix 3.3.5).

**Protein content determination**

The protein content of prepared (dry basis) and post prepared polymeric (dry basis) diet was determined using the Kjeltec system according to the Kjeldahl method (3.51). This method is based on rapid digestion in a heating block followed by steam distillation with automatic dilution and addition of an alkali. The distillate is collected in a receiver flask for subsequent titration and calculation (Appendix 3.3.6).

**Osmolality measurement**

In order to determine the osmolality of polymeric and partially digested diets, the Fiske Ms Cryoscope # 3,203,226 was used. The osmolality is measured as the magnitude of the freezing point depression in 0°C. The formula used is:

\[
\Delta T = K_f m
\]

Where \(\Delta T\) is the freezing point depression in °C,

\(K_f\) is a proportionality constant, the molal freezing point depression constant

and \(m\) is the molality of the solution that is the number of moles of solute dissolved in 1000 g of solvent". (Glasstone and Lewis 1964) (3.52).

The underlying principle is that the more molecules in a solution, the lower the freezing point will be. Using the same principle, freezing point of the enteral formulae (polymeric and partially digested diets) was determined to calculate the osmolality on the Cryoscope calibrated -.600 to -.408. The same calibration is used to determine the freezing point of milk which is isotonic (Appendix 3.3.7). To determine the osmolality, freezing point of the polymeric and partially digested diets were assessed on the cryoscope and osmolality was calculated using the following equation:

\[
\text{Osmolality} = \frac{\text{Freezing point of the diet}}{1.86} \times 1000
\]
**Trichloroacetic acid (TCA) Solubility Index**

This test was specifically used for enzyme treated enteral feeds (partially digested feeds) to estimate the percentage of protein hydrolysed to small peptides (Appendix 3.3.8).

**Microbiological screening**

Bacteriological tests were carried out to assess the safety of polymeric and partially digested diets using a procedure stated in Appendix 3.3.9. Preliminary testing of a prepared enteral formulae was undertaken to determine the microbiological status of the product and to assess whether hygienic, but not non-aseptic conditions would yield a safe product. Result of this initial testing demonstrated the need for a more stringent preparation methodology.

**Figure 3.3.2 Sampling plan for bacteriological testing of enteral feeds**
A second production run was undertaken and further testing was performed to assess microbiological status of the product at various stages of production. A flow chart of the formulae preparation methodology was drawn and three sampling points were chosen, which are indicated in Figure 3.3.2. Testing of these samples identified the critical control points (CCP) of the process, providing the basis for the Hazard Audit and Hazard Analysis Critical Control Point (HACCP) system (3.56).

A third set of tests were carried out to assess the efficacy of the HACCP system. Sampling of the products occurred at the same three sampling points (Figure 3.3.2) and results were compared to those obtained from the second test. Additional testing was carried out on the ingredients deemed to be especially prone to contamination. Of these lecithin is the most important because it is added after the final heating step and when the temperature has dropped to 40°C. Coliform and plate count tests were carried out using the procedure prescribed by NSW Dairy Corporation (3.53).
Chapter 3.4: Results

Polymeric diets

Protocol for the preparation of polymeric diets

Twenty nine diets were shortlisted for the preparation of indigenous enteral formula. Preparation methodologies for polymeric diets were carried out as per preparation protocol stated in Figure 3.4.1. Based on

Figure 3.4.1. Protocol used for shortlisting polymeric diets

- 29 diets originally shortlisted for enteral formulations
  ↓
  Preparation experiments for polymeric diets
  
  Preparation criteria
  
  * Ease of preparation
  * Minimum food handling
  * Safe to administer
  * Cost effective preparation method

Analysis

1. Nitrogen content
2. Moisture content
3. Viscosity measurement
   viscous enough to flow through 12 French tube. (Targeted flow rate: Not more than 120 mls/hr)
4. Microbiological count
5. Osmolality

Selected 7 diets

Selected 2 diets
Rejected 5 diets

Rejected 22 diets

Basis of rejection:
Lengthy preparation method
Food handling was maximum
Very viscous or too thin consistency
Expensive
certain preparation criteria and physical and chemical analysis of the final product, the polymeric diets were further shortlisted.

Rheological properties of polymeric diets

*Incubated diets*

Viscosity and flow properties of the four diets prepared by incubation techniques were assessed as shown in Table 3.4.1. Diets treated with malted barley were comparatively less viscous due to the saccharification of starch. However, the flow properties of the diets without malted barley treatment indicated that the diets can be passed through a 12 French tube without clogging. Therefore, polymeric diets do not necessarily need to be treated with malted barley. The incubation technique was cumbersome and involved considerable handling of food and extended cooking time. Consequently, the incubation technique for preparing polymeric diets was rejected.

**Table 3.4.1. Rheological characteristics of polymeric incubated feeds**

<table>
<thead>
<tr>
<th>Technique #</th>
<th>Viscosity</th>
<th>Flow status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spindle size</td>
<td>Speed (RMP)</td>
</tr>
<tr>
<td>*I.1</td>
<td>UL</td>
<td>30</td>
</tr>
<tr>
<td>*I.2</td>
<td>UL</td>
<td>30</td>
</tr>
<tr>
<td>*I.3</td>
<td>UL</td>
<td>30</td>
</tr>
<tr>
<td>*I.4</td>
<td>UL</td>
<td>30</td>
</tr>
</tbody>
</table>

* Incubation

*Cooked diets*

Rheological properties of these feeds were assessed (Table 3.4.2) and

**Table 3.4.2. Rheological properties of polymeric cooked feeds**

<table>
<thead>
<tr>
<th>Technique #</th>
<th>Viscosity</th>
<th>Flow status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spindle size</td>
<td>Speed (RMP)</td>
</tr>
<tr>
<td>*C.1</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>*C.2</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>*C.3</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>*C.4</td>
<td>1</td>
<td>30</td>
</tr>
</tbody>
</table>

* Cooked
found to be more viscous when compared with incubated feeds. The flow rate assessment revealed that clogging of the feed can be avoided by straining the feed. Hence reduction in viscosity was not required for the polymeric diets. In order to obtain homogenised feeds, 2 g of lecithin per 300 mL yield proved to be effective.

After conducting the series of experiment listed in Table 3.3.2 (pp14). The cooking methodology # 13 was found to meet the preparation criteria:

* Minimum food handling
* Minimum preparation time
* Simple cooking procedure
* Safe to administer
* Homogenous feed.

Therefore method # 13 is considered as standard method for the preparation of polymeric enteral formula (Figure 3.4.2).

**Figure 3.4.2. Cooking method # 13 for the development of polymeric diets**

1. All ingredients are weighed and put into a saucepan except egg.
2. Cook the mixture till it gelatinized (approx 10 -12 minutes at 90 - 100° C).
3. Cool to 30 - 40°C.
4. Add 2 g of lecithin and blend on a high speed mixer for 1 minute.
5. Add egg white and blend for 1/2 minute on medium speed.
6. Strain the mixture through sterile muslin strainer.

To select the most appropriate diet formulation with acceptable rheological characteristics, the cooking methodology # 13 was tried on 29 diets (Appendix 3.3.2). A Brookfield viscometer was used to determine the viscosity of the diets and 12 and 8 French size feeding tubes were used to assess the flow rate (Table 3.4.3).

The assessment of rheological characteristics of the feed revealed that food composition of some formulae are not suitable for enteral feeding either because the consistency was too thin and the flow rate could not be
controlled or the consistency was too viscous to pass through 12 French size nasogastric feeding tube. Feeds with flow rates greater than 120 mL/hour in an 8 French tube were eliminated from the list.

Since diets # 4a and 8a gave the desired flow rates, they were further tested as a half strength feeds. This led to a reduction in viscosity which in turn affected the flow rate. In order to control the flow rate, psyllium husk and guar gum were used to increase the viscosity. An attempt was made to add thickener after straining which resulted in curdling of feed. Hence, the use of a thickener for 1/2 strength polymeric diet was eliminated.

**Table 3.4.3. Rheological characteristics of prepared polymeric feeds**

<table>
<thead>
<tr>
<th>Option #</th>
<th>Viscosity</th>
<th>Flow rate</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet # 1</td>
<td>1</td>
<td>30</td>
<td>49</td>
</tr>
<tr>
<td>Diet # 2</td>
<td>1</td>
<td>30</td>
<td>52</td>
</tr>
<tr>
<td>Diet # 3</td>
<td>1</td>
<td>30</td>
<td>52</td>
</tr>
<tr>
<td>Diet # 3b</td>
<td>1</td>
<td>30</td>
<td>114</td>
</tr>
<tr>
<td>Diet # 4a</td>
<td>1</td>
<td>30</td>
<td>143</td>
</tr>
<tr>
<td>Diet # 8</td>
<td>1</td>
<td>30</td>
<td>38</td>
</tr>
<tr>
<td>Diet # 8a</td>
<td>1</td>
<td>30</td>
<td>89</td>
</tr>
<tr>
<td>Diet # 10</td>
<td>UL</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Diet # 12</td>
<td>1</td>
<td>12</td>
<td>200</td>
</tr>
<tr>
<td>Diet # 14</td>
<td>1</td>
<td>12</td>
<td>500</td>
</tr>
<tr>
<td>Diet # 25</td>
<td>1/UL</td>
<td>30/30</td>
<td>270/200</td>
</tr>
<tr>
<td>Diet # 30</td>
<td>1</td>
<td>30</td>
<td>109</td>
</tr>
<tr>
<td>Diet # 30 a</td>
<td>1</td>
<td>30</td>
<td>200</td>
</tr>
<tr>
<td>Diet # 40</td>
<td>1</td>
<td>30</td>
<td>252</td>
</tr>
</tbody>
</table>

*French, a unit used for feeding tube*
Further analyses were carried out to assess protein and moisture content and osmolality of the diets.

**Moisture content of polymeric feeds**

Moisture content of the post prepared polymeric diets in duplicate was determined using a hot air oven. The moisture content ± SD of all the post prepared polymeric diets ranged between 80-84% (Table 3.4.4).

<table>
<thead>
<tr>
<th>#</th>
<th>Diet #</th>
<th>Moisture content %</th>
<th>#</th>
<th>Diet #</th>
<th>Moisture content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>81 ± .74</td>
<td>8</td>
<td>14</td>
<td>82 ± .13</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>84 ± .37</td>
<td>9</td>
<td>14a</td>
<td>83 ± .42</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>82 ± .26</td>
<td>10</td>
<td>16</td>
<td>81 ± .89</td>
</tr>
<tr>
<td>4</td>
<td>3a</td>
<td>82 ± .26</td>
<td>11</td>
<td>25</td>
<td>81 ± 3.49</td>
</tr>
<tr>
<td>5</td>
<td>3b</td>
<td>82 ± .02</td>
<td>12</td>
<td>30</td>
<td>83 ± .00</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>83 ± .71</td>
<td>13</td>
<td>30a</td>
<td>83 ± .02</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>82 ± .18</td>
<td>14</td>
<td>38</td>
<td>82 ± .15</td>
</tr>
</tbody>
</table>

**Protein content of polymeric diets**

Protein content of pre-prepared (dry basis) and post prepared polymeric (dry basis) diets in duplicate was determined by Kjeldahl method (Kjeltic experiment). The purpose of this test was to determine the actual protein content of the diets and to ascertain the impact of preparation methodology on the protein content ± SD of post prepared polymeric diets. Results (Table 3.4.5) indicated protein loss in 3 post prepared polymeric diets which could be due to comparatively large quantity of remnants left after straining. Other 9 post prepared diets gave the higher reading upto 10.50%. The higher readings could be due to the loss of water in the process of cooking which in turn concentrated the protein content of prepared diets. The other reason could be the use of lecithin which was used in different quantities in the trials.

**Osmolality of polymeric diets**

Osmolality is one physical characteristic of enteral formulae that affects the feed tolerance. Hypertonic solutions require more care in
Table 3.4.5. Protein content of polymeric diets (Dry basis)

<table>
<thead>
<tr>
<th></th>
<th>Diet #</th>
<th>Pre-prepared diets</th>
<th>post prepared diets</th>
<th>Difference %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>21.6 ± 0.9</td>
<td>23.5 ± 0.70</td>
<td>1.8 †</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>16.3 ± 0.21</td>
<td>22.1 ± 0.54</td>
<td>5.7 †</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>20.2 ± 0.13</td>
<td>21.3 ± 0.23</td>
<td>1.1 †</td>
</tr>
<tr>
<td>4</td>
<td>3a</td>
<td>15.9 ± 0.47</td>
<td>22.8 ± 0.96</td>
<td>6.9 †</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>22.6 ± 0.17</td>
<td>26.6 ± 0.30</td>
<td>4.0 †</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>20.1 ± 0.04</td>
<td>20.9 ± 0.24</td>
<td>0.8 †</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>22.9 ± 0.06</td>
<td>27.2 ± 0.57</td>
<td>4.3 †</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>19.2 ± 1.55</td>
<td>30.4 ± 0.74</td>
<td>10.5 †</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>18.7 ± 0.46</td>
<td>19.6 ± 0.23</td>
<td>9 †</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>21.2 ± 0.12</td>
<td>19.5 ± 0.26</td>
<td>1.7 †</td>
</tr>
<tr>
<td>11</td>
<td>30a</td>
<td>20.9 ± 0.11</td>
<td>20.6 ± 0.50</td>
<td>0.2 †</td>
</tr>
<tr>
<td>12</td>
<td>38</td>
<td>17.1 ± 0.03</td>
<td>16.6 ± 0.00</td>
<td>.54 †</td>
</tr>
</tbody>
</table>

administration especially in patients with compromised digestive tract. The result in Figure 3.4.3 shows that polymeric feeds are isotonic at 3/4

Figure 3.4.3. Osmolality of polymeric diets
strength and are hyperosmolar at full strength.

Diet 4a and 8a were ultimately selected because these two diets satisfied all the criteria set for indigenous enteral formulation.

**Partially digested diets**

*Protocol for the preparation of partially digested diets*

Experiments were carried out to finalise the preparation methodology for partially digested diets as well. For this purpose, only 13 possible workable diets were selected. The protocol adopted for the preparation of partially digested diets is shown in Figure 3.4.4.

Experiments were carried out to identify the most appropriate preparation technique and enzymes for protein and carbohydrate hydrolysis. In addition, the most suitable lactase enzyme was identified for these diets. Trichloroacetic acid (TCA) solubility index test was conducted on partially digested diets to determine the degree of protein hydrolysis.

*Incubated diets (Table 3.3.3 on page 17)*

Experiment # 1 showed that the feeds were not homogenous. In experiment # 2, only protease enzyme was used and the need for starch breakdown became obvious due to gelatinisation of starch which led to experiment # 3. In this experiment, alcalase for protein hydrolysis and termamyl for saccharification of starch to dextrin was used. Another variable in experiment # 3 was the incubation time. All three incubation experiments were found to be very time consuming and underlined the need for simpler methodology. Thus the incubation technique was once again rejected for development of partially digested diets.

*Cooked diets*

In method # 1, the amount of both enzymes were insufficient to hydrolyse the protein and starch or alternatively the time available for hydrolysis was a limiting factor. Therefore in method # 2, length of hydrolysis time was increased. Although the desired result was achieved the method was too
time consuming. Methods #3, 4 and 5 were carried out to standardise the cooking method, amount of water and enzyme quantity required to yield 300 mL of feed and subsequently 600 mL of feed. Method #5 was ultimately short listed for preparing the partially digested diet.

Figure 3.4.4. Protocol for the preparation of partially digested diets

<table>
<thead>
<tr>
<th>29 partially digested diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 diets</td>
</tr>
<tr>
<td>* Selected</td>
</tr>
<tr>
<td>Selection criteria</td>
</tr>
<tr>
<td>Preparation criteria</td>
</tr>
<tr>
<td>Analysis</td>
</tr>
<tr>
<td>* Thin consistency</td>
</tr>
<tr>
<td>Ease of preparation</td>
</tr>
<tr>
<td>Selection of appropriate enzyme available for protein and carbohydrate hydrolysis</td>
</tr>
<tr>
<td>Minimum food handling</td>
</tr>
<tr>
<td>Safe to administer</td>
</tr>
<tr>
<td>Cost effective preparation method</td>
</tr>
<tr>
<td>13 diets</td>
</tr>
<tr>
<td>* Selected</td>
</tr>
<tr>
<td>2 diets</td>
</tr>
<tr>
<td>* Rejected</td>
</tr>
<tr>
<td>11 diets</td>
</tr>
<tr>
<td>Nitrogen determination</td>
</tr>
<tr>
<td>TCA* solubility index to determine the degree of hydrolysis</td>
</tr>
<tr>
<td>Moisture content</td>
</tr>
<tr>
<td>Viscosity</td>
</tr>
<tr>
<td>Viscous enough to flow through 12 French tube (Targeted flow rate: Not more than 120 mls/hr)</td>
</tr>
<tr>
<td>Microbiological count</td>
</tr>
<tr>
<td>Osmolality measurement</td>
</tr>
</tbody>
</table>

Basis of rejection
- preparation method is too time consuming
- more food handling required
Figure 3.4.5. Cooking method # 5 for the development of partially digested diets

1. All ingredients except egg were mixed and boiled.
2. Four mL of termamyyl was added and cooked for 10 minutes at temperature 90 - 100°C.
3. Cooled to 60°C and 2 mL of alcalase and egg were added and left in hot water sink maintaining the temperature between 55 - 65°C for 30 minutes.
4. Boil to inactivate the enzyme. Cooled to 40°C, 0.2 g of lecithin and .2 mL of lactase were added and blenderised for 2 minutes and strained through sterile muslin cloth.

Further experiments for standardising the use of thickener and emulsifier were carried out on diets # 4a and 8a, which were ultimately selected for human trial.

Rheological properties of partially digested diets

Table 3.4.6 shows the effect of different thickeners on the viscosity of partially digested diets. 1.5 g of psyllium husk was found to be effective in increasing the viscosity and controlling the flow rate.

Table 3.4.6. Rheological characteristics of partially digested diets treated with thickener

<table>
<thead>
<tr>
<th>Diets</th>
<th>Thickener treatments</th>
<th>Spindle size</th>
<th>Speed (RMP)</th>
<th>Centipoise (Cps)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>1.5 g P.h at the beginning</td>
<td>1</td>
<td>30</td>
<td>190</td>
<td>108 mL / hr /12 French</td>
</tr>
<tr>
<td>4a</td>
<td>1 g P.h at the beginning</td>
<td>1</td>
<td>30</td>
<td>46.8</td>
<td>Rapid flow</td>
</tr>
<tr>
<td>4a</td>
<td>.35 g G.g at the beginning</td>
<td>1</td>
<td>30</td>
<td>68</td>
<td>Rapid flow</td>
</tr>
<tr>
<td>4a</td>
<td>2 g C.f at the beginning</td>
<td>1</td>
<td>30</td>
<td>80</td>
<td>Rapid flow</td>
</tr>
<tr>
<td>4a</td>
<td>.35 g G.g at the time of inactivating the protein enzyme</td>
<td>1</td>
<td>30</td>
<td>83.2</td>
<td>Rapid flow</td>
</tr>
<tr>
<td>4a</td>
<td>4 g P.h at the beginning</td>
<td>1</td>
<td>30</td>
<td>43.6</td>
<td>Rapid flow</td>
</tr>
<tr>
<td>8a</td>
<td>5 g of V.g at the time of blenderising</td>
<td>1</td>
<td>30</td>
<td>16</td>
<td>Rapid flow</td>
</tr>
</tbody>
</table>

P.h. psyllium husk, G.g Guar gum, C.f. Corn flour, V.g. vegetable gelatin
Moisture contents of partially digested diets

In the preliminary stages, moisture content of partially digested diets were determined in order to assess the effect of enzyme treatment on moisture content of diets. Table 3.4.7. shows the moisture content ± SD of the diet # 11 under going treatment by the incubation and cooking techniques. Moisture content of partially digested diets did not vary much when compared with polymeric diets.

Table 3.4.7. Moisture content of partially digested diets

<table>
<thead>
<tr>
<th>Incubation technique</th>
<th>Cooking technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 2</td>
<td>Moisture content %</td>
</tr>
<tr>
<td>1</td>
<td>84.0 ± .11</td>
</tr>
<tr>
<td>2</td>
<td>85.4 ± .05</td>
</tr>
<tr>
<td>3</td>
<td>84.3 ± .24</td>
</tr>
</tbody>
</table>

Protein content of partially digested diets

Initially, protein content (dry basis) of pre-prepared and post prepared (dry basis) partially digested diets in duplicate was determined. In the pre-prepared state, the protein content of diets was 18.7 % ± SD (dry basis). When treated with enzymes, the hydrolysed protein content ± SD gave a lower reading. This could be due to the amount of water added to yield 300 mL. Since in the incubation technique there is a possibility of more water loss, water up to 400 mL was added which may have diluted the protein content. Another reason may be the preparation method in which the residue after straining was more than 3%. The cooking technique showed more consistent results for protein content and a minimal loss of protein even after straining. Table 3.4.8 shows the difference of protein content of partially digested diet 4a (dry basis).

Determination of protein hydrolysis of partially digested diets

Degree of protein hydrolysis of partially digested diets was determined in duplicate by Tricholoroacetic acid Index. The purpose of carrying out the
test was to assess the efficiency of enzyme in the development of partially digested diets for patients with compromised digestive tract.

Table 3.4.8. Difference of protein content of partially digested diet 4a (dry basis)

| Cooking technique (Diet # 4a) | Protein content (%) | | | |
|-------------------------------|---------------------|-----------------|-----------------|
| Variations                    | Pre preparation     | Post preparation | Difference %    |
| Method 4.1                    | 18.7 ± 1.22         | 16.6 ± .69      | 2.1 ↓           |
| Method 4.2                    | 18.7 ± 1.22         | 17.3 ± .96      | 1.3 ↓           |
| Method 4.3                    | 18.7 ± 1.22         | 18.0 ± .00      | .7 ↓            |
| Method 4.4                    | 18.7 ± 1.22         | 18.0 ± .21      | .7 ↓            |

For the purpose of this experiment, a number of NOVO brand enzymes (3.55) were used for starch and protein breakdown as well as lactase enzyme for patients with lactose intolerance. Qualitative assessment was carried out for starch hydrolysis by observing the liquification of starch into dextrin resulting in thinning of the feed mixture. Protein hydrolysis was monitored quantitatively due to its vital role in the maintenance of nitrogen balance of seriously ill patients. Table 3.4.9. shows the degree of hydrolysis of protein ± SD in the diet 4a, prepared by using cooking technique.

Table 3.4.9. Degree of protein hydrolysis of partially digested diet 4a

<table>
<thead>
<tr>
<th>Cooking technique (Diet # 4a)</th>
<th>DH* %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variations</td>
<td>DH* %</td>
</tr>
<tr>
<td>Method 4.1</td>
<td>43 ± 2.16</td>
</tr>
<tr>
<td>Method 4.2</td>
<td>41 ± 6.23</td>
</tr>
<tr>
<td>Method 4.3</td>
<td>37 ± 8.00</td>
</tr>
<tr>
<td>Method 4.4</td>
<td>59 ± .00</td>
</tr>
</tbody>
</table>

* Degree of hydrolysis

Table 3.4.10. shows hydrolysis of protein ± SD in the diets treated with enzymes in varying quantity. Enzyme ratio of 2:1 (termamyl: alcalase) used in method # 4.4 was found to be most appropriate to achieve more than 50% protein hydrolysis. The same ratio was used in cooking method # 5 for the development of partially digested diet. Moreover, the percentage of protein hydrolysed in the process of preparing 600 mL, 1200
Table 3.4.10. Protein hydrolysis of partially digested diets

<table>
<thead>
<tr>
<th>Variations (method #5)</th>
<th>Yield 600 mL (full strength)</th>
<th>Variations (method #5)</th>
<th>Yield 1200 mL (full strength)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DH %</td>
<td></td>
<td>DH %</td>
</tr>
<tr>
<td>4a</td>
<td>4 mL termamyl + 2 mL alcalase</td>
<td>46 ± 0.00</td>
<td>4a</td>
</tr>
<tr>
<td>8a</td>
<td>4 mL termamyl + 2 mL alcalase</td>
<td>46 ± 0.00</td>
<td>4a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4a</td>
</tr>
</tbody>
</table>

mL, full strength and half strength feed is also recorded here. Results show very little difference in the percentage of hydrolysis. Never the less, the use of enzymes in a hospital kitchen requires strict adherence to preparation protocol. The degree of hydrolysis is dependent upon:

* Quantity of enzyme.
* Length of treatment.
* Temperature.
* pH.

The percentage of protein hydrolysis ± SD of the diets with different thickeners was also assessed to determine any effect on enzyme activity (Table 3.4.11).

**Osmolality of partially digested diets**

Polymeric diets # 4a and 8a in partially digested state (treated with enzyme) were assessed in duplicate for osmolality. Results in the Figures 3.4.6 -3.4.8 indicate that these diets when polymeric were slightly hyperosmolar but for the post enzymatic treatment, the osmolality of these diets increased to 1000 mOsm /kg. Such a high osmolality necessitates the dilution of diets to 1/2 strength for better tolerance in bolus and intermittent feedings.
Table 3.4.11. Protein hydrolysis of partially digested diets treated with thickener

<table>
<thead>
<tr>
<th>Variations</th>
<th>DH %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method # 5: 1 g psyllum husk</td>
<td>57 ± 5.98</td>
</tr>
<tr>
<td>Method # 5: No thickener</td>
<td>57 ± 0.00</td>
</tr>
<tr>
<td>Method # 5: 5 g vegetable gelatin</td>
<td>47 ± 1.12</td>
</tr>
<tr>
<td>Method # 5: 10 g vegetable gelatin</td>
<td>50 ± 1.70</td>
</tr>
</tbody>
</table>

1 mL termamyl + .5 mL alcalase
1. .2 g guar gum                   | 23 ± 1.87 |
2. .3 g guar gum                   | 27 ± 2.26 |
3. .4 g guar gum                   | 35 ± 2.12 |

2 mL termamyl + 1 mL alcalase + .2 mL lactozyme
1. .35 g guar gum at the beginning | 71 ± 2.11 |
2. 1 g of psyllum at the beginning | 65 ± 8.49 |
3. 2 g corn flour at the beginning | 70 ± 2.51 |

Figure 3.4.6. Tonicity of polymeric versus partially digested diets at different stages of standardisation
Figure 3.4.7. Tonicity of polymeric versus partially digested diets at different stages of standardisation (Use of thickener)

(G.G. guar gum, P.H. Psyllium husk, C.F. Corn flour)

Figure 3.4.8. Tonicity of polymeric versus partially digested diets at different stages of standardisation (1200 mL yield)
Final selection of diets

A number of formulations were tried and diet # 4a and 8a were finally selected because these two diets best meet the criteria (Figure 3.4.1 and 3.4.4) for the preparation of enteral formula. The nutrition profile of both these diets is indicated in Figure 3.4.9. These two diets will be used for the pilot study to be carried out on hospital patients requiring enteral nutrition. Detailed composition of the selected diets are shown in Appendix 3.4.1.

Figure 3.4.9. Nutritional profile of selected diets

<table>
<thead>
<tr>
<th>Diet # 4a</th>
<th>Food composition (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice flour</td>
<td>20</td>
</tr>
<tr>
<td>Egg white</td>
<td>34</td>
</tr>
<tr>
<td>Milk whl. drd.</td>
<td>32</td>
</tr>
<tr>
<td>Sesame seed</td>
<td>3</td>
</tr>
<tr>
<td>Sugar</td>
<td>11</td>
</tr>
</tbody>
</table>

**Macronutrient ratio (g)**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>52</td>
<td>30</td>
</tr>
</tbody>
</table>

**NPC : N**

113 : 1

**Feed cost / 2000 mL (prepared) Rs / Aus $***

Rupees 44.00 / Aus $ 2.44 cents

<table>
<thead>
<tr>
<th>Diet # 8a</th>
<th>Food composition (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sago</td>
<td>6</td>
</tr>
<tr>
<td>Rice flour</td>
<td>16</td>
</tr>
<tr>
<td>Milk whl.drd.</td>
<td>29</td>
</tr>
<tr>
<td>Egg white</td>
<td>32</td>
</tr>
<tr>
<td>Sesame seed</td>
<td>4</td>
</tr>
<tr>
<td>Sugar</td>
<td>13</td>
</tr>
</tbody>
</table>

**Macronutrient ratio (g)**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>43</td>
<td>38</td>
</tr>
</tbody>
</table>

**NPC : N**

105 : 1

**Feed cost / 2000 mL (prepared) Rs / Aus $***

Rupees 40.00 / Aus $ 2.22 cents

*M Aus $ 1.00 = Pak Rs 18.00 (1-8-91)

Microbiological analysis

Results on the initially prepared enteral formulae indicated that extensive contamination occurred when aseptic techniques were not used. The initial high counts increased two to three orders of magnitude respectively when
formulae were stored at 5°C or 22°C over a 24 hour period, indicating rapid spoilage when initial bacterial load is high (Table 3.4.12).

Table 3.4.12. Microbial status of prepared enteral formula

<table>
<thead>
<tr>
<th>#</th>
<th>Time</th>
<th>Coliforms c.f.u</th>
<th>Total count/g</th>
<th>B.cereus</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 hrs</td>
<td>&gt;3 * 10^2</td>
<td>&gt;3 * 10^2</td>
<td>10^4</td>
<td>ND* in 0.1 g</td>
</tr>
<tr>
<td>2</td>
<td>6 hrs</td>
<td>1.7 * 10^3</td>
<td>2.5 * 10^2</td>
<td>10^4</td>
<td>ND* in 0.1 g</td>
</tr>
<tr>
<td>3</td>
<td>24 hrs</td>
<td>4.7 * 10^5</td>
<td>1.2 * 10^6</td>
<td>10^4</td>
<td>ND* in 0.1 g</td>
</tr>
<tr>
<td>4</td>
<td>24 hrs (22 °C)</td>
<td>2.4 * 10^8</td>
<td>6.0 * 10^8</td>
<td>10^4</td>
<td>ND* in 0.1 g</td>
</tr>
</tbody>
</table>

*Note: prepared formula was stored at 5°C, unless otherwise indicated.*

A systematic examination of the process was undertaken and hazard audit proposed to ameliorate the contamination problem (Table 3.4.13). Two further production runs were carried out. Run 2 was used as a control, and used normal hygienic but not aseptic preparation techniques. Run 3 used aseptic techniques as proposed in the hazard audit.

In-line testing of samples taken from runs 2 and 3 showed that counts were unacceptably high during all stages of production unless aseptic measures were employed (Table 3.4.14). This experiment did not examine formulae for growth of *S.aureus* or *B.cereus*. These results indicate the need for aseptic technique in minimising contamination of the formulae during preparation.

Enzyme were thought to represent a particular hazard since they must be purchased and stored in batch sizes far larger than that needed for immediate use. Lecithin was also considered a potential source of contamination, since it is added after the final heating step. Results indicated that lecithin, but not the enzymes, was likely to cause the contamination (Table 3.4.15).

Rigorous control of the microbial integrity of the formulae needs to be guaranteed. Precautions which must be taken during preparation are itemised in the hazard audit. It is recommended that a dedicated area be used for preparing the formulae and storing the ingredients. It is also recommended that the blender and muslin used should be sterilised by autoclaving at 110°C for 20 minutes. Attempts to sterilise the blender by
Table 3.4.13. Hazard Audit

<table>
<thead>
<tr>
<th>Operation</th>
<th>Potential risk</th>
<th>Control</th>
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<tbody>
<tr>
<td>Raw material storage</td>
<td>High temperature, high humidity, Vermin</td>
<td>Clean environment, appropriate temperature and humidity.</td>
</tr>
<tr>
<td>Sanitising of equipment</td>
<td>Bacterial contamination</td>
<td>Rinsing and soaking all equipment in sodium hypochlorite solution.</td>
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<tr>
<td></td>
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<td>Autoclaving of blender and muslin for 20 minutes at 110°C prior to use.</td>
</tr>
<tr>
<td>Addition of egg white and</td>
<td>Salmonella or other bacterial</td>
<td>Swab egg surfaces with 70% ethanol in water or soak egg in the same.</td>
</tr>
<tr>
<td>enzyme</td>
<td>contamination</td>
<td>Allow 5 minutes contact time. Subsequently handle with gloves which</td>
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<tr>
<td></td>
<td></td>
<td>have been swabbed with 70% ethanol. Use a sterile pipette to dispense</td>
</tr>
<tr>
<td></td>
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<td>enzyme to prevent contamination of unused portion.</td>
</tr>
<tr>
<td>Incubation of formula at 55</td>
<td>Growth of B. cereus</td>
<td>Keep temperature up to 60 to 65°C</td>
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<td>to 65°C</td>
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<tr>
<td>Cooling formula in fridge</td>
<td>Bacterial contamination</td>
<td>Ensure saucepan lid fits closely and is sanitised before use.</td>
</tr>
<tr>
<td>Addition of lecithin</td>
<td>Bacterial contamination</td>
<td>Use aseptic technique. Portion needed should be pre-weighed into a</td>
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<td></td>
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<td>sterile container.</td>
</tr>
<tr>
<td>Blending</td>
<td>Bacterial contamination</td>
<td>Blender must be autoclaved immediately prior to use and regularly</td>
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<tr>
<td></td>
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<td>inspected for deterioration of small component especially rubber and</td>
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<td>seal.</td>
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<tr>
<td>Straining formula through</td>
<td>Bacterial contamination</td>
<td>Muslin must be sterile. Gloves dipped in sanitizer or ethanol must be</td>
</tr>
<tr>
<td>muslin</td>
<td></td>
<td>worn and disposed of after each production run.</td>
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<td>Receiving vessel must be sanitised.</td>
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</table>

soaking in sodium hypochlorite sanitising solution were unsuccessful, presumably due to inaccessibility of the solution to some areas of the blender. The product must not be stored for too long, otherwise bacterial spoilage will occur even during refrigeration.
Table 3.4.14. Comparison of aseptic and non-aseptic processing

<table>
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<tr>
<th>#</th>
<th>Run 2 (Non-aseptic)</th>
<th>Run 3 (Aseptic)</th>
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<td>Coliform c.f.u</td>
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<td>2</td>
<td>$6.8 \times 10^3$</td>
<td>$3.3 \times 10^4$</td>
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<td>$5.1 \times 10^4$</td>
<td>$2.2 \times 10^5$</td>
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<td>4</td>
<td>$1.9 \times 10^4$</td>
<td>$3.1 \times 10^3$</td>
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Table 3.4.15. Microbial status of enzyme and lecithin

<table>
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<th>Ingredients</th>
<th>Coliform c.f.u</th>
<th>Total count / g</th>
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<tbody>
<tr>
<td>1</td>
<td>Alcalase</td>
<td>&lt; 10</td>
<td>&lt; 100</td>
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<tr>
<td>2</td>
<td>Termamyl</td>
<td>&lt; 01</td>
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<td>3</td>
<td>Lecithin</td>
<td>2</td>
<td>7,800</td>
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</table>
Chapter 3.5: Discussion

Initial experiments were conducted to develop a polymeric diet. Two preparation techniques were trialed: incubation and cooking technique. The incubation method did not work well because it was time consuming and involved massive handling of food as compare to cooking method. Moreover, this technique is not applicable in a home setup.

Subsequently, a cooking method was standardised for the development of the polymeric diet. Malted barley was used in the early stages of cooking with the view that starch saccharification is essential. However trials showed that saccharification is not essential if a proper cooking method and the correct amount of water is used for the preparation of polymeric feed.

Both incubation and cooking techniques were used for the development of partially digested diet. In the incubation technique malted barley was tried for the saccharification of starch but it did not yield the desired results. Other incubation techniques were found to be very time consuming and not workable in a hospital setting. After a series of cooking trials, cooking method # 5 was finalised for the preparation of partially digested diets. Termamyl for starch saccharification and alcalase for protein hydrolysis and lactozyme for lactase breakdown were shortlisted.

Initially experiments were carried out on 100 g of food items yielding 300 mL feed and furnishing 1 kcal/mL at full strength. Subsequent experiments were conducted to yield 600 mL and 1200 mL full strength and as well 1/2 strength feed.

During the course of experimental trials for the development of polymeric and partially digested diets, problems were encountered: The disassociation of particles resulted in settling down of feed which was over come by the use of lecithin. For partially digested diets lecithin is required in very small quantity.
The second problem was the thin consistency of half strength polymeric feed, for which thickener was tried but it gave poor results. Therefore the idea of using thickener for 1/2 strength polymeric diet was dropped. The use of a thickener in partially digested diets showed encouraging result. No curdling of the diet occurred probably due to the effect of enzyme on psyllium husk.

The protein content of various polymeric diets was determined and result showed a negligible difference. Some of the post prepared polymeric diets gave higher values, which could be due to the loss of moisture content during cooking or the addition of lecithin in varied quantity. This experiment showed that the cooking method had no negative impact on the protein content of the diet and could be used for the preparation of enteral feed. As opposed to polymeric feed, post prepared partially digested diets gave a lower protein content, which could be due to the dilution of feed. However the difference was very little. To overcome this problem, one can look at the ratio of water required to prepare partially digested diet.

Polymeric diets are isotonic at 4/5 strength and hyperosmolar at full strength. A study by Bell, et al (3.3) mentioned that mildly hyperosmolar liquids up to 400 mOsm/kg are as well tolerated as are isoosmolar solutions by patients who have impaired or limited gastrointestinal tolerance. Other studies (3.3, 3.7-3.8) have shown that hypertonic solution resulted in significantly better nitrogen intake and balance which indicated that these feeds can be infused by bolus without resulting in any intolerance. Further they may be taken orally because they are palatable. However, when the same feed is treated with protease enzyme, it becomes bitter due to the protein breakdown into peptide and amino acids. When treated with amylase the severity of bitterness was reduced because starch molecules when intact do not give sweet taste but when they breakdown into dextrin, they do give a slightly sweet taste.

Partially digested diets as compared to polymeric diets are hyperosmolar when full strength and isotonic at half strength. Hyperosmolality of the partially digested diet is due to the breakdown of protein and starch molecules which increases the solute load and in turn the osmolality. Intolerance which may occur due to hyperosmolality can be avoided by infusing at slow rate or intermittently.
Microbiological screening of these diets indicated that contamination does occur when aseptic techniques are not used. Contamination can occur at any step of preparation starting from raw items till the feed is infused. Hence, meticulous care in preparation needs to be taken to avoid contamination.

After trying numerous formulations, diets 4a and 8a were finally selected for pilot study because these diets best met the selection criteria. Diet 4a was one of the diets used in the protein quality assessment of indigenous enteral formulae.
Chapter 3.6: References


3.56 Novo *enzymes for starch industry.* Novo Nordisk

### Appendix 3.3.1.

**Food combinations for the development of indigenous enteral formulae**

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<th>Diets #</th>
<th>Rice</th>
<th>Sago</th>
<th>Semolina</th>
<th>Milk Whl.</th>
<th>Egg skd</th>
<th>Milk whl</th>
<th>Egg whl</th>
<th>Egg drd.</th>
<th>Mb</th>
<th>Sds</th>
<th>Peanuts</th>
<th>Ygts</th>
<th>Sugar</th>
<th>Honey</th>
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Appendix 3.3.2.

Combinations selected for the preparation of indigenous enteral formulae

Diet # 1

- S. seed 20%
- Honey 18%
- Rice flour 15%
- Milk skim 25%
- Egg white 25%

Diet # 1a

- S. seed 20%
- Honey 15%
- Rice flour 20%
- Milk skim 20%
- Egg white 25%

Diet # 2

- S. seed 10%
- Honey 15%
- Rice flour 20%
- Milk whole 40%

Continue...


Continue...

Diet # 2a

- S.seed: 9%
- Rice: 17%
- Egg: 43%
- Milk: 18%
- Honey: 13%

Diet # 3

- S.seed: 15%
- Rice flour: 20%
- Egg: 25%
- Milk skm: 25%
- Honey: 16%

Diet # 3a

- S.seed: 15%
- Rice flour: 19%
- Egg: 26%
- Milk skm: 25%
- Honey: 16%

continue...
Continue...

Diet # 3b

- S.seed: 16%
- Honey: 16%
- Egg whl: 25%
- Milk skm: 25%
- Rice flour: 20%

Diet # 3c

- S.seed: 16%
- Honey: 16%
- Egg whl: 26%
- Milk skm: 25%
- Rice flour: 18%

Diet # 4

- S.seed: 6%
- Honey: 13%
- Egg whl: 35%
- Milk wh.drd: 26%
- Rice flour: 20%

continue...
Continue...

Diet #4a
- S. seed: 3%
- Egg wht: 34%
- Milk wh.drd: 32%
- Sugar: 11%
- Rice flour: 29%

Diet #7
- S. seed: 16%
- Egg wht: 32%
- Milk skm: 26%
- Sugar: 10%
- Sago: 20%

Diet #8
- Sago: 5%
- S. seed: 4%
- Egg wht: 32%
- Milk whl: 32%
- Sugar: 12%
- Rice flour: 16%

continue...
Continue...

Diet # 8a
- Sago: 6%
- Rice flour: 16%
- Milk whey: 29%
- Egg white: 32%
- Sugar: 13%
- S. seed: 4%

Diet # 9
- Sago: 10%
- Rice flour: 16%
- Milk skm: 20%
- Egg white: 48%
- Honey: 20%

Diet # 10
- Sago: 15%
- Milk skm: 24%
- Egg white: 29%
- S. seed: 16%
- Honey: 15%
Continue...

Diet # 10a

- S.seed 15%
- Egg whl 25%
- Honey 15%
- Sago 20%
- Milk skm 25%

Diet # 11

- S.seed 10%
- Egg whl 25%
- Honey 20%
- Rice flour 20%
- Milk wh.drd 25%

Diet # 12

- S.seed 25%
- Milk skm 35%
- Rice flour 20%
- Honey

continue...
Continue...

Diet #14

- Egg whl: 33%
- Rice flour: 13%
- Milk whl: 23%
- Mg.beans: 22%
- Honey: 9%

Diet #14a

- Egg whl: 30%
- Rice flour: 11%
- Milk whl: 23%
- Mg.beans: 16%
- Honey: 15%

Diet #16

- Egg whl: 35%
- Rice flour: 16%
- Milk skim: 20%
- S.seed: 30%
Continue...

Diet # 25

- Sago: 10%
- Rice: 10%
- S. seed: 25%
- Milk skm: 35%
- Honey: 20%

Diet # 27

- Milk wh drd: 28%
- Rice flour: 16%
- Sago: 8%
- S. seed: 4%
- Egg wht: 33%
- Honey: 12%

Diet # 27a

- Milk wh drd: 29%
- Rice flour: 12%
- Sago: 18%
- S. seed: 5%
- Egg wht: 33%
- Honey: 3%

continue...
Continued...
Continue...
Appendix 3.3.3.

Viscosity measurement

**Procedure**

- Spindle was attached to the lower shaft. Then spindle was inserted in the test diet up to the immersion groove level (cut in the spindle’s shaft).
- Viscometer was leveled with the aid of the bubble level.
- The viscometer’s motor was turned on, depressing the clutch to prevent unnecessary wear. Clutch was released and diet was allowed to rotate till the pointer was stabilized at a fixed position on the dial.
- The viscosity of the test diets were calculated using the Factor finder indicated below:

```
Dial reading x Factor Viscosity in Centipoise (mpq.s)
```

**Factor Finder Table**

<table>
<thead>
<tr>
<th>Spindle 1</th>
<th>Spindle 2</th>
<th>Spindle 3</th>
<th>Spindle 4</th>
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</table>

* M = 1000

Note: Brookfield Synchroleum Dial-reading viscometer was used to measure the viscosity of formulae.
Appendix 3.3.4.

Flow rate assessment

Procedure

Start up

- Gravity clamp controlled bag (feed holding bag) was positioned at 6 feet height.
- A tube (12 French or 8 French caliber) was attached to the feeding set.
- End of the tube was placed in a flask to collect the fluid flowing through the tube.

Operation

- Prepared feed was poured into the feed holding bag.
- Gravity clamp was released to let out the air and left the feed flowing for a minute.
- Using a gravity clamp, flow was controlled to the maximum. The position of the holding bag was also adjusted to control the flow rate of the feeds.
- Using a stop watch, fluid pouring into the empty flask was recorded.
- Repeated after every subsequent half an hour for 90 seconds. This step was carried out to make sure that no clogging of tube occurred during the administration period.

Note: A feeding set (Kangaroo, Sherwood Medical) of 500 mls capacity and 8 to 12 French caliber tubes were used.
Appendix 3.3.5.

Moisture content determination

Procedure

- To ensure complete drying, sand was used to increase the surface area. Thus aluminium moisture dishes containing sand and a glass rod were placed into air oven at 105\(^\circ\)C for 1 hour, then removed and placed into desicators.
- On cooling, these dishes were weighed on an analytical balance and weight was recorded.
- Approximately 10 g of liquid samples in duplicate were weighed on analytical balance and mixed with sand using the glass rod.
- Means of the duplicate samples were calculated to determine the moisture content of the samples.
- Moisture dishes containing the samples and sand mixture and the glass rod were placed in an air oven at 105\(^\circ\)C for overnight.
- In the morning samples were removed from the oven and stored in desicators till cool.
- Samples were reweighed and moisture content was calculated using the equation below:

\[
\% \text{ Moisture} = \frac{\text{Loss of weight in samples}}{\text{Weight of samples}} \times \frac{100}{1}
\]
Appendix 3.3.6

Protein content determination

Digestion: Procedure

- Digestion block was preheated to 420°C (approx. 30 minutes on full).
- Diets (dry) were weighed analytically on an ashless filter paper. The filter paper was folded around the sample and placed into a digestion tube. Liquid diets were measured using 5 mL pipette and were poured into a digestion tube. Blank was also run simultaneously.
- One catalyst tablet and 12 mL of concentrated sulphuric acid were added to the tube.
- The contents were mixed by swirling.
- Digestion tubes were placed in the heating block (digester). Exhaust caps were fitted on to the tubes to avoid leakage of dangerous fumes and water was turned on.
- Fume cupboard was turned on and thermometer was put into the block and front heat shield was placed into the position. After 10 minutes, the water flow through the water suction pump was reduced to a minimum.
- The end of digestion was observed by the solution becoming clear. However, digestion was continued for 10 minutes passed this point.
- The digestion tube was removed with the caps still attached. Water flow was increased and tubes were allowed to cool.
- On cooling, the exhaust caps were removed and the water flow was stopped.

Distillation

Start up

- Water and alkali level were checked in the tank.
- The power was switched on and the tap was turned on.
• Steam button was pressed and after a few minutes steam was generated. Distillation was continued till some water was collected in the receiver flask. Steam valve was closed by pressing the steam button.
• Thumb wheel was checked for Alkali, Delay and Steam, which were set as follow: Alkali: 2, Delay: 0.2, Steam: 3.6.
• “Auto/man” button was pressed for automatic operation of distilling unit.

Operation

• Digested sample tube was placed in a position.
• Receiver flask containing 50 mls of Boric acid was placed in a position and platform was raised so the tip of the delivery tube is below the liquid surface.
• In order to start the distillation automatically, safety door was closed.
• When the distillation was stopped automatically, tube and flask were replaced with the next one.

Close down

• A tube half filled with water was connected to the distilling unit with a receiving flask.
• Distilled in manual mode for about 3 minutes to clean the system.
• Replaced with a clean digestion tube and receiver flask.
• Switch off the power and tap water and clean the spill tray in the bottom of the instrument.

Titration

Distilled samples were titrated in the receiver flask back to a neutral grey end point.

Calculation

Percentage of nitrogen was determined using the following equation:
\[ \% \text{ Nitrogen} = \frac{14.01 (\text{sample titrant} - \text{blank titrant}) \times \text{HCL Molarity} \times 0.1M}{\text{Sample weight} \times 10} \]

(\% \text{ protein} = N \times \text{Factor specific for different diets})

Note: Kjeltec system model # 1026 distilling unit was used to determine the protein content of formulae.
Appendix 3.3.7.

Osmolality Measurement

Procedure

- Two aliquots of 2 mL each were pipetted into clean dry cryoscope tubes
- Tubes were placed into the cryoscope and freezing point of the sample was determined within a minute.
- Samples reading less than -.408 were repeated after diluting with distilled water till the reading fell within the specified range.
- The osmolality was than calculated using the following equation:

\[
\text{mOsm} = \frac{\Delta T}{K_f} \times 1000
\]

Note: The Fiske Ms Cryoscope # 3.203.266 was used to determine the osmolality of polymeric and partially digested diets.
Appendix 3.3.8.

Trichloroacetic acid (TCA) Solubility Index

Procedure

- Ten mL of sample were pipetted out into an centrifuge tube.
- Five mL of Trichloroacetic acid was added and shake well.
- The content of the flask was centrifuged at 4000 x g for 10 minutes and nitrogen concentration (g/L) in the supernatent was determined using Kjeltec procedure.
- The trichloroacetic acid index was determined using the calculation below:

\[
\text{TCA index} = \frac{15 \times N \text{ (g/L) in supernatant}}{\% \text{ of total } N \text{ before TCA}} = \% 
\]

In the TCA index determination, all large proteins peptides are precipitated. One study noted “that the TCA soluble fraction of one egg albumin hydrolysate consisted mainly of peptides with a chain length of not more than four amino acid residue” (3.54). To what extent this observation can be generalized to other hydrolysates is uncertain (3.55).
Appendix 3.3.9.

Microbial screening

Procedure for coliform and plate count

Plate count agar was used for plate count and violet red bile agar was used for coliform count.

Start up

- For $10^{-1}$ dilution, 10 gms of sample was added in 90 g of .1 % peptone diluent.
- One mL of $10^{-1}$ mixture was added into 9 mL of peptone solution for $10^{-2}$.
- For $10^3$, 1 mL of $10^{-2}$ was added to 9 mL of peptone solution. Same procedure was followed for $10^4$ to $10^5$.

Procedure

- One mL of $10^{-2}$ - $10^{-1}$ was poured into petri dishes in duplicate and then plate count agar was poured. To distribute it evenly, petrie dishes were rotated in clockwise and anti clockwise position 5 times and 5 times up and down.
- For coliform count, step # 1 was followed but instead of plate count agar, violet red bile agar was used.
- Petri dishes for coliform were placed into the incubator in $30^0$C for 24 hours and total plate count were placed into the incubator at $30^0$C for 72 hours.
- For Steph. aureus, 0.1 mL aliquots of dilutions $10^1$, $10^2$ and $10^3$ were spread on to pre-poured Baird Parker Agar plates, effectively giving dilutions of $10^2$, $10^3$ and $10^4$ respectively. Sample aliquot was spread using a flammed glass “hockey stick”. Plates were inverted and incubated at $37^0$C, and checked for typical colonies after 24 hours. Plates negative after this time were re-incubated for a further 24 hours.
After the specified time, colonies were counted using Cuebec Dark Field Colony Counter.
Continue...
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Table 4.4.9. Initial and final total lymphocyte count value of patients in control and experimental groups

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4: Abstract

In order to assess the efficacy of indigenous enteral formula versus commercial formula in improving the nutritional status of hospital patients, a pilot study was carried out at the Aga Khan University Hospital, Karachi, Pakistan. Patients meeting the selected criteria were allocated randomly to either an experimental diet or a control diet.

Diet # 4a was selected to be the experimental diet. It was polymeric, suitable for the functionally intact digestive system. The same diet treated with enzyme designated a partially digested diet. Both diets were isocaloric with a caloric ratio of 53: 29: 18 from carbohydrate, fat and protein, respectively. The polymeric diet was isotonic at 4/5 strength while the partially digested diet was isotonic at 1/2 strength.

Isocal (Powdered), a Mead Johnson product which is available in Pakistan was used as a control diet with a calorie ratio of 50: 37: 13 from carbohydrate, fat and protein, respectively and isotonic at full strength.

Harris Benedict equation was used to calculate the energy requirement taking into account the activity and stress factor of the patients. The protein requirement was calculated using a factor of 1.25g/kg of body weight.

Patients were fed as per protocol and effectiveness of therapy was determined using three parameters: nitrogen balance, prealbumin and total lymphocyte count. Patients were also monitored for possible side effects. To ensure the safety of the patients, microbial screening of the formulae was conducted on a random basis.

There was no significant difference between the groups in terms of mean age and length of stay. Mean sepsis score of the control group was greater than in the experimental group but not statistically different. Calorie intake in both groups was 75% of the recommended intake. The protein intake was 63% in the control and 81% in the experimental group.
Nitrogen balance and total lymphocyte count in the experimental group comparatively showed a better positive trend than the control group while the prealbumin of the control group was better than the experimental group but not significant.

The most common side effect experienced by 6 patients on the control diet was elevated blood sugar and in the experimental group, hyponatremia was the main side effect experienced by 5 patients.

Bacteriological screening carried out on both formulae and in the preparation room showed contamination, which indicated the need for a revised method of formulae preparation. Strict adherence to preparation protocol, monitoring and regular bacterial testing of enteral formulae are warranted.

Preliminary results have shown that the experimental diet is at least as effective as the control diet in improving the nutritional status. The incidence of side effects was not different. The experimental diet is considerably cheaper and can be readily prepared in hospital setting.
Chapter 4.1: Introduction

Statement of the problem

The supply of commercially prepared enteral formulae is expensive and scarce when compared with demand in Pakistan. Hence it is imperative to look for indigenous sources of good nutrition support.

With the foregoing in consideration, formulae have been developed using indigenous food items with simple technology and tailored to provide good nutritional support to patients at an affordable cost. These formulae have been successfully constituted, achieving the desired energy to nitrogen ratio (NPC:N). Viscosity is comparable to commercial feeds enabling delivery through 8 French and 12 French caliber feeding tubes.

In order to prove the efficacy of the indigenous enteral formulae, a pilot study was carried out on hospitalised adult patients in need of enteral nutrition support. Approval was obtained from the University of Western Sydney, Hawkesbury Human Ethics Committee and the Aga University Hospital Human Subject Protection Committee. The study was conducted at the Aga Khan University Hospital (AKUH) in Karachi, Pakistan.

Aim

To determine the efficacy of indigenous enteral formulae on tube fed adult patients.

Objectives

1. To compare the efficacy of an indigenous enteral formula with a commercial formula by assessing the nutritional status of the patients.
2. To compare the cost of indigenous enteral formulae with the commercial formula.
3. To evaluate the feasibility of introducing indigenous enteral formulation in a hospital setting.
4. To anticipate the difficulties that might be associated with a large random control trial.

**Hypothesis**

The pilot study was based on the hypothesis that an indigenous enteral formula is as effective as a commercial formula in improving the nutritional status of enteraly fed patients.
Chapter 4.2: Literature Review

Indications for enteral nutrition (4.1)

I. Clinical settings where enteral nutrition via tube is a necessary part of routine care

1. Protein-calorie malnutrition with inadequate oral intake of nutrients for the previous 5 days. Protein-calorie malnutrition is defined as greater than 10% loss of usual weight or serum albumin less than 3.5 g/dL.

2. Normal nutritional status with less than 50% of required nutrient intake orally for the previous 7-10 days. Patients with normal nutritional status and functional gastrointestinal tract may be unable or unwilling to ingest adequate nutrients orally. Examples: cancer patients.

   a. Severe Dysphagia In patients with neurologic illnesses such as strokes, brain tumours, head injuries, multiple sclerosis, amyotrophic lateral sclerosis, and Guillain-Barre syndrome, enteral nutrition is warranted.

   b. Major Burns After a short period of postburn ileus, the intestinal tract functions normally, and enteral nutrition is the preferred route of nutrition intervention because it overcomes the severe anorexia that is often present with administration of large doses of narcotics. Patients with burns that are less extensive but involve the face and neck may also have difficulty in swallowing and require long-term administration of enteral nutrition.

   c. Massive Small Bowel Resection Patients with short gut (50-90% small bowel resection) should receive enteral nutrition when clinically stable to hasten regeneration of the remaining small intestine. Feeding should be initiated slowly into the stomach and should be given continuously. The presence of an ileocecal valve
greatly facilitates the tolerance of enteral nutrition in these patients.

**d** *Low Output Enterocutaneous Fistulae*  Patients with low output (less than 500 mL / day) enterocutaneous fistulas often will have complete closure of the fistula tract during administration of enteral nutrition. If the fistula is in the proximal gut, the feeding should be delivered distal to the fistula site. With distal enterocutaneous fistulas, sufficient proximal length of small intestine must be present to ensure adequate absorption of administered nutrients.

**II. Clinical conditions where enteral nutrition via tube is usually helpful**

1. *Major Trauma*  Patients sustaining major or penetrating traumatic wounds often have decreased oral intake and increased metabolic needs and enteral nutrition should be recommended for them.

2. *Radiation Therapy*  The use of enteral nutrition should be considered for patients receiving radiation therapy. Due to this therapy they often are anorexic and have mild gastrointestinal discomfort without vomiting or diarrhoea.

3. *Mild Chemotherapy*  Many patients receiving mildly toxic chemotherapeutic regimens have some anorexia; examples include chemotherapy for cancers of the lung, breast, colon, ovary and testis.

4. *Liver Failure and Severe Renal failure*  Patients with acute or chronic liver failure often have severe anorexia, despite functioning intestinal tracts. For such patients the need for salt, water and protein restriction requires very careful nutrient formulation. Patients with severe renal dysfunction (less than 5 - 10 % of normal glomerular filtration) often are anorexic and have problems of hyperkalemia, hypermagnesemia, hyponatremia, and hyperphosphatemia, thereby requiring frequent modifications of enteral formulae.

5  **III. Clinical settings where enteral nutrition via tube is of limited value**

*Intensive Chemotherapy*  Patients receiving intensive combined
chemotherapy often have stomatitis, anorexia, nausea, vomiting, diarrhoea and decreased oral intake.

6 *Immediate Postoperative Period or Post Stress Period* In the immediate postoperative period, if it is anticipated that the return of oral intake will be more than 5-7 days, enteral nutrition can be given to selected patients if the gastrointestinal tract can be used safely and effectively.

7 *Acute Enteritis* Enteritis secondary to radiation, acute infection, or active inflammatory bowel disease may be aggravated by enteral nutrition. Bowel rest and the delivery of parenteral nutrition is usually recommended in the acute setting. When the acute inflammatory process subsides, enteral nutrition should be initiated.

8 *Less Than 10% Remaining Small Intestine* Patients in this category are rarely able to tolerate enteral nutrition as the sole source of nutrients.

**IV. Clinical settings in which enteral nutrition should not be used**

1. *Complete Intestinal Obstruction* Patients with complete small or large bowel obstruction do not tolerate feeding into the gastrointestinal tract. Patients with obstruction of the proximal intestine may be fed via surgically placed tubes distal to the site of obstruction if there is sufficient length of nonobstructed intestine for adequate absorption.

2. *Ileus or Intestinal Hypomotility* Patients with ileus or intestinal hypomotility tolerate enteral nutrition poorly, and are at increased risk for aspiration and infectious enteritis. Such patients should be fed parenterally.

3. *Severe Diarrhoea* Patients with severe diarrhoea resistant to pharmacologic therapy are difficult to feed enterally. Hence these patients must be given total parenteral nutrition.
4. **High Output External Fistulae** Patients with high output (greater than 500 mL / day) external fistulas usually have increased output when fed enterally.

5. **Severe Acute Pancreatitis** Enteral nutrition usually is not tolerated in the presence of severely acute pancreatitis or acute complications of chronic pancreatitis such as haemorrhage, abscesses, or pseudocysts.

6. **Shock** Patients with hypovolemic or septic shock should not be fed enterally.

7. **Prognosis Not Warranting Aggressive Nutritional Support** Withhold enteral nutrition from patients with extremely poor prognosis with no hope for improvement, like those who have an imminently terminal illness and those with documented, irreversible coma.

**Relevance of nutrient absorption to enteral feeding**

Knowledge of the physiology of nutrient absorption can be of assistance in the selection of appropriate enteral formulae.

**Protein absorption** Hydrolysis of protein into small peptides and free amino acids occurs within the intestinal lumen (gastric, jejunal and ileal) and at the enterocyte brush border (4.2). Assimilation of exogenous protein occurs mainly in the proximal jejunum (4.3), although the ileum also has considerable digestive and absorptive capacity (4.4).

The functional reserve of the human gastrointestinal tract is such that considerable (4.2) impairment of organ function must occur before there is clinical malabsorption (4.5). Although patients with a normal gastrointestinal tract should be fed diets containing intact protein, there is controversy as to whether the nitrogen source for severely impaired gastrointestinal tracts should be of free amino acid or oligopeptides. Intestinal perfusion studies have (4.6) shown more efficient absorption of α-amino acid nitrogen from hydrolysates of whole protein than from equivalent free amino acid mixtures. Silk, et al (4.3) and Moriarty (4.7) observed no difference in overall absorption or metabolic utilisation of protein, protein hydrolysate or the equivalent amino acid mixtures in
subjects with normal bowel function. Even for patients with moderately impaired gastrointestinal function, there appears to be no nutritional superiority of protein hydrolysates over whole protein (4.8). McIntyre, et al (4.9) showed that intact protein and partially hydrolysed protein are equally well absorbed in patients with 60 - 150 cm of intact jejunum. O’Morain, et al (4.10) compared the nutritional efficacy of polymeric and predigested elemental diets in patients with moderately impaired gastrointestinal function. No difference in stool weight, nitrogen absorption or nitrogen balance and nutritional parameters were observed between the two groups.

**Carbohydrate absorption** Digestion of carbohydrate occurs both within the lumen of the small intestine and at the surface of the small bowel mucosa (4.11). The dietary carbohydrate is predominantly absorbed in the proximal jejunum (4.12, 4.13).

The luminal hydrolysis of starch is catalysed by two $\alpha$-amylases secreted by the pancreas and salivary glands. Pancreatic $\alpha$-amylases play the predominant role in the lumen of the small intestine. The end products of luminal starch hydrolysis are maltose, maltotriose and $\alpha$-limit dextrin. Initially elemental diets contained glucose as the sole source of energy (4.14, 4.15). This was replaced in part by sucrose and later by glucose polymer mixtures derived from the hydrolysis of starch by $\alpha$-amylase. Using gel permeation chromatography, Jones, et al (4.16) characterised the composition of starch hydrolysates commonly used in enteral diets: 50% of the glucose was contained in polymers of less than 10 glucose units and 50% in more than 10 glucose units. Intestinal perfusion studies performed in normal human volunteers in the absence of luminal $\alpha$-amylase activity showed the slower assimilation of higher molecular weight glucose polymers.

**Lactose** Havenberg (4.17) and Kwon (4.18) showed that the ingestion of 480 mL milk by individuals with confirmed lactose malabsorption resulted in a higher incidence of gastrointestinal side effects than when a similar quantity of lactose free milk was ingested. Patients with confirmed lactose intolerance did not have a higher incidence of gastrointestinal side effects on a lactose containing enteral diet (20.9 ± 5.3 g lactose / 24 hrs) than they did on a lactose free formulation. The reason may be the reduced
"load" of lactose (concentration of lactose) that is administered to lactose intolerant patients at any given time (4.19).

**Fat absorption** Normal lipid digestion and absorption is dependent on an adequate luminal level of pancreatic lipase and bile salts and sufficient absorption surface. In case of severe exocrine pancreatic insufficiency (chronic pancreatitis and cystic fibrosis), medium chain triglycerides have been proposed as an efficiently absorbed fat source. However, exclusive use of medium chain triglycerides may provoke essential fatty acid deficiency. Currently a mixture of medium chain triglycerides (MCT) and long chain triglycerides (LCT) is suggested as a lipid source for enterally fed patients with severely reduced digestion and absorptive function (4.20 - 4.21).

**Water and electrolyte absorption** The intestinal mucosa acts as a semipermeable membrane through which water flows in either direction in response to a difference in osmotic pressure. Mucosal permeability varies in the intestine. The jejunum is much more freely permeable permitting rapid equilibration of osmotic pressure gradient created by the digestion and absorption of nutrients. The pores through which water passes in the jejunum are about twice the diameter than of those in the ileum (4.22). The colon is the least permeable and prevents water from leaking back into the lumen.

**Nutrient requirements for enteral feeding**

To meet the nutritional requirement of patients receiving enteral feeding, a readily utilised energy substrate, nitrogen for protein synthesis, fluid, minerals and vitamins must be included.

**Energy requirement** The body surface area derived from height, weight, age and sex determines the energy requirements (4.23). Calorie needs can be predicted with reasonable accuracy by the Harris Benedict equation for normal man.

The equation is used to determine the basal energy expenditure (BEE). In order to determine the resting energy expenditure (REE) in bed ridden
patients, the thermogenic effect of food must be added which increases
BEE by 10% (4.24).

<table>
<thead>
<tr>
<th>Man: Kcal/day = 66.47 + (13.75 x W*) + (5 H*) - (6.76 x A*)</th>
<th>Women: Kcal/day = 655.10 + (9.56 x W*) = (1.85 x H*) - (4.86 x A*)</th>
</tr>
</thead>
</table>

*W = weight in kg, *H = height in cm, *A = age in yrs

Malnutrition reduces the BEE down to 35% by virtue of weight loss. Injury, sepsis and burns were once thought to increase energy requirement by approximately 30%, 60% and 100% respectively but studies have failed to prove that injured or septic patients are markedly hypermetabolic. On the contrary, Askanazi, et al (4.25) and Roullet and associates (4.26) showed increase in the metabolic rate of injured and septic patients.

Balance trials by Calloway (4.27) in which protein and energy intake were varied, concluded that “energy intake appears to have a greater effect on nitrogen balance than does protein intake in the marginally adequate range of intake”. Elwyn (4.24) expressed this somewhat differently “when N intake is adequate, increasing energy intake causes increased N balance. At low energy intake, the effect is very marked, an increase of 8 mg N / kcal. At about 1400 kcal intake, the curve breaks fairly sharply and above this level the improvement in nitrogen balance is only 2 mg N / kcal”. In a healthy young man protein sparing may be achieved within intake of 1400 kcal and 7 g N / day. Above this the yield per increment of intake is much less.

Source of energy A study by Gambles (4.28) showed that a reduction in the excretion of nitrogen in the urine of normal fasting subjects occurred when glucose was fed demonstrating a protein sparing effect. A controlled trial by Macfie and colleagues (4.29) showed that glucose alone does not result in protein sparing but that a glucose-lipid mixture promoted nitrogen retention. Nanni and associates (4.30) confirmed that septic patients are more dependent on lipids for oxidative metabolism. Jeejeebhoy (4.31) revealed that when glucose is used as the exclusive source of non-protein calories, excess glucose calories are converted to fat in the liver, resulting in liver steatosis. Furthermore, Askanazi and associates (4.32) have reported that when glucose is used as the sole source of non-protein
calories, there was elevated oxygen consumption, carbon dioxide production, resting energy expenditure and urinary norepinephrine excretion which may be detrimental to patients with marginal respiratory reserves. The conclusion is that injured and septic patients need a mixed substrate of glucose and fat to meet energy needs.

**Protein requirement** The protein component of the diet must provide the essential amino acids which the body cannot make, and nitrogen for the synthesis of other amino acids and physiologically important nitrogen containing compounds. According to Silk (4.33) in a normal active man, positive nitrogen balance is achieved with a nitrogen intake of 8-9 g / 24 hours and NPC:N ratio of 320: 1. Whereas, altered gastrointestinal function and changes in metabolism that accompany trauma, infections and chronic diseases increases protein requirements (4.34). Steffee, et al (4.35) showed that in order to promote tissue restitution in traumatised, catabolic or seriously undernourished patient, the essential amino acid intake should be at least 40% of the total amino acid intake which is similar to the requirements of a growing child.

Ideally, the nitrogen requirement should be estimated from direct measurement of nitrogen losses in urine, stool, stoma or fistula effluent. However, this is not done in practice because the methodology is cumbersome, time consuming and costly. With the exception of patients with protein losing enteropathies and fistulae, reliable estimations of nitrogen losses (NL) in g /24 hrs can be calculated using nitrogen equation balance. When patients have a high rate of protein catabolism, the increase losses of nitrogen are reflected in increased urinary excretion of urine and when combined with protein intake data give a rough estimate of nitrogen balance (4.36). The equation for nitrogen balance (4.37) is:

\[
\text{Nitrogen balance} = \frac{24 \text{ hours protein intake}}{6.25} - (UUN \times 4)
\]

*Urinary urea nitrogen

The factor of 4 is added to take into account constant non-urea urinary nitrogen as well as fecal and skin losses.
Electrolyte requirements The importance of fluid and electrolyte replacement for promoting tissue perfusion and ionic equilibrium is self evident. In the absence of any one single electrolyte. It is difficult to achieve optimal nutritional integrity. Rudman, et al (4.38) indicated that omission of sodium, potassium, phosphorous from TPN formula prevented the retention of the remaining elements, including nitrogen among emaciated hospitalised adults.

Intracellular ions

Potassium According to Cox (4.39) 90% of the total body potassium is located intracellularly in a concentration of 150 to 160 mEq/L. The potassium loss is partially due to increased cellular catabolism and partially due to loss of intracellular mass. In the injury state, cellular permeability may rise, giving rise to intracellular sodium shift and a fall in intracellular potassium. In anabolism, the direction of potassium flux is reversed which is rapidly enough to produce hypokalemia. In order to replenish stores and meet the daily requirements, 80-120 mEq/day of potassium is required (4.23).

Phosphorous Within cells, the concentration of phosphate is 75 mEq/litre. Following injury there is an increase of phosphorous loss in the urine (4.40). However, hypophosphatemia is not normally observed until nutritional repletion is initiated. Intravenous phosphorous containing solutions should not be given during the course of treatment for hypophosphatemia because rapid infusion may cause calcium phosphate to precipitate intravascularly and may produce symptomatic hypocalcemia. The total need for phosphorous is 14 to 16 mmol/day when a glucose / lipid source of non-protein energy is given. The requirements are increased when glucose is given as the sole source because high insulin levels increase the cellular uptake of phosphorous.

Magnesium Next to potassium, magnesium is the most abundant ion in cells with concentration of 58 mEq / litre. It is bound to protein and is necessary for a number of vital cellular functions which include membrane and mitochondrial integrity, enzyme activation and synthesis and stability of nuclear DNA (4.23). Like hypokalemia and hypophosphotemia, hypomagnesemia is also observed following the initiation of nutritional
supplementation which is treated by administering as much as 100 mmols in the initial 24 hrs period. Following this period, 30 mEq / day is generally considered adequate (4.41 - 4.42).

**Extracellular ions** In malnourished and critically ill patients due to the relative expansion of the extracellular space, ions are confined to the extracellular space if present above normal quantities.

**Sodium** In extracellular fluid, sodium is the principal ion in a concentration of about 140 mmols / litre. Total body sodium in the adult averages 30 mmol / kg body weight and is almost doubled, that is up to 53.9 mmol/kg, in surgical patients (4.43).

In stressed patients, occurrence of hyponatremia is very common due to increased secretion of antidiuretic hormone and to free water administration. In the average patient, about 100 to 120 mmol / day of sodium is recommended. Abnormal loss through the G.I tract requires additional sodium. In severe malnutrition and cardiopulmonary disease, the sodium intake should be reduced to 50 - 60 mmol / day.

**Calcium** More than 50% of total serum calcium is bound to protein, out of which 80% is normally bound to albumin and 20% to globulin. Normally when serum albumin is low, total serum calcium is also found to be low. Clinical signs of severe hypocalcemia include hypotension, myocardial depression, congestive heart failure, arrhythmias and seizures (4.43).

**Vitamins** Vitamins are essential nutrients that are active in a minute quantity. Dempsey, et al (4.44) noted improvement in nutritional status with a mixture that contained vitamins A and C and pyridoxine. Thiamine must be administered to all patients receiving nutritional support. Since pyridoxine is necessary for transamination of amino acid and may be deficient in patients with renal insufficiency, adequate pyridoxine should be administered.

**Selection of feeding mixture**

A variety of enteral mixtures are available for nutrition support which vary
in osmolality, digestibility, caloric density, lactose content, viscosity, taste and cost.

**Polymeric mixtures** are constituted of protein, fat and carbohydrate in high molecular weight forms. Formulae falling under these categories are mostly low in osmolality and require a functionally intact digestive system. The caloric density ranges from 1 to 2 kcal / mL. They are comparatively less expensive than other enteral solutions.

**Elemental mixtures** (monomeric) furnish protein in the form of amino acids and carbohydrate in the form of oligosaccharides and contain little or no starch or triglycerides. These formulas are hyperosmolar and moderately expensive. Elemental diets are indicated for patients with compromised digestive tract.

**Modular mixtures** are mixtures of commercially available protein, carbohydrate, fat and minerals to yield formulae for patients with special requirements.

**Enteral feeding routes**

Presently enteral feeding routes in practice are divided into two major categories as indicated in Figure 4.2.1:

**Figure 4.2.1. Routes for enteral feeding**

**Nasoenteric feeding** include nasogastric, nasoduodenal or nasojejunal instillation of a feeding tube in the G.I tract through the nose.

**Indications** Patients with oropharyngeal or oesophageal disorders who cannot eat, benefit from nasoenteric feeding. Patients with burns, certain gastrointestinal diseases, short gut or severe malabsorption and patients undergoing chemotherapy or radiation therapy all benefit from such feeding. Nasoenteric feeding is also used in the transition from total parenteral nutrition to combined parenteral and enteral nutrition and eventually to oral intake.

Nasoenteric feeding may provide nutritional therapy for patients with gastrointestinal fistulae. One study revealed that the placement of the nasoenteric tube beyond the fistulous site and feeding an enteral formula distal to the fistula was effective \((4.45)\). Humans studies have documented the rapid achievement of positive nitrogen balance and improved wound healing with immediate post operative feeding \((4.46)\).

The nasoenteric route is suggested for those patients who will be on this mode of enteral therapy for less than 4 weeks. However, nasoenteric tube feeding has been installed for long term alimentation in the hospital and at home with great success \((4.47-4.49)\).

**Contraindications:** Nasoenteric feeding should be avoided in instances when pancreatic or biliary stimulation is undesirable. Nasogastric in particular is contraindicated in patients with gastrooesophageal reflux.

**Enterostomy feeding**

Tube enterostomy is the surgical placement of the tube or catheter into any segment of the gastrointestinal tract. Tube enterostomy for feeding is indicated when access for nasoenteric feeding is not possible or long term feeding is anticipated (more than 4 weeks). Tube feeding by tube enterostomy consists of two major categories, primary and adjunctive (Figure 4.2.2). Primary enterostomy is the placement of a tube solely for feeding and is usually for patients in need of long term nutritional support where an adjunctive tube enterostomy is placed at the time of surgery and when delay in post operative intake is clinically anticipated and is usually
temporary. As a general rule, feeding tube enterostomy for more than six months is considered a permanent enterostomy (4.50).

**Figure 4.2.2. Categories of tube enterostomy**

<table>
<thead>
<tr>
<th>Primary tube enterostomy</th>
<th>Adjunctive tube enterostomy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a. Swallowing dysfunction</strong></td>
<td><strong>a. As an adjunct to surgical procedure</strong></td>
</tr>
<tr>
<td>central nervous system disorder</td>
<td>esophagostomy</td>
</tr>
<tr>
<td>collagen vascular disease</td>
<td>gastrectomy</td>
</tr>
<tr>
<td>myasthenia gravis</td>
<td>pancreatectomy</td>
</tr>
<tr>
<td><strong>b. Upper gastrointestinal obstruction</strong></td>
<td>hepatobiliary surgery</td>
</tr>
<tr>
<td>oropharyngeal neoplasm</td>
<td>small bowel resection</td>
</tr>
<tr>
<td>oesophageal structure or neoplasm</td>
<td>laparotomy</td>
</tr>
<tr>
<td>gastric, duodenal or pancreatic neoplasm</td>
<td></td>
</tr>
</tbody>
</table>


**Types of enterostomy**

Surgical tube enterostomies for feeding may be placed in the pharynx, oesophagus, stomach and jejunum. The stomach and jejunum are the most common location for feeding tube enterostomies (4.51).

**Esophagostomy** Ware, et al (4.52) indicated esophagostomy for patients requiring prolonged tube feeding such as patients with head and neck surgery. Esophagostomy performed at the time of operation has proven useful. Advantages of esophagostomy feeding are that it eliminates irritation of the nose and throat and causes less problem with secretions than a nasogastric tube. For esophagostomy, no drains are used since the tube itself acts as a drain.
Complications Infrequent and minor complications related to esophagostomy procedure are mild inflammation around the tube or tissue granulation.

Pharyngostomy is the placement of a tube in the oropharynx. Soon after pharyngostomy, patients can be started on feeding. The advantages of pharyngostomy are that it does not take long to construct; the abdomen is not opened, minimal drainage occurs at the stoma site; and only the smallest dressing is necessary. The tube is easily changed 5 to 6 days following placement. After removal of the tube, the fistulous tract closes rapidly leaving a minimal residual scar (4.51).

Indications Pharyngostomy for feeding is indicated for patients with:

1. Trauma or congenital anomalies of the maxillofacial region.
2. Cervical or maxillofacial procedures
3. Radiotherapy for partially obstructing oesophageal tumours.
4. Oral-pharyngeal lesions

Contraindications Pharyngostomy for feeding is contraindicated for patients with:

1. Complete obstruction of the thoracic oesophagus
2. Gastrointestinal obstruction
3. Neck trauma
4. Superior vena caval syndrome (4.51)

Complications: Very few complications of pharyngostomy feeding are reported (4.53-4.57). The complications of pharyngostomy are usually related to the pressure of the tube, which can cause irritation of the surrounding skin and lead to mild cellulitis. Other complications secondary to pharyngostomy are haemorrhage, aspiration pneumonia and injury to the surrounding structures.

Gastrostomy Gastrostomy is the placement of a tube in the stomach for feeding or decompression. It was the first operation ever performed on the human stomach (4.58). Due to its reservoir capacity, osmotic regulation
and prolonged transit, it is the preferred route for nutrition delivery. Easy access and flexibility of nutrient delivery further supports its use (4.51).

**Indications:** Gastrostomy is indicated for patients:

1. With oesophageal stricture and carcinoma.
2. Who refuse or cannot tolerate a nasoenteric tube.
3. With neurologic, traumatic and degenerative disease that interferes with swallowing.

**Contraindications:** Feeding by gastrostomy is contraindicated in the following condition:

1. Stomach with primary disease
2. Abnormal gastric and duodenal emptying
3. Significant oesophageal reflux
4. Lack of intact gag reflex (4.51)

**Complications:** Guaderer and Stellato (4.59) classified gastrostomy complications into three categories:

1. Complications related to operative technique:
   * separation of stomach from the abdominal wall.
   * wound infection
   * wound separation, ventral hernia
   * haemorrhage
   * improper placement of tube.

2. Complications related to stoma site:
   * skin irritation
   * tube plugging

3. Complications related to tube presence:
   * inadvertent removal of the tube
   * leakage of gastric content
   * excessive tissue granulation.
   * internal migration of the tube.
   * persistent gastrocutaneous fistula.
**Jejunostomy** is a surgical procedure which places a feeding tube in the jejunum. The decision for jejunostomy rather than a gastrostomy depends on the patients gastric emptying function, anticipated duration of enteral support and ambulatory status. The advantage of jejunostomy is that it reduces the gastroesophageal reflux (4.51) and the risk of aspiration associated with intragastric feeding. An additional advantage of jejunostomy is that feeding can be started within 12 to 24 hours following surgery (4.60).

**Indications (4.51)** Feeding jejunostomy is commonly performed as an adjunctive procedure during surgery and it is indicated for patients with:

1. Oesophageal and pancreatic surgery
2. Hepatic resection for trauma or tumour.
3. Reconstructive biliary surgery.

**Contraindications (4.51)** The major surgical complications associated with jejunostomy are:

1. Inadvertent removal or dislodgment.
2. Leakage of content into peritoneum.
3. Plugging of tube.
4. Volvulus.
5. Diarrhoea.

**Delivery of enteral formula**

A correct choice of the specific method for tube feeding whether it is bolus, intermittent, continuous or cyclic, ensures a safe delivery of desired nutrients as well as the patient’s tolerance. The other important factors, which support the provision of optimum nutrition are feeding initiation rate, concentration and systemic progression of a formula (4.60). The modes of enteral delivery include bolus, intermittent infusion or continuous drip.

1. **Bolus drip** involves rapid infusion of 300 to 400 mL of formula by syringe over 5 minutes. Bolus feeding into the stomach may cause abdominal distension and increase the risk of aspiration. However
healthy patients can tolerate bolus feeding. This mode of feeding is not recommended into the small intestine because of the risk of dumping syndrome (4.61 - 4.62).

2. **Intermittent feeding** involves dripping in formulae without the assistance of pump over a 30 minute period several time a day. This approach reduces the frequency of side effects noted during bolus feeding. The mode of delivery is suitable for out-patients with normal gastric emptying but should be avoided for jejunostomy feeding (4.61-4.62).

3. **Continuous feeding** is feeding patients continuously for 24 hours either by gravity drip or with an enteral feeding pump. It is the controlled delivery of a prescribed volume of formula at a constant rate over a period of 24 hours (4.62). Continuous infusion is utilised when bolus or intermittent feeding are not tolerated. Continuous feeding has an advantage over other methods of enteral feeding because it is better tolerated by the patients (4.63). Moreover, continuous feeding is associated with increased nitrogen retention when compared to intermittent feeding at night only (4.64). Continuous feeding is a preferred method especially when the availability of a feeding pump is not a problem. Otherwise a constant infusion rate cannot be assured with gravity feeding bag (4.62).

4. **Cyclic infusion** is usually pump assisted and can be administered to a patient over 8-16 hours per day. It is considered beneficial in transitional feeding. Krey (4.65) supported cyclic feeding by observing that it facilitates home enteral support and allows patients a more normal life style.

**Complications of tube feeding**

Complications which may arise during enteral feeding are either tube related, or due to composition or sterility of the feed. Most of these complications may be avoided by altering the feeding technique or composition of the formulae.
1. **Mechanical or tube related complications** include knotting or clogging of the tube, improper tube placement, nasopharyngeal erosion and discomfort, otitis, gagging, oesophageal reflux and esophagitis, tracheoesophageal fistulas and ruptured oesophageal varices (4.63, 4.66-4.67). These problems may be related to size and rigidity of the tube.

2. **Gastrointestinal complications** are among the most commonly reported problems in patients (over 25%) receiving enteral nutrition (4.68 - 4.69).

   a. *Nausea and vomiting* Nausea, vomiting, bloating and abdominal cramps may occur in 10 - 15% of tube fed patients. Common causes of such complications are the rate of infusion, large volumes, fat or lactose intolerance, hyperosmolality and delayed gastric emptying (4.69).

   b. *Diarrhoea* Diarrhoea develops in 2.3 -30.6% of enterally fed patients (4.67, 4.69). Among the causes are hypertonic feeding, volume and rate of infusion, bacterial contamination, lactose intolerance, medicinal elixir and concomitant antibiotic therapy (4.70 - 4.72). In the ICU setting, hypoalbuminemia, changes in the intestinal flora and drug side effects are also common causes of G.I problems (4.73, 4.78). Enteral formulae containing large quantities of fat, including MCT may occasionally produce diarrhoea (4.33, 4.79). Kagawa-Busby (4.80) reported abdominal cramps and diarrhoea upon the administration of a cold feed straight from the refrigerator.

   In most cases, diarrhoea due to the above mentioned causes may be managed by eliminating or reducing problem nutrients such as fats, lactose or by maintaining a reasonable osmolality and infusion rate (4.67).

   c. *Aspiration and regurgitation* Regurgitation of feeds up into the oesophagus, occasionally followed by pulmonary aspiration, is common in patients with gastric emptying problems and with neurological disease, particularly affecting the swallowing reflex.
The problem of gastric emptying is common in post operative patients, in patients with cerebrovascular accidents and with those who have suffered recent severe trauma (4.81).

d. **Constipation** It is considered a chronic problem in long term enterally fed patients and studies have been carried out to address this issue. Patients on long term enteral nutrition may develop constipation, particularly if they are dehydrated or on low lactose formula (4.82). Shankardass, et al (4.83) conducted a double blind randomised crossover study to evaluate the effect of two enteral formulae on stool frequency, fecal weight, laxative use, gastrointestinal tolerance and bowel function. The result showed that enteral formula with dietary fibre improved gastrointestinal tolerance and bowel function and reduced laxative use in long term enterally fed patients. Another study by Gibson, et al (4.84) on effectiveness of bran supplement on bowel management of elderly rehabilitation patients showed that patients on fibre supplements had significantly lower number of bowel agent per day as compared to the control patients.

**II. Metabolic complications** may arise during enteral feeding such as:

a. **Dehydration and hyperglycemia** Nasogastric enteral feeds of a very high osmolality (ie about 1000 mOsmol/ kg) with a high protein content have occasionally produced dehydration, hypernatremia and uraemia (4.63, 4.85).

Hyperglycemia may occur during enteral feeding due to either a high carbohydrate content or a relative insulin resistance that occurs with illness (4.86).

b. **Electrolyte abnormalities** particularly hypokalemia and hyperkalemia may occur during enteral feeding and are related not only to the feeding regimen, but also to the underlying medical or surgical disorders. Low levels of calcium, magnesium, zinc and phosphate can also occur, which highlights the need for careful monitoring and administration of supplements during enteral nutrition support (4.87).
c. Abnormalities of liver function have been reported in patients receiving enteral nutrition (4.88, 4.89). Elevation of alkaline phosphatase, gamma glutamyl transpeptidase and the hepatocellular enzymes as well as occasional and mild elevation in bilirubin levels are observed in such cases.

**Determination of the nutritional status of hospitalised patients**

Sufficient intake of diet will keep the body composition and function of the healthy individual within the normal range. The equilibrium may be disturbed by three processes: decreased intake, increased requirements and altered utilisation which prevents nutrients from being utilised for tissue repair (4.90). Proteins are the least dispensable. The loss of protein is minimised by reducing the need for its use as a source of energy (4.87). But protein wasting continues when fat stores are exhausted. Loss of body fat and protein results in loss of body weight, reduced thickness of subcutaneous fat folds and reduced bulk and reduced excretion of creatinine in the urine. Nutritional status is assessed by:

1. Historical data
2. Anthropometric measurement
3. Biochemical analysis (laboratory test)
4. Physical examination

**Historical data**

*Medical history* An accurate, complete health history can reveal conditions that place an individual at risk for malnutrition.

*Socioeconomic history* An individual’s ethnic background, economic and educational level may influence food availability and food choices.

*Diet history* A diet history provides a record of a person’s food intake. Information about what and how a person eats provides the background for realistic and attainable nutritional goals.

a. 24 hours recall The 24 hours recall provide data for one day only and is commonly used in nutrition survey. Its usefulness is limited in that it
does not provide enough accurate information to allow generalisations about an individual's usual food intake. This limitation is partially overcome when 24 hours recalls are collected on several non-consecutive days.

b. *Usual intake* A person’s usual intake pattern can provide much useful information especially in verifying food intake when the past 24 hours have been atypical.

c. *Food frequency checklist* The purpose of this record is to assess how often an individual eats a specific type of food per day, week or month.

d. *Food diary* Another tool for history taking is the food diary, which includes not only food eaten, but time of day and place. A food diary can help to determine factors associated with eating that may affect dietary balance and adequacy.

Nutrient intakes identified by diet histories are only pieces of a puzzle that must be put together with other indicators of nutrition status.

**Anthropometric measurement**

Anthropometrics are physical measurements that provide an indirect assessment of body composition and development. The primary purpose is to evaluate the progress of growth in pregnant women, infants, children and adolescents as well as to detect undernutrition and obesity in all age groups.

Among anthropometric measurements, height and weight are the most common. Other anthropometrics include fat fold measurement and various measures of lean tissue. A head circumference measurement may help to assess brain development in an infant and abdominal girth measurement supplies information about abdominal fluid retention in individuals with liver disease.

*Body weight* It is the most common variable considered in nutritional assessment and there is a definite correlation between significant weight loss and morbidity. In assessing weight loss, current body weight is
compared with the usual and ideal body weights. The limitation of overall weight loss as a nutritional assessment index is that the specific compartments of the body contributing to the lost mass are not identified. However, changes in body weight over a long period are probably the best indicator of a change in nutritional status. Never the less, weight loss measurements alone are not reliable for nutritional assessment and should be combined with other measures of nutritional status.

**Total body fat** Subcutaneous fat constitutes approximately 50% of the body fat stores and accurately reflects the total body fat content. Triceps skin fold thickness is used to measure subcutaneous fat and the value measured is compared to a table of norms (4.90).

**Skeletal muscle store** Skeletal muscle represents 60% of the total body protein pool and is the major source of amino acids during stress and starvation (4.90).

**Biochemical analysis**

Biochemical analysis or laboratory tests help to determine what is happening to the body internally. Most tests are based on analysis of blood and urine samples which contain nutrients, enzymes and metabolites that reflect nutrition. However, no single test is a reliable index because many factors influence laboratory tests.

**Creatinine height index (CHI)** is designed to measure skeletal muscle mass. Creatinine is the end product of creatinine metabolism. It is found mainly in skeletal muscle and is excreted in the urine and directly correlates with muscle mass. However, creatinine height index does not correlate well with measured changes in body composition (4.91).

**Methyl histidine excretion** “Histidine is an amino acid which is found in muscle protein. The histidine is methylated in the 3 position and after proteolysis, is excreted unchanged in the urine” (4.91). Thus, it is used as an index of the rate of muscle proteolysis (4.92).

**Plasma proteins** The circulating level of plasma protein depend on:
a. the rate of synthesis which in turn depends on the adequacy of substrate precursors and liver synthetic ability.

b. the volume of distribution which deals with the relative sizes of the intravascular and extravascular spaces and oncotic pressures.

c. the rate of catabolism which deals with plasma protein half life and losses of protein from renal and other sources.

**a. Serum albumin** Over 50% of the total serum protein is albumin. Albumin’s prime functions are to maintain plasma oncotic pressure and to transport other substances (4.93). An albumin level between 2.8 and 3.5 g/dL represents mild protein depletion, 2.2 to 2.7 g/dL moderate depletion and less than 2.2 g/dL severe depletion (4.92). Albumin level correlate with the degree of malnutrition and the increased risk for mortality and morbidity (4.94). Changes in serum albumin levels also have been used as an indicator of the efficacy of nutritional support regimens but cannot be used in hospitalised patients as discrete markers of protein nutrity (4.94). Starker, et al (4.93) as well demonstrated that serum albumin level or body weight alone cannot be used as an accurate indicator of nitrogen balance due to the variability in the response of body fluid compartments to underlying clinical conditions.

**b. Serum transferrin** Transferrin is a protein that transports iron between the intestine and sites of haemoglobin synthesis and degradation. It has a half life of 8 days and hence is considered a more sensitive indicator because it responds more promptly to changes in protein intake.

**c. Thyroxine binding prealbumin (TBPA)** Thyroxine binding pre-albumin has a small body pool and a short half life (2 to 3 days). It transports thyroid hormone and is a carrier protein for retinol binding protein. Synthesis of prealbumin is calorie and protein dependent and rapidly responds to appropriate feeding (4.91). According to Seltzer, et al (4.95), measurable changes occur in pre-albumin level with in 7 days of a change in nutrient intake. In several studies, pre albumin proved to be an early indicator of visceral protein anabolism in patients receiving nutritional support (4.96 - 4.98). The below mentioned levels indicate the level of depletion (4.99):
10 - 15 mg/dL  mild depletion
5 - 10 mg/dL  moderate depletion
< 5 mg/dL  severe depletion

d. Retinol binding protein (RBP)  Retinol binding protein has a small body pool and a very short half life (12 hours) and therefore is very sensitive to synthesis and utilisation changes. It is specific for vitamin A transport and is linked with thyroxine binding pre-albumin in a constant molar ratio (4.91).

Total lymphocyte counts and other tests of immune function

Total lymphocyte count  As protein depletion occurs the count decreases and therefore it is a useful index of nutrition status but not an absolute indicator. It is more reliable than skin testing. It is cheap, fast and easy to do (4.91). The equation used to calculate total lymphocyte count (4.100) is given below.

\[
\text{Total lymphocyte count (mm}^3\text{)} = WBC (mm}^3\text{) } \times \text{ % lymphocytes}
\]

Interpretation of TLC with regards to nutritional status is as follow (4.94):

1500 - 1800  mild depletion
900 - 1500  moderate depletion
< 900  severe depletion

Antigen skin testing  An organism to which most people are immune is injected just under the skin (mumps, second strength - purified protein derivatives of tuberculin (PPD), candida streptokinase - streptodonase (SKSD) and trichophytin). After 48 hours, the site of injection is inspected for raised hardened areas (induration) (4.91).

A study by Jeejeebhoy and Meguid (4.101) revealed alteration in reactivity, secondary to underlying disease or sepsis and not related to
malnutrition. The following factors have been shown to alter delayed cutaneous hypersensitivity in the absence of malnutrition:

- Infection (viral and bacterial)
- Trauma, uraemia, cirrhosis, hepatitis and haemorrhage.
- Steroids, immunosuppressant, cimitidine and warfarin.
- General anaesthesia and surgery.

Considering the likelihood of co-existence of one or more of these factors with malnutrition, delayed cutaneous hypersensitivity is not recommended for routine nutritional assessment.

**Nitrogen balance** It is a measure of the daily intake of nitrogen minus the excretion. The intake represents nutritional nitrogen and the excretion consists of measured urinary urea nitrogen (UUN) in a 24 hours urine collection plus a factor for increased gastrointestinal and cutaneous losses usually 2 to 4 g. Urinary nitrogen has several components, urea, uric acid, ammonia, amino acids and creatinine. Approximately 90% of the urinary nitrogen is excreted as urea except in chronic situations or severe stress and sepsis when it may fall to as low as 65% to 70%. Urinary urea nitrogen is thus an unreliable measure for nitrogen loss in stressed or post operative patients (4.102). Under such condition, total urinary nitrogen (TUN) gives a more accurate reflection of losses (4.91).

A positive nitrogen balance indicates an anabolic state with an over all gain in body protein for the day, where as a negative nitrogen balance indicates a catabolic state with a net loss of protein.

Urinary nitrogen excretion is considered a useful indicator of protein catabolism. During times of extreme stress, such as in trauma, it may rise to 30 to 50 g/day which is equivalent to a daily loss of 1 to 1.5 kg of lean body mass daily (4.90).

**Energy balance** Energy balance is the difference between energy intake and expenditure. The energy expenditure can be measured using indirect calorimetry or calculated using predictive formula such as the Harris Benedict equation (4.103).
Chapter 4.3: Methodology

To assess the efficacy of indigenous enteral formulae, a pilot study was carried out comparing the effectiveness of indigenous formulae versus commercial formulae in improving the nutritional status of patients. The study was conducted after being approved by the University of Western Sydney Human Research Ethics Committee and the Aga Khan University Human Subject Protection Committee (Appendix 4.3.1). The study was supported by the Australian International Development Assistance Bureau and the University of Western Sydney, Hawkesbury. The study commenced in March 93 and was completed in August 93.

Patients selection criteria

This study was conducted with adult patients having any one of the following selection criteria:

1. Post operative patients with upper G.I anastomosis whose suture line needed to be protected were fed through a jejunostomy.
2. Patients with oral surgery who were unable to take solid food were fed through a straw or a nasogastric tube.
3. Head injury patients who were unconscious were fed through a nasogastric tube.
4. Patients with enterocutaneous fistulae were fed orally or through a nasogastric tube or through a enterostomy.
5. Patients with short bowel syndrome who were fed orally or through a nasogastric tube.

Each category was allocated randomly to either an experimental diet or a controlled diet (Appendix 4.3.2).

Diet Characteristics

*Experimental diets* Diet # 4a was selected to be the experimental diet for the trial. It was a polymeric diet (PD), suitable for patients with functionally intact digestive system (criteria 1-3). The same diet treated
with enzyme was designated a partially digested diet (PDD) for patients with compromised digestive systems (criteria 3-4). The experimental diets (PD and PDD) were similar in composition. The polymeric diet was isotonic at 4/5 strength and 410 mOsm/kg at full strength. The partially digested diets ranged from 266-290 mOsm/kg at half strength. Both diets were isocaloric with a calorie ratio of approximately 53:29:18 from carbohydrate, fat, protein respectively and isonitrogenous with a non-protein calories to nitrogen ratio (NPC:N) of 113:1, furnishing 1 kcal per 1 mL at full strength.

**Control diet** The control diet was a Mead Johnson product called "Isocal". The protein in the control diet was in the form of calcium and potassium caseinate; carbohydrate was supplied as maltodextrin; and the fat component was comprised of MCT oil and corn oil. Isocal is easily digestible by patients with a compromised digestive system such as gastrointestinal fistulae and/or short bowel syndrome. It is isotonic (300 mOsm / kg water) and isocaloric with a caloric ratio of approximately 50:37:13 from carbohydrate, fat and protein respectively with a non-protein calories to nitrogen ratio (NPC:N) of 150:1.

**Baseline assessment of patients**

After selecting patients for the study, baseline assessment was carried out which included the below mentioned biochemical measures beside taking height and weight (where applicable):

1. **Nitrogen balance** objectively estimate the protein turnover, hence considered useful for evaluating the adequacy of nutrition therapy by estimating nitrogen retention or loss. In order to determine the nitrogen balance, 24 hours urine specimen of all study patients was collected as per study protocol. A 24 hours urine collection of camotose patient was collected through urine catheter tube. Whereas ambulatory patients were instructed to collect urine for 24 hours in a given bottle. Once the urine sample of study patient was ready, it was sent to the hospital laboratory. In the laboratory, urinary urea nitrogen (UUN) was determined by laboratory technician using BUN Reagent Kit, P/N 667530 for in vitro diagnostic use. BEKMAN INSTRUMENT, INC. GALWAY, IRELAND (4.109). The measurement of urea in the collected
urine sample was than combined with protein intake data to give rough estimate of nitrogen balance. The equation used for nitrogen balance (4.100) is given below:

\[
\text{Nitrogen balance} = \frac{24 \text{ hrs protein intake}}{6.25} - (\text{UUN}^* + 4)
\]

* Urinary urea nitrogen

2. **Prealbumin** has been shown to be more responsible to dietary change than albumin in clinical studies involving malnourished adults (4.97 - 4.98). Prealbumin is considered superior to albumin in neonates following surgery (4.104) and considered useful indicator to monitor the response to nutrition therapy (4.105). In this study, serum prealbumin was determined by the hospital laboratory technician, using M - Partigen®. Prealbumin immunodiffusion plates were used for quantitative determination of plasma protein. BEHRING (4.106).

3. **Absolute lymphocyte count** was used to provide an estimate of immune function because it is considered useful as a screening parameter for non-critical patients (4.99). Delayed hypersensitivity skin testing could not be used for this study because it is not a useful parameter in individual with infection, cancer and trauma (4.99). In order to determine the TLC count, a complete blood cell count (CBC) was obtained using BUN Reagent Kit, P/N 667530 for invitro diagnostic use. BEKMAN INSTRUMENT, INC. GALWAY, IRELAND (4.107). It also provided a differential count, which state the percentages of the different types of white blood cells that make up the total. For assessment purpose, total lymphocyte count was obtained by the following calculation (4.100):

\[
\text{Total lymphocyte count} = \frac{\% \text{ lymphocyte} \times \text{total WBC/mm}^3}{100}
\]

**Note:** All biochemical tests were carried out at the Aga Khan University Hospital laboratory.
Calculation of calorie and protein requirements

**Calories requirement** Using the Harris Benedict equation, basal energy expenditure (BEE) was calculated and then multiplied by activity and stress factors.

\[
\text{Men BEE} = 66.47 + (13.75 \, W^* + 5.0 \, H^*) - 6.76 \, A^* \times \text{activity factor} \times \text{stress factor} \\
\text{Women BEE} = 655.10 + (9.56 \, W^* + 1.85 \, H^*) - 4.86 \, A^* \times \text{activity factor} \times \text{stress factor}
\]

\*W = weight in kg,  \*H = height in cm,  \*A = age in yrs

**Activity factor**

- confined to bed 1.2
- out of bed 1.3

**Stress factor**

- minor operation 1.2
- skeletal trauma 1.35

**Protein requirement** The protein requirement for healthy individuals is calculated on the basis of ideal body weight. Precise recommendations for protein allowance for the seriously ill vary widely. Keeping this in view, protein requirement for each study patient was calculated on the basis of factor 1.25 g per kg of body weight which is the mean of factors 1 and 1.5 suggested for patients with varying degree of stress (4.91).

**Feeding regimen**

A written consent was obtained (Appendix 4.3.1) prior to determination of the patients’ protein and calorie requirements and random allocation to either the experimental or control diet. Once the patient was on the study, he or she was put on the assigned diet and assessed and monitored for a maximum period of 23 days.

A protocol was prepared (Appendix 4.3.3) for ordering the feed. The feed orders were registered at the diet office and the experimental (PD, PDD)
and control diets were both prepared in the hospital kitchen by the kitchen staff and delivered to the ward nurse incharge.

Enteral feeding was initiated using the following guidelines:

**Polymeric diet (PD)**

*Oral feeding* The amount of full strength polymeric diet to be administered initially was dependent on the patient’s condition.

*Tube feeding* Feeding initiation was by slow infusion (50 mL / hour) of full strength polymeric diet on the first day. On the following day, the rate of infusion was increased every eight hours until the desired amount was achieved.

**Partially digested diet (PDD)**

*Oral feeding* The amount and strength of diet to be infused initially was dependent on the patient’s condition.

*Tube feeding* Slow infusion (50 mL/hour) of 1/2 strength partially digested diet was given on the initial day. Full strength was given on the following day. Subsequently, the rate was increased every 8 hours until the desired amount was achieved.

*Note: Partially digested diet is hyperosmolar at full strength, therefore 1/2 strength on day one was used to avoid any gastrointestinal distress.*

The study was continued for 23 days or until one of the following occurred:

- the patient started consuming food regularly.
- the attending physician felt that the patient recovery would be impaired as a result of:

  a. Nausea
  b. Diarrhoea
  c. Abdominal distension
  d. Electrolyte imbalance
e. Hyper / hypoglycemia
f. Increased fistula output.

**Evaluating the effectiveness of therapy**

There is no good single test that provides a measure of nutritional status. Hence for the purpose of the study, following methods were used to evaluate the outcome of nutrition therapy.

The following outcomes were assessed on *day 1, 2, 4, 6, 8, 12, 16, 20, final day* to measure the effectiveness of therapy:

1. Nitrogen balance (Urinary urea nitrogen)
2. Pre albumin
3. Absolute lymphocyte

*final day refers to the day on which the feed was discontinued, if this did not coincide with one of the proposed days for the lab test*

The following parameters were monitored for any possible side effects experienced by the patients:

- Serum glucose  
  Day 1, 2, 4, 6, 8, 12, 16, 20, final day* (This regime applies to serum glucose and electrolytes only)
- Serum electrolytes (Na⁺,K⁺)
- Stool output daily
  - < 3 loose stools
  - 3-6 loose stools
  - > 6 loose stools:
    - mild
    - moderate diarrhoea
    - severe diarrhoea
- Nasogastric aspiration/vomiting in mL: daily
- Fistula output in mL: daily
- Other output: daily
- G.I distress: daily
  - (nausea, abdominal, cramps, bloating and or oesophageal reflux)

*final day refers to the day on which the feed was discontinued, if this did not coincide with one of the proposed days for the lab test.*
1. **Serum glucose** was determined using Glucose Reagent Kit, P/N 668300 (for in Vitro diagnostic use). BECKMAN INSTRUMENT, INC. GALWAY, IRELAND (4.108).

2. **Serum electrolytes** levels were assessed using Sodium / Potassium Reagent Kit, No. 668302. Sodium / Potassium Conditioning Solution Kit, , Sodium / Potassium Buffer Solution Kit, No. 668802. (for in Vitro diagnostic use). BECKMAN INSTRUMENT, INC. GALWAY, IRELAND (4.109).

*Note: All Biochemical tests were carried out at the Aga Khan University Hospital laboratory.*

**Information sheet**

An information sheet (Appendix 4.3.4) was designed to record data for every individual patient included in the study.

**Criteria for excluding patients from the study**

The adult patients with anyone of the below mentioned criteria were excluded from the study:

1. Patients with septic scores of $\geq 20$. (A sepsis severity scoring system designed by Elebute and Stoner (4.110) was used to differentiate the non septic patients from those with sepsis (Appendix 4.3.5).
2. Patients having less than two readings of each of the three parameters of nutritional status.
3. Patients not tolerating the randomly assigned diet.

**Microbial screening**

To ensure the safety of the patients, microbial screening of the formulae was carried out on a random basis.

**Statistical test**

Student $t$-test was used to look at the changes in the nitrogen balance,
serum prealbumin and total lymphocyte count during the study period.
Chapter 4.4: Result

Patients’ profile

Twenty four (24) patients who met the selection criteria were included in the study. All those included scored less than or equal to 19 points on

Table 4.4.1. Profile of patients in control and experimental group

<table>
<thead>
<tr>
<th>S #</th>
<th>Age yrs</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Sep. score</th>
<th>Total energy req. (kcal)</th>
<th>Total protein req. (g)</th>
<th># of days</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>61</td>
<td>M</td>
<td>Tumour excision</td>
<td>6</td>
<td>2614</td>
<td>95</td>
<td>11</td>
</tr>
<tr>
<td>02</td>
<td>83</td>
<td>M</td>
<td>CVA</td>
<td>6</td>
<td>1915</td>
<td>85</td>
<td>14</td>
</tr>
<tr>
<td>03</td>
<td>30</td>
<td>M</td>
<td>Post RTA contusion and concussion</td>
<td>1</td>
<td>2448</td>
<td>73</td>
<td>16</td>
</tr>
<tr>
<td>04</td>
<td>60</td>
<td>F</td>
<td>Oesophageal stricture</td>
<td>2</td>
<td>1223</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td>05</td>
<td>83</td>
<td>M</td>
<td>Squamous-cell Carcinoma of Larynx</td>
<td>1</td>
<td>1800</td>
<td>72</td>
<td>06</td>
</tr>
<tr>
<td>06</td>
<td>71</td>
<td>F</td>
<td>Old CVA, Meningitis Gastroenteritis</td>
<td>5</td>
<td>2400</td>
<td>120</td>
<td>09</td>
</tr>
<tr>
<td>07</td>
<td>68</td>
<td>M</td>
<td>CVA</td>
<td>18</td>
<td>1622</td>
<td>81</td>
<td>06</td>
</tr>
<tr>
<td>08</td>
<td>53</td>
<td>F</td>
<td>CVA</td>
<td>5</td>
<td>1852</td>
<td>78</td>
<td>07</td>
</tr>
<tr>
<td>09</td>
<td>43</td>
<td>M</td>
<td>Meningitis</td>
<td>6</td>
<td>2000</td>
<td>56</td>
<td>21</td>
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<tr>
<td>10</td>
<td>65</td>
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<td>CVA, Intracerebral bleed</td>
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<td>19</td>
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<td>80</td>
<td>M</td>
<td>CVA</td>
<td>4</td>
<td>1900</td>
<td>95</td>
<td>12</td>
</tr>
<tr>
<td>12</td>
<td>67</td>
<td>F</td>
<td>Meningitis</td>
<td>19</td>
<td>1800</td>
<td>81</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S #</th>
<th>Age yrs</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Sep. score</th>
<th>Total energy req. (kcal)</th>
<th>Total protein req. (g)</th>
<th># of days</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>63</td>
<td>M</td>
<td>CA Tongue</td>
<td>01</td>
<td>1900</td>
<td>75</td>
<td>11</td>
</tr>
<tr>
<td>02</td>
<td>62</td>
<td>F</td>
<td>Excision of vocal cord tumour</td>
<td>01</td>
<td>1905</td>
<td>69</td>
<td>11</td>
</tr>
<tr>
<td>03</td>
<td>60</td>
<td>M</td>
<td>CA Larynx</td>
<td>01</td>
<td>1800</td>
<td>69</td>
<td>10</td>
</tr>
<tr>
<td>04</td>
<td>57</td>
<td>M</td>
<td>Bacterial meningitis</td>
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<td>100</td>
<td>22</td>
</tr>
<tr>
<td>05</td>
<td>60</td>
<td>M</td>
<td>Meningioma, post,op. deterioration.</td>
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<td>2175</td>
<td>85</td>
<td>23</td>
</tr>
<tr>
<td>06</td>
<td>70</td>
<td>M</td>
<td>Squamous cell CA of lower oesophagus</td>
<td>03</td>
<td>2000</td>
<td>80</td>
<td>06</td>
</tr>
<tr>
<td>07</td>
<td>60</td>
<td>M</td>
<td>CVA</td>
<td>0</td>
<td>2100</td>
<td>85</td>
<td>05</td>
</tr>
<tr>
<td>08</td>
<td>60</td>
<td>M</td>
<td>CVA, meningitis</td>
<td>09</td>
<td>-</td>
<td>80</td>
<td>05</td>
</tr>
<tr>
<td>09</td>
<td>75</td>
<td>M</td>
<td>CVA</td>
<td>04</td>
<td>1900</td>
<td>83</td>
<td>06</td>
</tr>
<tr>
<td>10</td>
<td>53</td>
<td>M</td>
<td>CVA</td>
<td>04</td>
<td>2123</td>
<td>91</td>
<td>08</td>
</tr>
<tr>
<td>11</td>
<td>43</td>
<td>M</td>
<td>CA, stomach</td>
<td>0</td>
<td>2590</td>
<td>88</td>
<td>13</td>
</tr>
<tr>
<td>12</td>
<td>68</td>
<td>F</td>
<td>CVA</td>
<td>03</td>
<td>1500</td>
<td>63</td>
<td>06</td>
</tr>
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</table>
Elebute and Stoner scoring system which meant essentially that they did not have sepsis. Twelve patients were randomly assigned to the control diet and 12 to the experimental diet.

Table 4.4.1. shows the profiles of the patients in the two groups including their age, sex, diagnosis, sepsis score, caloric and protein requirements. A process related characteristic, length of hospital stay is also compared in this table. Out of 16 patients who had cerebrovascular accident (CVA), meningitis or head injury, nine were in the control group and seven in the experimental group. Similarly out of 8 patients with head and neck, oesophageal or gastric cancer, three were in the control group and five in the experimental group. As all the patients had intact gastrointestinal tracts, the indigenous partially digested formulae was not used. There was no significant difference between the groups in terms of mean age and length of stay. The mean sepsis score in the control group was greater than in the experimental group (6.5 vs 3.25 respectively) but the difference was not statistically significant (Table 4.4.2).

**Table 4.4.2. Comparison of means related to age, length of stay and sepsis score**

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Degree of freedom</th>
<th>2 Tail probability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control*</td>
<td>62.92</td>
<td>16.10</td>
<td>22</td>
<td>.705</td>
</tr>
<tr>
<td>Experimental*</td>
<td>60.92</td>
<td>8.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong># of days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13.00</td>
<td>5.61</td>
<td>22</td>
<td>.312</td>
</tr>
<tr>
<td>Experimental</td>
<td>10.50</td>
<td>6.20</td>
<td></td>
<td></td>
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<tr>
<td><strong>Sepsis score</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>6.50</td>
<td>5.90</td>
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<td>.133</td>
</tr>
<tr>
<td>Experimental</td>
<td>3.50</td>
<td>3.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Controlled (N=12), Experimental (N=12)*

Energy requirement was calculated for all but one patient in the experimental group, whose height and weight measurements were not available. The mean caloric requirement of the control group was marginally less than that of the experimental group. The difference may be explained by the fact that there were comparatively older patients in the control group. The metabolic rate slows down with increasing age. The mean protein requirement of the two groups was similar (Table 4.4.2).
Actual calorie intake

Data reveals that the energy intake of patients in both control and experimental group was as a rule less than the calculated requirement (Table 4.4.3). In two patients, one in each group, the caloric intake exceeded the calculated requirement.

Table 4.4.3. Energy and protein requirement versus intake of control and experimental groups

<table>
<thead>
<tr>
<th>Control group</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>S #</td>
<td>Calculated energy requirement (kcal)</td>
<td>Actual energy intake (kcal)</td>
<td>Calculated protein requirement (g)</td>
<td>Actual protein intake (g)</td>
</tr>
<tr>
<td>1</td>
<td>2614</td>
<td>1089</td>
<td>95</td>
<td>63</td>
</tr>
<tr>
<td>2</td>
<td>1915</td>
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<td>3</td>
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<td>1491</td>
<td>73</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>1223</td>
<td>2378</td>
<td>29</td>
<td>79</td>
</tr>
<tr>
<td>5</td>
<td>1800</td>
<td>1386</td>
<td>72</td>
<td>49</td>
</tr>
<tr>
<td>6</td>
<td>2400</td>
<td>1301</td>
<td>120</td>
<td>42</td>
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<td>7</td>
<td>1622</td>
<td>1525</td>
<td>81</td>
<td>50</td>
</tr>
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<td>8</td>
<td>1852</td>
<td>1203</td>
<td>78</td>
<td>39</td>
</tr>
<tr>
<td>9</td>
<td>2000</td>
<td>1997</td>
<td>56</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>1800</td>
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<td>1516</td>
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<tr>
<td>12</td>
<td>1800</td>
<td>1055</td>
<td>81</td>
<td>39</td>
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<table>
<thead>
<tr>
<th>Experimental group</th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>S #</td>
<td>Calculated energy requirement (kcal)</td>
<td>Actual energy intake (kcal)</td>
<td>Calculated protein requirement (g)</td>
<td>Actual protein intake (g)</td>
</tr>
<tr>
<td>1</td>
<td>1900</td>
<td>1750</td>
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<td>78</td>
</tr>
<tr>
<td>2</td>
<td>1905</td>
<td>1312</td>
<td>69</td>
<td>64</td>
</tr>
<tr>
<td>3</td>
<td>1800</td>
<td>1725</td>
<td>69</td>
<td>69</td>
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<td>1696</td>
<td>100</td>
<td>71</td>
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<td>5</td>
<td>2175</td>
<td>1300</td>
<td>85</td>
<td>62</td>
</tr>
<tr>
<td>6</td>
<td>2000</td>
<td>1350</td>
<td>80</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>2100</td>
<td>1291</td>
<td>85</td>
<td>67</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>1252</td>
<td>80</td>
<td>49</td>
</tr>
<tr>
<td>9</td>
<td>1900</td>
<td>1265</td>
<td>83</td>
<td>46</td>
</tr>
<tr>
<td>10</td>
<td>2123</td>
<td>1394</td>
<td>91</td>
<td>64</td>
</tr>
<tr>
<td>11</td>
<td>2500</td>
<td>2058</td>
<td>88</td>
<td>92</td>
</tr>
<tr>
<td>12</td>
<td>1500</td>
<td>1900</td>
<td>63</td>
<td>85</td>
</tr>
</tbody>
</table>

The mean caloric intake ± SD of both control and experimental groups met approximately 75% of the calculated requirement only (Table 4.4.4).
Table 4.4.4. Mean energy and protein requirement versus intake of control and experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Mean calculated energy requirement</th>
<th>Mean actual energy intake</th>
<th>Mean calculated protein requirement</th>
<th>Mean actual protein intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1948 ± 653</td>
<td>1453 ± 386</td>
<td>79 ± 22</td>
<td>50 ± 13</td>
</tr>
<tr>
<td>Experimental group</td>
<td>2028 ± 278</td>
<td>1524 ± 283</td>
<td>81 ± 10</td>
<td>65 ± 24</td>
</tr>
</tbody>
</table>

Actual Protein intake

The mean protein intake ± SD of the control group met approximately 63% of the calculated protein requirement (Table 4.4.4). In this group protein intake of one patient exceeded the recommended allowance (Table 4.4.3). The mean protein intake ± SD of the experimental group was 81% of the recommended allowance (Table 4.4.4). Protein intake of three patients in the experimental group exceeded the recommended allowance (Table 4.4.3).

Due to lack of availability of enteral feeding pumps, patients were given boluses. Patients who experienced intolerance were given continuous feeding until the symptoms resolved.

Evaluation of nutritional outcome of patients

Nitrogen balance

Table 4.4.5 shows the initial and final value of nitrogen balance of control and experimental groups. Out of 12 patients in the control group, 7 showed improvement in nitrogen balance and 5 showed a downward trend, whereas in the experimental group, out of 11 patients, 7 improved and 4 showed deterioration in nitrogen balance.

Student t. test analysis showed that patients in experimental group had better positive trend of nitrogen balance as compared to control group but was not statistically significant (Table 4.4.6).
Table 4.4.5. Initial and final nitrogen balance value of patients in control and experimental groups

<table>
<thead>
<tr>
<th>Control group</th>
<th>Experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial value (g/day)</td>
<td>Final value (g/day)</td>
</tr>
<tr>
<td>1</td>
<td>- 6.90</td>
</tr>
<tr>
<td>2</td>
<td>- 4.63</td>
</tr>
<tr>
<td>3</td>
<td>+ 1.56</td>
</tr>
<tr>
<td>4</td>
<td>+ 6.55</td>
</tr>
<tr>
<td>5</td>
<td>- 2.73</td>
</tr>
<tr>
<td>6</td>
<td>- 1.37</td>
</tr>
<tr>
<td>7</td>
<td>+ 4.04</td>
</tr>
<tr>
<td>8</td>
<td>- 5.80</td>
</tr>
<tr>
<td>9</td>
<td>+ 2.56</td>
</tr>
<tr>
<td>10</td>
<td>- 7.88</td>
</tr>
<tr>
<td>11</td>
<td>- 4.39</td>
</tr>
<tr>
<td>12</td>
<td>- 9.34</td>
</tr>
</tbody>
</table>

Table 4.4.6. Comparison of nitrogen balance

<table>
<thead>
<tr>
<th>Groups</th>
<th># of cases</th>
<th>Mean g/day</th>
<th>Std Deviation</th>
<th>Degree of freedom</th>
<th>2 Tail probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>+ 1.44</td>
<td>7.77</td>
<td>21</td>
<td>.378</td>
</tr>
<tr>
<td>Experimental</td>
<td>11</td>
<td>+ 5.47</td>
<td>13.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Prealbumin

Table 4.4.7. shows the initial and final prealbumin value of patients in the control and experimental group. Ten out of 12 patients in the control group had an increased serum prealbumin, one had a decline and in one the prealbumin remained the same. In the experimental group, 6 out of 10 showed improvement, 3 showed a decline and in one the prealbumin level remained the same.

Table 4.4.8. shows the mean serum prealbumin status of both control and experimental group. Student t. test analysis revealed no statistical difference in the trend between these two groups. Further, no correlation was found between the prealbumin level and nitrogen balance.
Table 4.4.7. Initial and final prealbumin value of patients in the control and experimental groups

<table>
<thead>
<tr>
<th>Control group</th>
<th>Experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial value (mg/dL)</td>
<td>Final Value (mg/dL)</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>4.6</td>
</tr>
<tr>
<td>3</td>
<td>9.3</td>
</tr>
<tr>
<td>4</td>
<td>12.8</td>
</tr>
<tr>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>6</td>
<td>&lt; 3.3</td>
</tr>
<tr>
<td>7</td>
<td>3.9</td>
</tr>
<tr>
<td>8</td>
<td>16.7</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>7.4</td>
</tr>
<tr>
<td>11</td>
<td>9.3</td>
</tr>
<tr>
<td>12</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Table 4.4.8. Comparison of serum prealbumin

<table>
<thead>
<tr>
<th>Groups</th>
<th># of cases</th>
<th>Mean</th>
<th>Std deviation</th>
<th>Degree of freedom</th>
<th>2 Tail probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>± 6.84</td>
<td>7.83</td>
<td>20</td>
<td>.194</td>
</tr>
<tr>
<td>Experimental</td>
<td>10</td>
<td>± 2.76</td>
<td>6.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total lymphocyte count

Table 4.4.9. shows the initial and final total lymphocyte count value of patients in control and experimental groups. Initial value of TLC showed mild to moderate depletion in three patients in control group. Final reading of the same patients showed improvement in two only. In the experimental group, initial TLC value showed mild to moderate depletion in 4 patients, which improved in 2 patients by the end of the study period. Final reading could not be obtained for the other 2 patients because they refused to give blood sample. Initial and final TLC value remained within normal in rest of the patients in both groups.

Table 4.4.10. reveals the mean value for total lymphocyte count calculated by student t-test. The result showed better TLC value of patients in experimental group as compared to control group.
Table 4.4.9. Initial and final total lymphocyte count value of patients in control and experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th></th>
<th>Experimental group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial value (mm$^3$)</td>
<td>Final value (mm$^3$)</td>
<td>Initial value (mm$^3$)</td>
<td>Final value (mm$^3$)</td>
</tr>
<tr>
<td>1</td>
<td>2070 (18%)</td>
<td>3040 (20%)</td>
<td>2520 (18%)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2176 (16%)</td>
<td>1846 (13%)</td>
<td>2033 (19%)</td>
<td>2835 (35%)</td>
</tr>
<tr>
<td>3</td>
<td>4620 (25%)</td>
<td>3813 (41%)</td>
<td>1826 (22%)</td>
<td>1575 (21%)</td>
</tr>
<tr>
<td>4</td>
<td>2214 (27%)</td>
<td>1980 (18%)</td>
<td>1608 (6%)</td>
<td>2420 (22%)</td>
</tr>
<tr>
<td>5</td>
<td>1820 (16%)</td>
<td>2540 (19%)</td>
<td>2255 (11%)</td>
<td>2420 (22%)</td>
</tr>
<tr>
<td>6</td>
<td>2200 (15%)</td>
<td>1170 (9%)</td>
<td>1360 (12%)</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>1224 (9%)</td>
<td>1393 (7%)</td>
<td>2250 (14%)</td>
<td>2700 (18%)</td>
</tr>
<tr>
<td>8</td>
<td>2425 (25%)</td>
<td>3465 (35%)</td>
<td>1380 (6%)</td>
<td>1680 (6%)</td>
</tr>
<tr>
<td>9</td>
<td>1368 (24%)</td>
<td>2574 (26%)</td>
<td>1980 (18%)</td>
<td>1679 (23%)</td>
</tr>
<tr>
<td>10</td>
<td>1738 (11%)</td>
<td>2002 (14%)</td>
<td>2842 (29%)</td>
<td>2480 (20%)</td>
</tr>
<tr>
<td>11</td>
<td>3060 (20%)</td>
<td>2955 (15%)</td>
<td>3266 (23%)</td>
<td>3712 (29%)</td>
</tr>
<tr>
<td>12</td>
<td>2190 (15%)</td>
<td>1883 (7%)</td>
<td>1105 (13%)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4.4.10. Comparison of total lymphocyte count

<table>
<thead>
<tr>
<th>Groups</th>
<th># of cases</th>
<th>Mean mm$^3$</th>
<th>Std deviation</th>
<th>Degree of freedom</th>
<th>2 Tail probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>+ 101.50</td>
<td>735.53</td>
<td>19</td>
<td>.667</td>
</tr>
<tr>
<td>Experimental</td>
<td>09</td>
<td>+ 222.89</td>
<td>448.61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Side effects

Table 4.4.11. shows the side effects experienced by patients in the control and experimental groups. Elevated random blood sugar (RBS) was

Table 4.4.11. Side effects experienced by control and experimental groups

<table>
<thead>
<tr>
<th>Side effects</th>
<th>Control group</th>
<th>Experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperglycemia</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Hypernatremia</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Constipation</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bloating, abdominal distension</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>No side effects</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
experienced by 6 patients in the control group. Of these, 2 patients had to stop nutrition therapy. In the experimental group, hyponatremia was the main side effect experienced by 5 patients.

**Cost of feed**

Table 4.4.12. shows the comparative cost of control versus experimental diets. Though the labour cost of preparing the experimental diet is twice that of the control diet, the overall cost of the experimental diet is 1/4 that of the control diet.

**Table 4.4.12. Cost comparison of control and experimental diets**

<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>Experimental diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of feed / 2000 mL</td>
<td>Rs 270.00</td>
<td>* A$ 12.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rs 36.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>* A$ 01.64</td>
</tr>
<tr>
<td>Cost of preparation*</td>
<td>Rs 5.18</td>
<td>* A$ 00.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rs 10.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>* A$ 00.47</td>
</tr>
<tr>
<td>Cost of prepared feed</td>
<td>Rs 275.18</td>
<td>* A$ 12.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rs 46.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>* A$ 02.11</td>
</tr>
</tbody>
</table>

*(A$ 1.00 = Pak Rs 22.00. (1-3-95)). See appendix 4.4.1.

**Bacteriological screening of the diet**

Bacteriological screening of both diets was carried out at random intervals. The initial tests isolated Klebsiella species, Bacillus (not Cereus) and enterococcus in both diets. This led to a bacteriological screening of the preparation room to determine the source of the bacteria (Table 4.4.13).

More stringent measures needed to be taken to minimise the contamination. A sanitising solution was prepared using baby safe tablets (Sodium Dichloro-o-triazinetrione). A protocol was developed whereby

**Table 4.4.13. Bacteriological screening of the enteral formulae preparation room**

<table>
<thead>
<tr>
<th>Location tested</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swab from sink</td>
<td>Klebsiella species, Streptococcus</td>
</tr>
<tr>
<td>Swab from refrigerator</td>
<td>Staphylococcus (Not aureus)</td>
</tr>
<tr>
<td>Swab from blender</td>
<td>Klebsiella, Streptococcus, Bacillus species (not Cereus)</td>
</tr>
<tr>
<td>Air of enteral formulae preparation room</td>
<td>Staphylococcus (Not aureus) Streptococcus, Bacillus species (not Cereus)</td>
</tr>
</tbody>
</table>
every day after preparing the feed, all utensils including the blender was soaked in the sanitising solution and the next day they were removed, air dried 1/2 an hour before the feed preparation. However elimination of bacteria to a satisfactory level did not occur.

Although Coliforms were eliminated, streptococcus, micrococcus and bacillus continued to be contaminants.
Chapter 4.5: Discussion

A pilot study was undertaken to compare the efficacy of experimental diet versus control diet. Another reason was to anticipate the difficulties that might be associated with a full fledged random control trial.

For this study, inclusion criteria was restricted to patients with medical problems such as cerebrovascular accident, meningitis, head injury and cancer as oppose to surgical patients. The former usually undergo longer period of nutrition therapy and have longer period of hospital stay as compare to latter ones. Further, surgical patients with enterocutaneous fistulae and short term bowel syndrome would have required calculation of fistulae and fecal losses. They were more likely to have sepsis which would have made calculation of calories and protein requirements more difficult. Therefore, it was considered advisable to exclude these patients from the study. Hence, by restricting the inclusion criteria, comparison of the groups receiving experimental and commercial dietary preparation became easier.

Although two indigenous formulae were short listed for the pilot study but only one formula was used throughout the study period. The need for partially digested diet also did not arise because of the full functional G.I tract of the study patients in both experimental and control group.

The main purpose of developing these experimental diets were to make the macronutrient composition comparative to commercial formulae so to offer a better substitute to those patients who cannot afford commercial formula for long term and rely on liquid diets with little nutritional value. Hence priority was given to calories to nitrogen ratio and micronutrient ratio of the formulae. Both diets appeared to be low in vitamins and mineral content due to the fact that the data base used to analyse the diets were incomplete and therefore underestimated the micronutrient content. Furthermore, no vitamins and mineral supplements were added to the formulae because that would have made preparation procedure cumbersome and costly.
The goal of nutritional assessment was to evaluate the outcome of the nutrition therapy. Since there is no single test capable of achieving this goal, numbers of tests were used to assess the outcome of nutrition therapy. The monitoring of patients involved the actual intake of calories and protein which was less than the calculated requirement in the majority of the patients in both experimental and control group. This indicate that most patients were getting substantially less then the RDI for many vitamins and minerals during the clinical trial. There are several reasons for this:

- Prolonged startup period of 2-4 days during which the feed was administered at a slow rate to ensure tolerance.
- Decrease in infusion rate and/or formula strength due to intolerance.
- Lack of compliance on part of the patients.
- Feed was on hold for radiological and biochemical tests.
- Delayed feed administration due to shortage of nursing staff.

To ensure administration of the diet according to calculated requirements frequent adjustment were made in the rate of infusion. This was particularly so when infusion pumps were not available.

Biochemical parameters were being used to compare the efficacy of experimental versus control diets. There was no significant difference in nitrogen balance between patients receiving the control and experimental groups. Other studies have shown that nitrogen dense formulae are more effective than standard formulae in achieving positive nitrogen balance (4.111). However this was not demonstrated in the present study, possibly due to the small sample size. The prealbumin showed improvement in six patients on the experimental diet as compared with ten on control diet but the sample size is too small to draw conclusions. Other factors such as hydration status and hepatic functional reserve also affect the serum prealbumin concentration (4.112). No correlation was found between the results of nitrogen balance and prealbumin level. The total lymphocyte count showed a similar response in both groups.

Dietary intolerance in the control group was evident when one of the patient experienced bloating and two developed diarrhoea (Table 4.4.11). Bolus administration that was the method utilised in this study may have
been responsible for these symptoms. Other researcher has indicated intolerance of bolus feeding versus continued administration through an infusion pump (4.63). However, there are several other possible causes of diarrhoea that should also be considered eg, hypoalbuminemia (4.67) and medical elixirs containing sorbitol (4.70).

Metabolic complications occurred in patients from both groups. Fifty-five percent of the patients in the control group and 25% of those in the experimental group experienced hyperglycemia. Six of the patients on control diet who developed hyperglycemia had suffered a cerebrovascular accident (CVA) and were between 60 - 83 years old and 4 of them were diabetic as well. The critically ill are at risk for developing hyperglycemia due to insulin resistance (4.67). In the experimental group, the most common side effect was hyponatremia which could be the result of the low sodium content of the experimental diet.

Initially both formulae showed bacterial contamination even though an exclusive pantry was used for the preparation of the feeds. This led to more stringent measures such as the use of baby safe tablets for sanitising the utensils and the use of sterile distilled water for preparation of the feeds. Despite these measures, complete elimination of micro-organisms could not achieve. Further efforts need to be taken to eliminate contamination. Many studies indicate that blenderised feeds and reconstitute feeds are more contaminated than ready to hang formulae (4.63, 4.113, 4.114, 4.115). However, the high cost of ready to hang formulae in developing countries and their availability is an impetus to prepare enteral formulae in hospitals.

As shown in table 4.4.5, the cost of indigenous enteral formulae is less than 1/4 of the commercially available formulae. In view of its high cost the use of commercial formulae may not be justified in patients who have intact digestive tracts.

**Conclusion**

The pilot study has shown that patients with cerebrovascular accident (CVA), head injury and meningitis stay longer enough in the hospital to allow completion of the nutritional therapy according to the study
protocol. Preliminary results have shown that the experimental diet is at least as efficacious as the control diet in improving nutritional status. The incidence of side effect is no different. The experimental diet is considerably cheaper and can be readily prepared in a hospital setting. More attention needs to be given to the preparation and administration of the diets with a view to avoid contamination.

A full fledged random control trial using an appropriate sample size is necessary to confirm these initial impressions. Restricted selection criteria in a future full scale study will simplify the comparison of the effectiveness of treatment between the two groups. However, such elective inclusion criteria will prolong the duration of the study. A research assistant would be necessary to ensure safe and steady administration of the diets according to the calculated requirement. The assistant would also help on recording the side effects and in the timely collection of sample.
Chapter 4.5: References


4.17 Havenberg, L., Kwon, P.H., Scrimshaw, N.S.: Comparative tolerance of adolescent of differing ethnic background to lactose containing and lactose free containing drinks. Initial experience


Appendix 4.3.1.

Consent form

Title of project: To compare the effectiveness of the hospital prepared enteral formula with commercial feeds on adult patients.

Responsible investigators: Ms Nelofar Athar and Dr Jim Bergan, University of Western Sydney, Hawkesbury, Australia and Dr Mushtaq Ahmad, Aga Khan University Hospital, Karachi, Pakistan.

The purpose of the project is to determine the effectiveness of an indigenous enteral formulae. My proposed study has been reviewed and approved by the University of Western Sydney, Hawkesbury Human Research Ethics Committee and the Aga Khan University Human Subject Protection Committee. I need volunteers to take part in the study and would like you to consider participating. Your involvement will be approximately for 23 days. This is entirely voluntary and you will not be penalised in any way for not volunteering or if you decide to withdraw any time during the study.

You will be randomly assigned to take either one of the two diets available in the hospital. Routine lab tests will be performed on an ongoing basis (See attached sheet). As a consequence of this study some side effects may result (see attached sheet) but treatment will be provided for managing the adverse effects incurred by you. The information obtained will be kept confidential and only investigators involved directly in conducting the study will have access to them.

Volunteer’s consent

I have been asked to participate in the above research project and give my consent by signing this form on the understanding that:

- The research project will be carried out in a manner confirming with the Australian National Health and Medical Research Council Code of Practice, and the University of Western Sydney, Hawkesbury Human Research Ethics Committee and the Aga Khan Medical Human Subject Protection Committee Terms of Reference.
- I have been fully informed about the general purpose, method and demands and the possible side effects I may experience during the study.
- I agree to participate with the understanding that I may withdraw at any time. Refusal to take part in this study will not affect the treatment of my condition.
- Confidentiality of the data obtained during the study will be maintained.

Full name

signature ___________________________ date ___________________________

of ___________________________________________ HREC approval ___________________________
Explanatory note

Lab tests:

- Serum glucose
- Urinary urea nitrogen
- Serum prealbumin
- Serum electrolytes
- Total lymphocyte count

Possible side effects:

- Diarrhoea
- Nausea
- G.I distress such as stomach ache
- Electrolyte imbalance
- Hyper and hypoglycemia
- Increased fistula output
Appendix 4.3.2.

Diet allocation

Adult patients

Non septic

Control Experimental

Note:
For a true randomization, a set of 20 cards with the experimental diet and a set of 20 cards the with the control diet was prepared. After thorough shuffling, all the card were placed in a box. Soon after the identification of patients for the study, a card was selected and the diet was assigned accordingly.
Appendix 4.3.3.

Protocol for ordering enteral feeds

Order written by physician

Transcribed on meal slip by the assigned nurse

Convey to diet office by 12 noon daily

Order specification written by diet clerks on order slip and delivered to assigned kitchen staff

Order registered with the assigned kitchen staff

Feed preparation and labelling by the assigned kitchen staff

Delivery at the ward level by 2 pm, daily
Continue...
Appendix 4.3.5.

Sepsis score of Elebute and Stoner (4.110)

SCORING OF LOCAL EFFECTS OF TISSUE INFECTION

Attribute

Wound infection with prulent discharge/ enterocutaneous fistula

- Requiring only light dressing changed not more than once daily. 2
- Requiring to be dressed with a pack, dressing needing to be changed more than once daily, requiring application of a bag and / or requiring suction. 4

Peritonitis

- Localised 2
- Generalised 6

Chest Infection

- Clinical or radiological signs of chest infection without productive cough 2
- Clinical or radiological signs of chest infection with a cough producing purulent sputum. 4
- Full clinical manifestations of lobar/ bronchopneumonia 6
- Deep-seated infection (eg. subphrenic abscess, pelvic abscess, empyema thoracic, acute or chronic osteomyelitis) 6

SCORING OF PYREXIA (ORAL TEMPERATURE)

Attribute

Maximum daily temperature (°C)

- 36 - 37.4 0
- 37.5 - 38.4 1
- 38.5 - 39 2
- > 39 3
- < 36 3

Minimum daily temperature > 37.5°C 1
If 2 or more temperature peaks above 38.4°C in 1 day 1
If any rigours occur in a day 1
Temperature should be recorded at least 4 times in 24 hours. The record for the 24 hrs period is assessed as above and “Pyrexia Score” computed.
SCORING OF SECONDARY EFFECTS OF SEPSIS

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obvious jaundice (in the absence of established hepatobiliary disease)</td>
<td>2</td>
</tr>
</tbody>
</table>

**Metabolic acidosis**

<table>
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<tr>
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</tr>
<tr>
<td>Uncompensated</td>
<td>2</td>
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</tbody>
</table>

**Renal failure**

Gross disturbance of mental orientation/level of consciousness (eg. delirium coma) and/or other focal neurological manifestations of pyemia/septicemia (having excluded other causes).

Bleeding diathesis (from disseminated intravascular coagulation)

3

SCORING LABORATORY DATA

<table>
<thead>
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</thead>
<tbody>
<tr>
<td><strong>Blood culture</strong></td>
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<tr>
<td>Single positive culture</td>
<td>1</td>
</tr>
<tr>
<td>Two or more positive cultures</td>
<td>3</td>
</tr>
<tr>
<td>separated by 24 hours</td>
<td></td>
</tr>
<tr>
<td>Single positive culture + history</td>
<td>3</td>
</tr>
<tr>
<td>of invasive procedure</td>
<td></td>
</tr>
<tr>
<td>Single positive culture + cardiac</td>
<td>3</td>
</tr>
<tr>
<td>murmur and/or tender large spleen</td>
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</table>

**Leucocyte count (x 10⁹ L)***

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<td>12 - 30</td>
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</tr>
<tr>
<td>&gt; 30</td>
<td>2</td>
</tr>
<tr>
<td>&lt; 2.5</td>
<td>3</td>
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</table>

**Haemoglobin level in the absence of obvious bleeding (g/dL)**

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</thead>
<tbody>
<tr>
<td>7-10</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 7</td>
<td>2</td>
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</tbody>
</table>

**Platelet count (x 10⁹ L)***

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</thead>
<tbody>
<tr>
<td>100 - 150</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>2</td>
</tr>
</tbody>
</table>
**Plasma albumin level (g/l)**

- 31-35  
- 25-30  
- < 25

**Plasma total bilirubin level in the absence of clinically obvious jaundice**

- > 25 (mmol/l)
Appendix 4.4.1.

Calculation of Labour cost

Food service worker 1. salary = Rs 2000.00 / month
Food service worker 1. salary = Rs 2000.00 / 30 days = Rs 66.00/day
Food service worker 1. salary = Rs 66 / 8 W. hours = Rs 8.30/hour

An average preparation time of 2000 mL takes one and one-half hour, which equates to Rs 10.37 (Aus $ 0.47 cents).

Note: Aus $ 1.00 = Pak Rs 22.00.
Stage 5: Microbiological screening of indigenous enteral formulae
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<th>Page</th>
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<td><strong>Table 5.4.4</strong></td>
<td>Microbiological analysis of indigenous enteral formula using sampling Plan 2 (Protocol B)</td>
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<td><strong>Table 5.4.5.</strong></td>
<td>Microbiological analysis of indigenous enteral formula using sampling Plan 3 (Protocol B)</td>
<td>5-13</td>
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<tr>
<td><strong>Table 5.4.6.</strong></td>
<td>Microbiological analysis of commercial enteral formula using sampling Plan 1(Protocol B)</td>
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<td>Figure 5.3.5.</td>
<td>Sampling plan 1 for bacteriological testing of indigenous enteral formulae</td>
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<td>Figure 5.3.6.</td>
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<tr>
<td>Figure 5.3.8.</td>
<td>Sampling Plan 3 for bacteriological testing of indigenous enteral formulae</td>
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<td></td>
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</tbody>
</table>
Abstract

This part of the study specifically dealt with the issue of formula contamination and what precautionary measures to be taken to produce safe and hygienic formula.

The initial production run involved formula preparation as per protocol A which was practised at the time of the pilot study. Three sampling plans were developed to determine the critical control points of formulae preparation. The results of all three sampling plans showed bacterial activity in the formula dry mix and in the post-blenderised formulae. Since pre-blenderised formula indicated that bacterial growth can be minimised by cooking, the formula dry mix was not further tested. Post-blenderised formula showed contamination that clearly indicated that thorough cleaning and washing of the blender and minimum handling was required. Hence another protocol was developed and in order to determine the bacterial activity, sampling plan 3 was used.

Results indicated a remarkable improvement in the elimination of fecal E.coli, streptococci and Coliforms, but complete elimination of environmental contamination was not achieved. This set of experiments increased awareness of factors which result in increased the contamination. In order to resolve this issue, critical analysis of each and every aspect of formulae preparation is required regardless of whether or not the formula is hospital or commercially prepared.
Chapter 5.1: Introduction

Statement of the problem

This section of the study deals with the issue of formula contamination and the measures which need to be taken to avoid contamination.

As mentioned in Stage 3, a detailed microbial analysis of the enteral formulae was conducted at the time of formulae preparation and a Hazard Audit was proposed to ameliorate the contamination problem. Unfortunately the aseptic techniques proposed in the hazard audit could not be followed during the pilot study due to the non-availability of an autoclave.

The control and experimental diets were prepared within the available existing resources. However, in order to minimize the contamination, formula were prepared in the designated place, disposable gloves were used to avoid direct contact of hands and sterile distilled water was used for formulae preparation. Feed contamination could not be completely eliminated. A critical need exists for the development of a preventive system that provides a more specific and critical approach in order to control microbial hazards during enteral formula preparation.

Aim

The purpose of this experiment was to develop a Hazard Audit and a protocol for the preparation of enteral formulae to minimize contamination.

Objectives

1. To trial various methods of preparing enteral formulae.
2. To develop an aseptic technique for preparing formulae within the resources available at the Aga Khan University Hospital.
3. To propose hazard audit which is practicable in the Aga Khan University Hospital.
Chapter 5.2: Literature Review

"The very first requirement of a hospital is that it should do the sick no harm" (Florence Nightingale) (5.1). Throughout the United Kingdom, in hospitals much attention is given to the safety of drugs but the provision of safe and wholesome food is often neglected (5.1). In Latin America the rate of nosocomial infection ranges from 10 to 26% with a severe impact on morbidity and mortality. Hence, nosocomial infection is a major public health problem which is yet not universally recognised. (5.21).

Enteral formulae not only provide nutrients to patients but are also ideal media for bacterial growth. Hence, the same formula can become a source of microbial infection and the impact of such infection is much more severe for immunity compromised patients than for healthy patients (5.2-5.3). Therefore the use of sterile liquid diet and an aseptic preparation technique is suggested to prevent bacterial contamination. As a result, ready to hang enteral formulae are preferred as and when possible. However, injudicious use of even these formulae can become a source of microbial infection.

Source of contamination

Regardless of whether or not, enteral formulae are commercially prepared or hospital made, they require aseptic preparation techniques to deliver safe and hygienic feed to patients. Many potential sources of contamination exist. In this section emphasis will only be given to the possible sources of enteral formulae contamination.

Kitchen utensils

Contamination of formulae often occurs during mixing and/or diluting (5.4-5.7) and the liquidizer or blender used for this purpose have been found to be contaminated (5.1, 5.4-5.9).
**Nasogastric feed ingredients**

Two main types of formulae are available: the commercially made and the hospital prepared formulae. Hospital using the commercially made formulae are not free from this problem particularly when powdered (reconstituted w/ water) formulae are used (5.4, 5.14). The contamination problem is more severe in home made formulae due to a variety of food used in the preparation, such as milk whole dried, milk skim, raw egg, sugar and water. Thompson, et al (5.10) found E.coli and B. cereus in spray dried skim milk. Although pasteurization of milk reduces the chances of contamination, B. cereus may remain even after pasteurization (5.11). Also the use of distilled water does not guarantee safe feed because pseudomonas serratia and E.coli have been found in distilled water (5.12-5.13).

**Hand washing** Unwashed hands may be another major source of contamination. It is a well accepted fact that hand washing is quite effective for preventing transmission of infection (5.22) but compliance by health care providers is estimated at approximately 40% (5.20).

Kitchen environment, air, dust, formula handling and time lapse between the preparation and administration are all potential sources of contamination (5.14-5.16).

The infection control status is even worse due to factors such as:

1. Lack of awareness among health care providers.
2. Lack of resources such as proper preparation facility and equipment.

Even if the optimal situation is reached whereby one complete ready to use system is available for tube feeding, the contamination will not be decreased to zero due to the following reasons:

1. Retrograde growth during continuous feeding.
2. Contact contamination after intermittent feeding (5.17-5.19).
To minimize the contamination problems in hospitals in developing countries, the fundamental needs are:

* Acknowledgment of the problem by hospital administration.
* Increased awareness by medical personnel of the contamination problem.
* Education of health care providers on infection control as well as sanitation and hygiene.
* Increased awareness among the population of their right to better health care.
* Funds provided by International Organisations to support a education and research program to address the contamination problem (5.21).
Chapter 5.3: Methodology

To determine the critical control points in the preparation of indigenous enteral formulae, a series of experiments were conducted in order to assess the microbiological status of formulae at different stages of preparation at the Aga Khan University Hospital, Karachi, Pakistan. For the purpose of this experiment, a formula dry mix was prepared in bulk (5 kgs), whereby ratio of food items except eggs for diet # 4a were mixed in a Hobart mixer and placed in a plastic bucket and stored at 4°C.

As indicated in Stage 4, bacterial contamination was found in the areas where both the experimental and control formulae were prepared for the pilot study. Therefore, for this experiment formulae were prepared in the diet bay located in the main kitchen.

Protocol A for enteral formulae preparation

Initially, both the indigenous and commercial formulae were prepared as per Protocol A (Figures 5.3.1, 5.3.2) This protocol was used to prepare the control and experimental diets for the pilot study. The formulae prepared by this method were tested for bacterial growth.

Figure 5.3.1. Protocol A for preparing indigenous enteral formulae

1. Spray the disinfectant (Diversol CX) on the work surface and wipe it clean with a cloth towel.
2. Remove all utensils and the blender from the sanitizing solution and allow to air dry.
3. Meanwhile place food dry mix, eggs, lecithin and distilled water on the work table.
4. Wash hands and dry them under hand dryer.
5. Wear hairnet and hand gloves.
6. Prepare feed as per preparation method and cool it to 30 to 40°C in refrigerator.
7. Add emulsifier and blend for 60 seconds.
8. Add raw egg white and blend for 30 seconds.
9. Using the metal strainer, strain the mixture directly into formula container for ward to use.
10. Label container and keep in refrigerator at 4°C till ready to be transported to ward.
11. Wash all utensils and the blender and immerse them in a sanitizing solution till next use.
**Figure 5.3.2. Protocol A for preparing commercial enteral formulae**

1. Spray the disinfectant (Diversol CX) on the work surface and wipe it clean with a cloth towel.
2. Remove all utensils and the blender from the sanitizing solution and allow to air dry.
3. Meanwhile place commercial powdered formula tin and distilled water on the work table.
4. Wash hands and dry them under hand dryer.
5. Wear hairnet and hand gloves.
6. As per specifications, reconstitute feed with distilled water and blend for 30 seconds.
7. Using the metal strainer, strain the mixture directly into formula container for ward to use.
8. Wash all utensils and the blender and immerse them in a sanitizing solution till next use.
9. Label container and keep in refrigerator at 4°C till ready to be transported to ward.

**Protocol B for enteral formula preparation**

The purpose for developing Protocol B (Figure 5.3.3, 5.3.4) was to minimize handling and to determine its impact on bacterial growth in the enteral formulae.

**Figure 5.3.3. Protocol B for preparing indigenous enteral formulae**

1. Clean the work surface with sanitizing solution (Diversol CX, Diversy product).
2. Pass all the required utensils and blender to be used for preparing formulae through dish washing machine.
3. Place them on a clean tray to allow them to air dry.
4. Collect the food dry mix, egg, lecithin and sterilized water on a clean and washed tray.
5. Wear hairnet, wash hands thoroughly with water and soap and wear hand gloves.
6. Prepare the formula per specification and cool it to 30 to 40°C.
7. Add emulsifier and blend for 60 seconds.
8. Add egg white and blend for 60 seconds. Using the metal strainer, strain the mixture directly into formula container for ward to use.
9. Label container and keep in refrigerator at 4°C till ready to be transported to ward.
10. Wash thoroughly all the soiled utensils, blender and strainer and place in a designated place for next use.

*Note:*
- Must not use duster cloth.
- Must not touch any other equipment while preparing formulae.
- Avoid talking to other staff during formula preparation.
Figure 5.3.4. Protocol B for preparing commercial enteral formulae

1. Clean the work surface with sanitizing solution (Diversol CX, Diversy product).
2. Pass all the required utensils and blender to be used for preparing formulae through dish washing machine.
3. Place them on a clean tray to allow to air dry.
4. Meanwhile place commercial powdered formula tin and distilled water on a clean and washed tray.
5. Wear hair net and wash your hands thoroughly with water and soap and wear hand gloves.
6. As per specifications, reconstitute feed with distilled water and blend for 30 seconds.
7. Using the metal strainer, strain the mixture directly into formula container for ward to use.
8. Label container and keep in refrigerator at 4°C till ready to be transported to ward.
9. Wash thoroughly all the soiled utensils, blender and strainer and place in a designated place for next use.

Note:
- Must not use duster cloth.
- Must not touch any other equipment while preparing formulae.
- Avoid talking to other staff during formula preparation.

Determination of critical control points

Initially, both commercial and experimental formulae were prepared as per Protocol A. Under this method, for experimental formula, three sampling plans for bacteriological testing were drawn. In sampling plan 1, three sampling points were chosen which are indicated in Figure 5.3.5.

In commercial enteral formula, only one sampling point was chosen which is indicated in Figure 5.3.6.

Sampling plan 2 and 3 were used to further investigate the points of contamination in indigenous enteral formulae as illustrated in Figure 5.3.7 and Figure 5.3.8.
Figure 5.3.5. Sampling plan 1 for bacteriological testing of indigenous enteral formulae

Dry mix formula items → Sample 1 was taken

Formula dry mix was mixed in water and cooked till it started getting thick (approximately 5 minutes)

→ Sample 2 was taken

Cooled in chiller to 40°C (preblenderized)

→ Sample 3 was taken

Lecithin was added

Blended for 60 seconds in blender

Raw egg white was added and blended for 30 seconds (Post blenderized and strained)

Figure 5.3.6. Sampling plan 1 for bacteriological testing of commercial formulae

Commercial formula (Powdered)

→ Sample 1 was taken (after straining)

Reconstituted the formula using distilled water

Strain mixture using strainer directly in a container to be taken to wards
Figure 5.3.7. Sampling Plan 2 for bacteriological testing of indigenous enteral formulae

- Dry mix formula items → Sample 1 was taken
- Formula dry mix was mixed in water and cooked till it started getting thick
- Cooled in chiller to 40°C (preblenderized)
- Lecithin was added
- Blended for 60 seconds in blender
- Raw egg white was added and blended for 30 seconds (Post blenderized and strained)
- Strained through strainer → Sample 2 was taken

Figure 5.3.8. Sampling Plan 3 for bacteriological testing of indigenous enteral formulae

- Dry mix formula items
- Formula dry mix was mixed in water and cooked till it started getting thick → Sample 1 was taken
- Cooled in chiller to 40°C (preblenderized)
- Lecithin was added
- Blended for 60 seconds in blender → Sample 2 was taken
- Raw egg white was added and blended for 30 seconds (Post blenderized and strained)
- Strained through strainer
Development of Hazard Audit

A systematic examination of the process was undertaken and hazard audit was proposed to ameliorate the contamination problem (Table 5.3.1).

Table 5.3.1. Hazard Audit

<table>
<thead>
<tr>
<th>Operation</th>
<th>Potential risk</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw material storage</td>
<td>High temperature, high humidity, vermin</td>
<td>Clean environment, appropriate temperature and humidity.</td>
</tr>
<tr>
<td>Sanitizing of equipment</td>
<td>Bacterial contamination</td>
<td>Passing through a dish washing machine</td>
</tr>
<tr>
<td>Addition of egg white and enzyme</td>
<td>Salmonella or other bacterial contamination</td>
<td>Swab egg surfaces with *Deogin. Allow 5 minutes contact time. Use a disposable syringe to dispense enzyme to prevent contamination.</td>
</tr>
<tr>
<td>Incubation of formula at 55 to 65°C</td>
<td>Growth of B. cereus</td>
<td>Keep temperature at 60 to 65°C.</td>
</tr>
<tr>
<td>Cooling formula in fridge / chiller</td>
<td>Bacterial contamination</td>
<td>Ensure metal saucepan lid fits closely and is sanitized before use.</td>
</tr>
<tr>
<td>Addition of lecithin</td>
<td>Bacterial contamination</td>
<td>Use aseptic technique. Portion needed should be pre-weighed into a sterile container.</td>
</tr>
<tr>
<td>Blending</td>
<td>Bacterial contamination</td>
<td>Pass through a dish washing machine and air dry.</td>
</tr>
<tr>
<td>Straining formula through metal strainer</td>
<td>Bacterial contamination</td>
<td>Strainer must be passed through a dish washer before and after use. Receiving vessel must be passed through dishwasher.</td>
</tr>
</tbody>
</table>

*Deogin: Quaternary ammonium compound
Chapter 5.4: Result

Microbiological analysis of formulae prepared as per Protocol A

Indigenous enteral formula  Results for sampling plan 1 of the indigenous enteral formula showed extensive contamination in the dry mix.. Bacteria found in dry mix were not present in the pre blenderized and post blenderized feeds, but Bacillus species (not cereus) were present indicating heavy environmental contamination (Table 5.4.1). Addition of lecithin did not increase bacterial contamination in this run.

Table 5.4.1. Microbiological analysis of indigenous formula using sampling Plan 1 (First run)

<table>
<thead>
<tr>
<th>Species</th>
<th>Dry mix</th>
<th>Pre blenderized</th>
<th>Post blenderized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Colony count</td>
<td>TNT*</td>
<td>100 cfu*/ml</td>
<td>75 cfu/ml</td>
</tr>
<tr>
<td>Fecal E.coli</td>
<td>Present</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Fecal Streptococci</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Klebsiella 50cfu/gm</td>
<td>150 cfu/ml</td>
<td>Nil</td>
</tr>
<tr>
<td>Other organisms</td>
<td>Mold 150 cfu/gm</td>
<td>Mold 5 cfu/ml</td>
<td>Bacillus 75 cfu/ml</td>
</tr>
<tr>
<td></td>
<td>Acinobactor 150 cfu/gm</td>
<td>Bacillus 95 cfu/ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flavobactrium 150 cfu/gm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* TNT = Too numerous to count. cfu = colony forming unit

Second production run was under taken following sampling plan 1. Result indicated heavy contamination in dry mix and in postblenderized feed.

Table 5.4.2. Microbiological analysis of indigenous enteral formula using sampling Plan 1 (Second run)

<table>
<thead>
<tr>
<th>Species</th>
<th>Dry mix</th>
<th>Pre blenderized</th>
<th>Post blenderized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Colony count</td>
<td>TNT*</td>
<td>100 cfu */gm</td>
<td>TNT</td>
</tr>
<tr>
<td>Fecal E.coli</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Fecal Streptococci</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Klebsiella 25 cfu/gm</td>
<td>Nil</td>
<td>Klebsiella TNT</td>
</tr>
<tr>
<td>Other organisms</td>
<td>Mold 25 cfu / gm</td>
<td>Bacillus 100 cfu/gm</td>
<td>Candida TNT</td>
</tr>
<tr>
<td></td>
<td>Bacillus TNT</td>
<td>(Not cereus)</td>
<td>(Not Albican)</td>
</tr>
</tbody>
</table>

* TNT = Too numerous to count. cfu = colony forming unit
Pre blenderized feed showed some environmental contamination (Table 5.4.2). Bacterial count of post blenderized feed indicated that either the lecithin or the blender were heavily contaminated.

**Commercial formula** Results on sampling plan 1 of commercial formula showed extensive contamination. Bacteria found in reconstituted feed were Klebsiella and Acinetobacter calcoaceticus (Table 5.4.3). Potential sources were the powdered formula itself, the distilled water or the vessel used for reconstituting the formula.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reconstituted commercial formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Colony count</td>
<td>TNT</td>
</tr>
<tr>
<td>Fecal E. coli</td>
<td>Nil</td>
</tr>
<tr>
<td>Fecal Streptococci</td>
<td>Nil</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Klebsiella TNT</td>
</tr>
<tr>
<td>Other organisms</td>
<td>Acinetobacter calcoaceticus TNT</td>
</tr>
</tbody>
</table>

*TNT= Too numerous to count. cfu= colony forming unit*

In sampling Plan 2 bacterial testing was carried out at two critical points in dry mix and post blenderized feed. Result showed the same pattern whereby dry mix was heavily contaminated and prepared formulae had only 200 cfu/gm of total colony count with different types of organisms (Table 5.4.4).

**Table 5.4.4. Microbiological analysis indigenous enteral formula using sampling Plan 2**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dry mix</th>
<th>Postblenderized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Colony count</td>
<td>TNT*</td>
<td>200 cfu* / ml</td>
</tr>
<tr>
<td>Fecal E. coli</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Fecal Streptococci</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Klebsiella TNT</td>
<td>Klebsiella 200 cfu / ml</td>
</tr>
<tr>
<td>Other organisms</td>
<td>Mold 100 cfu / gm</td>
<td>Enterobacter 50 cfu/ml</td>
</tr>
<tr>
<td></td>
<td>Bacillus 200 cfu / gm</td>
<td>bacillus 50cfu / ml</td>
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</tbody>
</table>

*TNT= Too numerous to count. cfu= colony forming unit*

A number of production runs were carried out following sampling plan 3, whereby only preblenderized and post blenderized feed were checked for
contamination. Post blenderized formulae were found contaminated with Klebsiella, Candida and mold as compared to pre blenderized feed which had only bacillus species. Thus, the blender was found to be the source of contamination and required deep cleaning for minimum contamination.

Microbiological analysis of formulae prepared as per protocol B

Indigenous enteral formula The results indicated a critical need to revise to preparation methodology. Hence protocol B was developed and formulae were prepared accordingly. Three production runs of indigenous enteral formula were carried out as per protocol B (Table 5.5.5). Only sample plan 3 was used for bacterial screening. The result showed remarkable improvement by eliminating Klebsiella, mold, Acinetobacter and Candida but environmental contamination was not completely eliminated.

Table 5.4.5. Microbiological analysis indigenous enteral formula using sampling Plan 3

<table>
<thead>
<tr>
<th>Species</th>
<th>Run 1</th>
<th></th>
<th>Run 2</th>
<th></th>
<th>Run 3</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre. blen</td>
<td>Post. blen</td>
<td>Pre. blen</td>
<td>Post. blen</td>
<td>Pre. blen</td>
</tr>
<tr>
<td>Total Colony</td>
<td>TNT</td>
<td>TNT</td>
<td>185 cfu / ml</td>
<td>100 cfu / ml</td>
<td>TNT</td>
</tr>
<tr>
<td>count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal E. coli</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>F. Streptococci</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Other organisms</td>
<td>Bacillus</td>
<td>Bacillus</td>
<td>Bacillus</td>
<td>Bacillus</td>
<td>Bacillus</td>
</tr>
<tr>
<td></td>
<td>TNT</td>
<td>TNT</td>
<td>180 cfu / ml</td>
<td>75 cfu / ml</td>
<td>TNT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Candida 5 cfu / ml</td>
<td>Candida 5 cfu / ml</td>
<td></td>
</tr>
</tbody>
</table>

Commercial formula Three production runs of commercial formula were carried out as per protocol B. Only plan 1 for commercial formulae was used whereby only reconstituted formula was screened for bacterial growth (Table 5.4.6). Initial run showed no growth. Second run showed environmental contamination and 3rd run had bacterial contamination. The reason could be the lack of compliance to follow preparation protocol.
Table 5.4.6. Microbiological analysis commercial enteral formulae using sampling Plan 1

<table>
<thead>
<tr>
<th>Species</th>
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<tr>
<td>Total Colony count</td>
<td>Nil</td>
<td>150 cfu/ml</td>
<td>TNT</td>
</tr>
<tr>
<td>Fecal E. coli</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>F. Streptococci</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Coliforms</td>
<td>Nil</td>
<td>Nil</td>
<td>Klebsiella 250 cfu/ml</td>
</tr>
<tr>
<td>Other organisms</td>
<td>Nil</td>
<td>Mold 5 cfu/ml</td>
<td>Pseudomonas 150 cfu/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ Bacillus (not cereus)</td>
<td>Acinetobacter 500 cfu/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pseudomonas 500 cfu/ml</td>
</tr>
</tbody>
</table>
Chapter 5.5: Discussion

Microbiological analysis of both formulae: commercial (reconstituted with water) and the hospital made indigenous one showed that complete elimination of bacteria is difficult to achieve within the present available resources.

However, a number of experiments have proved that E.coli., Coliforms and Streptococci can be eliminated from enteral feeds by strictly adhering to the protocol B.

In previous sections, much emphasis has been placed on aseptic techniques and the use of hazard audit to avoid contamination of formulae. Desired results should be achieved if they are strictly followed.

Result indicates that proposed hazard audit was not applied successfully in the hospital in Pakistan for the preparation of enteral formula. Further this series of experiment increased the awareness of other factors which were not recognised earlier such as:

1. The safety of food which is procured is questionable. Result showed heavy bacterial growth of formula dry mix.
2. No designated area for formulae preparation.
3. Non-availability of some basic equipment to produce safe formulae such as designated refrigerator, steriliser and laminar flow hood to avoid contamination.
4. Warm ambient temperature is another major factor. If the room temperature is between 25 to 30°C, it is extremely difficult to achieve the desired result.
5. Food handlers are not aware of the hazards of contamination.
6. Poor compliance in following instructions.
7. The problem is not acknowledge by the staff.

Developed countries are unable to completely eradicate this problem inspite of their continuous effort to look for better methodology and introduction of new techniques to offer safe and hygienic food. In
developing countries, where the provision of basic health care is questionable due to shortage of medical supplies and equipments, very little attention can be paid to this problem, which unfortunately does not have a simple solution.

Contamination in a hospital’s kitchen may occur at any stage from procurement of raw food till it reaches patients. It may not be easily eliminated by instituting few procedures. In order to minimise the contamination problem, some major steps need to be taken in terms of educating health care providers on infection control, making populations aware of contamination hazards and practice of hygiene and sanitation in the normal life style and particularly in health care institutions.
Chapter 5.5: References


6: Limitations

1. Limited food item selected for this project restricted the number of diet formulations.

2. Non-protein calorie: nitrogen ratio specification limited the number of diets.

3. Due to time and financial constraints more patients could not be included in the study.

4. Surgical patients could not be included in the study due to their short stay.

5. Timely collection of specimens of study patients was difficult to achieve.

6. Non-availability of autoclave or steriliser was one of the factors contributing to contaminated formulae.

7. Non-existence of designated place for feed preparation increased the risk of formulae contamination in this hospital based study.

8. Warm ambient temperatures were another problem contradicting the preparation of a safe and hygienic formulae.

9. Poor compliance to formulae preparation methodology was due to the lack of awareness among food handlers about the hazards of contamination.
7: Recommendations

1. There should be additional research using indigenous food items for enteral formulae preparation.

2. Non protein calorie: nitrogen ratio needs to be specified in the range of 100:1 - 150:1 to yield more theoretical formulations.

3. More time and funding needs to be allocated to conduct a large sample scale study.

4. Selection criteria should be limited to patients with neurological problems because they tend to be on tube feeding for a longer period of time and that will allow evaluation of the efficacy of treatment.

5. Designated Nurses should be hired for the collection of specimens and monitoring of side effects.

6. Regardless of whether it is a preparation of commercial or hospital formulae, there should be an autoclave or steriliser for minimum bacterial growth.

7. In the specific case of the hospital, there should be a separate formula room for the preparation of enteral feeds.

8. The enteral formulae room should be air conditioned to restrict the bacterial contamination.

9. An in-house training programme should be introduced for food handlers to be sensitised about the hazards of contamination and the preventive measures which need to be undertaken to minimise bacterial contamination and growth.
8: Conclusion

The main aim of this project was to develop an indigenous enteral formulae due to erratic supply and high cost of imported enteral products. The whole exercise of preparing indigenous enteral formulae was carried out in 4 stages.

The initial section particularly dealt with the theoretical development of indigenous enteral formulae whereby, a number of food items were selected. A master sheet was designed in which the identified food items, their nutritional profile and cost were specified. The purpose for developing the master sheet was to try different combinations in order to choose the best combinations for enteral formulae.

A computer aided program was specifically designed to work on formulae combinations and to reveal information on the nutritional profile, calorie ratio, nitrogen conversion factor, nitrogen ratio and nitrogen content of the diets.

One hundred combinations were developed from the Master sheet and were analysed using the main features of What If Problem Solver®, an attachment of Lotus spread sheet: **What if cell** was used to specify the quantity of each food item in ranges. **Constraint cell** was used to specify the desired calorie ratio for macronutrients, non-protein calorie to nitrogen ratio and nitrogen content of enteral formulae. **Target cell** was used to restrict the total amount of food desired. The exercise proved that theoretical formulation of indigenous enteral diet is possible. However, the efficacy of these formulation required scientific testing.

In order to assess the protein quality of indigenous enteral formulae, protein efficiency ratio (PER) was carried out on six diets. These six diets were selected out of 100 combinations and were designed to conduct the assay as per AOAC (1990) specification. In this study PER was modified with all the diets designed to be isonitrogenous but with slightly different carbohydrate and fat contents. This variation was due to the use of selected food items and their contribution in 100 g of food mix. Casein
diet was used for control group and despite an adequate amount of essential amino acids present in the control diet, the PER was low when compared to experimental diets. Protein digestibility corrected amino acid score data supported the PER results for experimental diets.

Since the main objective of this experiment was to demonstrate the nutritional efficiency of the formulated diet due for its ultimate use for seriously ill patients whose protein requirement is considered to be similar to that of growing child. This experiment indicated that the indigenous enteral formulae can be developed using the multimix approach to yield a good protein quality enteral formulae.

Further experiments were conducted to develop the polymeric and partially digested diets. Twenty nine diets out of 100 were selected for this experiment and two preparation techniques were trialed; incubation and cooking for both the polymeric and the partially digested diets. Incubation techniques were not satisfactory because they were time consuming, involved massive handling of food and could not be applied in a home situation.

Initial experiments were carried out on 100 g of food items, yielding 300 mL feed furnishing 1 kcal/mL. Subsequent experiments were conducted to yield 600 mL and 1200 mL of full strength as well as 1/2 strength feed. After going through series of cooking trials, method # 13 was identified as the best possible method for the preparation of polymeric diet and method # 5 for the preparation of partially digested diets.

These methodologies were tried on a number of formulations and diets # 4a and 8a were shortlisted as polymeric diet. The same two diets were treated with enzymes to make them partially digested. The reason for selecting these two diets was that they best met the criteria in terms of nutritional profile, preparation method and cost factor.

Indigenous polymeric enteral formulae are isocaloric and slightly hyperosmolar at full strength and isotonic at 4/5 strength, indicating that these feeds can be infused as a bolus without causing intolerance. Indigenous partially digested enteral formulae are isotonic at 1/2 strength
and hyperosmolar at full strength and furnish 1 kcal / mL which suggest that a slow infusion rate be used for improved tolerance.

Diet # 4a was used as a polymeric diet in the pilot study. The need for partially digested diet did not arise because all patients had intact digestive tracts. The indigenous diet was compared to a control diet of Isocal, a MeadJohnson product. The study was carried out on 24 hospitalised patients meeting the selection criteria. Nutritional outcome of these patients were assessed using 3 biochemical parameters: Nitrogen balance, serum prealbumin level and total lymphocyte count. The pilot study indicated that patients with cerebrovascular accident (CVA), head injury and meningitis stay long enough in the hospital to allow completion of nutritional therapy according to the protocol. Preliminary results have shown that the experimental diet is at least as efficacious as the control diet in improving nutritional status. The incidence of side effects was not different. However, a full fledged random control trial using an appropriate sample size is necessary to confirm these initial results impressions. The experimental diet is considerably cheaper and can be readily prepared in a hospital setting.

In order to minimise microbiological contamination, an aseptic technique was proposed for the preparation of these formulae. Further hazard audit was developed for the preparation of safe and hygienic formulae which could not be followed at the time of pilot study due to the non availability of an autoclave.

However, a series of experiments were carried out at the Aga Khan University Hospital, Karachi, Pakistan, to determine points where formulae may become contaminated. Results showed contamination in the food dry mix and in the post binderised feed indicating that thorough cleaning of the blender and minimum handling is warranted. A new protocol was developed and the hazard audit was revised on the basis of its applicability. A marked improvement was noted but complete microbial elimination could not be achieved because it is a multifaceted problem and does not have a single solution. Introduction of new procedures and strict adherence should minimise the growth but complete elimination would require acknowledgment of the problem, awareness among health care providers and education of the food handlers on infection control.
Development of indigenous enteral formulae

by

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M.Sc (Pak), M.S (US)

A thesis submitted to the
Faculty of Science and Technology,
University of Western Sydney, Hawkesbury
in fulfillment of the requirement for the degree of
Doctor of Philosophy

1995
PLEASE NOTE

The greatest amount of care has been taken while scanning this thesis,

and the best possible result has been obtained.
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- Actual protein intake
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Abstract

A procedure for preparing an enteral formula was developed, using Pakistani indigenous food items. The basis of development was that it would be nutritionally effective, easy to prepare and relatively cheap. The procedure was developed in 4 stages as illustrated in the flow chart.

Initially indigenous enteral diets were theoretically formulated involving a number of steps whereby a computer aided master sheet was developed and various combinations were analyzed using What if, Constraint and Target cells; which are the main features of What If Problem Solver® (Lotus spreadsheet). One hundred theoretical formulations were successfully developed with complete information on their nutrient content and cost.

In order to prove scientifically the efficacy of these diets, a modified PER was carried out on 6 diets and results indicated a higher PER for the experimental diet. Protein digestibility corrected amino acid score supported the PER results for experimental diets.

Out of 100 formulations, 29 were shortlisted for preparation trials. Two main techniques were applied: incubation and cooking techniques. Cooking method # 13 was shortlisted for the preparation of polymeric (for functional G.I tract) and cooking method # 5 for partially digested diets (for compromised G.I tract). Physical and chemical analyses were carried out to assess the effect of preparation. These cooking methodologies were tried on various diets and ultimately 2 diets: 4a and 8a were shortlisted for human trial.

To compare the efficacy of the indigenous enteral formula vs commercial formula, a pilot study was carried out at the Aga Khan University Hospital on 24 patients meeting the selection criteria. Patient nutritional outcomes were assessed using biochemical parameters: nitrogen balance, prealbumin and total lymphocyte count. Preliminary findings indicated that the experimental diet performed as well as control diet.

The problem of microbial contamination of enteral formulae could not be completely eliminated despite stringent measures in preparing enteral
formulae. This problem can be minimised through education and training of food handlers and instituting hygienic practice in health care institution.

**Flow Chart**

Identification of Pakistani food items

Using computer Lotus spread sheet (What If Problem Solver), 100 diets were theoretically formulated

Protein quality of six diets were determined using protein efficiency ratio (PER)

100 combinations

71 rejected

29 diets accepted
(for preparation of polymeric diets)

13 diets accepted
(for preparation of partially digested diets)

**Preparation criteria**

* Ease of preparation
* Minimum food handling
* Safe to administer
* Cost effective preparation method

Preparation experiments were based on the formulation of polymeric and partially digested enteral formulae

Two diets were shortlisted for clinical trial
(The same polymeric diets treated with enzymes to make them partially digested diets)

Polymeric diets
(for patients with functional G.I tract)

Partially digested diets
(for patients with compromised G.I tract)

Pilot study was conducted

Need did not arise to use partially digested diets
Declaration

The study presented in this thesis is original and was completed by the author, a post-graduate student in the Faculty of Science and Technology, University of Western Sydney, Hawkesbury, NSW, Australia, under the supervision of Dr Jim Bergan, Dr Goeff Skurray and Dr Mushtaq Ahmed.

I certify that the work presented in this thesis has not been submitted to any other University, or Institution for any degree or qualifications. Any help received in preparing this thesis and all sources used, have been acknowledged.

Nelofar Athar

April, 1995.
Publications from this work:


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