Amylase-Digested and Fermented Weaning Foods in the Dietary Management of Acute Diarrhoea in Malnourished Tanzanian Children

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Table of Contents

INTRODUCTORY PAGES ..................................................................................13

1 LITERATURE REVIEW ..................................................................................21

1.1 MALNUTRITION .......................................................................................21
  1.1.1 Defining and measuring malnutrition ..................................................21
  1.1.2 Importance of malnutrition .................................................................22
    1.1.2.1 Mortality due to malnutrition .......................................................22
    1.1.2.2 Morbidity due to malnutrition ....................................................23
    1.1.2.3 Mechanism for malnutrition-related morbidity and mortality .......23
  1.1.3 Effect of malnutrition on mental development ......................................25
  1.1.4 Causes of malnutrition .......................................................................25
    1.1.4.1 Immediate causes ........................................................................27
    1.1.4.2 Underlying causes ......................................................................29
  1.1.5 Addressing the malnutrition problem ..................................................29

1.2 WEANING FOODS AND THE PROBLEM OF DIETARY BULK .................30
  1.2.1 What is weaning? ...............................................................................30
  1.2.2 Good weaning practice ......................................................................31
    1.2.2.1 Biological determinants ...............................................................31
    1.2.2.2 Environmental determinants .......................................................32
  1.2.3 Problems with weaning ......................................................................34
    1.2.3.1 Low energy density and dietary bulk .........................................34
    1.2.3.2 Microbiological contamination ...................................................37
1.5.4 Use of intestinal permeability to evaluate dietary management of diarrhoea... 69
1.5.5 Conclusions .................................................................................................................. 69

1.6 ACUTE PHASE PROTEINS IN CHILDREN WITH DIARRHOEA ............................................ 70
1.6.1 Introduction .................................................................................................................. 70
1.6.2 Functions of acute phase proteins .................................................................................. 71
1.6.3 Control of acute phase proteins ...................................................................................... 72
1.6.4 Measurement of the acute phase protein response .......................................................... 72
1.6.5 Acute phase proteins in malnutrition and diarrhoea ......................................................... 72

1.7 TANZANIA - THE STUDY LOCATION .................................................................................. 73
1.7.1 Background .................................................................................................................. 73
1.7.2 Malnutrition in children ................................................................................................. 76
1.7.3 Administration and health facilities in Dar es Salaam ..................................................... 77
1.7.3.1 Local health statistics .................................................................................................. 78

2 METHODS .......................................................................................................................... 79

2.1 AIMS AND OVERVIEW OF STUDY .................................................................................. 79
2.1.1 Aims and objectives ........................................................................................................ 79
2.1.2 Collaborating institutions .............................................................................................. 79
2.1.2.1 The Tanzania Food and Nutrition Centre (TFNC) ......................................................... 79
2.1.2.2 The Muhimbili Medical Centre (MMC) ........................................................................ 80
2.1.2.3 The Centre for International Child Health (CICH) ...................................................... 80
2.1.3 Overview ......................................................................................................................... 80

2.2 STUDY METHODS .......................................................................................................... 82
2.2.1 Preparation, Planning and Ethical Approval ...................................................................... 82
2.2.2 Site and study population ............................................................................................... 83
2.2.3 Study Foods .................................................................................................................... 84
2.2.3.1 Feed Preparation and Composition .............................................................................. 84
2.2.3.2 Evaluation of feed characteristics ............................................................................... 84
2.2.4 Study Design .................................................................................................................. 87
2.2.4.1 Inclusion and Exclusion Criteria ................................................................................... 87
2.2.4.2 Entry to Study and Randomisation .............................................................................. 87
2.2.4.3 Questionnaire ............................................................................................................... 87
2.2.4.4 Anthropometry and Clinical Assessment ..................................................................... 88
2.2.4.5 Information about Stooling .......................................................................................... 89
2.2.4.6 Dietary Assessment ............................................................................................................. 90
2.2.4.7 Discharge and Follow up ..................................................................................................... 91
2.2.4.8 Intestinal Permeability Tests ............................................................................................... 92
2.2.5 Documentation and Data Processing ........................................................................................ 93
2.2.6 Statistics and Data Analysis .................................................................................................... 93

2.3 LABORATORY METHODS ........................................................................................................... 95
2.3.1 Storage and transport of samples .............................................................................................. 95
2.3.2 Intestinal permeability tests ..................................................................................................... 95
  2.3.2.1 Mannitol ................................................................................................................................. 95
  2.3.2.2 Lactulose and lactose ............................................................................................................ 96
  2.3.2.3 Validation of methods ........................................................................................................... 98
2.3.3 Acute phase proteins ............................................................................................................. 98
2.3.4 Thiocyanates .......................................................................................................................... 98

3 RESULTS ........................................................................................................................................ 100
3.1 PROBLEMS WITH DATA ACCURACY .................................................................................. 100
  3.1.1 Identification of invalid data ................................................................................................. 100
  3.1.2 Response ............................................................................................................................... 103
  3.1.3 Effect of the response ........................................................................................................... 103
  3.1.4 Patient Groups used for Data Analysis ............................................................................... 105

3.2 DESCRIPTIVE DATA ON ENTRY TO STUDY ....................................................................... 106
  3.2.1 General and illness-related characteristics ........................................................................... 106
  3.2.2 Acute phase proteins ........................................................................................................... 107
    3.2.2.1 Acute phase proteins as markers for systemic illness ..................................................... 107
  3.2.3 Feeding characteristics ........................................................................................................ 114
    3.2.3.1 Feeding history ............................................................................................................... 114
    3.2.3.2 Food and fluids offered during diarrhoeal illness .......................................................... 114
  3.2.4 Environmental factors ......................................................................................................... 114

3.3 DIETARY INTAKE ...................................................................................................................... 123
  3.3.1 Weight of food consumed ..................................................................................................... 123
  3.3.2 Energy intake ....................................................................................................................... 123

3.4 CLINICAL OUTCOMES AND FOLLOW UP ........................................................................... 128
  3.4.1 Diarrhoeal illness ................................................................................................................... 128
  3.4.2 Mortality ............................................................................................................................... 128
3.4.3 Weight changes ................................................................................................... 128
3.5 Intestinal Permeability .......................................................................................... 134
  3.5.1 Lactulose/Mannitol ratios during the study .................................................. 134
  3.5.2 Urinary lactose and intestinal permeability ................................................. 136
3.6 EVALUATION OF AMYLASE-DIGESTED FOODS ................................................. 140
  3.6.1 Knowledge, attitudes and practices relating to amylase-digested foods ....... 140
  3.6.2 Thiocyanates .................................................................................................. 140
  3.6.3 Cost .................................................................................................................. 140
  3.6.4 Variation of pH and viscosity in study foods throughout the study .......... 140
  3.6.5 Osmolalities .................................................................................................... 141
4 DISCUSSION ............................................................................................................. 147
  4.1 Problems with Data Accuracy ........................................................................... 147
  4.2 Patient Groups .................................................................................................... 148
  4.3 Descriptive Data ................................................................................................. 148
    4.3.1 Selection bias and randomisation ................................................................. 148
    4.3.2 General features .......................................................................................... 149
      4.3.2.1 Current and past illness ........................................................................... 150
      4.3.2.2 Home dietary management and rehydration ........................................... 154
      4.3.2.3 Early feeding practices .......................................................................... 154
      4.3.2.4 Socioeconomic factors .......................................................................... 155
      4.3.2.5 Acute phase proteins ............................................................................ 156
  4.4 Dietary Intake ....................................................................................................... 159
    4.4.1 Amylase-digested feed (AMD) .................................................................. 159
      4.4.1.1 Comparison with studies evaluating effect of viscosity reduction in healthy
            children ........................................................................................................... 159
      4.4.1.2 Comparison with studies evaluating effect of viscosity reduction in children
            with diarrhoea .................................................................................................. 160
    4.4.2 Fermented and amylase-digested feed (FAD) ............................................. 161
    4.4.3 Other factors ................................................................................................. 161
  4.5 Clinical Outcomes ............................................................................................... 165
  4.6 Intestinal Permeability ......................................................................................... 166
  4.7 Evaluation of Amylase-Digested Foods ............................................................. 169
  4.8 Optimum Feed Viscosities for Young Children ................................................... 171
CONTENTS

4.8.1 Feed viscosity for healthy children .................................................. 172
4.8.2 Feed viscosity for ill children .......................................................... 172

5 CONCLUSIONS ....................................................................................... 174

5.1 THE IMPORTANCE OF THE WEANING FOODS USED DURING DIARRHOEAL ILLNESS .... 174
5.2 MAIN FINDINGS OF THE STUDY .......................................................... 175
5.3 APPLICABILITY OF STUDY FINDINGS TO OTHER SITUATIONS .................. 177
  5.3.1 Applicability to other similar foods .................................................. 177
  5.3.2 Applicability to other illnesses ......................................................... 178
  5.3.3 Applicability to healthy children ..................................................... 178
5.4 SAFETY ISSUES AND CAUTIONS ....................................................... 179
5.5 PROMOTION OF AMYLASE-DIGESTED AND FERMENTED FOODS ............... 179
5.6 RECOMMENDATIONS FOR FURTHER RESEARCH .............................. 181
  5.6.1 Optimum feed viscosity for healthy and ill children .......................... 181
  5.6.2 Do amylase-digested and fermented foods reduce growth faltering? ........ 181
  5.6.3 Effect of fermented foods on intestinal permeability ......................... 181
  5.6.4 Combinations of AMD, FAD and other foods in the dietary management of diarrhoea and other illnesses .......................................................... 182
  5.6.5 Cultural acceptability of amylase-digested and fermented foods for dietary management of diarrhoea .......................................................... 182
5.7 PRACTICAL RECOMMENDATIONS FOR USE OF AMYLASE-DIGESTED AND FERMENTED FOODS IN THE DIETARY MANAGEMENT OF DIARRHOEA .................................................. 183
5.8 SUMMARY ............................................................................................ 184

6 REFERENCES ........................................................................................... 185

7 APPENDICES .......................................................................................... 206

APPENDIX A METHOD FOR VISCOSITY MEASUREMENT .......................... 206
APPENDIX B STANDARD WEIGHT CHECKS OF SCALES ............................. 208
APPENDIX C dBASE COMPUTER PROGRAMME USED TO ANALYSE FOOD RECORDS ........... 209
APPENDIX D PROGRAMME PARAMETERS FOR COBASFARA ANALYSER .......... 210
APPENDIX E QUESTIONNAIRES ................................................................ 211
APPENDIX F DATA COLLECTION FORMS ................................................ 224
**Tables**

**TABLE 1** Effect of diarrhoea on total energy intake in partially weaned children in four studies

**TABLE 2** Relationship between consistency and viscosity

**TABLE 3** Effect on viscosity of small quantities of ARF added to a thick rice porridge

**TABLE 4** Comparison of viscosities of porridges prepared from unmalted or fully malted cereal flours

**TABLE 5** Viscosities and energy intakes in feeding trials - Tanzania

**TABLE 6** Viscosities and energy intakes in feeding trials - India, Chile and Ethiopia

**TABLE 7** Examples of African lactic-fermented foods based on cereals and starchy tubers

**TABLE 8** International comparisons of national income and infant and child mortality rates 1991

**TABLE 9** Reasons for admissions and deaths in general paediatric wards at Muhimbili Medical Centre 1987/1988

**TABLE 10** Composition and characteristics of diets

**TABLE 11** Effect of introduction of enumerator coding on validity of raw dietary intake data

**TABLE 12** General characteristics of patients on admission by dietary group

**TABLE 13** Features of diarrhoeal illness on admission by dietary group

**TABLE 14** Past medical history by dietary group

**TABLE 15** Acute phase proteins by dietary group, compared to follow-up group

**TABLE 16** Early feeding practices by dietary group

**TABLE 17** Socioeconomic factors by dietary group

**TABLE 18** Household structure by dietary group

**TABLE 19** Weight of food consumed (grams per kg body weight per day) by dietary group and by day of study

**TABLE 20** Energy intake (kcal per kg body weight per day) by dietary group and by day of study

**TABLE 21** Outcome features of diarrhoeal illness by dietary group
TABLE 22  DETAIL OF CHILDREN WHO DIED DURING ADMISSION..............................................131
TABLE 23  CHANGE IN WEIGHT DURING FIRST 4 DAYS OF STUDY AND AT FOLLOW UP.....133
TABLE 24  MEAN L/M RATIOS ON DAYS 0 AND 3 AND AT FOLLOW-UP BY DIETARY GROUP137
TABLE 25  RESULTS OF ANOVA OF L/M RATIO AT 3 DAYS, WITH INITIAL L/M RATIO,
          AGE ON ADMISSION, AGE AT WEANING AND CLINICAL DIAGNOSIS AS COVARIANTS 138
TABLE 26  KNOWLEDGE, ATTITUDES AND PRACTICES RELATING TO AMYLASE-DIGESTED
          FOODS BY DIETARY GROUP, ON ENTRY TO STUDY.................................................................141
TABLE 27  OPINION AT DISCHARGE OF MOTHERS IN EACH TEST FOOD GROUP ON A)
          CHARACTERISTICS OF THEIR CHILD’S STUDY FOOD  B) SUITABILITY FOR HEALTHY AND
          ILL CHILDREN ............................................................................................................................142
TABLE 28  FREQUENCY OF MOTHER CITING EACH STUDY FOOD AS ONE OF CHILD’S MAIN
          FOODS ON INDIRECT QUESTIONING BY FOLLOW UP VISIT .................................................143
TABLE 29  RELATIVE COSTS OF STUDY FEEDS .............................................................................145
TABLE 30  COMPARISON WITH PREVIOUS STUDIES OF DIETARY MANAGEMENT OF
          DIARRHOEA: ADMISSION FEATURES..........................................................................................152
TABLE 31  COMPARISON WITH PREVIOUS STUDIES OF DIETARY MANAGEMENT OF
          DIARRHOEA: OUTCOME FEATURES............................................................................................153
TABLE 32  COMPARISON OF SOCIOECONOMIC FACTORS IN THE STUDY GROUP WITH THE
          1988 CENSUS DATA FOR THE DAR ES SALAAM URBAN POPULATION ..............................156
TABLE 33  COMPARISON OF THIS STUDY WITH THE JAMAICAN STUDY..............................158
TABLE 34  COMPARISON OF ENERGY INTAKE (TOTAL AND FROM BREAST MILK) DURING
          DIARRHOEA AND IN HEALTH IN THREE STUDIES .................................................................164
Figures

Figure 1 Effect of malnutrition on immunity ................................................................. 25
Figure 2 Causes of malnutrition ..................................................................................... 26
Figure 3 The malnutrition-infection cycle ..................................................................... 30
Figure 4 Determinants of weaning practice .................................................................. 32
Figure 5 Some factors affecting food intake and availability of energy and
    nutrients for absorption in a young child ............................................................... 33
Figure 6 Viscosity changes during cooking of a starch-based porridge ............... 35
Figure 7 Longitudinal sections through a grain prior to germination.............. 41
Figure 8 Preparation of cereal-based togwa ................................................................. 53
Figure 9 The principle of differential absorption ......................................................... 67
Figure 10 Cytokines and the acute phase response .................................................... 71
Figure 11 Changes in under-five mortality rates and infant mortality rates
    in Tanzania from 1960 to 1991 (UK data for 1991 given for comparison) . 74
Figure 12 Map of Tanzania ......................................................................................... 75
Figure 13 Number of cases of childhood diarrhoea per month admitted to
    Muhimbi Medical Centre 1990/91 ......................................................................... 77
Figure 14 Outline of study design ................................................................................ 81
Figure 15 Principle reactions in analysis of lactose and lactulose .................. 97
Figure 16 Frequency distribution of raw dietary intake data before and
    after introduction of enumerator coding on 30/06/92 ........................................ 101
Figure 17 Frequency distribution of meal amounts by enumerator after
    introduction of coding ............................................................................................ 104
Figure 18 Patient groups used for analysis of data ................................................... 105
Figure 19 Presenting symptoms in addition to diarrhoea ........................................ 110
Figure 20 Plasma concentrations of CRP, SAA and AGP in children with and
    without systemic infection ..................................................................................... 113
Figure 21 Foods or fluids other than breast milk by age of introduction... 116
Figure 22 Frequency of usage of different foods/fluids after three months
    of age .................................................................................................................... 117
Figure 23 Foods reported to be one of child’s main foods in week prior to
    becoming ill .......................................................................................................... 118
Figure 24 Foods normally mixed with thin maize porridge (Uji) ....................... 118
FIGURE 25 Foods offered to children with diarrhoea in the 24 hours prior to admission by dietary group .......................................................... 119
FIGURE 26 Fluids offered to children with diarrhoea in the 24 hours prior to admission by dietary group ......................................................... 120
FIGURE 27 Plots of individual daily energy intakes by dietary group and by day of study ............................................................. 125
FIGURE 28 Energy intake (KCAL per kg body weight per day) by dietary group and by day of study ........................................................... 127
FIGURE 29 Percentage of children still having diarrhoea by dietary group and by day of study (survival analysis) ........................................ 129
FIGURE 30 Percentage change in weight during first 4 days of study and at follow up ........................................................................ 132
FIGURE 31 L/M ratios during the study (including follow up) by dietary group compared to controls ...................................................... 137
FIGURE 32 Individual plots of change in L/M ratio from day 0 to day 3 by dietary group .................................................................................................................. 138
FIGURE 33 Correlation of lactose concentration and L/M ratio at admission for cases and controls ............................................................ 139
FIGURE 34 Urinary thiocyanate levels by dietary group after at least 12 consecutive study meals ................................................................. 144
FIGURE 35 Variation in pH by study food during the dietary study period ... 145
FIGURE 36 Variation in viscosity by study food during the dietary study period ................................................................................................. 146
FIGURE 37 Immediate causes of malnutrition ..................................................... 175
Photographs

PHOTOGRAPH 1  THICK MAIZE PORRIDGE BEFORE AND AFTER ADDING ARF .................. 44
PHOTOGRAPH 2  BROOKFIELD VISCOMETER AND SPINDLES ........................................ 85
PHOTOGRAPH 3  WHATMAN PHA PORTABLE pH METER ........................................... 86
PHOTOGRAPH 4  MODIFIED CHOLERA COT ............................................................. 90
PHOTOGRAPH 5  T SHIRT WITH HEALTH PROMOTION LOGO GIVEN TO MOTHERS

COMPLETING THE STUDY ............................................................................................ 92

Panels

PANEL 1  MAKING AND USING AMYLASE RICH FLOUR ........................................... 43
PANEL 2  METHODS FOR PREPARATION OF STUDY FOODS .................................. 88
Abstract


Month and Year of Submission: February 1997.

Developing countries need better weaning foods in order to combat malnutrition, which is an important underlying factor contributing to mortality and morbidity in children. Such foods are particularly necessary during and after episodes of diarrhoeal disease, an important cause of impaired growth. Amylase from germinating cereal grains in the form of an amylase-rich flour (ARF) enables preparation of porridges with a higher energy density than conventional weaning foods. This can be combined with lactic fermentation, which inhibits pathogen growth. These food technologies are inexpensive, can be implemented at the household level, and are therefore particularly appropriate for use in developing countries. There have been no previous randomised controlled trials comparing ARF-processed or lactic-fermented foods to conventional weaning foods in the dietary management of acute diarrhoea.

In a controlled clinical trial, 75 children age 6 - 25 months admitted to hospital with acute diarrhoea were rehydrated and then randomly allocated to 3 maize porridge dietary groups: conventional, amylase-digested (AMD), and fermented and amylase-digested (FAD). The study diets were given ad libitum five times daily and all intakes except breast milk were weighed. Comparison of admission characteristics and acute phase proteins between groups demonstrated that randomisation had been effective. Mean daily energy intakes over 4 days in the conventional, AMD and FAD groups respectively, were 32.4 (95% CI 28.7-36.6), 46.0 (39.6-53.4) and 37.3 (31.8-43.9) kcal/kg/day. The energy intake in the AMD group was 42% higher than the conventional group (p = 0.003). There were no significant differences between the groups for duration of diarrhoea, frequency of stooling or vomiting. The overall mortality during admission was 6%, with no significant difference between groups.

Lactulose:mannitol (L/M) permeability tests were performed on admission, at 3 days, and at follow up 2 and 4 weeks after discharge. The L/M ratios were compared between dietary treatment groups and to a group of age matched, healthy controls. Results show that children with diarrhoea had raised L/M ratios (geometric mean 0.85, 95% CI 0.68-1.05) compared to controls (0.14, 0.12-0.17) on admission. There was a significant difference in the change in L/M ratios between admission and 3 days between dietary treatment groups in favour of the FAD group (p < 0.05). Urinary lactose concentrations in spot urine samples taken prior to the permeability test were also measured. There was a significant correlation with the L/M ratio (correlation coefficient = 0.62, p < 0.001). The mean feed osmolalities measured in two samples for each study food were: conventional 22.5; AMD 62.0; and FAD 334.5 mmol/kg. Cyanide is present in unprocessed white sorghum, which was used to manufacture ARF, but is removed by normal processing. Urinary thiocyanate levels were measured in 27 consecutive study patients who had consumed at least 12 consecutive study meals. Geometric means were: conventional 14.7 (95% CI 9.1-23.8); AMD 16.7 (7.0-40.0); FAD 17.4 (9.1-33.4) μmol/l (normal range < 90 μmol/l) indicating that there had been no significant exposure to cyanide. The foods were received favourably by mothers.

Starch digestion using amylase from germination is a safe and effective way of improving energy intake in children with acute diarrhoea, while lactic fermentation improves intestinal permeability. Both technologies have a useful role in the dietary management of acute diarrhoea.
Declaration
No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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The author

Having graduated from Manchester Medical School with M.B. Ch.B. in 1986, I commenced my paediatric training 2 years later after house jobs and a year in general medicine, and gained my MRCP (UK) in 1990. Following paediatric jobs in Manchester, Oxford and London, I took up a 2 year Clinical Research Fellow post at the Centre for International Child Health, Institute of Child Health, London in 1991, which included a year in Tanzania where the field work for this thesis was carried out. I had no prior substantial research experience. I am currently Lecturer in Paediatrics (Honorary Senior Registrar) in the Academic Unit of Paediatrics and Child Health, St James’s University Hospital, Leeds.
List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AGP</td>
<td>$\alpha_1$-acid glycoprotein</td>
</tr>
<tr>
<td>AMD</td>
<td>Amylase-digested study food</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APP</td>
<td>Acute phase proteins</td>
</tr>
<tr>
<td>ARF</td>
<td>Amylase rich flour</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval (95 percent unless specified)</td>
</tr>
<tr>
<td>CICH</td>
<td>Centre for International Child Health</td>
</tr>
<tr>
<td>Conv</td>
<td>Conventional study food</td>
</tr>
<tr>
<td>cps</td>
<td>Centipoise (unit of viscosity measurement)</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>FAD</td>
<td>Fermented and amylase-digested study food</td>
</tr>
<tr>
<td>H/A</td>
<td>Height for age</td>
</tr>
<tr>
<td>HCN</td>
<td>Hydrocyanic acid</td>
</tr>
<tr>
<td>IP</td>
<td>Intestinal permeability</td>
</tr>
<tr>
<td>L/M ratio</td>
<td>Lactulose: mannitol ratio</td>
</tr>
<tr>
<td>MMC</td>
<td>Muhimbili Medical Centre</td>
</tr>
<tr>
<td>MUAC</td>
<td>Mid upper arm circumference</td>
</tr>
<tr>
<td>NCHS</td>
<td>National Center for Health Statistics</td>
</tr>
<tr>
<td>ORT</td>
<td>Oral rehydration therapy</td>
</tr>
<tr>
<td>PEM</td>
<td>Protein-energy malnutrition</td>
</tr>
<tr>
<td>SAA</td>
<td>Serum amyloid A</td>
</tr>
<tr>
<td>TFNC</td>
<td>Tanzania Food and Nutrition Centre</td>
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<td>WHO</td>
<td>World Health Organisation</td>
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<td>W/A</td>
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Introduction

This thesis has two principal subjects: weaning foods and diarrhoea. The amylase-digested weaning foods chosen for study are special because they are both ordinary and extraordinary. They are ordinary because they may be produced with simple ingredients and procedures (germination and fermentation) in any household in a warm climate; but they are extraordinary because a remarkable transformation is effected, changing poor quality weaning foods into those with many desirable properties.

Diarrhoea is the second principal subject because of its major contribution to global childhood morbidity and malnutrition, and the underlying thesis that amylase-digested weaning foods could reduce diarrhoea-associated growth faltering, and so help break the cycle of infection causing malnutrition, and malnutrition predisposing to further infection. This thesis addresses the question: What is the place of amylase-digested and fermented weaning foods in the dietary management of acute diarrhoea?

These two subjects are inextricably linked to malnutrition and its underlying causes. It is because malnutrition exists as a major public health issue for the majority of the world’s children that the study of alternative and inexpensive weaning foods is important, particularly in relation to infections such as diarrhoea.

This work is set in Tanzania, East Africa, where inadequate weaning foods, diarrhoeal disease, and malnutrition are all of great significance, and contribute to continuing high levels of child mortality and morbidity. In introducing and discussing these subjects, special reference will be made to the Tanzanian situation, both because it provides the local context for the work, and serves as an illustration of the wider issues which are raised.

The first section of the thesis will review the literature relevant to the research to be presented. This will include: an overview of malnutrition and infection; consideration of problems with weaning foods; the potential of the technologies of germination and fermentation to address these problems; the dietary management of diarrhoea, with reference to the possible role of these technologies; intestinal permeability tests and
acute phase protein measurements (both significant components of the research); and finally, the Tanzanian situation.

The research took the form of a single major study, comprising several components. The study was a hospital-based randomised controlled clinical trial comparing two amylase-digested weaning foods, one of which was also fermented, with the conventional food (control) in the dietary management of children with diarrhoea. The principal outcomes were dietary energy intake, clinical progress and weight gain, and change in intestinal permeability. An evaluation of the study foods, was also included within this framework. The second section will present an overview of the study and the study methods. The third section contains the results, and in the fourth section these are discussed in the light of the relevant literature. The final section will summarise the conclusions that may be drawn, and recommendations that follow from them.
1 Literature Review

1.1 Malnutrition

1.1.1 Defining and measuring malnutrition

Malnutrition is a state of nutritional inadequacy, whether of protein, energy or micronutrients [1]. Nutritional inadequacy exists when lack of a particular nutrient or nutrients prevents normal growth, development and biological function. There are two main categories of malnutrition: protein energy malnutrition (PEM), and micronutrient deficiencies. PEM is the category of particular concern in this thesis. However, there is considerable overlap, and PEM is often accompanied by deficiencies of micronutrients, particularly vitamin A, zinc and iron. PEM covers the spectrum of clinical pictures ranging from frank kwashiorkor to severe marasmus [2].

PEM is generally measured in anthropometric terms, using height, weight and arm circumference. A mid upper arm circumference (MUAC) of less than 12.5cm indicates severe, and 12.5 to 14.0 cm mild/moderate, malnutrition [3-5]. Weight and height are interpreted by comparison to international standards, usually either Harvard (Iowa 1930’s), or the more recent NCHS (National Center for Health Statistics - WHO 1983). Although these allow international population comparisons, they must be used cautiously since some populations are likely to have different genetic growth potentials than others. The main three indices are [6]:

- **weight-for-height (W//H²)** - a measure of wasting (i.e. thinness), and the best short term index, since it effectively measures recent malnutrition;
- **height-for-age (H//A)** - a measure of stunting (i.e. shortness), and used as a long term index of malnutrition, and thereby poor overall economic conditions and/or repeated exposure to adverse conditions;
- **weight-for-age (W//A)** - this is primarily a composite of the first two, and fails to distinguish tall, thin children from short, well proportioned children. Therefore, it is the least useful of the three, but it does obviate the need to measure height.

¹ The use of a double rather than a single slash is after Waterlow [2] to avoid the erroneous implication that these are ratios.
Z-scores are the best way to express comparisons with the reference population used, since they are comparable across ages and have the statistical property of being normally distributed. A commonly used cut-off point for the three main indices is a Z-score of -2, and the proportion of children below this cutoff is used to give the prevalence of wasting, stunting and underweight in different populations. These definitions will be used except where specified. Percentage of the median of the reference population has been widely used in the literature, but interpretation is age-dependent. The usual cutoff for W/A and W/H is 80 percent (to give proportions of undernourished and stunted respectively) and for H/A 90 percent. Percentiles are not useful because most malnourished children fall below the first percentile of NCHS standards [6,7].

PEM affects over one third of children under five, according to UNICEF [8]. In communities whose breast feeding rates are high most growth faltering occurs between 6 and 24 months of age, coinciding with the weaning period [9-12]. The most recent statistics from UNICEF show that 6 percent of children in developing countries suffer from moderate or severe wasting, 42 percent from moderate or severe stunting, and 35 percent from being moderately or severely underweight [13].

1.1.2 Importance of malnutrition

If malnutrition only caused poor growth, resulting in small children and small adults, one might contend that the smallness was a useful adaptation to adverse conditions (for instance due to less requirement for nutrients), and consider malnutrition itself (certainly mild-to-moderate malnutrition) of little concern [14,15]. But malnutrition is important because of three main consequences: death, disease, and impaired mental function.

1.1.2.1 Mortality due to malnutrition

Extreme protein energy malnutrition causes death. This is not contested in the literature. In a large prospective study in Zaire [16], extreme marasmus and kwashiorcor caused 16 percent of deaths in 0 - 5 year olds, even in a favoured area with a longstanding integrated development project. Controversy has arisen, however, regarding the contribution of lesser degrees of malnutrition to mortality. The issues are well summarised in reviews by Pelletier and Waterlow [17,18]. The central issue is whether there is a threshold effect, as advocated by Chen et al. [19], and supported by
the Zairean study mentioned above [16], or an effect at every level of malnutrition, as Pelletier argues [17]. The overall literature supports Pelletier's case. He makes the important point that malnutrition is much more often a potentiator of infection that causes death, than a cause of death on its own, and this potentiation is proportional to the severity of the malnutrition. He shows that in 53 developing countries, 56 percent of all child deaths are due to the potentiating effects of malnutrition on disease, of which 83 percent are due to mild-to-moderate malnutrition. Other surveys which have attempted to quantify the contribution of malnutrition to mortality have resulted in broadly similar figures [20,21]. Thus, it seems reasonable to conclude that about half of under-5 deaths in developing countries are caused or potentiated by malnutrition.

1.1.2 Morbidity due to malnutrition

There is clear evidence for increased incidence and severity of infections in extreme malnutrition [22-25]. The effect is less pronounced in mild-to-moderate malnutrition, but nevertheless it is clear that such children are at increased risk of morbidity, particularly from diarrhoea. Many of the relevant studies have been summarised by Briend [26] and Tomkins & Watson [1]. Taken together with the results of more recent studies, the main effect of malnutrition seems to be on duration of diarrhoea. There is no one anthropometric index that consistently shows the strongest association, although W/H is the most frequently reported as being associated. Two studies which showed no association are noted by Briend to have had excessively long follow up periods (1 - 2 years), which are likely to have allowed changing nutritional status in some children to have obscured the relationship with diarrhoea.

The information on whether malnutrition predisposes to diarrhoea can be summarised as follows:

- severe malnutrition undoubtedly predisposes to increased severity and duration of diarrhoea;
- the magnitude of this increase is in the order of 1.3 to 3.6 times that of normal children
- the strength of the association is weaker for lesser degrees of malnutrition, but nevertheless likely to be present;
- the effect on duration of diarrhoea is more pronounced than that on incidence.

Mechanism for malnutrition-related morbidity and mortality
Scrimshaw’s monograph in 1968 included a summary of human and animal data on the effects of malnutrition on immunity [27]. More recent useful reviews of this subject include those by Tomkins and Watson [1], and Chandra and Kumari [28]. Malnutrition erodes immune defences at almost all levels (See Figure 1, p25). At the level of external defences, malnutrition is known to reduce integrity of mucosal surfaces, stomach acidity, and glycoprotein synthesis (with consequent decrease in mucus and secretory IgA production) [25]. Innate immunity is also affected, with impaired production of cytokines, complement, and possibly acute phase proteins [28] and impaired phagocytosis [29]. Cell mediated immunity is markedly reduced [30]. Humoral responses are relatively spared, but antibody production is impaired in acute, severe malnutrition [25].

Mechanisms more specific to diarrhoea include depressed whole body protein turnover, of which that of the intestinal epithelium represents about one quarter and so repair is therefore likely to be compromised, and lower levels of digestive enzymes, which may prolong diarrhoea [26].
1.1.2.3 Effect of malnutrition on mental development

Despite difficulties in designing studies that adequately control for confounding factors, such as coexisting sociocultural deprivations, the balance of evidence indicates an adverse effect on mental development. Studies have either retrospectively compared survivors of severe malnutrition with siblings or controls, or prospectively supplemented at-risk groups and compared them to unsupplemented controls. The findings are helpfully reviewed by Grantham-McGregor [31]. These studies provide reasonably strong evidence that the poor levels of development commonly found in stunted children are partly mediated through poor nutrition.

1.1.3 Causes of malnutrition

It is clear that malnutrition is of major global importance, because of its impact on health and mental development. In order to formulate appropriate interventions that might alleviate malnutrition, it is necessary to consider its causes. A useful framework
Figure 2 Causes of malnutrition

![Diagram showing causes of malnutrition]

Source: Adapted from UNICEF 1990 [32]

is that used by UNICEF, shown in Figure 2. This illustrates that there are different levels of causes of malnutrition. The immediate causes are infection and inadequate dietary intake.
1.1.3.1 Immediate causes

1.1.3.1.1 Infection

Infection affects nutrition in the following ways:

- **reduced dietary intake**, due to anorexia or vomiting, reduction in the mother’s milk supply, intentional restriction of dietary intake by her (starving the fever), or substitution of the child’s regular food with a watery gruel [33];

- **reduced nutrient absorption**, particularly in intestinal infections, where there is decreased gut transit time (i.e. more rapid passage through the gut) and damage to the mucosal absorptive surface [25], but also to a lesser extent in other infections such as otitis media and pneumonia, which have been shown to affect intestinal permeability [34], or because of inappropriate administration of purgatives;

- **increased nutrient utilisation**, due to extra requirements related to fever (increased energy requirement), and for staging an appropriate acute phase and immune response (increased protein requirement) [25];

- **increased catabolism** - in an attempt to meet the “nutrient gap” caused by the above, there is increased protein breakdown (mainly muscle), and a switch to ketones as fuel instead of carbohydrates [1].

Both detailed case studies [35,36] and population studies [10,12,37-40] have demonstrated the impact of infection on growth. Although a review by Briend [26] indicates some weak links in the chain of evidence for diarrhoea as a major cause of malnutrition, it nevertheless seems extremely likely that the chain exists, given the overall literature, and the conclusions of other reviewers, such as Mata [41] and Black [42]. Black, in his review published in 1991 [42], concluded that all prospective studies of the adverse effect of paediatric infectious diseases on nutrition in the preceding 15 years demonstrated an effect of diarrhoea.

The information on infection as a cause malnutrition can be summarised as follows:

- infections undoubtedly cause growth faltering in individual children, and contribute to population malnutrition;
• the relative contribution of infections and poor dietary intake to malnutrition will vary in different populations, but both are important;
• of all childhood infections, diarrhoea is the most consistently implicated as a cause of malnutrition, with its contribution to weight and height deficits being in the order of 20 - 40 percent, and 10 - 20 percent respectively over the first few years of life;
• other infections less consistently implicated include respiratory infections (especially pneumonia), malaria, and measles.

1.1.3.1.2 Inadequate dietary intake

Inadequate dietary intake occurs in two ways, firstly acutely, through anorexia or vomiting during infections, and secondly on a chronic basis due to poor quality weaning foods which are low in energy density, compounded by inadequate meal frequency (see section 1.2.3.3, p38). Here the impact of infection on intake will be considered.

Several community studies have quantified the changes in intake that occur during infection. Mata in a small, detailed study of 30 Guatemalan children, found a strong inverse correlation between infections and energy intake in the second year of life [35]. Martorell et al. studied 477 Guatemalan village children between 15 months and 7 years of age with regular morbidity and dietary surveys, and found approximately a 20 percent reduction in dietary energy intake in sick children [43]. The deficit increased with age. Table 1 (p29) summarises the results of 4 studies of the effect of diarrhoea on total energy intake (including breast milk) in partially weaned children. Hospital studies showed a reduction in total intake of between 31 and 52 percent during diarrhoea, whereas, as might be expected, children with diarrhoea in the community study showed a smaller reduction in intake of 12 percent.

1.1.3.1.3 The malnutrition-infection cycle

It is clear that infections cause malnutrition, but also that malnutrition predisposes to infection. This close interrelationship has been called the malnutrition-infection cycle (see Figure 3, p30) [1]. Diarrhoea is not only the infection most likely to lead to malnutrition, but is also the one most likely to be potentiated by malnutrition. Thus within the larger cycle, there is an important diarrhoea-malnutrition sub-cycle.
Table 1 Effect of diarrhoea on total energy intake in partially weaned children in four studies

<table>
<thead>
<tr>
<th>Country and year of study</th>
<th>Location</th>
<th>Number of children</th>
<th>Age range (mo)</th>
<th>Total energy intake (kcal/kg/day)</th>
<th>% reduction in energy intake during diarrhoea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh, 1980</td>
<td>Hospital</td>
<td>41</td>
<td>6 - 35</td>
<td>During diarrhoea: 61 - 75</td>
<td>After recovery: 130†</td>
</tr>
<tr>
<td>Bangladesh, 1983</td>
<td>Hospital</td>
<td>17</td>
<td>11-24</td>
<td>72</td>
<td>104</td>
</tr>
<tr>
<td>Bangladesh, 1983</td>
<td>Hospital</td>
<td>63</td>
<td>&lt; 60</td>
<td>69 - 75</td>
<td>109 - 115</td>
</tr>
<tr>
<td>Nigeria, 1990</td>
<td>Community</td>
<td>45</td>
<td>5 - 28</td>
<td>85</td>
<td>96</td>
</tr>
</tbody>
</table>

Sources
1 Hoyle et al., 1980 [44]; 2 Sarker et al., 1983 [45]; 3 Molla et al., 1983 [46]; 4 Dicken et al., 1990 [47].
† This figure is for matched controls, rather than for the patient group after recovery from diarrhoea, as in the other studies.

1.1.3.2 Underlying causes

The bottom line is that structural inequality of resource distribution causes poverty, which leads to malnutrition [48]. This is mediated through many factors at different levels (Figure 2, p26), and these underlying causes are often interrelated [49,50].

1.1.4 Addressing the malnutrition problem

Ideally, the problem should be tackled at its root, which requires a global restructuring initiated by the rich industrialised countries who control the vast majority of the world’s resources. Any change at this root level is likely to be very slow, but yet such change must remain the goal. In the meantime, changes at intermediate levels have been advocated by UNICEF, on the basis that the actual cost of dealing with a large proportion of the ill effects of poverty throughout the world’s developing countries would be relatively small [51].
There remains a pressing need for interventions at the level of the immediate causes of malnutrition, which can be implemented by poor communities, and which will reduce the burden of malnutrition that they carry. Such interventions need to be simple and cheap. A key area to target at this level is the interface between diarrhoeal disease and dietary intake, since together they form the hub on which the malnutrition-infection cycle turns. Any intervention that can reduce the nutritional impact of diarrhoea is likely to alleviate the morbidity associated with malnutrition. This is the thrust of this thesis.

1.2 Weaning Foods and the Problem of Dietary Bulk

1.2.1 What is weaning?

The term wean is derived from the ancient Anglo-Saxon word “wenian” meaning “accustom”, and the weaning period has come to mean the period during which the infant gradually becomes accustomed to foods other than breast milk [52]. There is no standard definition of weaning [53]. In this thesis weaning will be defined entirely with reference to breast feeding:

- **weaning** - the process of leaving the breast
- **start of weaning** - the first time when any food or fluid (including water or bottle milk) other than breast milk is given to the infant;
- **end of weaning** - the time when breast feeding ceases;
- **weaning food** - any food other than breast-milk regularly given during the weaning period.
This definition is logical in the context of infant feeding in developing countries because in such countries almost all babies are breast fed, and any intake that is not from the breast carries significant microbiological hazards. In older children, breast milk has immunological and other benefits, even when contributing only a small proportion of dietary intake [54,55]. For industrialised countries where bottle feeding is prevalent, a much looser definition is required, with the change from liquids to solids being the key process [56].

1.2.2 Good weaning practice

Weaning practice is determined by two groups of factors: biological and environmental (Figure 4, p32). Good weaning will comprise environmentally determined practices which fall within the safe biological limits.

1.2.2.1 Biological determinants

Exclusive breast-feeding is the best way to feed infants in the early months of life. It reduces morbidity from diarrhoeal and other diseases, and enhances survival in infancy by a factor of between 2 and 5 times in developing countries [57]. Weaning foods should therefore only be introduced when breast milk can no longer satisfy the infant’s nutritional requirements, which is around 6 months of age, assuming that growth should be along international reference standards [58-60]. However, these are probably excessively demanding, being drawn from primarily bottle-fed infants who tend to be heavier at 1 year of age [61,62].
Solid foods cannot be introduced until the infant matures to the point of being able to consume them, digest and absorb them safely, and excrete the higher solute load via the kidneys [53,63]. This corresponds to an age of about 6 months, when there is a convergence of maturation across all the involved systems [53,63,64].

1.2.2.2 Environmental determinants

Prevailing cultural attitudes are an important determinant of weaning practices (Figure 4). Pelto has reviewed the range of sociocultural that influence infant feeding [65]. There are three main factors that lead to early supplementation: firstly, rejection of colostrum in many cultures leads to substitution with pre-lacteal feeds; secondly, the local staple or a particular formula milk may be perceived to be particularly nourishing, while breast milk alone is seen as inadequate; and thirdly, infants may often be left with another caretaker for some hours while mothers fulfill agricultural or other work commitments, requiring some other alternative to breast-feeds [66].
The amount of energy and nutrients available to the weaning child for absorption is determined by an interaction of child-characteristics with food-characteristics, which to a large extent is controlled by the mother, and her resources and education (Figure 5). In developing countries, the mother’s time and resources are constrained, and this results in three principal problems with weaning: low energy density and high dietary bulk; microbiological contamination; and infrequent child feeding.

Figure 5 Some factors affecting food intake and availability of energy and nutrients for absorption in a young child

Note that all factors shown in shaded ellipses (except child's stomach capacity) are influenced by the mother and her resources.
1.2.3 Problems with weaning

1.2.3.1 Low energy density and dietary bulk

1.2.3.1.1 Definitions

Energy density is the quantity of energy per unit mass of feed. It is a measurement that contains no reference to feed volume or viscosity. Typical energy densities of weaning foods in the UK are 0.9 to 1.4 kcal/g, while breast milk has an energy density of 0.7 kcal/g [67,68]. In contrast, typical energy densities of developing country weaning foods are 0.2 to 0.3 kcal/g. When such foods are manipulated to increase their energy density by addition of more solids, then their viscosity increases to a level that makes them difficult for the young child to consume. However, if the energy density is left unchanged, then the volume the child must consume to satisfy energy requirements is far too large relative to stomach size [69-71]. It is this combination of volume and viscosity characteristics that Svanberg has termed dietary bulk [52]. Developing country weaning diets are defined as bulky because either their volume or viscosity must be excessively high if adequate energy intake is to be achieved.

1.2.3.1.2 Food factors causing dietary bulk

In developing countries, most weaning foods are made by cooking flour from a starchy staple, such as maize, with water to form a porridge. Starch is a polysaccharide composed of long chains of glucose, present in plants as a storage material in the form of granules. When progressively heated in water, the granules begin to absorb water and swell when the temperature reaches about 55 - 70 °C (see Figure 6, p35). This is the initial gelatinization temperature. Swelling continues as the temperature rises, resulting in a marked increase in viscosity. At about 85 °C, the granules collapse and the chains disperse in the water, with a slight fall in viscosity. At this point, there exists a colloidal solution or sol. On cooling, the starch chains congeal into a three-dimensional matrix, with formation of a semi-liquid or semi-solid gel, and a further increase in viscosity. This gel has a high water content, which is due to the capacity of the starch chains to retain water, both by holding it within the matrix, and by binding it to the polar groups that have been exposed. The exact behaviour of starches from different food sources is
determined by granule size (large granules swell more than small ones), variations in the proportions of the two starch types (amylose swells more than amylopectin), and presence of other food constituents, such as lipids and proteins, which can modify the viscosity potential of starch [67,72,73]. These cooking characteristics of starch explain the problem of dietary bulk and low energy density.

1.2.3.1.3 Measuring viscosity of infant feeds

In studies of dietary intake where bulk is an important factor, it is important to measure both energy density and viscosity. Starch gels are viscoelastic materials, exhibiting simultaneously both the viscous properties of a liquid and the elastic properties of a solid [68]. For infant feeding, foods are usually liquid or semi-liquid, and so the viscosity is more relevant than the gel strength. Viscosity is the property of a fluid whereby it tends to resist relative motion within itself [74]. If a paddle is rotated within a fluid, its rate of rotation is the shear rate, whereas the force with which the rotation is resisted is the shear stress. In simple liquids and true solutions, there is a constant relationship between shear stress and shear rate. Thus, the faster the rotation of the paddle, the greater will be the resistance to its rotation. The ration of shear stress to
shear rate is the viscosity [68]. Units of measurement are usually milliPascal seconds (mPa s) or centipoise (cps), which are equivalent. Table 2 shows the approximate relationship between consistency and viscosity. Mosha and Svanberg define the viscosity appropriate for young child feeding as 1000 - 3000 cps [75], while Gopaldas et al. recommend a slightly higher range of 2000 - 6000 cps [76].

**Table 2 Relationship between consistency and viscosity**

<table>
<thead>
<tr>
<th>Consistency</th>
<th>Viscosity (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dough-like</td>
<td>&gt;40,000</td>
</tr>
<tr>
<td>Very thick, non-spoonable</td>
<td>10 - 40,000</td>
</tr>
<tr>
<td>Thick, batter-like</td>
<td>6 - 10,000</td>
</tr>
<tr>
<td>Easily spoonable</td>
<td>3 - 6,000</td>
</tr>
<tr>
<td>Soup-like</td>
<td>1 - 3,000</td>
</tr>
<tr>
<td>Free-flowing liquid</td>
<td>&lt; 1,000</td>
</tr>
</tbody>
</table>

Source: Ashworth & Draper, 1992 [77] and Gopaldas et al., 1988 [78]

Starchy porridges do not behave like simple liquids (which have a constant viscosity), except at low concentrations, when the large chains are widely separated and so behave as separate particles. As the concentration increases, there is increasing interaction between the starch chains, until at high concentrations the three-dimensional matrix becomes a solid gel. In the intermediate liquid state, viscosity varies with shear rate, due to the interaction between entwining starch chains, and the force disturbing them. Viscosity also decreases with time at a constant shear rate. These factors make viscosity measurements difficult to obtain, and interpretation of them complex [68].

**1.2.3.1.4 Intake studies in young children**

In theory, a child’s maximum eating capacity could be calculated from the physiological stomach volume, the gastric emptying rate, and the feeding frequency. In practice, however, this approach is not feasible due to the variability of the first two factors [72]. Instead, maximum eating capacity must be estimated from studies of actual
consumption in children offered food *ad libitum*. Svanberg reviewed a number of such intake studies in pre-school children (1 - 5 years), and concluded that their maximum daily capacity is about 900 - 1400 grams [52]. This assumes feed frequency is not a constraint. At about a year of age, this intake range corresponds to a required dietary energy density range of 0.7 to 1.0 kcal/g. This lower figure corresponds with the energy density of breast milk, and it would seem logical that the weaning foods which initially supplement breast milk should have at least the same energy density, becoming higher with increasing age and ability to chew more solid foods. Preference for liquid foods is higher in younger children, and also increases with severity of disease [69].

### 1.2.3.1.5 Increasing energy density and reducing bulk

The simplest way to increase energy density of traditional weaning foods, without a significant increase in viscosity, is to add energy-rich supplements [67]. Fats and oils are the most energy-dense food components (9 kcal/g), and so are often suggested as the most effective. However, addition of significant quantities of fat may compromise protein intake [68] and may in addition lead to micronutrient deficiency. Protein-rich foods, such as groundnuts, or carbohydrates (commonly sugar) are often added, and these have an energy density in the region of 4 kcal/g. Such additions may also improve palatability and influence viscosity, although the latter effect is relatively small. All suffer from the constraint of being relatively expensive, and availability for child feeding is often limited in poor communities.

The second way to increase energy density and reduce bulk is to modify the starch so that its water-binding and water-holding capacity is reduced. This can be done by physical means, for example by by brief exposure to intense dry heat, and/or physical force (e.g. parching, roasting, puffing, flaking) [77], or chemical means using amylase digestion (see section 1.3, p39). The latter is far more effective. Industrial-grade amylase is routinely used in the preparation of commercial weaning foods in developed countries in order to achieve high energy and nutrient levels in low viscosity feeds.

### 1.2.3.2 Microbiological contamination

In 1978, Rowland *et al.* identified several points at which weaning foods became contaminated with bacteria in a Gambian village. They showed that there was
significant contamination of the flour and water used (both around $10^2$ E. coli/g), which persisted even during brief cooking, and also of the apparently clean bowls used for serving portions [79]. When feeds were stored for 8 hours, as was common practice, total viable counts of aerobic bacteria rose from approximately $3 \times 10^3$ to $3 \times 10^7$ per gram. These high rates of contamination were in a setting where children frequently had diarrhoea, and began to growth falter from the age of 3 months, at the age when weaning foods were introduced. They concluded that where possible, infants should only receive freshly prepared food, but there is a need for foods that resist bacterial overgrowth for at least 1 - 2 hours. Hibbert and Golden performed microbiological “spot checks” on the bottle feeds (milk and thin porridges) being fed to well-nourished, compared to malnourished, Jamaican children, and found that there was no difference between rates of bacterial contamination, but that two-thirds of all feeds had more than $10^9$ faecal organisms per ml [80]. These feeds had been brought by mothers to a well baby clinic, and bacterial counts were not influenced by method of bottle sterilisation or time elapsed since the feed was made up. In the same study, milk feeds made up using standard hygienic kitchen precautions and clean water were not found to have any bacterial contamination. In a study in rural Bangladesh, Black et al. found similar high levels of contamination with E. coli (57 percent of non-rice weaning foods), which rose markedly with increasing environmental temperature [81]. Storage at ambient temperature for several hours was again found to be common practice, and encouraged growth of organisms. For each child, the proportion of contaminated food samples (obtained monthly over a 10 month period) was significantly related to the child’s incidence of diarrhoea due to enterotoxogenic E. coli. In rural Thailand, the mean total bacterial count of 130 feeds sampled was $3.8 \times 10^4$ organisms per gram, but 10 percent had counts at or above $10^9$/g, which is the maximum permissible viable total count suggested by the International Association of Microbiological Societies [82]. From this brief survey, it can be seen that weaning foods in developing countries have a high level of bacterial contamination which is increased further by storage practices.

1.2.3.3 Infrequent child feeding

In Rutishauser’s study of 45 pre-school Ugandan children living in a normal rural environment and attending a child health clinic monthly, 15 percent were only having 1 meal a day, 70 percent 2 meals per day, and the remaining 15 percent 3 meals per day, not including breast feeds, although the majority were not breast fed, since a large
number were between 2 and 3 years of age [71]. Very little information was given on the nutritional status of these children. In Kenya, the frequency of feeding weanlings at various ages was at maximum 4 meals per day at 7 - 12 months [83]. The main reason for low feeding frequency is lack of time. Preparation of each meal is a time-consuming process, and in rural areas collection of wood for fuel may take several hours. For a busy mother, who may have agricultural or other work in addition to childcare, there are economic reasons to minimise time spent preparing children’s meals [84,85]. Thus either the meal frequency must be kept low, or meals once prepared must be stored (usually without refrigeration) for later feeds.

1.2.4 Conclusions

In developing countries, good weaning practice consists of exclusive breast-feeding until at least age 4 - 6 months, followed by gradual introduction of weaning foods. Ideally, these should have an energy density of at least 0.7 kcal/g in the latter half of infancy, increasing to 1 - 2 kcal/g by 2 - 3 years, but with a low viscosity, especially for younger or ill children. They should have adequate micronutrient levels and protein quality, and be microbiologically safe. Children should be fed frequently (at least 4 times per day in addition to breast feeds). In practice, these ideals are rarely achieved because weaning foods are usually of low nutrient density and high dietary bulk, are often contaminated with pathogenic bacteria, and are given too infrequently. The next section will review the traditional technologies of germination and fermentation, and their role in improving weaning foods.

1.3 Germination and Fermentation - Technologies at the Household Level

Two weaning food technologies lie at the heart of this thesis: germination and fermentation. Both are technically simple, can be performed at the household level by those preparing children’s food, and effect a transformation on the foods on which they act. Germination is particularly effective in reducing viscosity and thus enabling an increase in energy density, while fermentation is useful for reducing risk of contamination with pathogenic bacteria, especially when foods need to be stored for some hours before serving. A useful collection of papers on these technologies is contained in the proceedings of an international workshop held in Nairobi, Kenya, in 1987, and published the following year [86]. A thorough and helpful review of the
subject was published in 1992 by Ashworth and Draper, during the period of the present study [77]. The sponsorship/commissioning of both these works by major international development agencies (including UNICEF for the former and WHO the latter), along with an editorial in *The Lancet* in 1991 [87] reflects the high degree of interest that has been generated by research in this area.

1.3.1 Germination

1.3.1.1 Biology of germination

When a cereal grain or legume is soaked in water, the process of germination is initiated, where the embryo within it grows and develops into a new plant. During the initial stages, the embryo is entirely dependent on nutrient stores contained within the grain for its metabolism and growth, until a functioning root and photosynthetic system is established. Early germination is therefore essentially a process of mobilisation and processing of nutrient stores so that they optimally meet the needs of the growing embryo. In many instances, these in-built processing systems may also improve the nutritional quality of the grain for human consumption.
During germination, complex chemical changes occur within the grain which are of three basic types: a) the breakdown of materials, particularly storage components such as starch and protein, into a more useable form for the growing embryo; b) the transport of breakdown products from one part of the grain to another; and c) the synthesis of new substances such as vitamins, especially niacin, riboflavin, and vitamin C [89]. Dormant enzyme systems become active on hydration, with an increase in the rate of respiration. Starch is the major reserve material in cereals, and is stored in granules within the endosperm (see Figure 7). β-amylase is also present in the endosperm, and is activated on hydration [90]. β-amylase hydrolyses amylose to maltose almost completely, by acting on 1:4 glycosidic linkages. However, it only partially hydrolyses amylopectin chains, producing maltose and high-molecular-weight dextrins, because it is unable to break the 1:6 linkages at chain branching points. During germination, α-amylase is synthesized in the aleurone layer and scutellum, and is secreted into the endosperm, producing a rapid rise in amylolytic activity [90]. α-amylase acts on 1:6
linkages, thus further breaking down large dextrins into small ones. β-amylase completes the amylolytic process by hydrolysing these to maltose. Maltose is converted to glucose by α-glucosidase. This moves across the scutellum to the embryo to sustain the growth of the seedling roots and shoot [91].

Germination involves the breakdown of other reserve materials in addition to starch. These include proteins, lipids, hemicellulose and polyphosphates. Proteins are stored in protein bodies, both in the endosperm, and also in the cotyledons of the embryo. These are hydrolysed by peptidases to form an amino acid pool which may be modified by synthesis and interconversion. They are then transported to the growing points of the embryo, initially the hypocotyl, and later the plumule and radicle [90].

Although there is some leaching of minerals and water-soluble vitamins to the surrounding water during germination, the overall nutritional value is increased because anti-nutritional factors such as lectins and phytate are broken down [90]. Flavour and cooking properties are also affected.

1.3.1.2 Use of germination for food-processing

Germination is a traditional technology employed to process foods and drinks in many developing countries. In this process, a grain or legume is soaked in water overnight, and then allowed to germinate for several days at 20 - 30 °C in damp, dark conditions. Optimal duration of germination varies for different grains and according to the purpose for which it is being carried out. Worldwide, germinated grains or legumes are used in a variety of ways, from beansprouts consumed as a raw vegetable in China, to deep-fried germinated grains in Indian curries, and (when ground to flour) as a malt for beer-brewing in Africa and Mexico [77]. A malt refers to grain that has been germinated and then dried. Grinding of a malt produces an amylase-rich flour (ARF). Wheat, sorghum or millet are usually used to prepare ARFs, and the sprouted grains are often simply sun-dried. This ARF, when added in small amounts to a thick cereal porridge, dramatically reduces its viscosity (see Photograph 1, p44) by digesting starch to its constituent dextrins and sugars. In Tanzania, this flour is known as “kimea” or “power flour”. Panel 1 gives practical details for preparation and use of an amylase rich flour. Use of ARF enables preparation of cereal feeds with a much higher energy and nutrient density, because the starting flour concentration can be as much as tripled without the
Panel 1  Making and Using Amylase Rich Flour

1. Select whole grains of sorghum, millet, maize or wheat, inspecting several sample handfuls carefully to check there is no evidence of damage (e.g. broken grains, or small holes due to pests), since this will impair germination potential. Clean to get rid of debris, and wash in plenty of clean water several times until the wash water becomes clear.

2. Add water (about three times the volume of the grains), cover and leave to soak overnight (6 - 12 hours).

3. Pour off the water, and spread the grains out on a clean wet cloth or banana leaves, cover (e.g. by folding the cloth over), and leave to germinate for 48 - 72 hours in damp, dark conditions at 25 - 30 °C. Keep moist by sprinkling with water 3 - 4 times per day. Do not use if mould forms (regular washing with water during germination prevents this).

4. Dry the germinated grains completely, either in bright sunshine for 8 hours, or by light-roasting over a low flame. Devegetate the grains by rubbing in a sieve (devegetation is not necessary if the final porridge is reboiled as in Step 8).

5. Mill to flour (hand pounding or electric grinder), and store in a dry, airtight container. This is ARF.

6. Make up child feed as normal (e.g. maize porridge) using ordinary flour (not ARF), but use 2 - 3 times as much flour, so that the porridge is very thick (a spoon will stand up in it).

7. Allow the porridge to cool for about an hour until it is warm, not hot (about 40°C), and then add a small amount of the ARF (approximately 10% of the weight of flour used to make the porridge), and stir until the porridge becomes thin (3 - 4 minutes).

8. Bring the porridge back to the boil, then serve as normal. Note that other foods such as groundnut flour, beans, egg, fish etc. can be added at this point or at stage 6.

...final feed viscosity being increased. The cost of this increase in energy density is mainly that of the extra flour used, and is usually a cheaper way of providing extra calories than use of energy rich supplements. A variety of other foods can be added in order to ensure a balanced nutrient intake.
Initial research in use of ARF for weaning foods took place in the early 1980's, and used feeds made entirely from malted flour, but it subsequently became clear that almost the same viscosity reduction could be achieved using only a small amount of ARF (e.g. 4% of wet weight) to process a feed made from normal flour (see Table 3, p45) [52,92]. In practice, even smaller amounts of ARF (around 2% of wet weight) have been found to produce an adequate viscosity reduction. Use of such small amounts of ARF is more labour-efficient, and so has become the preferred method of preparation. The procedure for producing ARF is familiar to many communities in Tanzania, since it is traditionally used in the production of local beverages.
Table 3  Effect on viscosity of small quantities of ARF added to a thick rice porridge

<table>
<thead>
<tr>
<th>Amount of ARF added ( % of total weight)</th>
<th>Amount of ARF added ( % of dry matter)</th>
<th>Viscosity (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>37,200</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>12,400</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>6,800</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>6,400</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>6,400</td>
</tr>
</tbody>
</table>

Data adapted from Gopaldas et al. 1986 [92]

1.3.1.3 Effects of germination

1.3.1.3.1 Viscosity reduction

α-amylase and β-amylase are both present in the germinating grain, and have complementary effects (see section 1.3.1.1, p40). α-amylase produces a more rapid decrease in viscosity [93]. Enzyme activity varies with grain type and length of germination, but for most begins to plateau between 2 and 5 days [77].

1.3.1.3.2 Nutritional effects

Germination generally increases the nutritive value of seeds, with increased synthesis of vitamins, particularly vitamin C, niacin and riboflavin [94,95]. Germination has also been shown to improve protein quality by increasing lysine, and the availability of amino acids [96]. Germinated products are good sources of protein, energy, B-vitamins, iron and phosphorus [95]. These nutritional advantages mainly apply when fully malted flours are used for weaning food preparation, but there will nevertheless be some such benefits even when small quantities of ARF are used. Feeds produced using ARF have a sweeter taste, due to the sugars produced from starch digestion, and this may improve their appeal to children.
Table 4 Comparison of viscosities of porridges prepared from unmalted or fully malted cereal flours

<table>
<thead>
<tr>
<th>Cereal</th>
<th>Viscosity (cps)</th>
<th>Viscosity ratio Unmalted: malted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unmalted</td>
<td>Malted for 48 h</td>
</tr>
<tr>
<td>Rice</td>
<td>1460</td>
<td>360</td>
</tr>
<tr>
<td>Wheat</td>
<td>1700</td>
<td>210</td>
</tr>
<tr>
<td>Maize</td>
<td>2200</td>
<td>580</td>
</tr>
<tr>
<td>Sorghum</td>
<td>1980</td>
<td>310</td>
</tr>
<tr>
<td>Finger millet</td>
<td>2100</td>
<td>120</td>
</tr>
<tr>
<td>Pearl millet</td>
<td>1810</td>
<td>85</td>
</tr>
</tbody>
</table>

Source: Malleshi and Desikachar 1986, cited in Ashworth and Draper 1992 [77]

Porridges were made with 10 percent dry matter

1.3.1.4 Factors influencing efficacy of germination

The amylolytic activity of different cereals is very variable (Table 4). Germinated millet has the greatest activity, and produces a sweeter and less viscous product than other grains. However, most of the research has concentrated on wheat (India) or sorghum (Tanzania). The choice of sorghum in Tanzania is because it is a hardy cereal that grows well in semi-arid conditions, and is therefore one of the cheapest. However, some varieties (particularly brown ones) are unsuitable because of their high tannin content, which inactivates amylases and also reduces protein digestibility and iron absorption [77]. White sorghum has an optimum germination time of 48 - 72 hours. Since above 70°C, amylase activity is considerably reduced, porridges must be cooled below this temperature before ARF is added [52,97]. The shelf-life of sun-dried ARFs is 10 - 21 days (21 days for sorghum) if kept in a dry, airtight container, when based on tight specifications such as used in industry, including acidity and bacterial counts
However, applying more practical standards such as adequate enzyme activity and effect of storage on taste, gives a shelf-life of over 4 weeks [78,92].

1.3.1.5 Dietary intake studies

Nine dietary intake studies using ARF for feeding healthy children have been located. Three were performed in Tanzania (see Table 5, p48, studies I - III) [73,98-100], four in India (see Table 6, p49, studies IV - VII) [76,78,92,101-103], and the other two in Chile [104] and Ethiopia [105] (Table 6, studies VIII and IX). All have methodological weaknesses which limit interpretation.

An ideal study should be a randomised controlled clinical trial of dietary intakes over at least a 24 hour period, with adequate data presented to allow the effectiveness of randomisation to be assessed, and control for potential confounding factors within the data analysis. Most of the studies (except II in Table 5, and IX in Table 6) confined intake measurements to a single test meal (either once only or daily for a period ranging from a few days to a few months), without reference to the time elapsed.
### Table 5: Viscosities and energy intakes in feeding trials - Tanzania

<table>
<thead>
<tr>
<th>First author</th>
<th>Age</th>
<th>Test meala</th>
<th>Viscosity (cps)</th>
<th>Energy density (kcal/g)</th>
<th>Amount consumed (g)</th>
<th>Energy intake (kcal)</th>
<th>% ▲ energy intake by ARF c.f. this diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Mosha</td>
<td>5 - 12 m</td>
<td>20 M/N</td>
<td>50 000</td>
<td>0.74*</td>
<td>153</td>
<td>114*</td>
<td>+ 6</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>20 M/N + ARF</td>
<td>3 000</td>
<td>0.74*</td>
<td>163</td>
<td>121*</td>
<td>+ 332</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>5 M/N</td>
<td>400</td>
<td>0.48*</td>
<td>154</td>
<td>28*</td>
<td>+ 26</td>
</tr>
<tr>
<td></td>
<td>13 - 24 m</td>
<td>20 M/N</td>
<td>50 000</td>
<td>0.74*</td>
<td>277</td>
<td>206*</td>
<td>+ 332</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>20 M/N + ARF</td>
<td>3 000</td>
<td>0.74*</td>
<td>347</td>
<td>259*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>5 M/N</td>
<td>400</td>
<td>0.48*</td>
<td>330</td>
<td>60*</td>
<td>+ 332</td>
</tr>
<tr>
<td></td>
<td>24 - 48 m</td>
<td>20 M/N</td>
<td>50 000</td>
<td>0.74*</td>
<td>405</td>
<td>230*</td>
<td>+ 43</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20 M/N + ARF</td>
<td>3 000</td>
<td>0.74*</td>
<td>445</td>
<td>325*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5 M/N</td>
<td>400</td>
<td>0.48*</td>
<td>491</td>
<td>88*</td>
<td>+ 274</td>
</tr>
<tr>
<td></td>
<td>48 - 65 m</td>
<td>20 M/N</td>
<td>50 000</td>
<td>0.74*</td>
<td>517</td>
<td>382*</td>
<td>+ 9</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20 M/N + ARF</td>
<td>3 000</td>
<td>0.74*</td>
<td>565</td>
<td>418*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5 M/N</td>
<td>400</td>
<td>0.48*</td>
<td>544</td>
<td>98*</td>
<td>+ 327</td>
</tr>
<tr>
<td>II. Lukmanji</td>
<td>6 - 12 m</td>
<td>8 M/Su/N</td>
<td>same</td>
<td>0.43*</td>
<td>128</td>
<td>55</td>
<td>+ 109</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>14 M/Su/N + ARF</td>
<td>same</td>
<td>0.85*</td>
<td>135</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25 M/R</td>
<td>stiff</td>
<td>1.11*</td>
<td>185</td>
<td>200</td>
<td>- 43</td>
</tr>
<tr>
<td></td>
<td>13 - 24 m</td>
<td>8 M/Su/N</td>
<td>same</td>
<td>0.43*</td>
<td>150</td>
<td>64</td>
<td>+ 120</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>14 M/Su/N + ARF</td>
<td>same</td>
<td>0.85*</td>
<td>166</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>25 M/R</td>
<td>stiff</td>
<td>1.11*</td>
<td>223</td>
<td>248</td>
<td>- 43</td>
</tr>
<tr>
<td></td>
<td>25 - 36 m</td>
<td>8 M/Su/N</td>
<td>same</td>
<td>0.43*</td>
<td>151</td>
<td>72</td>
<td>+ 108</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>14 M/Su/N + ARF</td>
<td>same</td>
<td>0.85*</td>
<td>176</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>25 M/R</td>
<td>stiff</td>
<td>1.11*</td>
<td>300</td>
<td>334</td>
<td>- 55</td>
</tr>
<tr>
<td></td>
<td>37 - 60 m</td>
<td>8 M/Su/N</td>
<td>same</td>
<td>0.43*</td>
<td>169</td>
<td>72</td>
<td>+ 115</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>14 M/Su/N + ARF</td>
<td>same</td>
<td>0.85*</td>
<td>172</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>25 M/R</td>
<td>stiff</td>
<td>1.11*</td>
<td>222</td>
<td>247</td>
<td>- 37</td>
</tr>
<tr>
<td>III. Kingamkono</td>
<td>9 - 24</td>
<td>33 M/fitc.</td>
<td>v. stiff</td>
<td>1.18</td>
<td>105</td>
<td>124</td>
<td>+ 7</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>17 M/Su</td>
<td>thick</td>
<td>0.60</td>
<td>377</td>
<td>88</td>
<td>+ 51</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>14 M/Su</td>
<td>soft</td>
<td>0.50</td>
<td>161</td>
<td>81</td>
<td>+ 64</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>27 M/Su + ARF</td>
<td>thick</td>
<td>0.96</td>
<td>138</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 - 36</td>
<td>33 M/fitc.</td>
<td>v. stiff</td>
<td>1.18</td>
<td>281</td>
<td>332</td>
<td>- 10</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>17 M/Su</td>
<td>thick</td>
<td>0.60</td>
<td>377</td>
<td>228</td>
<td>+ 31</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>14 M/Su</td>
<td>soft</td>
<td>0.50</td>
<td>482</td>
<td>242</td>
<td>+23</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>27 M/Su + ARF</td>
<td>thin</td>
<td>0.96</td>
<td>329</td>
<td>259</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37 - 56</td>
<td>33 M/fitc.</td>
<td>v. stiff</td>
<td>1.18</td>
<td>344</td>
<td>407</td>
<td>- 12</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>17 M/Su</td>
<td>thick</td>
<td>0.60</td>
<td>472</td>
<td>285</td>
<td>+ 25</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>14 M/Su</td>
<td>soft</td>
<td>0.50</td>
<td>501</td>
<td>251</td>
<td>+ 42</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>27 M/Su + ARF</td>
<td>thin</td>
<td>0.96</td>
<td>370</td>
<td>337</td>
<td></td>
</tr>
</tbody>
</table>

---

After Ashworth & Draper, 1992 [77]

a Studies cited are as follows: I Mosha & Svanberg, 1987 [98]; II Lukmanji et al, 1988 [99]; III Kingamkono, 1988 [100].

b Notation for test meals: initial figure is percentage dry matter; initial letter is staple (M=maize); abbreviations following slash are added foods (N= groundnuts; Su = sugar; R = relish; F= fish); +ARF indicates that amylase rich flour added after cooking. Rows are shaded where test meals included ARF.

c Values denoted by an asterisk have been deduced.

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since the previous meal, or what had been consumed at that meal. None of the studies measured intakes over a 24 hour period, and so there is the possibility that the increased intakes observed were not representative of the overall 24 hour intake. Although some of the studies mention randomisation, only one (study VII, Table 6) gives any information about the procedure, and none demonstrate that it has been effective. None control sufficiently for variables such as age, weight and health status.
### Table 6 Viscosities and energy intakes in feeding trials - India, Chile and Ethiopia

<table>
<thead>
<tr>
<th>Country and first author</th>
<th>Age</th>
<th>Total tests</th>
<th>Test meal¹</th>
<th>Viscosity (cps)</th>
<th>Energy density (kcal/g)</th>
<th>Amount consumed (ml)</th>
<th>Energy intake (kcal)</th>
<th>%  t energy intake by ARF c.f. non-ARF diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV. India Gopaldas</td>
<td>6-12 m</td>
<td>90</td>
<td>10 R/Su/O + ARF</td>
<td>10 R/Su/O</td>
<td>2.780</td>
<td>0.68*</td>
<td>56 ml</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>10 R/Su/O</td>
<td>3.120</td>
<td>0.67*</td>
<td>108 ml</td>
<td>72</td>
<td>+70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>10 S/Su/O + ARF</td>
<td>30</td>
<td>0.70*</td>
<td>66 ml</td>
<td>46</td>
<td>+70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>15 S/Maize/O + ARF</td>
<td>3.750</td>
<td>0.68</td>
<td>115 ml</td>
<td>50</td>
<td>+50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>15 S/Maize/O + ARF</td>
<td>440</td>
<td>0.68</td>
<td>210 ml</td>
<td>113</td>
<td>+126</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>25 R/Su/O + ARF</td>
<td>37200</td>
<td>1.85</td>
<td>56 ml</td>
<td>104</td>
<td>+67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>25 R/Su/O + ARF</td>
<td>6800</td>
<td>1.85</td>
<td>94 ml</td>
<td>174</td>
<td>+67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13-36</td>
<td>90</td>
<td>10 S/Su/O + ARF</td>
<td>3.100</td>
<td>0.70*</td>
<td>99 ml</td>
<td>69</td>
<td>+90</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>10 S/Su/O + ARF</td>
<td>300</td>
<td>0.70*</td>
<td>186 ml</td>
<td>131</td>
<td>+75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>15 S/Maize/O + ARF</td>
<td>3750</td>
<td>1.13*</td>
<td>86 ml</td>
<td>97</td>
<td>+75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>15 S/Maize/O + ARF</td>
<td>440</td>
<td>1.09*</td>
<td>156 ml</td>
<td>170</td>
<td>+75</td>
<td></td>
</tr>
<tr>
<td>V. India Gopaldas</td>
<td>6-12 m</td>
<td>?</td>
<td>20 W/Su/O + ARF(b)</td>
<td>0.500</td>
<td>1.75*</td>
<td>62 g</td>
<td>109</td>
<td>+95</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>?</td>
<td>3.500</td>
<td>1.65*</td>
<td>131 g</td>
<td>213</td>
<td>+95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>?</td>
<td>3.500</td>
<td>1.65*</td>
<td>160 g</td>
<td>126</td>
<td>+124</td>
<td></td>
</tr>
<tr>
<td>VI. India John</td>
<td>6-24 m</td>
<td>45</td>
<td>20 W/Su/O + ARF(b)</td>
<td>10 000</td>
<td>1.62*</td>
<td>55 g</td>
<td>89</td>
<td>+136</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>20 W/Su/O + ARF(b)</td>
<td>4320</td>
<td>1.68*</td>
<td>125 g</td>
<td>120</td>
<td>+136</td>
<td></td>
</tr>
<tr>
<td>VII. India Gopaldas</td>
<td>6-12</td>
<td>1800</td>
<td>20 W/Su/O + ARF(b)</td>
<td>25 000</td>
<td>1.63</td>
<td>26</td>
<td>42</td>
<td>+252</td>
</tr>
<tr>
<td></td>
<td>2340</td>
<td>20 W/Su/O + ARF(b)</td>
<td>3.500</td>
<td>1.63</td>
<td>71 g</td>
<td>116</td>
<td>+252</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1710</td>
<td>20 W/Su/O + ARF(b)</td>
<td>4400</td>
<td>1.63</td>
<td>160 g</td>
<td>126</td>
<td>+252</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1260</td>
<td>20 W/Su/O + ARF(b)</td>
<td>3 500</td>
<td>1.63</td>
<td>158 g</td>
<td>128</td>
<td>+252</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19-24</td>
<td>1620</td>
<td>20 W/Su/O + ARF(b)</td>
<td>25 000</td>
<td>1.63</td>
<td>41</td>
<td>67</td>
<td>+529</td>
</tr>
<tr>
<td></td>
<td>1640</td>
<td>20 W/Su/O + ARF(b)</td>
<td>3.500</td>
<td>1.63</td>
<td>146 g</td>
<td>238</td>
<td>+529</td>
<td></td>
</tr>
<tr>
<td>VIII. Chile. Alvina</td>
<td>3-4</td>
<td>244</td>
<td>16 P&amp;R + ARF</td>
<td>0.900</td>
<td>0.8</td>
<td>273 g</td>
<td>218</td>
<td>+218</td>
</tr>
<tr>
<td></td>
<td>279</td>
<td>20 P&amp;R + ARF</td>
<td>9 000</td>
<td>1.2</td>
<td>304 g</td>
<td>365</td>
<td>+67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>244</td>
<td>25 P&amp;R + ARF</td>
<td>9 000</td>
<td>1.6</td>
<td>337 g</td>
<td>540</td>
<td>+148</td>
<td></td>
</tr>
<tr>
<td>IX. Ethiopia Svanberg</td>
<td>2-5</td>
<td>200</td>
<td>20 S + ARF</td>
<td>same</td>
<td>1.0</td>
<td>272 g</td>
<td>272*</td>
<td>+20</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>25 S + ARF</td>
<td>same</td>
<td>1.2</td>
<td>272 g</td>
<td>336*</td>
<td>+20</td>
<td></td>
</tr>
</tbody>
</table>

After Ashworth & Draper, 1992 [77]


b Notation for test meals: initial figure is percentage dry matter; initial letter is staple (M=maize; R = rice; W = wheat; So = Soya; S = sorghum; P = pea flour); abbreviations following slash are added foods (Su = sugar; R = relish; O = oil); ‘+ARF’ indicates that amylase rich flour added after cooking; ‘+ARF(b)’ added before cooking. Rows are shaded where test meals include ARF.

c Values denoted by an asterisk have been deduced.

Given these limitations, the overall results (see Table 5 & Table 6) suggest that ARF-processing consistently increases energy intake, at least for single meals. The two main approaches have been to use ARF either to manipulate viscosity while fixing energy density, or vice versa. When viscosity is fixed, but energy density increased by using an
increased amount of dry matter in combination with ARF, then total dietary intakes are similar (although the ARF food is usually preferred). This results in increased energy intakes that are roughly proportional to the ratio of the energy density of the test food to the control, with most groups achieving at least a doubling (study II in Table 5, and studies VII and VIII in Table 6). When feeds of the same energy density but different viscosities are compared, a greater amount of the lower viscosity (ARF-treated) feed is usually consumed, with the resulting increase in energy intake being around 50 - 200 percent (studies IV, V, VI, and VII in Table 6), although this was much less marked in study I (Table 5). In study VII (Table 6), ARF and conventional feeds were given once daily to Indian slum children for a 6 month period. Given the impressive increases in energy intake reported over such a time period, the omission of any anthropometrical data is surprising. However, in an abstract referring to a similar 6 month study, one of the same authors indicates a significant gain in weight, height and MUAC in the ARF group [106]. These studies also suggest that even very young children can consume large amounts of very stiff porridge when healthy, and that the viscosity range of 1000 - 3000 cps recommended by Mosha and Svanberg [75] may be unnecessarily low. However, feeds of such low viscosity are much more likely to be necessary for ill children [69].

1.3.2 Fermentation

1.3.2.1 Description and background

Fermentation is a process in which complex carbohydrates, such as starches, are partially broken down by the action of micro-organisms, with the production of lactic, acetic and other acids [107]. This technology has been used for food preparation and preservation across the world since ancient times, and examples of its use occur in almost every culture. The main fermenting organisms used are yeasts (for bread and alcoholic beverages), moulds (for cheese production) and bacteria. Bacterial fermentations for food processing in Africa are usually due to lactic acid bacteria, which are a broad group of Gram-positive, catalase-negative, non-sporing rods and cocci, that utilize carbohydrates fermentatively and form lactic acid as the major end product [108]. These fermentations are classified as homolactic (lactic acid produced), or heterolactic (resulting in lactic acid, acetic acid, ethyl alcohol, carbon dioxide and other sugars), according to the metabolic pathways used [108]. Lactic-acid bacteria from four major genera, namely Leuconostoc, Lactobacillus,
**Lactococcus**, and **Pediococcus**, are responsible for most non-alcoholic fermented cereal porridges and drinks, and it is these that are most often suitable for use as weaning foods [107,108]. However, in most fermentations, a variety of other organisms including other bacteria, moulds and yeasts, make some contribution to the fermentation process, influencing the flavour and characteristics of the final product. In Africa there are many different lactic-fermented cereal products, some examples of which are shown in Table 7 (p52). Fermentation may be carried out either before or after cooking, or both. It may be allowed to occur spontaneously, or be initiated by inoculation with a starter culture or other source of organisms, such as flour. The fermentation process is usually initiated by a mixed flora of microorganisms, but with increasing acid production the non-lactic bacteria are eliminated. There is a microbial succession trend among the surviving lactic-acid bacteria, so that different organisms predominate at different stages of the process [108].

In Tanzania, the most common lactic-fermented product is a fermented maize beverage known as togwa [109]. Figure 8 (p53) shows a standard method of preparation, although there are significant variations across the country. Fermentation is usually combined with the use of ARF, and is initiated either by microbes contained within this flour, or by addition of a starter culture. During fermentation, the pH drops, and this is associated with the production of a characteristic tangy taste. After 12 - 24 hours at room temperature (25 - 30 °C), the pH of the porridge falls below 4.0, a level at which studies have shown that growth of enteropathogenic bacteria is inhibited (see section 1.3.2.2.4, p55), and the porridge is suitable for consumption over at least the next 12 hours without refrigeration or reheating.
<table>
<thead>
<tr>
<th>Product name(s)</th>
<th>Country</th>
<th>Staple</th>
<th>Lactic-acid Bacteria implicated</th>
<th>Other fermenting microorganisms</th>
<th>Starter inoculation</th>
<th>pH range of final product</th>
<th>Description</th>
<th>Usage (adults)</th>
<th>Usage as a weaning food*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ogi</td>
<td>Nigeria</td>
<td>Sorghum or millet</td>
<td><em>Lactobacillus plantarum, Lactobacillus delbrueckii, L. fermentum</em></td>
<td><em>Saccharomyces busae, Sacch. cerevisiae, Candida krusei, Corynebact. spp., Acetobacter spp., Bacillus spp.</em></td>
<td>-</td>
<td>3.6 - 3.7</td>
<td>Porridge</td>
<td>Breakfast and snacks</td>
<td>++</td>
</tr>
<tr>
<td>Uji, Togwa, Obusera</td>
<td>Kenya, Uganda, Tanzania</td>
<td>Millet, maize, sorghum or cassava</td>
<td><em>Lactobacillus plantarum, Leuconostoc mesenteroides</em></td>
<td>-/+ (ARF or starter culture, or both)</td>
<td>3.8 - 4.4</td>
<td>Thin porridge</td>
<td>Breakfast and snacks</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Mahewu</td>
<td>South Africa</td>
<td>Maize</td>
<td><em>Streptococcus lactis, Lactobacillus delbrueckii, L. bulgaricus, L. acidophilus</em></td>
<td>+ (wheat flour)</td>
<td>3.7 - 4.0</td>
<td>Beverage</td>
<td>Popular drink</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Kenkey/Koko</td>
<td>Ghana</td>
<td>Maize, sorghum or millet</td>
<td><em>Pediococcus cerevisiae, Lactobacillus plantarum, Leuconostoc mesenteroides, Lactobacillus delbrueckii, L. lactis</em></td>
<td><em>Saccharomyces cerevisiae, Acinetobacter sp., Enterobacter cloacae</em></td>
<td>-</td>
<td>3.5 - 4.1</td>
<td>Dough or dumplings</td>
<td>Staple food</td>
<td>Porridge prepared from the dough ++</td>
</tr>
<tr>
<td>Gari</td>
<td>Nigeria, Burkina Faso</td>
<td>Cassava</td>
<td><em>Lactobacillus plantarum</em></td>
<td><em>Streptococcus spp</em></td>
<td>-</td>
<td>3.6 - 3.9</td>
<td>Dumpling</td>
<td>Staple food</td>
<td>-</td>
</tr>
</tbody>
</table>

*Usage as weaning food: ++ common; + at least occasional; - no reports

Sources: Tomkins et al., 1988 [107]; Mensah et al., 1991 [110]; Ashworth & Draper, 1992 [77], Oyewole, 1995 [108].
1.3.2.2 Effects of fermentation

1.3.2.2.1 Improved taste and acceptability

The main acid produced by fermentation is lactic acid, which imparts a clean, sour flavour. Other products which may be present in smaller quantities include acetic acid, butyric acid, and ethyl alcohol. Organoleptic acceptability (i.e. taste, flavour, smell) of fermented products is reported to be greater than for corresponding non-fermented products, and the 'tangy' taste is reputed to appeal to ill children [86,111]. The nature of the fermentation is influenced by the substrate, the organisms present, the duration, and the ambient conditions such as temperature and pH [107]. Traditional fermentation processes have evolved to balance these aspects to achieve the characteristic acid taste.

Figure 8 Preparation of cereal-based togwa

Source: Lorri, 1993 [109]
which is most desirable. This nearly always corresponds to a pH of a very narrow range (3.4 - 3.8), which is usually reached over a period of 1 - 3 days [107].

1.3.2.2.2 Viscosity reduction

Ashworth & Draper stated in their review that no firm conclusion could be drawn regarding the effect of fermentation on viscosity and energy density, although there is a possibility that some fermentations reduce viscosity [77]. Low pH may itself reduce viscosity, due to an effect on starch characteristics [112], but may also inhibit any amylolytic activity produced by the fermentation [77]. Mlingi demonstrated a reduction in viscosity in porridge made from air-fermented but not wet-fermented cassava, and speculated that this was due to yeasts and moulds detected in the former [113]. Lorri found that fermentation with pure cultures of lactic-acid bacteria did not reduce viscosity, but if a mixed culture was used that included other organisms, the effect could be considerable [114]. This again suggests that it is the presence of other organisms, such as amylolytic moulds and yeasts that effect a viscosity reduction. Some fermented foods, such as togwa in Tanzania, are prepared using ARF, thus combining germination and fermentation. Since there is usually no reboiling after addition of ARF, the added amylase is presumably active throughout the fermentation, except for inhibition by low pH. This may explain why some fermented foods are widely perceived to be low in viscosity [107]. It can be concluded that there is no clear evidence that lactic fermentation reduces viscosity, although this may occur when certain other organisms are present.

1.3.2.2.3 Nutritional effects

Fermentation has been called “biological ennoblement”, and has a number of beneficial effects on the nutritional quality of foods. Protein digestibility is increased [107,115], and this effect has been shown to apply to lactic-fermented cereal gruels suitable for child feeding [116]. The percentage relative nutritive value (protein quality) has also been shown to improve [110]. In vitro studies indicate that bioavailability of iron and other minerals is increased due to hydrolysis of phytates, which reduce mineral solubility and absorption [117,118]. Fermentation has also been found to remove cyanide-yielding glycosides [119] (see section 1.3.3.1, p56).
1.3.2.2.4 Antimicrobial effects

One of the most important effects of fermentation in relation to child feeding, is that of inhibition of enteropathogen growth. Mensah et al., in a study of foods prepared by mothers in a Ghanian village, found that fermented dough was contaminated less often with gram-negative bacilli than the unfermented equivalent, and that after storage for 6 - 12 hours this difference persisted, with lower levels of organisms in the fermented food [120]. When samples of a fermented maize gruel (mahewu) were heavily innoculated with enteropathogens, no strains of *Campylobacter jejuni* survived after 30 minutes, and numbers of enteropathogenic and enterotoxigenic *Escherichia coli* remained static or decreased, suggesting that growth was inhibited [121]. Studies by Kingamkono et al. [122] and Svanberg et al. [123] on lactic-fermented cereal gruels compared to unfermented maize gruels have shown a reduction in enteropathogen colony counts in the fermented gruels, compared to static or increased counts in the control gruels. The inhibition of enteropathogen growth is primarily mediated by low pH, since most such organisms have a narrow pH tolerance, and do not survive when the pH is below 4.0 [110,122]. It is also likely that some fermentations produce bacteriocins which further inhibit pathogen growth. Mensah et al. showed that antimicrobial effect of fermented dough was reduced by cooking, despite a persisting acidic pH of 3.3 [124]. The presence of a heat-labile bacteriocin was postulated. Further studies showed the presence of an antimicrobial substance optimally active at pH 3.1 [125]. Svanberg et al. have also shown a similar loss of antimicrobial activity following cooking [123].

1.3.2.3 Clinical studies

There are very few clinical studies comparing dietary intake, growth or incidence of diarrhoeal disease in children fed fermented food versus unfermented equivalents. In a small longitudinal study of growth in 30 Ghanian children, those supplemented early (during the first month) with fermented porridge were reported to have a greater weight gain than exclusively breast fed infants, although no statistical tests were used to compare the groups, and the differences were small [126]. Lorri & Svanberg compared incidence of diarrhoea in two groups of about 100 children under 5 years of age living in adjacent villages in Tanzania, one of which used fermented foods for their children daily, while the other not at all [127]. Children in the fermented group had a 40 percent lower incidence of diarrhoea over a 9 month period. However, this was not a randomised trial, and little information was given to show that the groups were similar.
in respect of potential confounding factors, such as socio-economic status and water supply. Several studies have considered the use of fermented foods in the management of diarrhoea, and these will be reviewed in section 1.4.3.1.2 (p62).

1.3.3 Safety issues

1.3.3.1 Cyanide toxicity

Cyanide precursors are present in the roots and leaves of certain plants, particularly cassava, which is a staple for 400 million people in the Tropics [128]. They impart a bitter taste, which protects the plant from being eaten by animals, but are removed during normal food processing. Inadequate processing (e.g. as may occur during famine) risks toxicity, particularly when dietary sulphur is low, since sulphur is necessary for detoxification to thiocyanate [128]. Toxicity is due to inhibition of cytochrome oxidase, with prevention of cell respiration and oxidative phosphorylation.

Germinated sorghum contains dhurrin, a cyanogenic glycoside, which yields hydrocyanic acid (HCN) on hydrolysis. Panasuik and Bills drew attention to a theoretical risk of cyanide toxicity from germinated sorghum in 1984 [129]. They showed that the average yield of HCN from sprouts of eight sorghum cultivars germinated at 30 °C for 3 days was 61.3 mg per 100g seed. This compares with an estimated inhaled fatal dose of HCN of 1mg /kg body weight, this being rapidly absorbed from the lungs over a few minutes [128]. When cyanide is ingested, however, a much larger dose is required due to slower absorption from the gastrointestinal tract [128]. An infant is able to detoxify around 1.5mg/kg body weight of HCN per 24 hours [128]. Dada and Dendy studied the effects of processing germinated sorghum grains on cyanide content, and found that boiling, fermentation, or devegetation, all reduced the cyanide content by between 70 and 100 percent, to levels between 0 and 11.9 mg per 100g seed [119]. A child satisfying his energy requirements entirely from a standard amylase-digested diet such as used in this study (see Table 10, p83) would consume around 4 g/kg/day of sorghum malt. At a cyanide level of 11.9mg per 100g (the highest level found), the amount of HCN consumed would be 0.5 mg/kg/day, which is well below the detoxification capacity of an infant, even if this was compromised by malnutrition. Even if the cyanide content of the sorghum malt was as high as 20mg per 100g, which is the maximum permissible level of HCN in lima beans in the USA [129],
the amount of HCN consumed would still only be 0.8 mg/kg/day. Gopaldas et al. have advocated routine devegetation for preparation of ARF, since cyanide precursors are largely confined to the roots and shoots [76]. Tanzanian practice has instead been to reboil or ferment the porridge after addition of the ARF, which effectively removes any risk of cyanide poisoning [130]. There have been no reports of cyanide toxicity from use of germinated sorghum.

It can be concluded that although there is a theoretical risk of cyanide toxicity with unprocessed germinated sorghum, normal cooking practices effectively remove cyanide precursors and render the foods safe for consumption. Tanzanian porridges prepared using sorghum ARF have not been found to have any appreciable levels of cyanide.

1.3.3.1.1 Assessing exposure to cyanide

When foods containing cyanogenic glycosides such as dhurrin are consumed, a proportion of the glycoside is absorbed and excreted intact in the urine, while the remainder is hydrolysed to hydrocyanic acid (HCN) [128]. On entry to the blood stream, this is immediately trapped by the methaemoglobin fraction by reversible binding, and is then gradually released and detoxified by reaction with a sulphur donor. The main detoxification product is thiocyanate, which is excreted in the urine [128]. Cyanide exposure can therefore be assessed by measurement of serum thiocyanate, urinary thiocyanate, and urinary cyanogenic glycosides. Measurement of serum thiocyanate is the most sensitive method of assessing low levels of exposure, but urinary thiocyanate measurement is a non-invasive alternative that will detect any significant cyanide exposure [131]. Although in theory there might be limited conversion of cyanide to thiocyanate if there was a lack of sulphur-containing amino acids, in practice adults and children with low urinary inorganic sulphate levels due to such a lack produce high levels of urinary thiocyanate in response to cyanide exposure [132]. Thus, cyanide is converted to thiocyanate even in the face of low sulphur availability.

1.3.3.2 Other safety issues

Contamination of germinating grains with aflatoxin-producing moulds (*Aspergillus flavus* and *Aspergillus parasiticus*) is a potential risk, and can affect any type of grain [77]. Aflatoxins have been incriminated as a cause of human hepatoma, and possibly
Regular washing (2 - 3 times daily) of germinating grains prevents mould growth, and mouldy grains should not be used in preparation of ARF. Grains that do not appear mouldy are not at risk of contamination.

Trials of shelf-life of ARFs referred to above have been criticised because they have been carried out under laboratory conditions that are substantially different to those applying at the household level [77]. There may be some increase in enteropathogen counts during storage. Reboiling the porridge after addition of ARF, or fermentation, minimises any such risk.

Because both fermentation and amylase-digestion of starchy porridges produce dextrins and sugars, the osmolality of such porridges is increased, with a theoretical risk of osmotic diarrhoea. However, this has not been documented in practice [77]. Osmolality measurements have not previously been reported in such porridges.

Bongrekic acid toxicity is a rare occurrence during fermentation, when insufficient acidity develops, and contamination with \textit{Pseudomonas cocovenenans} occurs. This organism produces the toxin bongkrekic acid which can cause fatal poisoning due to hypoglycaemia [77]. There have been no reports of such contamination in Tanzania.

1.3.4 Summary

Germination and fermentation are household-level food technologies which have complementary actions in improving weaning foods. Germination (and to a lesser extent fermentation) reduces viscosity and dietary bulk, enabling the production of feeds of higher energy density. Fermentation reduces feed contamination with enteropathogens, and allows feeds to be stored safely for limited periods of time without refrigeration or reheating. This facilitates more frequent child feeding. Germination and fermentation can be used together. Foods produced in this way have the combined advantages of viscosity reduction and improved microbiological safety.

1.4 Dietary Management of Acute Diarrhoea in Young Children

The interface between diarrhoeal disease and dietary intake is a key target area in addressing the problem of malnutrition (see section 1.1). The purpose of the research described in this thesis was to evaluate the role of germination and fermentation in the
dietary management of diarrhoea. Literature relevant to use of these technologies in this role will be considered in this section, but first it is necessary to briefly review the mechanisms and other therapy of acute diarrhoea in children.

1.4.1 Mechanisms of diarrhoea

The small intestine is not only an organ of absorption (via the villi), but also of secretion (from the crypts) [134]. These normal bidirectional fluxes are balanced in favour of absorption. Acute diarrhoea is due to decreased absorption, or increased secretion, of fluid by the small intestinal mucosa, such that there is an increased fluid load to the colon which exceeds its maximum absorptive capacity [135,136].

1.4.1.1 Reduced absorption

Absorption is reduced when there is damage to the mucosa, for instance by infection or immunological insult. This may be initially histologically subtle, for example when the epithelium is immature due to increased rates of cell loss. In this situation, cells are prematurely promoted from the crypts in a relatively undifferentiated state, and so tend to secrete rather than absorb [136]. Rotavirus, which is the commonest cause of gastroenteritis in children under the age of 2 years in both developed and developing countries, causes grossly perturbed enterocyte renewal and migration which may lead to villous atrophy [135,137,138]. Enteropathogenic *Escherichia coli* (EPEC), another major cause of infantile diarrhoea in developing countries, causes particular damage to the brush border by its characteristic attaching and effacing lesion, which compromises absorption [135,137]. Epithelial damage from any cause may lead to secondary disaccharidase deficiency, particularly of lactase, with consequent solute malabsorption and an osmotic diarrhoea. Recovery in all instances of mucosal damage depends on adequate nutrient intake to enable epithelial regeneration [139], coupled with avoidance or removal of factors perpetuating the injury [135].

1.4.1.2 Increased secretion

Increased secretion is the main functional disturbance in diarrhoeas due to toxins, which are produced by many organisms, the best known being *Vibrio cholerae* and Enterotoxigenic *E. coli* (ETEC) [134]. Both these organisms are important causes of childhood diarrhoea in developing countries [140,141]. Their toxins bind enterocyte receptors and stimulate secretion from crypt cells by increasing intracellular cAMP or cGMP levels [135,142]. Secretory diarrhoea is typically high volume and extremely
watery, and since it does not depend on ingested solute, it continues unabated with fasting, in contrast to osmotic diarrhoea. Increased secretion is also caused by inflammatory mediators such as eicosanoids secreted by mast cells and phagocytes in the lamina propria, particularly in invasive diarrhoeas due to organisms such as shigella [134,135]. Most diarrhoeas have at least some component of increased secretion.

1.4.2 Rehydration and drug therapy

The cornerstone of management is oral rehydration therapy (ORT), a low-cost remedy that is effective in about 90 percent of cases, and has been hailed as the medical advance of the century [135,143-145]. Glucose-sodium cotransport may be impaired but is not abolished in most diarrhoeal states, and thus oral administration of a glucose-electrolyte solution converts a state of net intestinal secretion into one of net absorption [135,146,147]. Thus death and morbidity due to dehydration is prevented. Practical guidelines on use of ORT in diarrhoea have been widely disseminated by WHO [148]. Although there has been debate about the optimal composition of ORT [147], risk of bacterial contamination [149,150], who should be targeted and how much should be provided [151], it is clear that this effective and simple therapy remains under-used [144,152]. Solutions where glucose is substituted with complex carbohydrates from rice or other cereals reduce the osmotic load and may prove to be superior to conventional ORT, with the added benefit of providing some nutrition [153-155].

Drugs have a limited role in the management of acute diarrhoea [152]. Antibiotics are indicated for certain organisms (e.g. Shigella), and in certain clinical settings, but these are a minority of cases [135,146]. Antidiarrhoeal drugs have no place in the routine treatment of diarrhoea in children, and may have dangerous side-effects [156].

1.4.3 Dietary Management

Over the last decade, as ORT has become established in the management of acute diarrhoea, there has been a shift of emphasis towards dietary management. This aims to: a) maximise nutrient absorption during diarrhoeal episodes, both to promote enterocyte renewal and to reduce growth faltering; and b) minimise morbidity, by reducing stool volumes and duration of diarrhoea. Recent useful reviews include those by Torin and Chew [157], Brown [158], Booth [159], and Rivin and Santosham [145]. Attention to dietary management is particularly important in the developing world.
where children have frequent episodes of diarrhoea against a background of marginal nutrition. Without correct dietary management, many of the lives so dramatically saved by ORT will be later lost due to malnutrition and its potentiating effects on infection (see section 1.1, p21).

The previous conservative approach of a gradual reintroduction to milk and solid feeds following an episode of diarrhoea has been found unnecessary, and considerably reduces nutrient intake and absorption. Breast feeding should be continued wherever possible, since it reduces stool outputs in diarrhoea [160]. Lactose-free formulae are probably unnecessary, especially when standardised ORT protocols are followed and mixed diets are reintroduced quickly, according to a meta-analysis by Brown et al. [161].

Torin and Chew have summarised the results of several studies performed in developing countries in children under 3 years of age, and show that a wide variety of common local foods can be used to feed children with diarrhoea, without an exacerbation of symptoms, and with greater apparent absorption of energy and protein [157]. In a detailed study, Brown et al. found that continued oral feeding with a lactose-free formula during diarrhoea improved energy intake and weight gain [162]. Alarcon et al. then found that a mixed diet produced similar results to such a formula, but with a shorter duration of diarrhoea [163]. Brown's group have proposed that this effect is due to the dietary fibre content [158,164]. They have also shown that the incorporation of beans in a mixed diet for children with mild or moderately severe acute diarrhoea results in a shorter duration of diarrhoea, although this is at the cost of greater stool outputs, and therefore some caution is required in their use [165]. In a community study, Lanata et al. found that cereals consumed during acute diarrhoea were associated with a shortened acute episode and seemed to protect against subsequent development of persistent diarrhoea [166]. Of a wide variety of foods consumed during acute diarrhoea, none were significantly associated with development of persistent diarrhoea.
1.4.3.1 **Use of amylase-digested and fermented foods in diarrhoea**

1.4.3.1.1 **Amylase-digested foods**

At the time of setting up of the current study, there were no published reports of the use of amylase-digested weaning foods in children with diarrhoea. Reports on two such studies have been published since [167,168], and these will be discussed later (see 4.4.1.2, p160).

1.4.3.1.2 **Fermented foods**

A few studies have evaluated the use of fermented foods, or lactic-fermenting organisms, in the management of diarrhoea. The fermenting organisms are thought to assist in the restoration of the ecological balance in the normal intestinal flora. A study by Isolauri et al. compared the use of a *Lactobacillus*-fermented milk product, the same *Lactobacillus* given as a freeze dried powder, and a placebo in 71 children aged 4 - 45 months with acute diarrhoea [169]. Both groups receiving the *Lactobacillus* had a shorter duration of diarrhoea than the placebo group. However, an earlier study in 94 children under 3 years of age, comparing the oral administration of *Lactobacilli* to placebo, found no difference in outcome [170]. Both were randomised, but neither defined how diarrhoea was deemed to have stopped, and the former gave no information about antibiotic usage. A preliminary study has suggested that fermented soya bean (tempeh) may shorten the duration of persistent diarrhoea in children [171]. However, this was not a randomised study, the intervention and control groups were very different, both in size (79 versus 32 respectively) and proportion malnourished (80 versus 53% respectively), and the data provided was inadequate, both regarding description of patients and statistical tests performed. There are other unpublished reports from Indonesia suggesting a beneficial effect of tempeh in acute diarrhoea, but these are also methodologically weak. There are no randomised controlled studies of the use of fermented cereal porridges in the dietary management of diarrhoea, and none have considered dietary energy intake in this context.

Dietary fibre polysaccharides are normally fermented by bacteria in the colon, with the production of short chain fatty acids (SCFA), particularly butyric acid, which are important for mucosal nutrition [172,173]. Such acids are also produced by lactic fermentation used for food processing, and may therefore help to improve mucosal
function and recovery when fermented foods are given as part of the dietary management of diarrhoea.

### 1.4.3.2 Micronutrient supplements

The increased severity of diarrhoea and mortality associated with Vitamin A deficiency is largely prevented by periodic universal population supplementation [174-176]. From the data on universal supplementation, it would seem reasonable to hope that high-dose supplements given to children presenting with acute diarrhoea in at-risk areas might be effective in reducing illness severity [177]. However, the only randomised trial of vitamin A administration in acute diarrhoea did not find any benefit [178].

Zinc deficiency has been demonstrated in moderate and severe PEM, and further depletion of this mineral occurs in acute diarrhoea [179]. Zinc is likely to play a role in maintenance and function of mucosal surfaces, since severe deficiency causes dramatic skin changes and chronic diarrhoea [180], while low plasma levels have been shown to be associated with increased risk of diarrhoea and respiratory morbidity [181]. Supplementation during acute and persistent diarrhoea has been found improve intestinal permeability in some groups of malnourished children [182], and to reduce stool volume and shorten the duration of illness in persistent diarrhoea [183]. In a double-blind, randomised, controlled trial in New Delhi, India, involving 937 children aged 6 - 36 months with acute diarrhoea being treated in the community, Sazawal et al. found a 23 percent reduction in the risk of continued diarrhoea in children who received zinc supplementation, and a 39 percent reduction in the mean number of watery stools per day [184]. It seems likely that zinc therapy is beneficial in diarrhoeal illness, although the best dose, and duration and timing of treatment remain to be clarified [185].

### 1.4.4 Conclusions

Walker-Smith has emphasised that the malnourished child with gastroenteritis is in a very different situation to that of a previously well-nourished child with the same illness, because the former tolerates caloric restriction so poorly [143]. However, it is becoming increasingly clear that for both malnourished and well-nourished children, optimal dietary management of diarrhoea consists of early reintroduction of feeds (both milk and non-milk) following rehydration, but this is especially important in
populations at risk of malnutrition. Most weaning diets are tolerated, but foods used should have a high nutrient density to minimise the nutritional effects of diarrhoea. The role of amylase-digested and fermented foods in this area has been little explored. Micronutrient supplements are likely to benefit some children presenting with acute diarrhoea, but need further research.

1.5 Intestinal Permeability Tests

The study of diarrhoeal diseases, and other conditions affecting the gastrointestinal mucosa, has been hampered by lack of non-invasive methods of ascertaining the nature and severity of damage to the mucosa. The concept of intestinal permeability has provided a basis for a new approach to studying the gut mucosa. Mucosal integrity is assessed by passing inert molecules through the intestine and observing their relative permeation through the mucosal surface by measuring urinary excretion. In a key paper, Menzies defined intestinal permeability (IP) as “the facility with which the intestinal mucosal surface can be penetrated by the unmediated diffusion of specified constituents” [186].

1.5.1 Physiological basis

Although IP tests are now well established in both clinical and research practice, surprisingly little is known about the physiological basis for what has been observed. The essential principles are that: i) the mucosal surface is “probed” by inert molecules of a known size, and the extent of their permeation is indicative of the size and number of presumed trans-mucosal “channels”; ii) a change in permeation is due to a change at the level of the mucosa, due to effects on these channels; and iii) diseases affecting the mucosa may affect the permeation of molecules of different sizes in different ways, and this may be informative about the disease process.

The majority of literature on IP has assumed the existence of pores of differing dimensions throughout the intestinal epithelium, whose relative numbers change with mucosal damage [186-188]. The larger pores have been said to be paracellular, and the smaller transcellular, and this has seemed a logical way to explain the observed correlation of IP tests with histological appearances [189-192]. However, Hollander has challenged the pore theory because in the field of membrane biology there is no experimental evidence for it (e.g. pores of a similar size to probe molecules have never
been seen in enterocyte membranes) [193]. His alternative hypothesis is that all permeability probes traverse the epithelium via paracellular tight junctions, and that these are tightest in villus tips, and 'loosest' in the crypts. Large molecules, such as lactulose, may only cross via the looser crypt junctions, to which under normal circumstances they have little access, but which become more exposed in mucosal damage (and perhaps more leaky). Small molecules like mannitol permeate through the villus tip tight junctions, which are reduced in number by mucosal damage. This is consistent with current understanding of epithelial membranes, and explains why large molecule permeation increases and small molecule permeation falls during mucosal damage [193-195].

1.5.2 Dual-sugar permeability tests

1.5.2.1 Criteria for selection of probe molecules

The characteristics of the probe molecule should minimise intrusion of other factors in the measurement of permeability [186]. Firstly, they should be inert. Thus, their permeation across the absorptive surface should be unmediated, and they should not be metabolised. They should also be resistant to the action of intestinal enzymes and bacteria. Their size should be such that clinically relevant changes in the mucosa are detected by changes in permeation. This has been shown experimentally to be between 5 and 15 nanometres (nm) [186,193]. Usually, urinary excretion is used to evaluate intestinal permeation, and therefore renal clearance of the marker should be quantitative. The probe should not be naturally excreted in the urine in any significant amounts, since this would confuse interpretation of the test results. The three main categories of probes used are sugars, polyethylene glycols, and ethylene diamine tetra-acetate labelled with $^{51}$Cr ($^{51}$Cr-EDTA).

1.5.2.2 Advantages of dual-sugar permeability tests

Recognition that reduced absorption of monosaccharides in coeliac disease is accompanied by a paradoxical increase in permeability to disaccharides prompted the development of the dual-sugar absorption test [186,187,196]. The probe combination most commonly used is lactulose as the disaccharide (size 9.5 nm), and mannitol as the monosaccharide (size 6.7 nm) [188], and the result is expressed as the lactulose: mannitol (L/M) ratio. In the presence of mucosal disease, urinary recovery of the monosaccharide is decreased whilst that of the disaccharide is increased. This means
that if the two molecules are given together, and their urinary excretion compared as a ratio, the measured permeability change is amplified. Not only does this give the test greater power to detect mucosal disease, but the effect of extraneous factors, such as transit times and renal function, is minimised, since both molecules are affected similarly [187] (Figure 9, p67). This means that only a relatively short collection time (usually 5 hours) is necessary, and loss of some urine is not critical, which for children is a major advantage over $^{51}$Cr-EDTA, where complete and more prolonged collection is necessary [188,197]. The information from $^{51}$Cr-EDTA tests is limited because only a single molecule (size 11nm) is used. Polyethylene glycols are attractive because of their great range of molecular sizes, but complex mathematical treatment is required before urinary excretion reflects intestinal absorption, which casts doubt on interpretation [187].
Figure 9  The principle of differential absorption

Simultaneous administration of two test probes, A and B, chosen to respond in an identical way to each variable except that selected for investigation, provides a non-invasive method of studying specific aspects of mucosal function. The A/B excretion ratio provides a specific index of the state of the function selected for investigation, remaining unaffected by the other variables enumerated.

From Menzies 1983 [186]
1.5.3 Intestinal permeability in diarrhoea and malnutrition

Since IP is a measure of small intestinal mucosal integrity, raised permeability is seen wherever this is compromised, including patients with small intestinal villous atrophy, coeliac disease, Crohn’s disease, or food allergy; and following necrotising enterocolitis [188,198-201]. Intestinal permeability is also increased in preterm neonates, probably due to gut immaturity, but perhaps also as a developmental stage of the gut [202,203]. Of more relevance to this thesis however, are the effects of diarrhoea and malnutrition.

1.5.3.1 Diarrhoea

IP is more elevated in diarrhoea where an organism is identified [204], particularly rotavirus [182,205]. In acute diarrhoea, it rises early so that most hospitalised children have raised permeability by the time of admission [182,190,206], shows some recovery by the time of symptom resolution [190], and complete recovery within a few weeks [190,206], although this progression has to be deduced from mainly cross-sectional, rather than longitudinal, studies. In malnourished Bangladeshi children between 3 and 24 months of age, Roy et al. found L/M ratios of 0.50 on Day 1 for acute diarrhoea in their placebo group, falling to 0.18 on Day 8 and 0.13 on Day 15, while for persistent diarrhoea the respective values were 0.35, 0.14 and 0.15 [182]. The highest values for L/M ratio at admission were found in children with rotavirus, where mean L/M ratios were 0.70 in both acute and persistent diarrhoea. In non-malnourished children of a similar age range, Isolauri et al. found a mean L/M ratio of 0.12 on admission with acute diarrhoea, compared to around 0.02 for controls [205]. In a prospective community study of children between 0 and 6 months of age in Tyneside, children with symptoms of diarrhoea and vomiting had a mean L/M ratio of about 0.82 when organisms were identified in the stool, and 0.35 when no organisms were found [204]. Controls had a mean L/M ratio of 0.25. Some studies have found higher intestinal permeabilities in acute compared to persistent diarrhoea [182,190], but other studies have suggested the opposite [34]. However, none of these studies have demonstrated a significant difference between acute and persistent diarrhoea.

1.5.3.2 Malnutrition

IP has been found to be chronically raised in infants in developing countries, even when well-nourished (e.g. mean L/M ratio 0.42 in children aged 0 - 18 months in a study by Behrens et al. [34]), but is higher in malnourished children (mean L/M ratio 1.30 for
malnourished children < 60 percent W/H/A in the same age range in the same study).
Lunn et al. calculated that raised IP explained 43 percent of the growth faltering occurring in 2 - 15 month old Gambian infants [207]. However, the chronically raised IP may have been a result of the underlying malnutrition, rather than a cause of it [208]. It has been suggested that one mechanism for poor growth related to increased intestinal permeability is an increased requirement for dietary sulphur-containing aminoacids to detoxify pathogens from the gut, which reduces their availability for growth [209].
Short periods of starvation impair enterocyte renewal and migration in mice [139] and increase permeability in humans [210], and it is likely that some of the diarrhoea occurring in severely malnourished children is due to mucosal malnutrition [211].

1.5.4 Use of intestinal permeability to evaluate dietary management of diarrhoea

IP tests allow repeated, non-invasive assessments of mucosal integrity, and in children with diarrhoea may therefore be used to compare rate of disease resolution in response to different dietary therapies. Few trials of this nature have been carried out, and none have used sequential IP measurements to evaluate the dietary management of diarrhoea. Isolauri et al. performed a cross-sectional study in 41 children 3 - 25 months old with acute diarrhoea, and found that those who had been fed without interruption had normal permeabilities, while the non-fed children had markedly elevated permeabilities [205]. Other aspects of therapy have been evaluated using IP tests. Zinc treatment was found to improve IP only in certain groups of children with diarrhoea [182]. Diosmectite (an antidiarrhoeal drug thought to act both by adsorbing toxin and bacteria, and reinforcing the mucosal barrier by binding mucus), was associated with a significant reduction in IP at 2 days [212]. Neither study examined whether these reductions were associated with clinical improvement, and subsequent growth. However, it seems likely that therapies that improve IP will also have beneficial clinical effects, even if the latter are not apparent in small studies.

1.5.5 Conclusions

IP tests are a useful, non-invasive tool that enable evaluation of the course of diarrhoeal disease at the mucosal level, and thus comparison of the effect of therapies at this level. Dietary management of diarrhoea with fermented and amylase-digested foods could be usefully evaluated in this way.
1.6 Acute Phase Proteins in Children with Diarrhoea

1.6.1 Introduction

In order to more objectively quantify and describe the presence of systemic infection in addition to diarrhoea, three acute phase proteins were measured: C-reactive protein (CRP), serum amyloid A (SAA) and alpha-1-acid glycoprotein (AGP, orosomucoid). The acute phase protein response will therefore be reviewed briefly here, with particular reference to children with diarrhoea.

The acute phase protein response is one component of the total acute phase response. This is the rapid systemic response of the body to infection, trauma, local disease or inflammation which results in mobilisation of defence and healing processes, with the goal of restitution of homeostasis [213-215]. It encompasses the whole range of physiological events which follow tissue damage or inflammation, such as fever, endocrine changes, changes of fluid and electrolyte balance, and changes in protein synthesis by the liver. There are at least 36 acute phase proteins, which are grouped as either positive (the majority) or negative reactants according to whether they rise or fall in the acute phase [214,216]. CRP and SAA are classed as major positive reactants because they have particularly large and rapid increases, rising as much as a thousand fold from as early as 6 hours after the initial injury or other stimulus, and peaking at 24-72 hours [213,214,217]. In contrast, AGP has a more subdued reaction, rising only after 24-48 hours, with an increase of 2-fold or less, but with a longer half life [217]. CRP is most responsive to bacterial infections, whereas SAA is elevated during viral infections as well [218,219]. Both rise during malarial parasitaemia, with CRP showing correlation with the degree of parasitaemia [220,221].

Human CRP is a pentameric protein comprised of five identical, noncovalently bound subunits, arranged in cyclic symmetry in a single plane [213], and together with serum amyloid P component (SAP), which is closely related in structure and function, has been classified as belonging to the "pentaxin" protein family [222]. Similar proteins have been identified in virtually all mammals tested, indicating the likely functional importance of this type of protein [213]. Only one pentaxin has the role of a major reactant in any given species [214]. Thus, although SAP is present in humans, its
concentration shows little change during inflammation, while in mice it is the major pentaxin reactant [222].

1.6.2 Functions of acute phase proteins

The acute phase protein response may be regarded as a complex damage limitation and repair exercise, spearheaded by CRP and SAA. The precise roles of these major proteins remain uncertain, but they are almost certainly of fundamental importance, since they (or closely related proteins) exist in virtually all mammals, and individuals without them have not been described [213,214]. CRP’s main functions are related to its binding capability, which allows it to recognise and bind to invading organisms and damaged tissue, and to activate immune defences [213,214]. This involvement with finding, binding and clearing debris have led to it being dubbed a “garbage man” [223], clearing up the cellular mess in the wake of injury or infection. SAA appears to have a complementary clear-up role, effecting cholesterol and lipid clearance after tissue damage, and possibly its recycling for use in tissue repair [214,222]. AGP is thought to be involved in steroid and drug binding, platelet activation, and modulation of T cell

Figure 10 Cytokines and the acute phase response
function [215]. Functions of other acute phase proteins include activation of the complement cascade, coagulation and wound healing, damage limitation by proteinase inhibitors, and metal binding to prevent iron loss and scavenge oxygen free radicals [214].

1.6.3 Control of acute phase proteins

The acute phase response is controlled by a complex signalling network of cytokines. These are generally low molecular weight polypeptide molecules released by activated cells, and include interleukins, tumour necrosis factors, colony stimulating factors, growth factors and soluble cytokine receptors [224]. IL-1 principally mediates fever, while IL-6 mediates acute phase protein production, but there considerable overlap (Figure 10) [225,226].

1.6.4 Measurement of the acute phase protein response

Measurements may be broadly categorised as integrated, specific protein, and cytokines. Integrated tests, such as ESR, measure the contributions of several different acute phase proteins, and are mainly influenced by large plasma proteins such as fibrinogen [227]. Such tests show a slow response, are insensitive to changes in inflammatory activity, and lack specificity [228]. CRP is the most widely used specific protein measurement, with a range of relatively cheap assays available, and is especially useful in discriminating bacterial from viral infections in high-risk groups [227,228]. However, because of its rapid rise and fall, measurements may be difficult to interpret because the exact duration of illness is often uncertain. SAA is not yet suitable for routine use, due to lack of a suitable standard and difficulties with antibody production [227]. It is a very sensitive indicator of inflammation, showing a rise with even trivial viral infections such as the common cold [228]. AGP is a useful marker of chronicity. The most promising cytokine measurement is IL-6, which seems to be a sensitive predictor of subsequent clinical course in patients with suspected acute sepsis [225].

1.6.5 Acute phase proteins in malnutrition and diarrhoea

Protein deficiency has been shown to alter the pattern and magnitude of the acute phase response in rats [229], but there is little clear evidence that malnutrition attenuates the response in humans. Studies supporting this are methodologically weak, for example giving little information about presence of infection [230,231], or using excessively lenient criteria to define infection [232]. However, such an effect would not be
surprising, in view of animal experiments, and the known effects of malnutrition on the liver and other aspects of the immunity [2].

There is very little published literature on changes in acute phase proteins during diarrhoea. It is generally assumed that acute watery diarrhoea does not provoke an acute phase protein response, whereas invasive diarrhoea does, but there are no studies that confirm this. If this is the case, then measurement of acute phase proteins in acute diarrhoea (excluding dysentery) should provide a good objective indicator of the presence of systemic infection.

1.7 Tanzania - the Study Location

1.7.1 Background

Tanzania lies on the eastern side of the African continent, just south of the equator (Figure 12, p75). In area, it is the largest country in East Africa, and its population in 1991 was 26.9 million [234]. The official language is Kiswahili, which is spoken almost

| Table 8 International comparisons of national income and infant and child mortality rates 1991 |
|---------------------------------|-----------------|-----------------|-----------------|
| Country                        | GNP per capita  | Infant mortality Rate | Under 5 mortality rate |
|                                | (US $)          | (per 1000 live births) | (per 1000 live births) |
| Countries with world's lowest GNP's per capita: |
| Mozambique                     | 80              | 170              | 292              |
| United Republic of Tanzania    | 100             | 112              | 178              |
| Ethiopia                       | 120             | 125              | 212              |
| Somalia                        | 160             | 125              | 211              |
| Other countries for comparison: |
| Uganda                         | 170             | 110              | 190              |
| Bangladesh                     | 220             | 101              | 133              |
| India                          | 330             | 84               | 126              |
| Kenya                          | 340             | 52               | 75               |
| Angola                         | 610             | 170              | 292              |
| Indonesia                      | 610             | 61               | 86               |
| Zimbabwe                       | 650             | 61               | 88               |
| South Africa                   | 2,560           | 54               | 72               |
| Brazil                         | 2,940           | 55               | 67               |
| UK                             | 16,550          | 7                | 9                |
| USA                            | 22,240          | 9                | 11               |
| Sweden                         | 25,110          | 4                | 5                |
| Switzerland                    | 33,610          | 7                | 9                |

universally, but in addition there are about 120 tribal languages. Tanzania is one of the world's poorest countries in terms of Gross National Product (GNP) per capita (Table 8), but has achieved lower infant and child mortality rates than many countries having higher incomes [49]. Although there has been a steady fall in infant and child mortality over recent decades (Figure 11, p74), mortality rates are approximately 20 times higher than developed countries, with more than 1 in 6 children dying before the age of 5.

Figure 11 Changes in under-five mortality rates and infant mortality rates in Tanzania from 1960 to 1991 (UK data for 1991 given for comparison)

Source: UNICEF 1993[234], Leach 1990 [235]
Figure 12  Map of Tanzania

Location of research (Dar es Salaam) is arrowed
Table 9 Reasons for admissions and deaths in general paediatric wards at Muhimbili Medical Centre 1987/1988

<table>
<thead>
<tr>
<th>Disease</th>
<th>Admissions (%)</th>
<th>Deaths (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>40.8</td>
<td>23.0</td>
</tr>
<tr>
<td>Diarrhoea and vomiting</td>
<td>23.4</td>
<td>16.0</td>
</tr>
<tr>
<td>Malnutrition: severe PEM and anaemia</td>
<td>14.1</td>
<td>24.6</td>
</tr>
<tr>
<td>Respiratory tract infections</td>
<td>7.7</td>
<td>16.4</td>
</tr>
<tr>
<td>Immunecable diseases ‡ (mainly measles, TB and tetanus)</td>
<td>3.1</td>
<td>6.6</td>
</tr>
<tr>
<td>Others</td>
<td>10.9</td>
<td>13.4</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Number of cases</td>
<td>6,925</td>
<td>888</td>
</tr>
</tbody>
</table>

Source: Leach, 1990 [238]

† Note that the categories in this Table are presumed to be the primary diagnosis, although this is not made explicit in the source. No information is given in the source about the amount of overlap between groups.

‡ Diseases which can be immunised against

Dar es Salaam is Tanzania's largest city, and one of the most important ports and commercial centres in East Africa. Its population has grown rapidly from around 450,000 in 1973 to 1.2 million in the 1988 census, with a projected figure of 3.6 million by the year 2000 [236,237].

The region has a hot, humid, tropical climate, with average temperatures around 23°C in the cool season (June to September) and 27°C in the hot season (December to March), and two rainy seasons.

1.7.2 Malnutrition in children

Reliable data for nutrition for the whole country are not available, but community surveys have consistently shown rates of 40 - 60% of under-5s less than 80% of the standard W//A, and 4 - 9% less than 60% of this standard (severely malnourished).
These figures imply that over 2 million children under five years of age in mainland Tanzania are malnourished (less than 80 percent of the standard W/H/A), and nearly 300,000 children under five years of age are severely malnourished (less than 60 percent standard weight-for-age). Rates of malnutrition in Dar es Salaam are lower than the national average.

### 1.7.3 Administration and health facilities in Dar es Salaam

Administratively, Dar es Salaam is divided up into 3 districts, each comprising about 15 wards, which are then divided into 10 cell units. The 10 cell unit is the smallest administrative unit, comprising 10 or so households, each unit having its own leader. Each ward has one or more dispensaries, usually in combination with a mother and child health clinic, which provide primary care facilities including community child programmes. Each district has its own hospital, with in-patient beds, and facilities to diagnose and treat common conditions. In addition, there are several private hospitals. The Muhimbili Medical Centre is a large tertiary referral centre, both for Dar es Salaam, and the rest of the country.
1.7.3.1 Local health statistics

Malnutrition together with anaemia caused 14.1 percent of all admissions and 24.6 of all deaths in general paediatric wards at Muhimbili Medical Centre in 1987 (Table 9, p76). The other major causes of morbidity and mortality in children in Dar es Salaam are malaria and diarrhoeal diseases. Admissions with diarrhoea have a seasonal variation, which approximately follows the pattern of rainfall. Figure 13 (p77) shows the distribution of admissions with diarrhoea for 1990 and 1991, and illustrates that there are fewer admissions during the dry months of August to October.
2 Methods

2.1 Aims and Overview of Study

2.1.1 Aims and objectives

The overall aims of the study were to determine whether the use of amylase-digested and fermented weaning foods in the dietary management of diarrhoea would:

- improve dietary energy intake
- hasten recovery, both clinical and as measured by intestinal permeability tests
- reduce growth faltering.

To achieve these aims, the following objectives were defined:

- to determine an appropriate site for the study and set up collaboration with local institutions
- to select a fermented and a non-fermented amylase-digested weaning food, and a control food, each representative of its class
- to run a hospital-based randomised controlled clinical trial comparing amylase-digested weaning foods to a conventional weaning food in the dietary management of diarrhoea

2.1.2 Collaborating institutions

Informal links were already in place between the Centre for International Child Health, the Tanzania Food and Nutrition Centre, and the Muhimbili Medical Centre. These were strengthened into a formal collaboration for the purposes of this project. The three institutions each had particular strengths, which in combination provided an ideal basis for the accomplishment of the aims set out above.

2.1.2.1 The Tanzania Food and Nutrition Centre (TFNC)

The Tanzania Food and Nutrition Centre is a parastatal organisation based in Dar es Salaam with great expertise in nutrition, ranging from laboratory food science work to large scale community surveys, and an advisory role to both governmental and non-
governmental organisations. It has had a longstanding interest in amylase-digested products for use as weaning foods.

2.1.2.2 The Muhimbili Medical Centre (MMC)

This tertiary referral centre has a large paediatric department with an ongoing and active research program. There are 4 general paediatric wards, one of which is a Diarrhoea Treatment Unit, and is a WHO training centre for the program for Control of Diarrhoeal Diseases. Large numbers of children with diarrhoea are admitted each year.

2.1.2.3 The Centre for International Child Health (CICH)

This is a department of the Institute of Child Health, London, within the Division of Public Health. It is committed to excellence in teaching and research in international child health, and its staff act as consultants for many projects worldwide. There is considerable experience and interest in the area of weaning food technologies for developing countries.

2.1.3 Overview

The study protocol is summarised in Figure 14 (p81). The study was restricted to: a) hospitalised children admitted to a single ward to minimise sources of error or bias in data collection, overall treatment, and food preparation; and b) acute watery diarrhoea because of the relatively small numbers of children admitted with persistent diarrhoea or dysentery. Children with acute diarrhoea were randomly allocated to one of three dietary groups, and several outcomes were compared: dietary intakes; clinical course; weight gain; and change in intestinal permeability.
**Figure 14 Outline of study design**

**Child with diarrhoea**

**Admission**
History, examination and rehydration as normal

**After rehydration**
(4 - 24 hrs after admission)

**Entry to study if:**
1. Informed consent
2. Entry criteria satisfied (see box)
3. Study bed available

**Randomisation**
To three dietary groups
Numbered envelopes

**Conventional**
(Control)

**AMD**
(Intervention)

**FAD**
(Intervention)

**Study entry procedure**
1. Baseline IPT - sugar drink, urine sample
2. Questionnaire Part 1
3. Examination
4. Anthropometry (weight, height, MUAC)
5. Commence study diet, record intakes
6. Commence stool collection

**Acute phase proteins**
2ml blood for acute phase proteins (within 24 hrs of entry to study)

**During admission**

**Daily procedures**
1. Weigh all study food consumed (to nearest gram)
2. Complete physical examination, with initiation of any appropriate treatment
3. Daily weight and MUAC
4. Recording of time of all stools passed, consistency, and measurement of volume

**Once**
1. Questionnaire Part 2
2. 2nd IPT at 3 days

**At discharge**
1. IPT (if admission longer than 3 days)
2. Teach mother how to prepare child’s study food

**Follow up (approx 14 & 28 days)**
1. IPT
2. Questionnaire, examination, and anthropometry

**Inclusion criteria:**
1. Acute diarrhoea (<14 days)
2. Age between 6 and 25 months
3. Already commenced weaning

**Exclusion criteria:**
1. Any congenital or chronic condition interfering with food intake
2. Kwashiorkor
3. Dysentry

**Further exclusion criteria:**
1. Well enough for discharge next day
2. NG feeding tube required within 24 hours

**Abbreviations**

AMD Amylase-digested feed
FAD Fermented and amylase-digested feed
IPT Intestinal permeability test
MUAC Mid upper arm circumference
NG Nasogastric
2.2 Study Methods

2.2.1 Preparation, Planning and Ethical Approval

In addition to the author, the study authoring group comprised the following people: Dr Jesse Kitundu, Specialist in Paediatrics, MMC; Professor Abel Msengi, Professor of Paediatrics and Director of Diarrhoea Treatment Unit MMC; Ms Rose Kingamkono, Senior Nutritionist, TFNC; Dr Benedicta Mduma, Director of Department of Community Health and Nutrition, TFNC; and Professor Andrew Tomkins, Professor of International Child Health, Director CICH, and supervisor to the author.

A preliminary visit was made by the author and supervisor in October 1991, following the writing of provisional protocols, and discussion of these by correspondence. During this visit, detailed discussions took place between all of the study team, and nursing staff on the ward where it was proposed to carry out the study. A final protocol was then prepared which was submitted to, and approved by, the Ethical Committees of the Hospital for Sick Children, London, and Muhimbili Medical Centre, and the Tanzania Commission for Science and Technology. Arrangements were made to purchase and ship necessary equipment not available in Tanzania to Dar es Salaam from the UK. The author was resident in Dar es Salaam from January 1992 to January 1993 in order to run the study. During the preparation phase, equipment was purchased and prepared, study food preparation methods were finalised, and administrative details were set in place. Participants, including study ennumerators (9 of the ward nursing staff), study cook, and questionnaire administrators were identified and trained. All received a small honorarium for their participation in the study. Training was initially through a one-day workshop for all participants, followed by ongoing training on the ward. A pilot study preceded the main study. Throughout the pilot and main study, weekly meetings of the study team (study authoring group, ennumerators, and other personnel) were held, to ensure smooth running of the project, resolve any difficulties, and maintain enthusiasm. There was regular communication between the author and supervisor (Professor Tomkins) throughout the study period, and two visits were made by the supervisor during the study.
Table 10 Composition and characteristics of diets

<table>
<thead>
<tr>
<th></th>
<th>Conventional a</th>
<th>AMD</th>
<th>FAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize flour</td>
<td>66</td>
<td>123</td>
<td>123</td>
</tr>
<tr>
<td>Groundnuts</td>
<td>28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amylase-rich flour</td>
<td>-</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>(ARF)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>906</td>
<td>858</td>
<td>858</td>
</tr>
<tr>
<td>Evaporative loss</td>
<td>117</td>
<td>131</td>
<td>117</td>
</tr>
<tr>
<td>during cooking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Characteristics*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy density (kcal/100g)</td>
<td>37.8 ±5.2</td>
<td>57.6 ±5.2</td>
<td>58.0 ±4.1</td>
</tr>
<tr>
<td>Protein (g/100g)*</td>
<td>1.2</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Fat (g/100g)*</td>
<td>1.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Percentage dry matter</td>
<td>8.0 ±1.0</td>
<td>13.6 ±1.1</td>
<td>13.9 ±1.4</td>
</tr>
<tr>
<td>Viscosity at serving temperature (cps)</td>
<td>798 ±282</td>
<td>597 ±528</td>
<td>543 ±416</td>
</tr>
<tr>
<td>pH</td>
<td>6.3 ±0.25</td>
<td>5.8 ±0.32</td>
<td>3.6 ±0.24</td>
</tr>
</tbody>
</table>

* Grams per kg total starting ingredients.

* * * Plus-minus values are means ±SD.

* These values calculated from food tables

In the preparation methods used during the study, the experimental foods were covered during cooking, while the conventional was not. The measured evaporative loss for the conventional food was 376g. In order to allow comparison, the composition figures for the conventional food have been adjusted to those that would give the same final composition when covered during cooking. The evaporative loss was assumed to be 117g, the same as measured for FAD (boiled once for same duration as conventional, rather than AMD which was reboiled). The actual starting composition was 47g maize, 20g groundnuts, and 933g water.

2.2.2 Site and study population

The study was conducted on the Paediatric Diarrhoea Treatment Unit of the Muhimbili Medical Centre (MMC), Dar es Salaam, between 30th March and 19th December 1992. The MMC is a tertiary referral centre. The majority of children with diarrhoea (about 80%) are referred from other city hospitals and health care facilities, the remainder being self referrals.
2.2.3 Study Foods

2.2.3.1 Feed Preparation and Composition

The experimental foods were chosen to be representative of their class, and were either amylase-digested (AMD), or fermented and amylase-digested (FAD). AMD is known locally in Tanzania as "kimea" porridge and FAD as "togwa". Although there is some variation countrywide, and even within Dar es Salaam, in mode of preparation of these foods, the methods chosen were widely known, and foods produced in this way had already been the subject of extensive research at TFNC.

The feeds used in the study are compared in Table 10 (p83). The methods of preparation are shown in Panel 2 (p88). The conventional feed was prepared as a thin porridge in the traditional manner; the other two were prepared to a thicker consistency using a higher proportion of flour. After cooling, their viscosity was reduced by the addition of ARF. The ARF was prepared by germinating white sorghum grain for 48 hours, sun-drying, and then grinding to a flour. For AMD, the porridge was then reboiled, while for FAD, it was allowed to ferment for 24 hours at room temperature (27-28°C) and was then used for feeding in the subsequent 18 hours. It was served without reheating. Both the conventional and AMD feeds were kept in thermos flasks and served hot.

2.2.3.2 Evaluation of feed characteristics

Feed characteristics are also shown in Table 10 (p83). Viscosity at the serving temperature (60°C for conventional and AMD feed, and room temperature (28°C) for FAD), was determined in a representative sample of 17 specimens using a Brookfield viscometer, Spindle RV2, Speed 20rpm [241] (Photograph 2, p85). The details of the method are given in Appendix A. pH was measured using a Whatman PHA 250 portable meter (Photograph 3, p86) in 112 samples. A representative sample of 32 food specimens were stored at -70°C for later determination of dry matter and energy density. They were transported to London under dry ice, and immediately after thawing were oven-dried to steady weight. Ballistic bomb calorimetry was performed according to the method of Miller [242]. A Gallenkamp bomb calorimeter was used, where the peak temperature rise of the bomb body is read from a thermocouple and galvanometer system to give a measure of heat release. A known weight of sample was burned in an excess of oxygen, and the peak temperature reached was compared with that achieved when a standard
material (sucrose) was burned. This was performed by the author at the Department of Nutrition and Dietetics in Kings College, London. Protein and fat concentration were calculated from food tables [243]. Osmolality was measured by freezing point depression in two samples for each study food using an Advanced 3MO Osmometer, by Chemical Pathology Services at the Hospital for Sick Children, Great Ormond Street, London. Samples for osmolality measurement were stored at -70°C, transported to London under dry ice, and analysed immediately after thawing.
Throughout the study, regular measurements (approximately weekly) of viscosity and pH were performed by the author on all study foods, as part of quality control. The pH meter was recalibrated against known standards at each use. The viscometer was zeroed to an internal standard on switching on, and several times during each period of use. Viscosity measurements were made for the hot foods (conventional and AMD) in samples obtained from thermos flasks between 1 and 6 hours after preparation, and for FAD in samples obtained between 24 and 28 hours after preparation. These samples were therefore representative of the foods being served to the children. Duration of cooking and evaporative water losses (measured by subtraction of the final weight from the starting weight) were regularly measured. The cost of all ingredients was recorded to enable comparison of costs of study foods to be made.

Photograph 3  Whatman PHA portable pH meter
2.2.4 Study Design

2.2.4.1 Inclusion and Exclusion Criteria

Inclusion criteria were as follows: acute diarrhoea, severe enough to require admission (acute diarrhoea was defined as stools more watery and more frequent than usual, for less than 14 days on parent's history); age between 6 and 25 months; already receiving solid foods. Exclusion criteria were: presence of kwashioorkor (because interpretation of body weight difficult); any congenital or chronic condition interfering with food intake; any evidence of dysentery (bloody diarrhoea or stool positive for dysenteric organisms); well enough for discharge the day after admission; insertion of a naso-gastric feeding tube required within 24 hours of admission because of very poor appetite or severe systemic illness.

2.2.4.2 Entry to Study and Randomisation

The parent(s) or guardian of all eligible children were invited to allow their child to participate in the study, providing that a study cot was available. Study cots were limited to six, which were specially modified for the purpose of the study (see 2.2.4.5, p89). Children were entered into the study after rehydration, between 4 and 24 hours after admission. Rehydration was performed according to WHO guidelines [148]. After obtaining consent from the parent or guardian, children were assigned to the dietary groups by block randomisation, using groups of 9 sealed envelopes.

2.2.4.3 Questionnaire

A questionnaire (Appendix E) was used to elicit information about the current illness and other relevant information, past medical history, feeding practices, environmental factors, and knowledge and attitudes relating to AMD and FAD. This was written in English, translated into Swahili, and then backtranslated by an third party to ensure consistency of meaning. It was administered (in Swahili) by two members of the study team and two nutritionists. The questionnaire comprised three parts. Part I consisted of the following: general information about the child; the current illness; past medical history; and knowledge, attitudes, and practice with regard to AMD and FAD. This was completed on the day of entry to the study. Part II was completed on the same day or the following day, and elicited information about feeding practices and environmental factors. Part III was completed at discharge, and enquired about attitudes to AMD and FAD after exposure to
Panel 2 Methods for preparation of study foods

All measurements should be made by weighing to the nearest gram using the study food scales (Soehnle).

**Conventional**

1. Measure out the exact quantity of water, and put most in saucepan to boil, keeping back about 100 ml to mix with flour.

2. Measure out the exact amount of maize flour, and mix with the remaining cold water in a 500 ml cup.

3. When the water has boiled, pour some into the flour/water mix until the cup is nearly filled, mix, and then pour the contents into the saucepan of water. After stirring, pour a further amount into the cup, stir, and pour back, in order to retrieve all the sediment. There should be no more than 3 grams left in the cup. For the conventional feed (but not AMD or FAD) add the measured amount of groundnut flour at this point, and stir in thoroughly.

4. Bring back to the boil on a medium heat, boil for 10 minutes, stirring constantly, and then cover and leave on heat for a further 5 minutes, stirring occasionally. The conventional diet is now ready for serving or storing in vacuum flask.

**FAD**

5. After completing steps 1-4, remove the lid and allow to cool to about 40°C (about 40 mins).

6. Measure out the exact amount of ARF, and add to the porridge, stirring in thoroughly until the porridge has stopped thinning (about 3 - 4 mins).

7. To make the fermented diet (FAD, togwa), leave to ferment at 24 hours at room temperature. It is then ready for serving for a further 18 hours.

**AMD**

8. After completing steps 1 - 6, bring the porridge back to the boil, and continue to boil for 5 minutes, stirring vigorously. The porridge is now ready to serve or store in a vacuum flask.

their use on the ward. The child's health card was consulted to establish the exact age, and confirm immunisation history.

2.2.4.4 Anthropometry and Clinical Assessment

Children were weighed daily on Soehnle baby scales accurate to 10g, length was measured on admission on a locally made board, accurate to 1mm, and mid upper arm circumference using a TALC tape [244] accurate to 2mm. The baby scales were checked monthly against standards of known weights of 1.50, 5.00 and 10.00 kg. The maximum variation was 0.01 kg (see Appendix B).
A complete physical examination was performed on admission and daily until discharge (by J.D or J.K.), appropriate investigations performed, and any necessary treatment provided. Blood films were done routinely on admission for malarial parasitaemia. Blood for analysis of CRP, SAA and AGP, was taken within 24 hours of entry to the study. Complicated diarrhoea was defined as the presence of co-existing systemic infection, such as pneumonia, meningitis, septicaemia, acute otitis media, urinary tract infection, malaria, or the presence of a fever of >38°C during the first 4 days. Pneumonia was defined as any two of the following: cough or difficulty breathing on history, intercostal recession, crepitations, respiratory rate > 50/min or positive radiograph. Malaria was defined as the presence of malarial parasites on blood film, together with fever of >38°C.

2.2.4.5 Information about Stooling

The time of each stool passed was recorded by the study ennumerators. Stools were collected via modified “cholera cots” manufactured locally (Photograph 4) and the total daily volume calculated after subtraction of flush volume. The duration of diarrhoea was the number of hours from the time of entry into the study until the earliest of one of the following: a) passage of a formed stool, or b) 12 hours elapsed without passing any stool, or c) 24 hours elapsed with no watery stools and less than four semisolid or formed stools; provided that no watery stools, and less than five semisolid or formed stools, were passed in the subsequent 24 hours. Recurrence of diarrhoea during admission was defined as more than six stools in any subsequent 24 hour period up to discharge, and after discharge if there was any history of diarrhoea during the subsequent 4 weeks. Dietary management was classified as having failed if the child required nasogastric tube feeding or intravenous fluids after entering the study, had a recurrence of diarrhoea during admission, or died during admission.
2.2.4.6 Dietary Assessment

The study foods were prepared in the ward kitchen by specially trained staff, and served *ad libitum* in 300 gram portions in plastic cups five times per day. The trial was not blind, since temperature and taste differences between the foods could not be disguised. Nearly all mothers were breast feeding, and this was encouraged, along with use of oral rehydration therapy after watery stools. Children only consumed food that was provided by the diet kitchen, according to the normal ward treatment regimen. All intakes of study foods were weighed to 1g on Soehnle food scales by nine trained nurse enumerators. Special care was taken to avoid food spillage. Breast milk intake was not measured. The food scales were checked monthly against standards of known weights of 100, 300, and 1500 grams. The maximum variation was -2 g at 300g, and -7g at 1500g (Appendix B). The study design allowed children in the AMD and FAD groups to be changed to the conventional food group at the mother’s request (subsequent food intake data was not included in the analysis), but not vice versa.

Photograph 4 Modified cholera cot
2.2.4.7 Discharge and Follow up

Children were discharged when they were considered medically fit. In the 48 hours prior to discharge, mothers or guardians were taught how to prepare their child’s study food, and encouraged to continue to use it regularly. Mothers of children in the experimental groups were provided with 2 weeks supply of ARF. During admission, children were followed until discharge. In this thesis, most of the data relating to admission will be limited to the first 4 days after entry, since the majority of children were discharged by the end of the 4th day. Children were seen on the ward for follow up visits at approximately 14 and 28 days after discharge. Attempts were made to trace those that did not attend, and visit them at home. Mothers’ travel expenses were reimbursed, and those completing both follow up visits received a T shirt with a health promotion slogan (see Photograph 5, p 92). This served as a token of appreciation for their assistance, and also encouraged a higher attendance rate. At follow up visits, information about recent health and feeding practices was obtained by questionnaire (Appendix E) and a full physical examination was performed. 25 of the children had blood taken at the 28 day visit for repeat analysis of acute phase proteins. These children were clinically well, with no recent history of diarrhoea, otitis, or pneumonia in the preceding two weeks, although some had minor upper respiratory symptoms.
Photograph 5  T shirt with health promotion logo given to mothers completing the study

Caption reads: “Save the life of your child! Has he/she got diarrhoea? Keep on breast feeding. Give extra food and fluids”

2.2.4.8  Intestinal Permeability Tests

Intestinal permeability tests were carried out at entry into the study, approximately 3 days after admission (or at discharge if sooner), and at both follow-up visits. Children were given 20 ml of water containing 5 g lactulose and 1 g mannitol. Sugar drinks were prepared by pipetting 7.5 mls of Lactulose Solution BP (3.35g per 5 ml, Duphar) and 5 mls of 20% Mannitol Intravenous Infusion BP (Baxter) into 7.5 mls of boiled and
filtered water. These were kept refrigerated and administered within 7 days of preparation. Urine samples were collected into a bag containing one drop of 20% wt/vol chlorhexidine gluconate (to prevent bacterial degradation of the sugars) before and for 5 hours after the administration of the dual sugar solution. Intestinal permeability tests were also carried out on 30 healthy children of the same age as the cases, at a local Well Child Clinic. Urine sample volumes were recorded.

2.2.5 Documentation and Data Processing

Standardised forms were used to record dietary intakes, stool outputs, clinical findings, anthropometrical measurements, and investigation results. These are shown in Appendix F. Data entry was performed using EpiInfo and dBase software programmes [245,246]. The majority was entered by the author, but a proportion of the food intake data was entered by a trained assistant. Range checks and consistency checks were built into the data entry programme throughout, using the CHECK programme feature [245]. All data was validated by the author against the original records. The food intake data was entered as individual meal records, and then processed by a programme written in dBase IV into daily intakes in g/kg/day for each day of the study (Appendix C). Data was entered, and periodic data validation performed, as the study proceeded (see section 3.1.1, p100) for further details of data validation).

2.2.6 Statistics and Data Analysis

Sample size calculations made using the formula according to Kirkwood [247], for a probability of 5% and a power of 80%, estimated that 22 children would be needed in each group in order to show significance for a 20% increase in energy intake in one of the dietary groups.

Data was analyzed using EpiInfo and SPSS/PC+ [248] statistical software packages. Tests were considered significant at the 5 percent probability level. For comparison of categorical variables, Chi squared tests were used, and p values were determined.

For continuous variables, each was examined graphically (using scatter, stemleaf, normal probability and box-plots) in SPSS/PC+ to check for outliers, and to determine whether the variable was normally distributed. Where extreme outliers were present, the individual cases were checked for errors, and the data was analysed with and without the outliers.
For the purpose of presenting the data, outliers have been included, since none produced a significant difference in the results. For the normal probability plot of each variable, the K-S (Lilliefors) statistic and significance level was calculated, and a p value of greater than 0.2 was accepted as indicating that the distribution of the variable was not significantly different from a normal distribution. For p values of ≤ 0.2, the variable was log-transformed using logarithms to the base 10, and retested for normality of distribution. Most variables required log-transformation in order to achieve a normal distribution, so all means are geometric, except where specified. Confidence intervals at the 95 percent probability level have been calculated for all means.

Parametric statistical tests have been used on all normally distributed variables (log-transformed if necessary) to compare means between the dietary groups. In almost all cases, the comparison has been between three groups, and therefore one-way analysis of variance has been used. For each test, homogeneity of variances between groups was tested using the Cochran C and Bartlett-Box F statistics. When variances were not homogenous, the results were rejected and non-parametric tests were used instead. Where there was a significant difference between groups, Scheffe’s multiple range test was used to determine which groups were different from each other. For occasions where only two groups were being compared, independent-sample t-tests have been used, again with checks for homogeneity of variance.

For variables that could not be log-transformed to normality, other standard transformations were applied, but in no case were these effective. For these variables, non-parametric tests were used. Where three groups were being compared, the Kruskal-Wallis test was used to rank cases and compare the mean ranks for each group. For comparison of two groups, the Mann-Whitney test was used.

Mean daily dietary intakes, and total intakes over the first 4 days of the study, for both weight of food consumed and energy intake, were compared between dietary groups using one-way ANOVAs. Potential confounding factors were controlled for in the analysis of variance of dietary energy intake between groups by including them as single covariates, and determining whether they influenced the result. A multiple
ANOVA model was also used to determine whether acute phase proteins on entry to the study influenced dietary intake.

In the analysis of the change in intestinal permeability over the first three days of the study, analysis of variance was performed for the L/M ratio on Day 3, using initial L/M ratio and other potential confounding variables as covariants.

Survival analysis was used to compare duration of diarrhoea between groups, which had been determined for each patient in hours from time of entry to the study. A life table was calculated for each dietary group using SPSS/PC+, using intervals of 24 hours, and the survival curves for each group were compared by calculating the significance level for the hypothesis that the three curves were identical (the Lee-Desu statistic) [248].

### 2.3 Laboratory Methods

#### 2.3.1 Storage and transport of samples

Blood samples were centrifuged within 2 hours, and serum stored at -40°C until transportation to London on dry ice. Samples were then stored at -70°C until analysis. Aliquots of urine were also stored at -40 °C, until transportation to London on dry ice, where they were then stored at - 20°C until analysis of lactulose and mannitol. Urinary thiocyanates were measured in a subset of samples in Dar es Salaam, by the Department of Food Science and Technology, TFNC.

#### 2.3.2 Intestinal permeability tests

These assays were set up by the author and Juana Willumsen (Centre for International Child Health) at the London School of Hygiene and Tropical Medicine. All the assays for the study were performed by Juana Willumsen.

##### 2.3.2.1 Mannitol

The mannitol assay uses Blood's method [249], which is based on the method of Lunn et al. [250], using a CobasFara centrifugal analyzer (Roche). Program parameters are shown in Appendix D. The assay is based on the oxidation of mannitol to fructose by NAD-specific D-mannitol dehydrogenase from *Leuconostoc mesenteroides* (Biocatalysts Ltd.). The change in absorbance at 340 nm is measured to indicate the rate
of reduction of NAD to NADH. Hexokinase is added to the coenzyme reagent together with Mg and ATP to abolish the inhibitory effect of fructose.

2.3.2.2 Lactulose and lactose

The lactulose and lactose assays use the method of Northrop, Lunn and Behrens [251] using the CobasFara centrifugal analyzer (Roche). Programme parameters are shown in Appendix D. The amount of NADPH measured by the increase in absorption at 340 nm would be directly proportional to the amount of lactulose in the urine sample, if this were the only sugar present (Figure 15, p97). However, the quadruple enzyme mix required to produce NADPH from lactulose will also act on any glucose, fructose and lactose also in the urine sample. Therefore, in order to measure lactulose, it is necessary to incubate the sample with and without β-galactosidase and subtract the amount of NADPH produced by free monosaccharides in the urine from that produced by monosaccharides derived from lactose and lactulose.

After incubation with and without β-galactosidase for 1 hour, the lactulose assay is performed in two stages, with an incubation of 30 seconds after the addition of the enzyme mix, during which free glucose and glucose from lactose react to form NADPH. The addition of phosphoglucose isomerase (PGI) causes fructose to react and form NADPH, and this subsequent additional increase in absorbance is measured, to give the fructose concentration in the sample. Subtraction of the value for the sample not treated with β-galactosidase from the treated sample gives the lactulose concentration in the original sample.
Figure 15 Principle reactions in analysis of lactose and lactulose

It can be seen that the amount of NADPH, obtained by measuring the increase in absorption at 340nm, is directly proportional to the lactulose concentration if this is the only sugar present. Because other sugars may be present which could interfere with the assay (lactose, glucose and fructose), the contribution of all four to the rise in NAPDH must be measured separately. An enzyme mixture of hexokinase and glucose-6-phosphate dehydrogenase, together with ATP and NADP, will allow measurement of glucose alone. Running the same reaction with addition of phosphoglucoisomerase allows measurement of fructose by subtraction of the first result from the second. If the same procedures are performed following incubation with β-galactosidase, further glucose and fructose are produced in proportion to the quantities of lactose and lactulose respectively. This allows the amounts of lactose and lactulose to be deduced.

The lactose assay is based on the same principle as the lactulose assay, without the second step involving PGI. In this way, it aims to measure only the free glucose and glucose from lactose. The lactose concentration was measured in spot urine samples taken before the administration of the differential sugar absorption test solution in order
to test the hypothesis that this could be used as a simple alternative test of intestinal permeability.

2.3.2.3 Validation of methods

The CobasFara sample and reagent pipetting precision coefficient of variation (c.v.) was less than 1% for 20 measurements made using the Roche precision kit and technique. The intra-assay c.v. was 0.85 for duplicate tubes of 6 urine samples spiked with mannitol. Mean recoveries of lactulose and mannitol at a concentration of 0.125 mg/ml were 100.80% and 99.98% respectively, and at a concentration of 0.250 mg/ml were 95.17% and 97.25% respectively. Mean recovery of lactose at a concentration of 0.250 mg/ml was 98.23%.

2.3.3 Acute phase proteins

Serum CRP, SAA and AGP were measured using a competitive enzyme-linked immunosorbent assay (ELISA) as previously described [220,252]. Microtitre plates were coated with affinity purified antibodies to human CRP, SAA, and AGP. Diluted sample (100μl) was added to a microlitre well together with 100μl of the appropriate enzyme-conjugated acute phase protein. After an incubation period, the plates were washed, p-nitrophenyl phosphate substrate was added, and the resulting colour change measured using an ELISA reader. The concentrations of acute phase proteins were determined by calculation from a series of standard concentrations of highly purified proteins tested in parallel on the same plate.

Concentrations considered to be in the normal range were CRP < 10mg/l, SAA < 5mg/l and AGP < 0.75g/l, as previously published [220,252]. Samples from patients later found to have HIV infection were excluded from analysis due to the increased laboratory time and hazard not deemed justified for the small number involved.

2.3.4 Thiocyanates

The literature on use of germinated sorghum grain indicated that preparation of study foods in the manner described made cyanide toxicity very unlikely (see section 1.3.3.1, p56). The processes of reboiling for AMD, and fermentation for FAD, following addition of ARF effectively destroy any cyanide present. However, during the period of the study, a WHO review was published which again raised cyanide toxicity as an issue [77]. Since urine samples were already available on study children consuming
sorghum-amylase-digested foods, it therefore seemed appropriate to measure urinary 
thiocyanate levels in a small sample, in order to obtain reassurance of the safety of these 
foods.

The method used was that of Lundquist et al. [253]. The method is based on the high 
affinity of thiocyanate to certain weakly basic anion-exchange resins [254]. 
Thiocyanate is thus separated from interfering compounds, and is then eluted from the 
resin using sodium perchlorate. In a modification of the König reaction, it is then 
halogenated with sodium hypochlorite, and coupled to pyridine using barbituric acid as 
the coupling agent. Absorption is then measured using a spectrophotometer at 580nm.

Only urine samples from children who had consumed at least 12 study meals were 
selected for analysis. Thiocyanates were measured in the first 27 such samples. 
Analyses were performed in the Department of Food and Nutrition in TFNC by Mr 
N.Mlingi, who was blind to the dietary group represented by each sample. Quality 
control for this assay is provided by the Swedish laboratory from where it originates. 
Subsequently, the same method was set up in London by the author and Suzanne Filteau 
(Centre for International Child Health), with the intention of analysing a larger number 
of samples, but this was prevented by lack of time.
3 Results

3.1 Problems with Data Accuracy

During the study, it became clear that at least one of the study enumerators was not recording food intake data accurately. This prejudiced the dietary intake results for the whole study up to that point.

3.1.1 Identification of invalid data

Nine of the ward nursing staff were trained to be enumerators for the study, and received a small honorarium for this work. Nursing shifts were organised so that there was always at least one study enumerator on duty. In practice, there were usually two to three study nurses working during the early and late shifts, and one during the night shift. For each meal, the enumerator recorded the starting weight (usually 350g) and the finishing weight, and calculated the amount consumed by subtraction. All measurements were recorded on a standard Dietary Intake Chart (see Appendix F). Any spillage was weighed and subtracted from the total, but in practice, this was found to be minimal. The enumerators calculated each day’s total intake and recorded it on the form. However, in order to validate data, reduce opportunity for error, and give greater flexibility for data analysis, raw data were entered and checked for every meal. A validation programme in EpiInfo checked each enumerator-calculated-value, and any discrepancy was flagged and checked by the typist. Once on computer, all data was rechecked against the original forms. A computer programme in dBase was used to calculate total daily intakes in g/kg (Appendix C). These calculations were checked by hand in a sample of records. Statistical calculations were then performed using SPSS/PC.

Entry of the raw data onto computer allowed evaluation of its validity by use of bar charts of frequency of each measurement. Figure 16 (a) shows such a frequency distribution for all single meal raw data up to 30/6/92. Unexpectedly high frequencies were observed for meal amounts of 50, 100, 150, 200 and 250 grams, and the overall distribution did not correspond to the skewed curve that would be predicted in such a study. This was taken as strong evidence that the dietary intakes were being either rounded up or estimated, and that this data was therefore invalid.
Figure 16 Frequency distribution of raw dietary intake data before and after introduction of enumerator coding on 30/06/92

(a) Before enumerator coding

<table>
<thead>
<tr>
<th>Amount consumed (g)</th>
<th>Frequency</th>
</tr>
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<tbody>
<tr>
<td>25-60</td>
<td></td>
</tr>
<tr>
<td>61-100</td>
<td>7</td>
</tr>
<tr>
<td>101-140</td>
<td>11</td>
</tr>
<tr>
<td>141-180</td>
<td>14</td>
</tr>
<tr>
<td>181-220</td>
<td>17</td>
</tr>
<tr>
<td>221-260</td>
<td>21</td>
</tr>
<tr>
<td>261-300</td>
<td>36</td>
</tr>
<tr>
<td>301-340</td>
<td>62</td>
</tr>
<tr>
<td>341-380</td>
<td>92</td>
</tr>
<tr>
<td>381-420</td>
<td>121</td>
</tr>
<tr>
<td>421-460</td>
<td>149</td>
</tr>
<tr>
<td>461-500</td>
<td>177</td>
</tr>
<tr>
<td>501-540</td>
<td>206</td>
</tr>
<tr>
<td>541-580</td>
<td>235</td>
</tr>
<tr>
<td>581-620</td>
<td>276</td>
</tr>
</tbody>
</table>

n = 754; Mean = 145; Median = 142; Mode = 100; S.D. = 59.7; Range = 25 - 310.

(b) After enumerator coding

<table>
<thead>
<tr>
<th>Amount consumed (g)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-60</td>
<td></td>
</tr>
<tr>
<td>61-100</td>
<td>12</td>
</tr>
<tr>
<td>101-140</td>
<td>21</td>
</tr>
<tr>
<td>141-180</td>
<td>34</td>
</tr>
<tr>
<td>181-220</td>
<td>42</td>
</tr>
<tr>
<td>221-260</td>
<td>60</td>
</tr>
<tr>
<td>261-300</td>
<td>84</td>
</tr>
<tr>
<td>301-340</td>
<td>107</td>
</tr>
<tr>
<td>341-380</td>
<td>135</td>
</tr>
<tr>
<td>381-420</td>
<td>163</td>
</tr>
<tr>
<td>421-460</td>
<td>192</td>
</tr>
<tr>
<td>461-500</td>
<td>220</td>
</tr>
<tr>
<td>501-540</td>
<td>249</td>
</tr>
<tr>
<td>541-580</td>
<td>276</td>
</tr>
<tr>
<td>581-620</td>
<td>306</td>
</tr>
</tbody>
</table>

n = 1754; Mean = 124; Median = 118; Mode = 150; S.D. = 59.1; Range = 4 - 320

Meals where the quantity was restricted in any way were excluded.
Further evidence for this was obtained by examining the last digit of every recorded meal amount. Since all meals were to be weighed to the nearest gram, a good test of data validity in a large data set is that the proportion of all observations ending in each digit will be equal, and will be approximately ten percent. Table 11 shows that meals ending in zero were grossly over-represented before introduction of ennumerator coding.

**Table 11  Effect of introduction of ennumerator coding on validity of raw dietary intake data**

<table>
<thead>
<tr>
<th>Last digit of meal amount</th>
<th>Before ennumerator coding</th>
<th>After ennumerator coding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All meals</td>
<td>Coded meals only</td>
</tr>
<tr>
<td></td>
<td>(100%)</td>
<td>(100%)</td>
</tr>
<tr>
<td>0</td>
<td>205 (27.2%)</td>
<td>239 (13.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>143 (10.5%)</td>
</tr>
<tr>
<td>1</td>
<td>52 (6.9%)</td>
<td>165 (9.4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>135 (9.9%)</td>
</tr>
<tr>
<td>2</td>
<td>74 (9.8%)</td>
<td>147 (8.4%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>126 (9.2%)</td>
</tr>
<tr>
<td>3</td>
<td>55 (7.3%)</td>
<td>153 (8.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123 (9.0%)</td>
</tr>
<tr>
<td>4</td>
<td>64 (8.5%)</td>
<td>186 (10.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>156 (11.4%)</td>
</tr>
<tr>
<td>5</td>
<td>65 (8.6%)</td>
<td>194 (11.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>149 (10.9%)</td>
</tr>
<tr>
<td>6</td>
<td>44 (5.8%)</td>
<td>157 (9.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>129 (9.5%)</td>
</tr>
<tr>
<td>7</td>
<td>56 (7.4%)</td>
<td>184 (10.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>144 (10.5%)</td>
</tr>
<tr>
<td>8</td>
<td>88 (11.7%)</td>
<td>170 (9.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>134 (9.8%)</td>
</tr>
<tr>
<td>9</td>
<td>51 (6.8%)</td>
<td>159 (9.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>126 (9.2%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>754 (100%)</strong></td>
<td><strong>1754 (100%)</strong></td>
</tr>
</tbody>
</table>
3.1.2 Response

Once this problem was identified, the implications were discussed with the enumerator team, and enumerator coding was introduced. Each enumerator was given a number, and the dietary intake forms were modified so that the enumerator responsible for each meal record could be identified by number and initials. Subsequent records were then periodically analysed by comparison between enumerator of frequency distribution and last digit proportions. Enumerators were aware that their records were being analysed.

Records prior to the introduction of enumerator coding were analysed in further detail to determine whether there was any pattern that would allow exclusion of dubious records while salvaging other results. Meal records from 11pm and 5am, given during the night shift period when only one study enumerator was on duty, were analysed for last digit proportions. Two enumerators had 68 and 56 percent of their records ending in zero respectively, while for the other enumerators the proportion was approximately 10 percent. It therefore seemed likely that these two enumerators were responsible for the majority of the invalid records. However, it was not possible to identify all daytime records for which they might have been responsible. Therefore, for analysis of dietary intake, all records prior to the introduction of enumerator coding were excluded, and were considered to have been part of the pilot study. This meant that the pilot study, which initially only included 10 patients, now contained 52 patients.

3.1.3 Effect of the response

Data used for calculation of dietary intakes was from after the introduction of enumerator coding on 30/06/92. The frequency distribution of the raw data is shown in Figure 16(b) (p101). The distribution curve was accepted as approximating to what would be expected. Last digit proportions were approximately equal, although there was still a slight excess of records ending in zero (Table 11, p102). This was felt to be within acceptable limits. There was a lag phase between the introduction of coding and compliance by all enumerators with the new arrangements. When analysis was restricted to coded meals only, the last digit proportions all lay within the range 9.2 to 11.4 percent (Table 11). Frequency distributions for each enumerator after the introduction of coding are shown in Figure 17. Numbers of observations for some of the enumerators were not large enough to allow comparison of the distribution curves.
Figure 17 Frequency distribution of meal amounts by enumerator after introduction of coding

**Enumerator 1**
- Std. Dev = 39.96
- Mean = 115.4
- N = 27.00

**Enumerator 2**
- Std. Dev = 54.41
- Mean = 124.5
- N = 124.00

**Enumerator 3**
- Std. Dev = 51.43
- Mean = 108.6
- N = 86.00

**Enumerator 4**
- Std. Dev = 53.28
- Mean = 122.4
- N = 265.00

**Enumerator 5**
- Std. Dev = 56.62
- Mean = 121.4
- N = 191.00

**Enumerator 6**
- Std. Dev = 50.05
- Mean = 102.3
- N = 130.00

**Enumerator 7**
- Std. Dev = 57.01
- Mean = 115.6
- N = 326.00

**Enumerator 8**
- Std. Dev = 55.40
- Mean = 110.0
- N = 152.00

**Enumerator 9**
- Std. Dev = 55.47
- Mean = 112.8
- N = 182.00

**All enumerators**
- Std. Dev = 59.15
- Mean = 124.0
- N = 1754.00

Vertical axes are frequencies, horizontal axes meal amounts (g). Normal distribution curves are superimposed for comparison.
3.1.4 Patient Groups used for Data Analysis

Patients enrolled into the study were divided into three groups for data analysis (see Figure 18). Group A (n=112) consisted of all eligible patients entering the study from 30/3/92 to 19/12/92. Since only the dietary intake data had been compromised on patients entering before the introduction of ennumerator coding on 30/6/92, this group has been used for analysis of descriptive and outcome data. This larger group was used in order to maximise the power of the study to detect significant differences between the groups for outcome and other features of interest.

**Figure 18 Patient groups used for analysis of data**

![Diagram showing patient groups]

Although urine samples for intestinal permeability tests were collected on all patients, resource constraints limited the number that could be analysed. Since serum samples for acute phase protein measurements had been collected from patients entering the study from 19/6/92, it was decided to also restrict intestinal permeability analyses to patients entering after that date. This would then maximise the power of study to detect a relationship between the APP response and intestinal permeability. This subset is termed Group B (n=86).
Finally, Group C (n=75) consisted of all eligible patients entering the study after the introduction of enumerator coding. This group was used for analysis of dietary intake data. The three groups were similar for all major descriptive parameters on entry to the study.

3.2 Descriptive Data on Entry to Study

The study commenced on 30/3/92, and the last patient was entered on 19/12/96. During this period, 1168 children were admitted to the ward with diarrhoea (acute and persistent). 695 (60%) were aged 6 - 25 months, and of these 399 (57%) were male. 131 patients were entered into the study. These were selected on the basis that they satisfied the entry criteria (see section 2.2.4.1, p87), consent could be obtained, and a study cot was available (these were limited to six). Of these, 18 were subsequently excluded (10 with persistent diarrhoea, 8 with dysentery, and 4 because they were later found to be over- or underage). The remaining 112 patients will be described in this section. These represent 16.1% of all admissions in this age group. From the available data, it was not possible to calculate the number of eligible patients who did not enter the study because of lack of consent or available study cot.

The admission characteristics of the three dietary groups were compared to determine whether randomisation had been effective.

3.2.1 General and illness-related characteristics

Table 12 (p108) shows the admission characteristics by dietary group. There were no significant differences between the groups for any of the features, except for mode of referral. As a group, the children were moderately malnourished. 25% had a Z score for W/H of less than - 2.0, with the corresponding figures for H/A and W/A being 28% and 51% respectively. With regard to features of diarrhoeal illness on admission (Table 13, p109), there were no significant differences between groups on any of the parameters. Figure 19 (p110) shows the presenting symptoms in addition to diarrhoea by dietary group, and Table 14 (p111) compares features of the past medical history between groups. The only significant differences were that the conventional group had a lower mean birthweight, while there were more children who were one of twins in the FAD group. Immunisation status was similar in each group, with 81 (72%) fully
immunised for age for DPT, 86 (77%) for polio, 96 (86%) for measles, and 102 (91%) for BCG.

3.2.2 Acute phase proteins

Acute phase protein (APP) concentrations were measured in 57 patients. The geometric mean concentrations by dietary group compared to controls are shown in Table 15, (p112) (see section 2.3.3 on p98 for details of cut-off levels used). The concentrations for the whole group were (geometric means (95% CI); CRP, mg/l: 14.5 (10.0-21.1); SAA, g/l: 8.6 (5.5 - 13.5); AGP, g/l: 1.11 (0.96 - 1.29). There were no significant differences between the dietary groups for any of the three proteins on oneway analysis of variance testing, nor was there a difference between the patients and the follow-up group. 34 patients (60%) had elevated CRP levels, compared to 44 percent of the follow-up group. 37 (65%) had raised SAA levels (follow-ups 56%), and 34 (60%) raised AGP levels (follow-ups 64%). There was no significant difference between dietary groups on Chi-squared tests, nor between patients and the follow-up group. 49 patients (86%) had at least one raised APP, with no significant difference between proportions in each dietary group.

3.2.2.1 Acute phase proteins as markers for systemic illness

35 of the 57 patients had systemic infection (presence of malaria or other systemic infection, as defined in section 2.2.4.4, p88). APP concentrations were compared between patients with and without systemic infection, and these are shown in Figure 20 (p113). Means for CRP and SAA were significantly higher in children with systemic infection compared to those without (geometric means (95% CI); CRP, mg/l: 22.1 (13.6-35.5) versus 7.4 (4.4-12.4); SAA, mg/l: 12.2 (6.8-22.1) versus 4.9 (2.5-9.7)). Levels of AGP were similar in both groups (1.16g/l (0.95-1.43) versus 1.04 (0.83-1.29) respectively). CRP >30 mg/l had a positive predictive value of 95%, sensitivity 51% and specificity 96% for correctly identifying systemic infection. The clinical outcome of diarrhoea was worse in children with systemic infection, in that they were significantly more likely to have a longer duration of diarrhoea (geometric means (95% CI); hours: 59.4 (47.6-74.3 versus 34.2(28.0-41.7)), and also had a greater number of stools on days 1 and 3 (day 1: 6.2 (5.1-7.5) versus 4.3 (3.0-6.1); day 3: 4.8 (3.8-6.0) versus 2.4 (1.5 to 4.1)).
Table 12 General characteristics of patients on admission by dietary group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conventional (n = 39)</th>
<th>AMD (n = 37)</th>
<th>FAD (n = 36)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>10.6 (9.3 to 12.0)</td>
<td>10.4 (8.9 to 11.9)</td>
<td>10.8 (9.5 to 12.1)</td>
<td>0.92</td>
</tr>
<tr>
<td>Sex (males)</td>
<td>25 (64%)</td>
<td>22 (60%)</td>
<td>26 (72%)</td>
<td>0.51</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>7.1 (6.7 to 7.5)</td>
<td>7.2 (6.7 to 7.7)</td>
<td>7.2 (6.7 to 7.7)</td>
<td>0.91</td>
</tr>
<tr>
<td>Weight for age (Z score)</td>
<td>-2.2 (-2.5 to -1.9)</td>
<td>-2.0 (-2.4 to -1.5)</td>
<td>-2.2 (-2.6 to -1.8)</td>
<td>0.58</td>
</tr>
<tr>
<td>Height for age (Z score)</td>
<td>-1.5 (-1.8 to -1.2)</td>
<td>-1.6 (-2.1 to -1.0)</td>
<td>-1.5 (-1.9 to -1.1)</td>
<td>0.98</td>
</tr>
<tr>
<td>Weight for height (Z score)</td>
<td>-1.5 (-1.7 to -1.2)</td>
<td>-1.1 (-1.4 to -0.8)</td>
<td>-1.5 (-1.9 to -1.1)</td>
<td>0.12</td>
</tr>
<tr>
<td>Mid upper arm circumference (cm)</td>
<td>12.6 (12.2 to 12.9)</td>
<td>12.6 (12.1 to 13.1)</td>
<td>12.5 (12.0 to 13.0)</td>
<td>0.92</td>
</tr>
<tr>
<td>Cholera positive</td>
<td>0</td>
<td>2 (5%)</td>
<td>3 (10%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9 (5.7 to 8.3) [24]</td>
<td>6.7 (5.7 to 7.8) [27]</td>
<td>6.9 (6.1 to 7.8) [29]</td>
<td>0.92</td>
</tr>
<tr>
<td>Transfused on admission</td>
<td>2 (5%)</td>
<td>6 (16%)</td>
<td>5 (14%)</td>
<td>0.28</td>
</tr>
<tr>
<td>History of fever</td>
<td>23 (59%)</td>
<td>23 (62%)</td>
<td>23 (64%)</td>
<td>0.91</td>
</tr>
<tr>
<td>Mean temperature on admission °C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.5 (37.1 to 37.8)</td>
<td>37.5 (37.2 to 37.9)</td>
<td>37.8 (37.4 to 38.2)</td>
<td>0.46</td>
</tr>
<tr>
<td>Temperature ≥ 37.5°C on admission</td>
<td>17 (44%)</td>
<td>16 (43%)</td>
<td>18 (50%)</td>
<td>0.65</td>
</tr>
<tr>
<td>Temperature ≥37.5°C during first 4 days</td>
<td>24 (62%)</td>
<td>21 (57%)</td>
<td>29 (81%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Malaria (any malarial parasites on blood film)</td>
<td>9 (30%) [30]</td>
<td>8 (29%) [28]</td>
<td>13 (39%) [33]</td>
<td>0.61</td>
</tr>
<tr>
<td>Systemic infection (except malaria)</td>
<td>6 (15%)</td>
<td>9 (24%)</td>
<td>9 (33%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Antibiotics prescribed on admission</td>
<td>10 (26%)</td>
<td>17 (49%)</td>
<td>16 (44%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Antimalarials prescribed on admission</td>
<td>24 (62%)</td>
<td>15 (41%)</td>
<td>21 (58%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Mode of referral:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>from other hospital</td>
<td>8 (22%) [36]</td>
<td>13 (41%) [32]</td>
<td>16 (49%) [33]</td>
<td></td>
</tr>
<tr>
<td>from clinic</td>
<td>22 (61%) [36]</td>
<td>10 (31%) [32]</td>
<td>16 (48%) [33]</td>
<td>0.01*</td>
</tr>
<tr>
<td>self referral</td>
<td>6 (17%) [36]</td>
<td>9 (28%) [32]</td>
<td>1 (3%) [33]</td>
<td></td>
</tr>
</tbody>
</table>

For continuous variables, means are shown, with 95% confidence intervals in parentheses; for categorical variables, values in parentheses are group percentages; values in square brackets are numbers in groups, if less than n.

<sup>a</sup>p values (or equivalent significance levels) for One way ANOVAs (continuous variables) and Chi squared tests (categorical variables). Values indicating a significant difference between groups are denoted with an asterisk.

<sup>b</sup>Geometric means and confidence intervals.
Table 13  Features of diarrhoeal illness on admission by dietary group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conventional (n = 39)</th>
<th>AMD (n = 37)</th>
<th>FAD (n = 36)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated degree of dehydration [n]</td>
<td>[38]</td>
<td>[33]</td>
<td>[32]</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5 (13%)</td>
<td>6 (18%)</td>
<td>6 (19%)</td>
<td></td>
</tr>
<tr>
<td>Some</td>
<td>28 (74%)</td>
<td>18 (55%)</td>
<td>20 (56%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Severe</td>
<td>5 (13%)</td>
<td>9 (27%)</td>
<td>6 (17%)</td>
<td></td>
</tr>
<tr>
<td>Median reported duration of diarrhoea (days)</td>
<td>3.0 (2.4 to 3.8) [38]</td>
<td>2.8 (2.2 to 3.5) [35]</td>
<td>2.4 (2.0 to 3.1) [35]</td>
<td>0.38</td>
</tr>
<tr>
<td>Reported number of stools in previous 24 h</td>
<td>3.9 (3.2 to 4.9) [34]</td>
<td>4.2 (3.3 to 5.4) [27]</td>
<td>4.7 (3.6 to 6.1) [27]</td>
<td>0.59</td>
</tr>
<tr>
<td>Median stool index</td>
<td>2.2 [34]</td>
<td>2.0 [26]</td>
<td>2.0 [27]</td>
<td>0.89</td>
</tr>
<tr>
<td>History of vomiting in previous 24 h</td>
<td>29 (83%) [35]</td>
<td>27 (82%) [33]</td>
<td>24 (75%) [32]</td>
<td>0.69</td>
</tr>
<tr>
<td>Of those vomiting, no of vomits in previous 24 h</td>
<td>2.5 (1.9 to 3.2)</td>
<td>2.6 (2.2 to 3.1)</td>
<td>3.6 (2.6 to 4.9)</td>
<td>0.08</td>
</tr>
<tr>
<td>Reported appetite in previous 24 h:</td>
<td>[38]</td>
<td>[34]</td>
<td>[34]</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>8 (21%)</td>
<td>16 (47%)</td>
<td>8 (23%)</td>
<td></td>
</tr>
<tr>
<td>Reduced</td>
<td>24 (63%)</td>
<td>14 (41%)</td>
<td>22 (65%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Not eating</td>
<td>6 (16%)</td>
<td>4 (12%)</td>
<td>4 (12%)</td>
<td></td>
</tr>
</tbody>
</table>

For continuous variables, geometric means are shown (unless median specified), with 95% confidence intervals in parentheses; for categorical variables, values in parentheses are group percentages; values in square brackets are numbers in groups, if less than n.

* p values (or equivalent significance levels) for One-way ANOVAs (continuous variables); Chi squared tests (categorical variables); Kruskal Wallis nonparametric One way ANOVA (continuous variables where median is presented). There were no significant differences between groups

WHO classification

*a Stool index = (reported stool frequency in previous 24 hours / reported usual stool frequency in 24 hours)
Figure 19 Presenting symptoms in addition to diarrhoea

Responses to the question “Which symptoms made you bring your child to hospital?” (Qn 24)
### Table 14 Past medical history by dietary group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conventional (n = 39)</th>
<th>AMD (n = 37)</th>
<th>FAD (n = 36)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight (kg)</td>
<td>2.7 (2.5 to 2.8)</td>
<td>3.1 (2.8 to 3.4)</td>
<td>3.0 (2.7 to 3.2)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Gestation &lt; 37 weeks</td>
<td>2 (5%)</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
<td>0.83</td>
</tr>
<tr>
<td>Twin</td>
<td>0</td>
<td>1 (3%)</td>
<td>4 (11%)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Born in hospital rather than at home</td>
<td>35 (90%)</td>
<td>29 (83%)</td>
<td>30 (83%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Fully immunised</td>
<td>26 (67%)</td>
<td>21 (57%)</td>
<td>19 (53%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Ever ill before</td>
<td>29 (83%) [35]</td>
<td>23 (70%) [33]</td>
<td>23 (74%) [31]</td>
<td>0.44</td>
</tr>
<tr>
<td>Of those previously ill:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever admitted</td>
<td>9 (31%)</td>
<td>7 (26%)</td>
<td>5 (22%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Ill in last 6 months</td>
<td>27 (93%)</td>
<td>16 (70%)</td>
<td>19 (83%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Ill more than once in last 6 months</td>
<td>4 (14%)</td>
<td>3 (13%)</td>
<td>3 (16%)</td>
<td>0.96</td>
</tr>
<tr>
<td>Diarrhoea in last 6 months</td>
<td>7 (24%)</td>
<td>2 (9%)</td>
<td>4 (17%)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

For continuous variables, means are shown, with 95% confidence intervals in parentheses; for categorical variables, values in parentheses are group percentages; values in square brackets are numbers in groups, if less than n.

* p values (or equivalent significance levels) for One way ANOVAs (continuous variables) and Chi squared tests (categorical variables). Values indicating a significant difference between groups are denoted with an asterisk.
Table 15 Acute phase proteins by dietary group, compared to follow-up group

<table>
<thead>
<tr>
<th>Acute phase protein</th>
<th>Conventional (n=20)</th>
<th>AMD (n=20)</th>
<th>FAD (n=17)</th>
<th>Follow-up group (n=25)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean CRP (mg/l)</td>
<td>9.7 (5.1 - 18.5)</td>
<td>22.5 (11.6 - 43.6)</td>
<td>13.8 (6.7 - 28.4)</td>
<td>8.3 (5.3 - 12.8)</td>
<td>0.17</td>
</tr>
<tr>
<td>Mean SAA (g/l)</td>
<td>6.4 (3.1 - 7.9)</td>
<td>11.1 (5.5 - 22.5)</td>
<td>9.1 (3.0 - 27.1)</td>
<td>5.2 (3.5 - 7.9)</td>
<td>0.58</td>
</tr>
<tr>
<td>Mean AGP (g/l)</td>
<td>0.88 (0.69-1.12)</td>
<td>1.26 (0.99-1.60)</td>
<td>1.26 (0.93-1.72)</td>
<td>1.09 (0.88-1.34)</td>
<td>0.07</td>
</tr>
<tr>
<td>Number (%) with raised CRP</td>
<td>10 (50)</td>
<td>14 (70)</td>
<td>10 (59)</td>
<td>11 (44)</td>
<td>0.43</td>
</tr>
<tr>
<td>Number (%) with raised SAA</td>
<td>12 (60)</td>
<td>15 (75)</td>
<td>10 (59)</td>
<td>14 (56)</td>
<td>0.50</td>
</tr>
<tr>
<td>Number (%) with raised AGP</td>
<td>14 (70)</td>
<td>16 (80)</td>
<td>12 (71)</td>
<td>19 (25)</td>
<td>0.73</td>
</tr>
<tr>
<td>Number (%) with any APP raised</td>
<td>16 (80)</td>
<td>20 (100)</td>
<td>13 (77)</td>
<td>21 (84)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Geometric means are given, with 95% confidence intervals given as ranges within parentheses; other figures in parentheses indicate percentages. There were no significant differences between groups on ANOVA testing. Concentrations considered to be in the normal range were CRP < 10mg/l, SAA < 5g/l and AGP < 0.75 g/l.

*p value for ANOVA testing between the three dietary groups
NSI = no systemic infection (n = 22); SI = systemic infection (n = 35); FU = follow up group (n = 25).

Horizontal bars are geometric means, whiskers represent 95% confidence intervals; dotted lines indicate upper limit of normal.

There were significant differences for t tests between the SI and the NSI groups for CRP (p = 0.004) and SAA (p = 0.047). There were no significant differences for any of the other parameters.
3.2.3 Feeding characteristics

3.2.3.1 Feeding history

Early feeding practices by dietary group are shown in Table 16 (p115). There were no significant differences between groups. 59 children (53%) had received food or fluid other than breast milk within the first week of life, and this had risen to 80 (71%) by 3 months of age. Only 10 children (9%) were no longer being breast fed at time of entry to the study. Figure 21 (p116) indicates that supplementation within the first month is mainly water, from 1 to 3 months mainly thin maize porridge (uji), and after 3 months a mixture of thin maize porridge and starchy staples. The frequency of usage of different foods and fluids after 3 months of age is shown in more detail in Figure 22 (p117). Information on recent diets, within the week prior to becoming ill, is given in Figure 23 (p118). 104 mothers (93%) considered thin maize porridge to be one of their child's main foods, 81 (72%) named breast milk, and 55 (49%) ugali (solid maize porridge). Groundnuts were the food most commonly reported to be mixed with thin maize porridge (Figure 24, p118). Seven mothers (6%) were using AMD for child feeding, and ten (9%) were using FAD, with no significant differences between groups (see section 3.6, p140).

3.2.3.2 Food and fluids offered during diarrhoeal illness

Foods and fluids offered to children in the 24 hours prior to admission are shown in Figure 25 & Figure 26 (pp119-120). These were primarily a combination of thin maize porridge and breast milk. There were no significant differences between dietary groups. Only 39 children (35%) had received oral rehydration therapy (ORT). For 32 of these, a commercially produced sachet was used to make the ORT, while the remainder received a home-made solution.

3.2.4 Environmental factors

Socioeconomic factors by dietary group are compared in Table 17 (p121), and household structure in Table 18 (p122). There were no significant differences between groups for any factor.
Table 16 Early feeding practices by dietary group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conventional (n = 39)</th>
<th>AMD (n = 37)</th>
<th>FAD (n = 36)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received colostrum in neonatal period</td>
<td>30 (88%) [34]</td>
<td>29 (85%) [34]</td>
<td>27 (84%) [32]</td>
<td>0.69</td>
</tr>
<tr>
<td>Age when first received food/fluid other than breast milk:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First week</td>
<td>20 (57%)</td>
<td>21 (62%)</td>
<td>18 (56%)</td>
<td></td>
</tr>
<tr>
<td>After first week up to first month</td>
<td>2 (6%)</td>
<td>0</td>
<td>1 (3%)</td>
<td>0.61</td>
</tr>
<tr>
<td>After first month up to three months</td>
<td>8 (23%)</td>
<td>4 (12%)</td>
<td>6 (19%)</td>
<td></td>
</tr>
<tr>
<td>After three months</td>
<td>5 (14%)</td>
<td>9 (26%)</td>
<td>7 (19%)</td>
<td></td>
</tr>
<tr>
<td>Bottle used for feeding</td>
<td>4 (11%)</td>
<td>2 (6%)</td>
<td>4 (13%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Spoon used for feeding</td>
<td>28 (80%)</td>
<td>29 (85%)</td>
<td>29 (91%)</td>
<td>0.47</td>
</tr>
<tr>
<td>No longer breast feeding</td>
<td>3 (8%) [38]</td>
<td>5 (14%) [36]</td>
<td>2 (6%) [33]</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Values in parentheses are group percentages; values in square brackets are numbers in groups, if less than n.

* p values for Chi squared tests. There were no significant differences between groups for any of the parameters.
Figure 21  Foods or fluids other than breast milk by age of introduction

Percentage of all respondents

- High energy/protein foods
- Other starchy staples
- Thin maize porridge
- Powdered/cows milk
- Water only

n = 99 respondents
Each category includes foods/fluids from the categories below
Starchy staples principally include thick maize porridge (ugali), potatoes and rice
High energy/protein foods principally include groundnuts, beans, eggs, dagaa (small fish), and oil
Figure 22  Frequency of usage of different foods/fluids after three months of age
Figure 23  Foods reported to be one of child's main foods in week prior to becoming ill

Figure 24  Foods normally mixed with thin maize porridge (uji)

Number of respondents = 99
Figure 25 Foods offered to children with diarrhoea in the 24 hours prior to admission by dietary group

No child had received any form of AMD or FAD
Dagaa = a small fish, with a slightly sour taste which is reasonably cheap
Plantains = a non-sweet variety of banana which is cooked as a starchy staple
There were no significant differences between groups
Figure 26  Fluids offered to children with diarrhoea in the 24 hours prior to admission by dietary group

There were no significant differences between groups.
Table 17 Socioeconomic factors by dietary group

<table>
<thead>
<tr>
<th></th>
<th>Dietary Group (n = 112)</th>
<th></th>
<th></th>
<th></th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conv (n = 39)</td>
<td>AMD (n = 37)</td>
<td>FAD (n = 36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age ≤20 yrs</td>
<td>13 (38%) [34]</td>
<td>7 (22%) [32]</td>
<td>6 (20%) [30]</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Single mother</td>
<td>5 (13%) [33]</td>
<td>8 (24%) [33]</td>
<td>5 (16%) [31]</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Mother illiterate</td>
<td>6 (17%) [35]</td>
<td>5 (15%) [33]</td>
<td>6 (19%) [32]</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Mother education</td>
<td>7 (21%) [33]</td>
<td>8 (25%) [32]</td>
<td>7 (23%) [30]</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Mother not in paid</td>
<td>22 (65%) [34]</td>
<td>26 (76%) [34]</td>
<td>29 (91%) [32]</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Previous child(ren)</td>
<td>5 (13%) [34]</td>
<td>6 (18%) [33]</td>
<td>9 (27%) [33]</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Living in rented or</td>
<td>19 (54%) [35]</td>
<td>24 (71%) [34]</td>
<td>24 (75%) [32]</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Relative's housing</td>
<td>5 (14%) [35]</td>
<td>5 (15%) [34]</td>
<td>5 (16%) [32]</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>House has earth floor</td>
<td>8 (23%) [35]</td>
<td>6 (18%) [34]</td>
<td>9 (28%) [32]</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>House has earth walls</td>
<td>17 (49%) [35]</td>
<td>14 (41%) [34]</td>
<td>13 (42%) [31]</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>No electric light</td>
<td>30 (86%) [35]</td>
<td>27 (79%) [34]</td>
<td>25 (81%) [31]</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Pit latrine toilet</td>
<td>28 (82%) [34]</td>
<td>30 (88%) [34]</td>
<td>28 (90%) [31]</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Toilet shared with</td>
<td>17 (49%) [35]</td>
<td>26 (76%) [34]</td>
<td>20 (65%) [31]</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Other families</td>
<td>[35]</td>
<td>[34]</td>
<td>[31]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water source:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside tap</td>
<td>13 (37%) [35]</td>
<td>13 (38%) [34]</td>
<td>9 (29%) [31]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Community tap</td>
<td>19 (54%) [35]</td>
<td>20 (59%) [34]</td>
<td>21 (68%) [33]</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>3 (9%) [35]</td>
<td>1 (3%) [34]</td>
<td>1 (3%) [31]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubbish disposal:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buried</td>
<td>26 (74%) [35]</td>
<td>25 (74%) [34]</td>
<td>19 (61%) [31]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burnt</td>
<td>5 (14%) [35]</td>
<td>3 (9%) [34]</td>
<td>7 (23%) [31]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubbish dump</td>
<td>0</td>
<td>2 (6%) [34]</td>
<td>1 (3%) [31]</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Collection by council</td>
<td>3 (9%) [35]</td>
<td>2 (6%) [34]</td>
<td>1 (3%) [31]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrown in street</td>
<td>1 (3%) [35]</td>
<td>1 (3%) [34]</td>
<td>2 (6%) [31]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are group percentages; values in square brackets are numbers in groups, if less than n. p values are for Chi squared tests. There were no significant differences between groups for any of the variables.
Table 18 Household structure by dietary group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Dietary Group (n = 112)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional (n = 39)</td>
<td>AMD (n = 37)</td>
<td>FAD (n = 36)</td>
<td>p value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 or more children in the household</td>
<td>11 (31%) [35]</td>
<td>11 (33%) [33]</td>
<td>8 (25%) [32]</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 or more adults and children in household</td>
<td>12 (34%) [35]</td>
<td>11 (33%) [33]</td>
<td>13 (41%) [32]</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children comprising more than 50% of household occupants</td>
<td>10 (29%) [35]</td>
<td>9 (27%) [33]</td>
<td>9 (28%) [32]</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of males: females ≥2:1</td>
<td>9 (26%) [35]</td>
<td>5 (15%) [33]</td>
<td>6 (19%) [6]</td>
<td>0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean number of persons per sleeping room ≥ 4</td>
<td>12 (35%) [34]</td>
<td>6 (18%) [32]</td>
<td>13 (41%) [32]</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are group percentages; values in square brackets are numbers in groups, if less than n. p values are for Chi squared tests. There were no significant differences between groups for any of the variables.
3.3 Dietary intake

3.3.1 Weight of food consumed

Table 19 (p124) compares the geometric mean weights of food consumed in grams per kg body weight in each dietary group, for the first 4 days of the study. There was no significant difference on any day between the amounts consumed by the conventional and AMD groups; but on days 2 and 4, the FAD group consumed significantly less weight of food than the conventional group. Over the 4 days, the mean amount consumed by the FAD group was 64.4 g/kg body weight, as compared with 85.8 g/kg in the conventional group.

3.3.2 Energy intake

Individual energy intakes from study foods are compared by dietary group and by day of study in Figure 27 (p125). Geometric means for energy intake are shown in Table 20 (p126) and shown graphically in Figure 28 (p127). The AMD group consumed significantly more energy than the conventional group for each of the first three days of the study. Over the 4 day period, the mean daily energy intake was 46.0 kcal/kg/day in the AMD group, as compared to 32.4 kcal/kg/day in the conventional group, which represents an increase of 42% (p = 0.003). The energy intake in the FAD group did not differ significantly from the other 2 groups on any day, and the overall mean for the 4 days of the study was 37.3 kcal/kg/day.

The following factors were controlled for in the analysis of variance of the 4 day energy intake results by including them as single covariates: age; sex; Z scores for weight/age, height/age and weight/height; presence or absence of systemic illness; whether received antibiotics or not; temperature during admission; and whether received blood transfusion or not. In each case, the p value for differences in energy intake between the groups remained ≤0.005. In a multiple ANOVA model, acute phase proteins on entry to the study were not found to influence dietary intake.
# Table 19: Weight of food consumed (grams per kg body weight per day) by dietary group and by day of study

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Days 1 - 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>73.8 (63.1 - 86.1)</td>
<td>89.2 (76.7 - 103.8)</td>
<td>90.3 (77.3 - 105.3)</td>
<td>103.7 (81.1 - 132.7)</td>
<td>85.8 (75.9 - 92.8)</td>
</tr>
<tr>
<td>n</td>
<td>26</td>
<td>26</td>
<td>17</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>AMD</td>
<td>68.4 (54.9 - 85.2)</td>
<td>80.7 (67.6 - 96.3)</td>
<td>88.7 (75.6 - 104.1)</td>
<td>82.9 (66.7 - 103.1)</td>
<td>79.9 (68.7 - 92.8)</td>
</tr>
<tr>
<td>n</td>
<td>25</td>
<td>24</td>
<td>19</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>FAD</td>
<td>62.1 (51.4 - 75.1)</td>
<td>60.2 (48.7 - 74.3)</td>
<td>72.8 (61.0 - 86.8)</td>
<td>59.8 (43.9 - 81.4)</td>
<td>64.4 (54.8 - 75.7)</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td>20</td>
<td>18</td>
<td>7</td>
<td>24</td>
</tr>
</tbody>
</table>

Total number in group: 72 70 54 28 75

Oneway ANOVA p value: p = 0.434 p = 0.007 † p = 0.105 p = 0.020 † p = 0.015 †

Values are geometric means (95% confidence intervals)

† Significant difference between conventional and FAD groups
Figure 27 Plots of individual daily energy intakes by dietary group and by day of study.

Energy intake (kcal/kg/day)

Day of study

FAD

AMD

Conventional

1000 100 10
### Table 20: Energy intake (kcal per kg body weight per day) by dietary group and by day of study

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Days 1 - 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>27.9 (24.3 - 33.0)</td>
<td>33.7 (29.4 - 39.7)</td>
<td>34.1 (29.6 - 39.9)</td>
<td>39.2 (30.7 - 50.0)</td>
<td>32.4 (28.7 - 36.6)</td>
</tr>
<tr>
<td>n</td>
<td>26</td>
<td>26</td>
<td>17</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>AMD</td>
<td>39.4 (32.6 - 49.9)</td>
<td>46.4 (39.5 - 56.0)</td>
<td>51.1 (43.9 - 60.3)</td>
<td>47.8 (39.1 - 59.3)</td>
<td>46.0 (39.6 - 53.4)</td>
</tr>
<tr>
<td>n</td>
<td>25</td>
<td>24</td>
<td>19</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>FAD</td>
<td>36.0 (30.6 - 43.8)</td>
<td>34.9 (29.1 - 43.4)</td>
<td>42.2 (35.8 - 50.7)</td>
<td>34.7 (26.0 - 56.5)</td>
<td>37.3 (31.8 - 43.9)</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td>20</td>
<td>18</td>
<td>7</td>
<td>24</td>
</tr>
</tbody>
</table>

Total number in group: 72 70 54 28 75

One-way ANOVA p value:
- p = 0.016 †
- p = 0.014 †
- p = 0.002 †
- p = 0.109
- p = 0.003 †

Values are geometric means (95% confidence intervals)

† Significant difference between AMD and conventional groups
Figure 28  Energy intake (kcal per kg body weight per day) by dietary group and by day of study

Energy intake (kcal/kg/day)

Days after entry

p = 0.016  p = 0.014  p = 0.002  p = 0.109

p values refer to One-way ANOVA tests between groups. The significant p values on Days 1-3 are due to differences between the AMD and the conventional food groups.
3.4 Clinical Outcomes and Follow Up

Results in this section are presented with reference to the whole study group of 112 patients.

3.4.1 Diarrhoeal illness

Time until cessation of diarrhoea was compared between groups using survival analysis; results are shown in Figure 29 (p129). Techniques used for the analysis are described in the Methods (see section 2.2.6, p93). Data were used until the 9th day, when all had stopped having diarrhoea. There was no difference between the groups (Lee-Desu statistic $p = 0.90$). Other outcome features are shown in Table 21 (p130). There were no significant differences between the groups for any of the parameters.

3.4.2 Mortality

Information about the seven children who died during admission is given in Table 22 (p131). Two children died on the fourth day (1 in the AMD, 1 in the FAD group), and a third on the fifth day (AMD group); each had pneumonia and marasmus (all had a weight for age less than -3.3). Two further children died on the eighth day, both with a clinical picture of septicaemia, although this was not confirmed on blood culture. One of these was only mildly malnourished, but in addition to diarrhoea had perineal ulceration, oral thrush, malaria, and otitis media. Two further children died 12 and 25 days after admission, both severely malnourished with probable septicaemia. The overall mortality was 6%.

3.4.3 Weight changes

Geometric mean weight changes for all dietary groups, as percentage of admission body weight, were between -1.2 and +0.7% on Day 4 of the study, -1.2 and +2.8% at first follow up, and +5.5 and 7.3% at second follow up. These figures correspond to absolute weight changes of between -50 and +12g on Day 4, +26 and +237g at first follow up, and +415 and +550g at second follow up. There were no significant differences between the groups on any day of the study for either parameter. Individual weight changes by dietary group are shown graphically in Figure 30 (p132).
Figure 29  Percentage of children still having diarrhoea by dietary group and by day of study (survival analysis)

Overall comparison between groups (Lee - Desu statistic) $p = 0.90$
### Table 21  Outcome features of diarrhoeal illness by dietary group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conventional (n = 39)</th>
<th>AMD (n = 37)</th>
<th>FAD (n = 36)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time until cessation of diarrhoea (h after entry)</td>
<td>42.3 (31.1 to 57.4) [39]</td>
<td>46.2 (35.4 to 60.2) [33]</td>
<td>47.3 (38.4 to 54.6) [33]</td>
<td>0.84</td>
</tr>
<tr>
<td>Number of stools day 1</td>
<td>4.7 (3.8 to 5.7) [37]</td>
<td>5.4 (4.2 to 7.0) [37]</td>
<td>5.1 (4.1 to 6.4) [35]</td>
<td>0.63</td>
</tr>
<tr>
<td>Number of stools day 2</td>
<td>3.8 (3.0 to 4.7) [37]</td>
<td>4.6 (3.5 to 6.0) [35]</td>
<td>4.9 (3.9 to 6.3) [35]</td>
<td>0.28</td>
</tr>
<tr>
<td>Number of stools day 3</td>
<td>3.6 (2.9 to 4.5) [31]</td>
<td>5.0 (3.7 to 6.8) [27]</td>
<td>4.3 (3.4 to 5.2) [30]</td>
<td>0.17</td>
</tr>
<tr>
<td>Number of stools day 4</td>
<td>4.3 (3.2 to 5.9) [16]</td>
<td>4.7 (3.3 to 6.9) [21]</td>
<td>3.6 (2.6 to 5.0)</td>
<td>0.51</td>
</tr>
<tr>
<td>Vomited during ward study period</td>
<td>26 (67)</td>
<td>27 (73)</td>
<td>22 (61)</td>
<td>0.34</td>
</tr>
<tr>
<td>Of those vomiting, percentage of meals vomited</td>
<td>16.7 (11.5 to 21.8)</td>
<td>15.0 (10.8 to 19.1)</td>
<td>19.2 (11.7 to 26.6)</td>
<td>0.54</td>
</tr>
<tr>
<td>Meals vomited out of total meals documented in study</td>
<td>101 /831 (12.2)</td>
<td>81/797 (10.2)</td>
<td>82/605 (13.6)</td>
<td>0.14</td>
</tr>
<tr>
<td>Change of study diet to conventional</td>
<td><strong>b</strong></td>
<td>1 (3)</td>
<td>4 (11)</td>
<td></td>
</tr>
<tr>
<td>Recurrence of diarrhoea on ward</td>
<td>3 (8)</td>
<td>3 (8)</td>
<td>1 (3)</td>
<td>0.58</td>
</tr>
<tr>
<td>Recurrence of diarrhoea in month after discharge</td>
<td>9 (23)</td>
<td>9 (24)</td>
<td>5 (14)</td>
<td>0.46</td>
</tr>
<tr>
<td>Required iv fluids</td>
<td>1 (3)</td>
<td>7 (19)</td>
<td>4 (11)</td>
<td>0.07</td>
</tr>
<tr>
<td>Required ng tube feeding</td>
<td>3 (8)</td>
<td>4 (11)</td>
<td>8 (22)</td>
<td>0.16</td>
</tr>
<tr>
<td>Died during admission</td>
<td>0</td>
<td>3 (8)</td>
<td>4 (11)</td>
<td>0.12</td>
</tr>
<tr>
<td>Median duration of admission (days)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0.68</td>
</tr>
<tr>
<td>Failed dietary management</td>
<td>6 (15)</td>
<td>13 (35)</td>
<td>11 (31)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

For continuous variables, geometric means are shown (unless median specified), with 95% confidence intervals in parentheses; for categorical variables, values in parentheses are group percentages; values in square brackets are numbers in groups, if less than n.

*p values (or equivalent significance levels) for Oneway ANOVAs (continuous variables); Chi squared tests (categorical variables); Kruskal Wallis nonparametric One way ANOVA (continuous variables where median is presented). There were no significant differences between groups.

**b** The study design did not allow children in the conventional group to change to the other study foods.

**c** Dietary management was classified as having failed if the child required ng tube feeding or iv fluids after entering the study, had a recurrence of diarrhoea during admission, or died during admission.
### Table 22 Detail of children who died during admission

<table>
<thead>
<tr>
<th>Age</th>
<th>Dietary group</th>
<th>Z scores</th>
<th>Day of death</th>
<th>Cause of death</th>
<th>Other problems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>W/A</td>
<td>H/A</td>
<td>W/H</td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>AMD</td>
<td>-0.20</td>
<td>+1.02</td>
<td>-1.15</td>
<td>8</td>
</tr>
<tr>
<td>8.6</td>
<td>AMD</td>
<td>-3.38</td>
<td>-2.56</td>
<td>-1.77</td>
<td>4</td>
</tr>
<tr>
<td>11.4</td>
<td>AMD</td>
<td>-3.40</td>
<td>-3.27</td>
<td>-1.34</td>
<td>5</td>
</tr>
<tr>
<td>6.9</td>
<td>FAD</td>
<td>-3.22</td>
<td>-0.48</td>
<td>-3.85</td>
<td>12</td>
</tr>
<tr>
<td>8.0</td>
<td>FAD</td>
<td>-2.91</td>
<td>-1.30</td>
<td>-2.57</td>
<td>8</td>
</tr>
<tr>
<td>9.3</td>
<td>FAD</td>
<td>-4.68</td>
<td>-3.49</td>
<td>-2.80</td>
<td>25</td>
</tr>
<tr>
<td>16.6</td>
<td>FAD</td>
<td>-4.38</td>
<td>-2.43</td>
<td>-4.23</td>
<td>4</td>
</tr>
</tbody>
</table>
Figure 30  Percentage change in weight during first 4 days of study and at follow up
Table 23 Change in weight during first 4 days of study and at follow up

<table>
<thead>
<tr>
<th>Weight change parameter</th>
<th>Conventional (n = 39)</th>
<th>AMD (n = 37)</th>
<th>FAD (n = 36)</th>
<th>p value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percentage weight change:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>-0.5 (-1.5 to +0.6) [34]</td>
<td>-0.5 (-1.7 to +0.9) [31]</td>
<td>-0.8 (-1.6 to -0.1) [34]</td>
<td>0.84</td>
</tr>
<tr>
<td>Day 3</td>
<td>-0.3 (-1.8 to +1.2) [33]</td>
<td>+0.2 (-0.9 to +1.4) [35]</td>
<td>+0.7 (-1.5 to +0.1) [35]</td>
<td>0.53</td>
</tr>
<tr>
<td>Day 4</td>
<td>-1.1 (-2.7 to +0.8) [27]</td>
<td>-0.3 (-1.5 to +1.1) [26]</td>
<td>-1.2 (-2.4 to +0.2) [31]</td>
<td>0.63</td>
</tr>
<tr>
<td>At 1st follow up</td>
<td>+2.8 (+0.7 to +5.1) [23]</td>
<td>-1.2 (-4.9 to +3.3) [22]</td>
<td>+3.2 (1.4 to 5.2) [21]</td>
<td>0.07</td>
</tr>
<tr>
<td>At 2nd follow up</td>
<td>+7.3 (+5.2 to +9.6) [22]</td>
<td>+5.5 (+2.6 to +8.7) [18]</td>
<td>+5.5 (+2.9 to +8.3) [18]</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Absolute weight change (grams):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>-26 (-101 to -50) [34]</td>
<td>-7 (-95 to -81) [31]</td>
<td>-48 (-96 to -1) [34]</td>
<td>0.71</td>
</tr>
<tr>
<td>Day 3</td>
<td>+2 (-98 to +103) [33]</td>
<td>+43 (-41 to +126) [35]</td>
<td>-28 (-80 to +25) [35]</td>
<td>0.45</td>
</tr>
<tr>
<td>Day 4</td>
<td>-50 (-154 to +53) [27]</td>
<td>+12 (-80 to +104) [26]</td>
<td>-52 (-127 to +23) [31]</td>
<td>0.52</td>
</tr>
<tr>
<td>At 1st follow up</td>
<td>+237 (+76 to +397) [23]</td>
<td>+26 (-204 to +255) [22]</td>
<td>+169 (-50 to +388) [21]</td>
<td>0.30</td>
</tr>
<tr>
<td>At 2nd follow up</td>
<td>+550 (+395 to +705) [22]</td>
<td>+441 (+210 to +672) [18]</td>
<td>+415 (+237 to +593) [18]</td>
<td>0.51</td>
</tr>
</tbody>
</table>

For continuous variables, geometric means are shown, with 95% confidence intervals in parentheses; for categorical variables, values in parentheses are group percentages; values in square brackets are numbers in groups, if less than n.

<sup>a</sup> Weight on Day 1, on entry to the study and after rehydration, is used as reference for all weight change parameters.

<sup>b</sup> p values (or equivalent significance levels) for One-way ANOVAs (continuous variables). There were no significant differences between the groups for any of the parameters.
3.5 Intestinal Permeability

Urinary lactulose:mannitol ratios were measured in 86 patients, including the 75 patients used in the dietary intake study. The remaining patients were the 12 entered into the study immediately prior to these, for whom acute phase protein data was also available. Samples from the preceding patients were not analysed because of time restrictions. The 86 patients were not significantly different from the larger group of 112 patients with respect to clinical parameters, duration of diarrhoea and nutritional status.

3.5.1 Lactulose/Mannitol ratios during the study

The 30 control children had a mean L/M ratio of 0.14 (0.12-0.17) as compared with the 86 cases with a mean of 0.85 (0.68-1.05) on admission. Table 24 (p137) shows the geometric mean L/M ratio (95% CI) for each dietary treatment group on admission, after 3 days and at the first and second follow-up examinations, carried out at approximately 14 and 28 days after discharge. These results are shown graphically in Figure 31 (p137). Analysis of Variance showed there to be a significant difference in L/M ratio between cases and controls at admission (p < 0.001) but no significant difference between the treatment groups at any point during the study.

Figure 32 (p138) shows the individual changes in L/M ratios by dietary treatment group during the first 3 days of the study for all children for whom paired samples were available. Comparison of the L/M ratios for each patient on Day 0 and Day 3 showed that there had been a fall in the L/M ratio in 11/25 (44%) of the conventional group, 15/20 (75%) of the AMD group, and 17/19 (89%) of the FAD group (χ²= 10.9, p = 0.004).

When the variation of lactulose and mannitol were analysed separately, the geometric mean urinary concentrations of lactulose on Day 0 were found to be 161, 184, and 403 on Day 0 and 124, 187 and 116 mg/l on Day 3 in the conventional, AMD and FAD groups respectively. The respective values for mannitol were 189, 231, 363 on Day 0 and 190, 309, and 265 mg/l on Day 3. The values for controls were 115mg/l for lactulose and 794 mg/l for mannitol.
When the change in L/M ratio between admission and day 3 was analysed, there was a significant difference between treatment groups ($p = 0.037$). This had been lost by the first and second follow-ups. Since the L/M ratios at the first and second follow up are near to the control levels, and as there was no means of ensuring that the children were still being fed on the treatment diet after discharge from the hospital, these have not been analysed further.

Analysis of variance also showed there to be no significant difference in L/M ratio for each clinical diagnosis group on admission and no significant difference attributable to age at weaning.

The ANOVA for L/M ratio at day 3 was repeated, including as covariants factors which were known causes of variation and increase in the error term. Factors such as sex and anthropometric data did not significantly affect L/M ratio at day 3. Table 25 (p138) shows the results of the analysis of L/M ratio at 3 days, using initial L/M ratio, age on admission and age at which foods other than breast milk were introduced as covariants.

Dietary treatment and initial L/M ratio significantly influenced L/M ratio at day 3 ($p < 0.05$). Age on admission and age at introduction of foods other than breast milk were not significant, but both contribute to the overall model when added sequentially.

FAD was shown to be significantly different from both conventional porridge ($p = 0.009$) and AMD ($p = 0.006$) by the t-test on means, which had been adjusted for the covariants by a least squares technique [255]. Intestinal permeability 3 days after admission was influenced by both the dietary treatment given, and the initial severity of damage. FAD was significantly better than the conventional porridge diet or AMD, and children on this diet had reduced intestinal permeability after 3 days, compared to others.

Age on admission was included in the analysis shown in Table 25 as there was a borderline significant difference in initial L/M ratio between children less than 15 months and those older than 15 months. Age at which foods other than breast milk were introduced was also included as covariant, as early weaning seems to have an effect on
intestinal permeability at day 3 and greatly increased the effect of FAD on mucosal recovery in other analyses. It would seem from other ANOVAs that clinical diagnosis has less effect on the L/M ratio at day 3 than age at weaning.

3.5.2 Urinary lactose and intestinal permeability

Regression analysis of the correlation between the log lactose concentration in spot urine samples and log L/M ratio gives $r = 0.62$ and $r^2 = 0.38$, as shown in Figure 33 (p139). This indicates that there is a positive relationship between the lactose concentration found in spot urine samples and the L/M ratio ($p < 0.001$). However, only 38 percent of the variation in L/M ratio is explained by the variation in lactose. Using a cut-off for L/M ratio of 0.4 (equivalent to a moderate degree of villous atrophy), and for lactose of 150mg/L, lactose has a sensitivity of 78% and a specificity of 76% for predicting raised intestinal permeability.
Table 24 Mean L/M ratios on Days 0 and 3 and at follow-up by dietary group

<table>
<thead>
<tr>
<th>Day of study</th>
<th>Conventional (n=33)</th>
<th>AMD (n=28)</th>
<th>FAD (n=25)</th>
<th>Controls (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0 (entry)</td>
<td>0.78 (0.56 - 1.10)</td>
<td>0.74 (0.53 - 1.05)</td>
<td>1.10 (0.75 - 1.61)</td>
<td>0.14 (0.12 - 0.17)</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.60 (0.42 - 0.86)</td>
<td>0.60 (0.41 - 0.88)</td>
<td>0.38 (0.21 - 0.67)</td>
<td></td>
</tr>
<tr>
<td>Day 14 (Approx time of first follow-up)</td>
<td>0.17 (0.10 - 0.29)</td>
<td>0.23 (0.16 - 0.33)</td>
<td>0.31 (0.18 - 0.55)</td>
<td></td>
</tr>
<tr>
<td>Day 28 (Approx time of 2nd follow-up)</td>
<td>0.17 (0.09 - 0.30)</td>
<td>0.20 (0.11 - 0.44)</td>
<td>0.16 (0.08 - 0.33)</td>
<td></td>
</tr>
</tbody>
</table>

Values are geometric means with 95% confidence intervals in parentheses. There was no significant difference between L/M ratios for the three dietary groups at any point. There was a significant difference between controls and cases on Day 0 (ANOVA p < 0.001).

Figure 31 L/M ratios during the study (including follow up) by dietary group compared to controls
Figure 32  Individual plots of change in L/M ratio from day 0 to day 3 by dietary group

Table 25  Results of ANOVA of L/M ratio at 3 days, with initial L/M ratio, age on admission, age at weaning and clinical diagnosis as covariants

<table>
<thead>
<tr>
<th>Source</th>
<th>F value</th>
<th>p value</th>
<th>% of variation explained*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary treatment</td>
<td>4.19</td>
<td>0.0206</td>
<td>4.4</td>
</tr>
<tr>
<td>L/M on admission</td>
<td>4.27</td>
<td>0.0439</td>
<td>13.5</td>
</tr>
<tr>
<td>Age at weaning</td>
<td>1.40</td>
<td>0.2415</td>
<td>19.8</td>
</tr>
<tr>
<td>Age on admission</td>
<td>0.73</td>
<td>0.3971</td>
<td>21.9</td>
</tr>
</tbody>
</table>

* The cumulative percentage of variation explained by each variable, taken from $r^2$, when each variable was added to the model sequentially.
Figure 33 Correlation of lactose concentration and L/M ratio at admission for cases and controls

Lactose concentration (g/L)
3.6 Evaluation of amylase-digested foods

3.6.1 Knowledge, attitudes and practices relating to amylase-digested foods

Mothers' knowledge, attitudes and practices relating to amylase-digested foods are shown at entry to the study in Table 26 (p141), and their opinions at discharge in Table 27 (p142). Table 28 (p143) shows the frequency of mothers citing each study food as one of the child's main foods at follow-up. This information was obtained by indirect questioning about the child's main foods, rather than directly asking whether the child was consuming a particular food.

3.6.2 Thiocyanates

Urinary thiocyanate levels are shown by dietary group in Figure 34 (p144). These were measured in urine obtained after at least 12 consecutive study meals had been consumed, in 27 consecutive patients. Geometric means (95 percent confidence intervals) for urinary thiocyanate levels by dietary group were: conventional 14.7 (9.1 - 23.8); AMD 16.7 (7.0 - 40.0); FAD 17.4 (9.1 - 33.4). There was no significant difference between groups (One-way ANOVA p = 0.91). These levels were well within the normal range of up to 90 µmol/l cited for children under 1 year of age [256].

3.6.3 Cost

The relative costs of the study foods are shown in Table 29 (p145). At the prices prevailing in Dar es Salaam during the study period, there was little difference in cost per gram, but the amylase-digested foods were between 31 (AMD) and 33 (FAD) percent cheaper than the conventional food in terms of cost per kcal. No adjustment was made for preparation and cooking times, or for fuel costs.

3.6.4 Variation of pH and viscosity in study foods throughout the study

The variation in pH of study foods throughout the period of the dietary intake study is shown in Figure 35 (p145). During this period, the mean pH measurements (95% CI) for each study food were: conventional 6.1 (6.0-6.3), n = 35; AMD 5.9 (5.7-6.0), n = 35; FAD 3.9 (3.8-4.0), n = 38. There was a significant difference between the groups on One-way ANOVA (p < 0.0001). The variation in viscosity measurements is shown in Figure 36 (p146). All measurements across a range of temperatures (57-89°C) were included. Mean viscosities for the study foods were: conventional 791 (616-1015), n =
35; AMD 777 (579-1044), n = 38; FAD 482 (400-583), n = 38. There was a significant difference between the groups (p = 0.006). When only measurements taken at below 75°C were included, the difference between the groups became less prominent, but remained significant (p = 0.048).

### 3.6.5 Osmolalities

Mean osmolalities for the study foods (2 measurements for each food) were: conventional 22.5 ± 0.7 mosmol/kg; AMD 62.0 ± 5.7 mosmol/kg; FAD 334.5 ±3.5 mosmol/kg. See section 2.2.3.2 in Methods (p84) for details regarding measurement.

#### Table 26 Knowledge, attitudes and practices relating to amylase-digested foods by dietary group, on entry to study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Dietary Group (n = 112)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional (n = 39)</td>
<td>AMD (n = 37)</td>
</tr>
<tr>
<td><strong>AMD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aware of kimea (ARF)</td>
<td>20 (65) [31]</td>
<td>25 (74) [34]</td>
</tr>
<tr>
<td>Uses for any purpose (inc beer)</td>
<td>4 (13) [31]</td>
<td>11 (32) [34]</td>
</tr>
<tr>
<td>Used within last month</td>
<td>0 [31]</td>
<td>3 (9) [34]</td>
</tr>
<tr>
<td>Makes kimea flour herself</td>
<td>2 (6) [31]</td>
<td>8 (26) [34]</td>
</tr>
<tr>
<td>Heard about use in porridge</td>
<td>10 (33) [30]</td>
<td>18 (53) [34]</td>
</tr>
<tr>
<td>Knows how to make AMD</td>
<td>3 (10) [30]</td>
<td>3 (9) [34]</td>
</tr>
<tr>
<td>Heard AMD useful for child feeding</td>
<td>8 (27) [30]</td>
<td>15 (44) [34]</td>
</tr>
<tr>
<td>Uses for child feeding</td>
<td>0 (0) [31]</td>
<td>3 (9) [34]</td>
</tr>
<tr>
<td><strong>FAD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aware of togwa (FAD)</td>
<td>21 (70) [30]</td>
<td>26 (76) [34]</td>
</tr>
<tr>
<td>Uses it for any purpose</td>
<td>12 (40) [30]</td>
<td>18 (53) [34]</td>
</tr>
<tr>
<td>Used within last month</td>
<td>0 [30]</td>
<td>1 (3) [34]</td>
</tr>
<tr>
<td>Own children allowed to drink it</td>
<td>9 (30) [30]</td>
<td>13 (38) [34]</td>
</tr>
<tr>
<td>Knows how to make it</td>
<td>4 (13) [30]</td>
<td>9 (28) [34]</td>
</tr>
<tr>
<td>Uses for child feeding</td>
<td>3 (10) [30]</td>
<td>5 (15) [34]</td>
</tr>
</tbody>
</table>

*Values in parentheses are group percentages; values in square brackets are numbers in groups, if less than n.

* p values for Chi squared tests. There were no significant differences between groups.
Table 28 Frequency of mother citing each study food as one of child’s main foods on indirect questioning by follow up visit

<table>
<thead>
<tr>
<th>Study food</th>
<th>1st follow up</th>
<th>2nd follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conv (n = 23)</td>
<td>AMD (n = 23)</td>
</tr>
<tr>
<td>Conv</td>
<td>19 (83)</td>
<td>7 (30)</td>
</tr>
<tr>
<td>AMD</td>
<td>0</td>
<td>15 (65)</td>
</tr>
<tr>
<td>FAD</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td></td>
<td>19 (86)</td>
<td>6 (32)</td>
</tr>
<tr>
<td>AMD</td>
<td>0</td>
<td>10 (53)</td>
</tr>
<tr>
<td>FAD</td>
<td>0</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

Shaded areas indicate the study food used by the dietary group in that column.
Table 28 Frequency of mother citing each study food as one of child's main foods on indirect questioning by follow up visit

<table>
<thead>
<tr>
<th>Study food</th>
<th>1st follow up</th>
<th>2nd follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dietary group</td>
<td>Dietary group</td>
</tr>
<tr>
<td></td>
<td>Conv (n = 23)</td>
<td>AMD (n = 23)</td>
</tr>
<tr>
<td>Conv</td>
<td>19 (83)</td>
<td>7 (30)</td>
</tr>
<tr>
<td>AMD</td>
<td>0</td>
<td>15 (65)</td>
</tr>
<tr>
<td>FAD</td>
<td>0</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

Shaded areas indicate the study food used by the dietary group in that column.
Figure 34  Urinary thiocyanate levels by dietary group after at least 12 consecutive study meals

For comparison, mean urinary thiocyanate levels in 17 normal Swedish schoolchildren were 31 μmol/l [132], and 90 μmol/l is cited as the upper limit of the normal range in children under 1 year of age [256].
Table 29  Relative costs of study feeds

<table>
<thead>
<tr>
<th></th>
<th>Conv</th>
<th>AMD</th>
<th>FAD</th>
<th>Thin maize porridge only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per gram of feed</td>
<td>0.017</td>
<td>0.018</td>
<td>0.018</td>
<td>0.008</td>
</tr>
<tr>
<td>Energy density (kcal/g)</td>
<td>0.378</td>
<td>0.576</td>
<td>0.580</td>
<td>0.220</td>
</tr>
<tr>
<td>Cost per kcal</td>
<td>0.046</td>
<td>0.032</td>
<td>0.031</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Costs are in Tanzanian shillings (T.sh), and do not include preparation and cooking times, or costs of fuel. They are based on the following prices, which were typical in Dar es Salaam at the time of the study: maize flour 105 T.sh/kg; sorghum grain 150 T.sh/kg; groundnuts 300 T.sh/kg. Costs of feeds were very dependent on the price and availability of groundnuts, which varied considerably.

Figure 35  Variation in pH by study food during the dietary study period
Figure 36  Variation in viscosity by study food during the dietary study period

All viscosity measurements made using RV2 spindle at 20rpm at approximate serving temperature.
4 Discussion

4.1 Problems with Data Accuracy

Dietary intake studies are notoriously difficult, with many opportunities for error. This is the case even in environments where such research is frequently performed, and all the more so where it is unusual. Twenty-four hour intakes over several days are either assessed by recall, which is inevitably approximate, or by measuring all intakes. This is time consuming, but should provide accurate intake data as long as there are no transcription or processing errors. However, it depends on reliable and obsessional enumerators. Even with careful selection a study team may be disabled by one or two unreliable members. These may be difficult to identify, especially when from a different culture. It is therefore necessary to develop tools and procedures that allow objective assessment of data validity. In this study, which ran over several months and required 24 hour intakes throughout, 9 enumerators were required to ensure that at least one trained person would be always be present, and two of these were found to be unreliable. Examination of the raw dietary intake data, by plotting frequency distributions and calculating last digit proportions, allowed detection and an appropriate response. Without such procedures, the invalid data would have been undetected.

Several important points can be drawn from the discovery of this problem, and its solution. Firstly, the importance of examination of the raw data is emphasised. Had summary data (e.g. total daily intakes) or manipulated data (e.g. amounts/kg) been examined instead, the presence of rounding up and estimation would have been undetectable. Examination of raw data in this way should be performed regularly throughout similar studies so that problems can be identified early. Secondly, when more than one enumerator is used, there should be a system of accountability for data. In this case, enumerator coding was used. This alone is an incentive to accuracy, but especially when combined with a publicised programme of periodic data examination. With such a system, enumerator errors can be more quickly identified and corrected, and affected records excluded from analysis. The initial lack of coding permitted slackness, and meant that when a problem was discovered, the source was difficult to trace and the unaffected data could not be salvaged. Thirdly, the techniques of data validation used have been shown to be appropriate and effective in the context of a
dietary intake study. To be accepted as valid, a large data set produced by several enumerators should meet the following criteria: a) a relatively smooth frequency distribution curve; b) last digit proportions should be approximately equal; c) individual enumerators should produce data with similar distributions to each other and to the overall distribution, and equal last digit proportions, provided each has contributed a large enough number of observations. Data from an enumerator who fabricates or estimates data is most unlikely to satisfy both criteria, since even if fabrication is systematic enough to produce equal last digit proportions, it would be almost impossible to produce a satisfactory frequency distribution without knowledge of the whole data set. In this study, the number of observations from each enumerator was not great enough to allow criterion (c) to be met, but the almost-equal last digit proportions of the coded records is strong evidence of validity.

Application of such a system of data validation is recommended in all dietary intake studies of similar size.

4.2 Patient Groups

The decision to analyse the data in three separate groups for three major aspects of the study was a compromise between maximising the information gained from available data, ensuring that only reliable data was used, and working within the resource constraints which limited the number of intestinal permeability samples analysed. An alternative option would have been to limit the whole dataset to patients enrolled after the introduction of enumerator coding. However, this would have reduced the power of the study to detect an effect of the study foods on intestinal permeability, or to detect significant differences in outcome such as diarrhoeal morbidity or weight gain. Since the groups were all complete sequences of consecutive patients enrolled (except in the case of exclusions), there should have been no effect on randomisation. This was found to be the case for each group of the major groups used.

4.3 Descriptive Data

4.3.1 Selection bias and randomisation

Analysis for selection bias was limited because of lack of information on those not selected for the study. There was a greater excess of male patients in the study group
(65%) compared to that for all patients admitted to the ward during the study period (57%). This may indicate some selection bias towards male patients. Although there was no reason to believe that patients selected for the study were different from the total population of eligible patients in any important way, the available data does not allow selection bias to be confidently excluded.

Effectiveness of randomisation was assessed by comparison of descriptive characteristics on admission by dietary group. Groups were similar in almost all respects, including general characteristics, features of diarrhoeal illness, presenting symptoms, past medical history, early and recent feeding practices, and socioeconomic factors. This demonstrated that randomisation had been effective. Significant differences between groups were in areas of minor importance with respect to the findings of the study: mode of referral, birthweight, and number of twins. Statistical comparisons were performed for the subgroups used for the intestinal permeability (Group B) and the dietary intake analyses (Group C). Similar results were obtained, confirming that randomisation had remained effective within these subgroups. In addition, there were no significant differences between dietary groups for levels of acute phase proteins, or intestinal permeability on entry to the study.

4.3.2 General features

The children in the study group as a whole were moderately malnourished, with mean mid upper arm circumferences around 12.5cm, and all Z scores being in the region of -1.5 to -2. Prevalence of moderate and severe wasting, stunting and underweight within the group as a whole was 25, 28 and 51 percent respectively (using Z score cutoffs of below -2 for all three indices). These levels are similar to the UNICEF figures for Tanzania as a whole over the period 1980 - 1992 of 29, 10 and 58 percent respectively [49]. Immunisation rates were relatively low (only 59% fully immunised for age) considering the well developed child health system that exists within the city, and the rates of 70-80% achieved nationally [257,258]. This may reflect lower socioeconomic status in the study group, resulting in diminished likelihood of accessing preventative health care. The low mean haemoglobins are probably due to the effect of chronic malaria.
4.3.2.1 Current and past illness

Admission features in this study are compared with those in five other studies of dietary management of acute diarrhoea in similar-aged children in Table 30. Only randomised controlled clinical trials in children less than 36 months in developing countries, published in English-language journals since 1985 (following widespread introduction of standardised ORT treatment protocols [161]), have been selected from a literature search. For purposes of comparison, studies using solid foods have been selected preferentially. No such studies were found for African countries. The small number of studies selected were all performed in Peru, and were published between 1988 and 1993 [162-165,167]. There were two important differences between these studies and the current one under discussion: firstly, these studies excluded moderately to severely malnourished children (based on weight-for-height criteria); and secondly, in most of the studies, children were no longer breast fed for more than one breast-feed per day.

Stooling frequency was about twice as frequent as normal in this study, with a mean of 4.2 stools reported in the 24 hours prior to admission. This is less than stooling rates reported in the other studies, which range from 7.6 to 12.0. This suggests that children in this study had less severe diarrhoea. However, the duration of diarrhoea (2-3 days) was similar or longer to that reported in the other studies, and the severity of dehydration was similar as far as can be assessed.

Vomiting, fever, and cough were the main presenting symptoms in addition to diarrhoea. The majority of children (70%) had a reduced appetite or were not eating, 80% had been vomiting, and 46% had a fever of ≥ 37.5°C. The studies cited in Table 30 reported similar rates of vomiting (range 59 - 85%) and fever (21 - 46% for temperature > 38°C).

Although there were no significant differences between acute phase protein levels between the groups, there was a trend for the experimental groups (particularly AMD) to have higher levels. This is in keeping with the trend for more antibiotics to have been prescribed in these groups, which also did not reach significance, and the difference in referral patterns, with a greater number in these groups being referred from other hospitals suggesting these patients were more ill. However, proportions found to have
either systemic infection or malaria were very similar in each group. The overall interpretation of the data relating to severity of illness, and presence of complicated diarrhoea is that the groups were not significantly different.

Most children (76%) had been ill previously, and of these 82% had been ill during the previous 6 months, but only 13% (13 children of the 99 in whom this information is available) had had a diarrhoeal illness in the past 6 months. Although incidence figures cannot be calculated from the available data, if an average attack frequency of 2 episodes in 6 months is assumed for the 13 affected children, the corresponding incidence would be 0.26 episodes per 6 months. This suggests a relatively low incidence of diarrhoea, particularly in view of the prevalence of malnutrition. Tomkins et al. reported a diarrhoeal attack rate corresponding to between 2.5 and 3.8 episodes per child in 6 months (the higher figure for wasted children (W/H < 80%)) for children aged 6 - 36 months in Nigeria [259]. Chowdhury et al. reported incidences corresponding to a range of 0.4 - 1.0 episodes over 6 months for children 6 - 60 months in Bangladesh (again higher in malnourished children) [260], while Mata et al. found incidences of 1.9 to 2.0 per 6 months in children aged 6 - 23 months [35].
### Table 30 Comparison with previous studies of dietary management of diarrhoea: admission features

<table>
<thead>
<tr>
<th>Study</th>
<th>Dietary group</th>
<th>No</th>
<th>Age range (mo)</th>
<th>Mean Z scores*</th>
<th>Degree of dehydration (%)</th>
<th>Duration of diarrhoea before admission (days)</th>
<th>No of stools in 24 h prior to admission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peru 1988*</td>
<td>Casein, sucrose, oil (CSO)</td>
<td>31</td>
<td>3-36</td>
<td>-0.98 -1.37</td>
<td>- 16 -</td>
<td>1.6</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Dilute (CSO)</td>
<td>29</td>
<td>3-36</td>
<td>-1.03 -1.16</td>
<td>- 21 -</td>
<td>1.7</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>ORT 48h then dilute CSO</td>
<td>34</td>
<td>3-36</td>
<td>-1.03 -1.54</td>
<td>- 18 -</td>
<td>1.6</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>iv fluids 48h then dilute CSO</td>
<td>34</td>
<td>3-36</td>
<td>-0.60 -1.42</td>
<td>- 21 -</td>
<td>1.5</td>
<td>8.6</td>
</tr>
<tr>
<td>Peru 1991*</td>
<td>Soy formula</td>
<td>29</td>
<td>5-24</td>
<td>-0.50 -0.96</td>
<td>79 21</td>
<td>2.0</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>Wheat-peas</td>
<td>28</td>
<td>5-24</td>
<td>-0.62 -1.10</td>
<td>71 28</td>
<td>2.7</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>Potato-milk</td>
<td>28</td>
<td>5-24</td>
<td>-0.92 -0.65</td>
<td>71 28</td>
<td>2.4</td>
<td>9.3</td>
</tr>
<tr>
<td>Peru 1992*</td>
<td>Rice-beans</td>
<td>25</td>
<td>6-24</td>
<td>-1.19 -1.02</td>
<td>36 64</td>
<td>2.4</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>Rice-soy isolate</td>
<td>25</td>
<td>6-24</td>
<td>-1.07 -0.99</td>
<td>42 57</td>
<td>2.5</td>
<td>9.4</td>
</tr>
<tr>
<td>Peru 1993*</td>
<td>Soy formula + fibre</td>
<td>19</td>
<td>2-24</td>
<td>-0.83 -1.90</td>
<td>- -</td>
<td>2.2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Soy formula</td>
<td>15</td>
<td>2-24</td>
<td>-0.94 -1.69</td>
<td>- -</td>
<td>2.5</td>
<td>12</td>
</tr>
<tr>
<td>Peru 1993*</td>
<td>Liquid amylase digested</td>
<td>29</td>
<td>9-20</td>
<td>0.20 -1.4</td>
<td>- - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>potato-milk-sugar-oil</td>
<td>27</td>
<td>9-20</td>
<td>0.20 -1.7</td>
<td>- - -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>This study</td>
<td>Conv</td>
<td>39</td>
<td>6-25</td>
<td>-2.2 -1.5 -1.5</td>
<td>13 74 13</td>
<td>3.0</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>AMD</td>
<td>37</td>
<td>6-25</td>
<td>-2.0 -1.1 -1.6</td>
<td>18 55 27</td>
<td>2.8</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>FAD</td>
<td>36</td>
<td>6-25</td>
<td>-2.2 -1.5 -1.5</td>
<td>19 56 17</td>
<td>2.4</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Studies cited:

- a Brown et al., 1988 [162]
- b Alarcon et al., 1991 [163]
- c Alarcon et al., 1992 [165]
- d Brown et al., 1993 [164]
- e Marquis et al., 1993 [167]

* Studies a - e excluded malnourished children variously defined as W/H Z score < -2 (a-c), < -2.5 (d), or W/H < 3rd percentile (e).
Table 31 Comparison with previous studies of dietary management of diarrhoea: outcome features

<table>
<thead>
<tr>
<th>Study</th>
<th>Dietary group</th>
<th>No</th>
<th>Time to cessation (hrs)</th>
<th>Mortality</th>
<th>Dietary energy density (kcal/100g)</th>
<th>Energy intake from study food days 1-4 kcal/kg/day †</th>
<th>Breast fed children included ‡</th>
<th>Weight change (days 1-4)</th>
<th>Treatment failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peru 1988</strong></td>
<td>Casein, sucrose, oil (CSO)</td>
<td>31</td>
<td>144</td>
<td>0</td>
<td>73</td>
<td>-</td>
<td>No</td>
<td>+175g</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Dilute (CSO)</td>
<td>29</td>
<td>134</td>
<td>0</td>
<td>37</td>
<td>-</td>
<td>No</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>ORT 48h then dilute CSO</td>
<td>34</td>
<td>129</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>No</td>
<td>-75g</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>iv fluids 48h then dilute CSO</td>
<td>34</td>
<td>135</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>No</td>
<td>-75g</td>
<td>3</td>
</tr>
<tr>
<td><strong>Peru 1991</strong></td>
<td>Soy formula</td>
<td>29</td>
<td>154</td>
<td>0</td>
<td>73</td>
<td>97</td>
<td>No</td>
<td>-20g</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Wheat-peas</td>
<td>28</td>
<td>57</td>
<td>0</td>
<td>73</td>
<td>102</td>
<td>No</td>
<td>+70g</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Potato-milk</td>
<td>28</td>
<td>55</td>
<td>0</td>
<td>73</td>
<td>99</td>
<td>No</td>
<td>+25g</td>
<td>7</td>
</tr>
<tr>
<td><strong>Peru 1992</strong></td>
<td>Rice-beans</td>
<td>25</td>
<td>60</td>
<td>0</td>
<td>80</td>
<td>128</td>
<td>No</td>
<td>No change</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Rice-soy isolate</td>
<td>25</td>
<td>121</td>
<td>0</td>
<td>80</td>
<td>125</td>
<td>No</td>
<td>Declined 100-200g</td>
<td>14</td>
</tr>
<tr>
<td><strong>Peru 1993</strong></td>
<td>Soy formula</td>
<td>15</td>
<td>163</td>
<td>0</td>
<td>68</td>
<td>117</td>
<td>No</td>
<td>+50g</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Soy formula + fibre</td>
<td>19</td>
<td>43</td>
<td>0</td>
<td>71</td>
<td>117</td>
<td>No</td>
<td>+50g</td>
<td>13</td>
</tr>
<tr>
<td><strong>Peru 1993</strong></td>
<td>Liquid amylase digested potato-milk-sugar-oil</td>
<td>29</td>
<td>103</td>
<td>0</td>
<td>100</td>
<td>41 (in 18 breast fed children)</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Semi-solid potato-milk-sugar-oil</td>
<td>27</td>
<td>103</td>
<td>0</td>
<td>100</td>
<td>62 (in 12 breast fed children)</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>This study</strong></td>
<td>Conv</td>
<td>39</td>
<td>42</td>
<td>0</td>
<td>38</td>
<td>32</td>
<td>Yes</td>
<td>-50g</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>AMD</td>
<td>37</td>
<td>46</td>
<td>8</td>
<td>58</td>
<td>46</td>
<td>Yes</td>
<td>+12g</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>FAD</td>
<td>36</td>
<td>47</td>
<td>11</td>
<td>58</td>
<td>37</td>
<td>Yes</td>
<td>-52g</td>
<td>31</td>
</tr>
</tbody>
</table>

Studies cited:

a Brown et al., 1988 [162]
b Alarcon et al., 1991 [163]
c Alarcon et al., 1992 [165]
d Brown et al., 1993 [164]
e Marquis et al., 1993 [167]

† Energy intake from daily means averaged over 1st 4 days
‡ Breast feeding defined as more than one breast feed per day
4.3.2.2 **Home dietary management and rehydration**

Thin maize porridge (uji) and breast milk comprised the most common feeding regime during the 24 hours prior to admission, with few (around 14%) mothers offering thick porridge (ugali) to their child during this period. This indicates that low viscosity feeds are preferred by the mother-child dyad during diarrhoeal illness. It is also of note that most children received thin maize porridge (uji) without any high energy suplementation. This would be likely to have an energy density of around 0.2 kcal/gram. Thus, home dietary management largely consisted of low viscosity, low energy feeds. However, feeds of high energy density should be encouraged instead [145,157] (see section 1.4.3, p60). Many children were being given thin maize porridge supplemented with higher-energy foods such as groundnuts, oil and fish, immediately prior to becoming ill (e.g. 44% were receiving groundnuts). This would imply that in many cases the change to low energy density foods during diarrhoea is due to a belief that higher-energy foods are not appropriate during this illness, rather than due to lack of resources. There remain a large number of children who do not receive high-energy supplements either when ill or well.

WHO figures for Sub-Saharan Africa in 1992 showed that 59% of all diarrhoeal episodes in children were treated with ORT in 1992 (quoted by UNICEF [261]). In our group, only 35% of children had been offered oral rehydration therapy (ORT), of which the majority was a commercially prepared solution. All these children were already partially weaned, and all should have been offered ORT. This suggests a failure of primary health care either to educate this group of mothers about the importance of this therapy, or to make it accessible. The importance of continued promotion of oral rehydration cannot be overemphasized [144,145,152]. This is the cornerstone of management of acute diarrhoea, and without it dietary management is largely irrelevant.

4.3.2.3 **Early feeding practices**

Fifty-eight percent of infants were also given food or fluid other than breast milk within the first week of life, and this figure had risen to 78% by the age of 3 months. Supplementation within the first month of life was usually with water. Although this often had been boiled, the practice nevertheless constitutes an unnecessary infection
hazard, and should be discouraged. In some cultures, there is a belief that colostrum is harmful and therefore water or formula milk must be given instead. In this community, however, there seemed to be little cultural resistance to infants being given colostrum in the neonatal period, since 86% received it. The most likely reasons for supplementation of breast feeding are: a) a perception that breast milk or colostrum is inadequate, perhaps particularly in a hot climate, and b) working mothers are unable to exclusively breast feed. Several studies have shown that additional water is not needed for healthy breast-fed infants in hot climates [262], and it seems that this message needs to be propagated further.

4.3.2.4 Socioeconomic factors

Socioeconomic factors in the study group are compared with the 1988 Census data for the Dar es Salaam population in Table 32. Although there is a broad similarity between households represented in this study and those in the 1988 Census data, the overall data suggests that the study children came from households of below-average socioeconomic class. In the study, more of the mothers were less educated, and more of the families did not own their own accommodation and used pit latrine toilets. However, the households in the study were slightly better off for electricity and piped water supplies, possibly due to improved provision throughout the city in between the Census and the study.
Table 32 Comparison of socioeconomic factors in the study group with the 1988 Census Data for the Dar es Salaam urban population

<table>
<thead>
<tr>
<th>Factor</th>
<th>Study group (%)</th>
<th>Census data (%) †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother illiterate</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Mother’s education standard 4 or less</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td>Mother not in paid employment</td>
<td>77</td>
<td>61</td>
</tr>
<tr>
<td>Family in rented accommodation or housing belonging to a relative</td>
<td>66</td>
<td>52</td>
</tr>
<tr>
<td>No electricity in the home</td>
<td>44</td>
<td>58</td>
</tr>
<tr>
<td>Pit latrine toilet</td>
<td>87</td>
<td>80</td>
</tr>
<tr>
<td>No piped water inside</td>
<td>65</td>
<td>69</td>
</tr>
</tbody>
</table>

† Data extracted from the 1988 Population Census for Dar es Salaam, United Republic of Tanzania [236]. Maternal characteristics have been compared to figures for females aged 15 - 39 years.

4.3.2.5 Acute phase proteins

A high proportion (86%) of children had an acute phase protein response, with similar proportions for complicated and uncomplicated diarrhoea. The children with uncomplicated diarrhoea had clinically non-invasive diarrhoeal disease, which is not recognised to cause an APP response [227,263]; children with bloody/invasive diarrhoea, which does provoke an APP response [264,265], were excluded. There are three possible explanations for the high prevalence of an APP response in apparently uncomplicated diarrhoea. Firstly, these children may have had concomitant subclinical or unrecognised systemic infection. Secondly, non-invasive diarrhoea may selectively induce an APP response (which is mainly activated by the interleukin IL-6 [266], without the fever that would normally accompany it (mediated largely via IL-1 [267]). In this study, presence of fever influenced SAA in a regression model, but otherwise there was no correlation between fever and acute phase proteins, suggesting some separation of the pathways of activation of these responses. Thirdly, the use of AGP, which is relatively “slow” marker, may have meant that the residual effects of recent infections which had clinically resolved were being seen. AGP may be chronically elevated in children in developing countries experiencing frequent infections. Fifty
seven percent of asymptomatic children in a population study in Ghana had an elevated AGP; many of these had malarial parasitaemia [268]. The lack of an APP response in some of the children in the complicated group was not related to nutritional status, although malnutrition is thought to impair ability to mount an APP response [232]. CRP was found to be the best marker for systemic infection. SAA was less useful, and AGP had no discriminative power. A CRP threshold of 30 mg/l gave high specificity (96%), and could correctly predict the presence of complicated diarrhoea in 95% of cases.

Children with systemic infection complicating their diarrhoea had nearly twice the duration of diarrhoea, with significantly increased stool frequencies on days 1 and 3. The more severe diarrhoea seen in association with complicated diarrhoea may be due to a systemic mechanism. Prostaglandins and other inflammatory mediators (such as interleukin-4) which circulate during systemic illness may directly modulate epithelial function resulting in net intestinal secretion [137,269,270]. A better understanding of these mechanisms may in the future lead to specific therapies for complicated diarrhoea.

The majority of children with systemic infection had pneumonia, malaria, otitis media or urinary tract infection. These are often amenable to diagnosis by careful clinical examination, and some basic investigations. The APP results in this study emphasise the need for a high index of suspicion for systemic infection in malnourished children hospitalised with acute diarrhoea. Diarrhoeal management therefore needs appropriate rehydration measures, adequate dietary management, and effective treatment of bacterial and malarial infection, which is often present. CRP is a useful marker for systemic infection in community and clinical studies and can play a valuable role in studies among children with diarrhoeal disease who are at high risk of systemic infection.
Table 33 Comparison of this study with the Jamaican study

<table>
<thead>
<tr>
<th>Study frame</th>
<th>Jamaican study†</th>
<th>This study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study frame</td>
<td>Energy intake in healthy children</td>
<td>Energy intake in ill children</td>
</tr>
<tr>
<td></td>
<td>&quot;Laboratory-style&quot; study maximises control but at expense of reducing applicability to normal clinical practice</td>
<td>Clinical study of interventions that might be reasonably expected to be used in normal practice</td>
</tr>
<tr>
<td></td>
<td>Focus entirely on child-food interaction (ignoring mother's influence) by isolating child from mother; child fed by university graduates following highly standardised feeding protocol</td>
<td>Focus on mother-child-food interaction by keeping mother and child together; child fed by mother, as would normally occur on ward</td>
</tr>
<tr>
<td></td>
<td>Exclude breast-fed and malnourished children (W/H &lt; 90 percentile)</td>
<td>Include breast-fed and malnourished children</td>
</tr>
<tr>
<td></td>
<td>Assume thick or thin food may be suitable for well children</td>
<td>Assume only thin food suitable for ill children</td>
</tr>
<tr>
<td>Main question addressed</td>
<td>Which diet achieves greatest energy intake in well children: thin, thick or thinned?</td>
<td>Which diet achieves greatest energy intake in children with diarrhoea: thin (Conv), thinned (AMD) or thinned/fermented (FAD)?</td>
</tr>
<tr>
<td>Description of study design</td>
<td>Randomised crossover trial on metabolic paediatric ward</td>
<td>Randomised controlled trial on diarrhoea treatment unit</td>
</tr>
<tr>
<td>Study subjects</td>
<td>15 healthy children aged 6 - 15 months who have recently completed inpatient treatment programme for malnutrition</td>
<td>75 children aged 6 - 25 months admitted with acute diarrhoea</td>
</tr>
<tr>
<td>Study diets</td>
<td>Diets comparable for most parameters; some ingredients expensive or not available locally; diets prepared using microwave oven</td>
<td>Diets different for several major parameters; all ingredients available locally; prepared using standard local electric stove</td>
</tr>
<tr>
<td>Maize flour</td>
<td>Thin Thick Thinned</td>
<td>Conv AMD FAD</td>
</tr>
<tr>
<td>Dried skimmed milk + sugar</td>
<td>+ + +</td>
<td>- - -</td>
</tr>
<tr>
<td>Oil</td>
<td>- + +</td>
<td>- - -</td>
</tr>
<tr>
<td>Industrial amylase</td>
<td>- - +</td>
<td>- - -</td>
</tr>
<tr>
<td>Vanilla</td>
<td>+ + +</td>
<td>- - -</td>
</tr>
<tr>
<td>Sorghum ARF</td>
<td>- - -</td>
<td>- + +</td>
</tr>
<tr>
<td>Groundnuts</td>
<td>- - -</td>
<td>+ - -</td>
</tr>
<tr>
<td>Viscosity (cps)</td>
<td>280-480 3000-4000 280-480</td>
<td>798 597 543</td>
</tr>
<tr>
<td>Energy density (kcal/100g)</td>
<td>51 98 97</td>
<td>38 58 58</td>
</tr>
<tr>
<td>Feeding</td>
<td>Fed ad libitum; no information on initial amount offered; thin feeds drunk from cup, thick fed using spoon</td>
<td>Fed ad libitum; 350g initially offered (always more than adequate); spoon used for all diets</td>
</tr>
<tr>
<td>Intake measurements</td>
<td>Increment in body weight (to 1g) with adjustment for insensible losses</td>
<td>Subtraction of weight of remaining food from starting weight</td>
</tr>
<tr>
<td>Results</td>
<td>Amylase-digested (thinned) feed achieved 48% higher energy intake than thin feed; thick feed 35% higher than thin feed</td>
<td>Amylase-digested feed achieved 42% higher energy intake than thin (conventional feed); FAD 15% higher than thin feed</td>
</tr>
<tr>
<td>Main conclusion</td>
<td>Thick and thinned equally good, but thin simpler to promote</td>
<td>AMD best for improving energy intake, but FAD also has a place in dietary management</td>
</tr>
</tbody>
</table>

† Stephenson et al., 1994 [271]
4.4 Dietary intake

4.4.1 Amylase-digested feed (AMD)

The energy intake data shows that use of an amylase-digested feed (AMD) results in a significantly greater energy intake during acute diarrhoea than use of a conventional maize porridge. Weight of food consumed was similar, suggesting that both foods were equally acceptable to each mother-infant pair, but the increased energy density of AMD enabled a much higher energy intake to be achieved. The increased mean intake over the first 4 days of the study was 42 percent.

4.4.1.1 Comparison with studies evaluating effect of viscosity reduction in healthy children

Where feeds of similar viscosities but different energy densities have been compared, increases in energy intake of between 20 and 148 percent have been reported [99,104,105] (see Table 5 & Table 6, pp48-49). In studies where feeds of differing viscosities but similar energy densities have been compared, results have been less consistent, with most studies finding increases in energy intake for the amylase-digested feeds of around 100 percent, but with large extremes in both directions (from +529% in one study to -55% in another) [73,76,78,92,98-103]. All these studies were in healthy children. However, all these studies were methodologically weak, and lacked scientific rigor (see section 1.3.1.5, p47).

A study of particular relevance to this one was a Jamaican feeding trial reported by Stephenson et al. in 1994 [271]. The two studies are compared in Table 33 (p158). There is an important degree of overlap in the main questions being addressed by both studies, in that both examine whether an amylase-digested feed will improve energy intake compared to a conventional thin feed. Stephenson’s group have chosen to address this question in the context of the healthy child, while in this study, children with diarrhoea have been the focus. The differences between the studies are instructive because they highlight the importance of what might be called the “study frame”. This is the perspective used to view the underlying problem, and what defines the components of the problem that have been chosen for inclusion within the study. Thus, both studies are located around the same problem of low energy density feeds and dietary bulk. This study has focused on children with diarrhoea, because this represents
a crucial area in the genesis of malnutrition (see section 1.1.3), but what children eat when they are healthy is also of great importance. Use of ill as opposed to well subjects has required basic differences in the study design, in particular because a crossover design would not be appropriate in children with diarrhoea, since dietary intake is not in "steady state". Although the decision by Stephenson et al. to isolate children from their mothers for the purposes of feeding seems logical with respect to allowing maximal standardisation, it could be argued that this creates an unduly artificial situation. In most hospitalised children in developing countries, the mother remains the child feeder, and it is likely that the behaviour of the mother-child dyad is of far greater practical importance than that of the isolated child. This is particularly the case in children under 2 years of age, where attachment behaviour is maximal, and a child may react very differently to its mother than to another feeder. Although the two iso-viscous thin feeds in the Jamaican study were of lower viscosity than the feeds used in this study, the findings with respect to improved energy intake for the amylase-digested feed are similar: 48% increase in that study compared to 42% in the current study. Stephenson et al. found a marginally lower intake in the thick diet group, but suggested that such a feed would be simpler to promote, and therefore favoured use of thicker feeds as a means of increasing energy intake. However, it should be noted that the thick feed used had a viscosity of 3000 - 4000 cps (i.e approximately soup-like consistency), and therefore was actually quite thin. In this study it was assumed that ill children (and their mothers on their behalf) prefer thin feeds, on the basis of normal practice in Tanzania, and literature to this effect [69]. This assumption is supported by the fact that very few mothers had given thick porridge to their child in the 24 hours prior to admission. However, there are no randomised trials examining the use the influence of varying viscosity on dietary intake during illness.

4.4.1.2 **Comparison with studies evaluating effect of viscosity reduction in children with diarrhoea**

In the year following this study, two reports of studies on non-hospitalised children with acute diarrhoea were published. The first was performed in Peru by Marquis et al., and used an industrial α-amylase and full-fat dried milk, in addition to local ingredients, to produce feeds of the same high energy density (1.0 kcal/g) but different viscosities (1,350 versus 5,900 cps) [167]. In a community-based randomised trial, these were fed for two days to children between 9 and 20 months of age with acute diarrhoea being
treated at home, and viscosity was not found to influence either total energy intake or energy intake from the study diet. The lack of an effect of viscosity in this study is likely to be due to a threshold effect, where bulk properties become relatively unimportant once a certain energy density level is reached. This threshold level is probably around 0.7 - 1.0 kcal/g, which is the level that may be considered an adequate weaning food energy density (see section 1.2.3.1.4). Since in Marquis' study mother’s recall, rather than direct weighing, was used to estimate dietary intakes for one-third of the study period, the study was inevitably more open to recording bias.

The second study of amylase-digested foods in the dietary management of diarrhoea was performed in Bangladesh by Mahalanabis et al., and published as a short communication which lacked important details such as feed energy density [168]. In this study, two feeds of the same energy density but very different viscosities (1,700 versus 81,300 cps) were produced by the addition of wheat ARF to one of them. Children between 5 and 12 months of age were randomised to receive a single meal of one or other food, and 40 percent more of the low viscosity feed (both in terms of weight and energy) was consumed. Although the energy density of the feeds is not given, they are described as “energy-dense”, and it is likely that their energy density was in the region of 0.7 - 1.0 kcal/g. Although this is above the postulated threshold, it is probable that a viscosity effect has been demonstrated because of the large difference in feed viscosity (around 50-fold, compared to approximately 5-fold in the study by Marquis et al.).

4.4.2 Fermented and amylase-digested feed (FAD)

Children on FAD consumed a lower mean weight of food per day than the conventional group, which may indicate that it was less acceptable. This study therefore does not support previous anecdotal reports that ill children prefer fermented foods [107]. Possible reasons include temperature (served cold), taste, and the perception of it by some mothers as a drink containing little nourishment. Despite this, FAD performed at least as well as the conventional group with regard to energy intake.

4.4.3 Other factors

Although there were some data suggesting an increased burden of systemic illness in the experimental groups, no indicators for systemic illness (including final diagnosis of
complicated or uncomplicated diarrhoea, whether received antibiotics, temperature during admission, and acute phase proteins) were found to influence dietary energy intake. Therefore, this slight difference between groups, which was not statistically significant, did not influence the findings of the study with respect to energy intake.

The use of groundnuts in only one of the three groups (the conventional), instead of all three, was for several reasons. Normal ward management is to use a maize feed with groundnuts, so this was used as the control. Addition of groundnuts to FAD would have resulted in a rancid product. Their omission from the amylase-digested feeds allowed comparison of two different methods of improving energy intake (amylase digestion and high energy supplementary food). When both can be used together the effect on energy intake would be additive.

In this study breast milk intakes were not measured. This was because the normal pattern of very frequent breast feeds in Tanzanian culture precluded meaningful weighings of separate feeds. Consideration of several studies that have measured breast milk intake during diarrhoea show that it varies little during illness and health. A carefully performed longitudinal study of infants in Peru showed that breast milk energy intake did not change during diarrhoea or fever, while intake from other sources fell by 20 - 30% [272]. Results drawn from three clinical studies in developing countries are compared in Table 34 [44,47,167]. The measured reduction in total energy intake during diarrhoea of between 11 and 49 percent is due to reduced calorie intake from weaning foods, while there is little change in breast milk intake. Brown and Perez have reviewed a number of other studies, with a similar conclusion that breast milk consumption remains constant during illness, and it is the dietary component from other foods that is compromised [273]. Thus, in our study, the increased energy intake in the AMD group is likely to represent a real increase in total energy intake.

The energy intakes from the study foods are relatively low compared to the studies cited in Table 30 & Table 31. This is likely to be due to two factors: most of these studies were in non-breast fed children, and therefore the child’s complete dietary intake comprised study food, whereas in this study breast milk undoubtedly comprised a significant proportion; secondly, the energy densities of foods used in the other studies were relatively high, therefore enabling a greater energy intake. In this study, the foods
chosen were much more likely to be representative of what could be achieved for normal hospital dietary management using locally available foods, but were therefore lower in energy density. Non-breast-milk energy intakes in our non-modified feed group were similar to those in the studies of children with diarrhoea managed with basic, locally available foods, reviewed in Table 34. The benefits of amylase addition in our study suggests that this technology might be of value in other communities.
<table>
<thead>
<tr>
<th>Author, Year and Country of study</th>
<th>Groupings (number in group)</th>
<th>Age range (months)</th>
<th>Mean weight for age Z score *</th>
<th>Energy intake (breast milk/total kcal/kg/day)</th>
<th>Change in energy intake during diarrhoea (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoyle et al, 1980 Bangladesh [44]</td>
<td>No dietary education (15)</td>
<td>6 - 35</td>
<td>-3.2</td>
<td>47/75 t</td>
<td>-5 -42</td>
</tr>
<tr>
<td></td>
<td>Dietary education (15)</td>
<td>6 - 35</td>
<td>-3.3</td>
<td>49/61 t</td>
<td>-4 -49</td>
</tr>
<tr>
<td></td>
<td>Healthy controls (11)</td>
<td>6 - 35</td>
<td>-3.1</td>
<td>† 54/130 t</td>
<td>† 54/130 t</td>
</tr>
<tr>
<td>Dicken et al, 1990 Nigeria [47]</td>
<td>No grouping (45)</td>
<td>5 - 28</td>
<td>-1.9</td>
<td>53/85 51/96 t</td>
<td>+2 -11</td>
</tr>
<tr>
<td>Marquis et al, 1993 Peru [167]</td>
<td>Liquid diet (with amylase) (18)</td>
<td>9 - 20</td>
<td>-1.0</td>
<td>25/66 25/93</td>
<td>0 -29</td>
</tr>
<tr>
<td></td>
<td>Semisolid diet (12)</td>
<td>9 - 20</td>
<td>-1.3</td>
<td>22/84 18/109</td>
<td>+4 -23</td>
</tr>
</tbody>
</table>

* Weight for age Z score was included in Dicken et al’s paper, but not in the other two papers, and an approximate figure has been derived from available anthropometrical indices.

† Intakes during diarrhoea were compared to the control group.
4.5 Clinical Outcomes

In view of the hydrolysis of starches to simple sugars and dextrins in AMD and FAD, resulting in a sweeter taste and a higher osmolality, there are theoretical grounds for proposing that consumption of these feeds could result in an osmotic diarrhoea. However, our results show that there were no significant differences in stool frequency or duration of diarrhoea between the groups. The geometric mean duration of diarrhoea in this study was 45 hours (from time of entry to the study). Outcome features for the studies already cited in Table 30 (p152) are compared with this study in Table 31 (p153), and it can be seen that duration of diarrhoea was usually longer in these studies.

There are several possible explanations for the shorter duration of diarrhoea in this study. Firstly, these children may have had less severe diarrhoeal disease. The lower reported stool frequency on admission supports this. Secondly, the dietary regimes used in all three groups in this study may have been superior to those used in the other cited studies. These studies used a variety of feeds (see Table 30). Feeds containing solids tended to achieve a shorter duration of diarrhoea in these studies. There are no published studies of the use of a groundnut-maize feed in the dietary management of diarrhoea, and it may be that this is effective in shortening the duration of diarrhoea. Thirdly, the different definitions used for cessation of diarrhoea may have resulted in a shorter apparent duration in this study. The definition most commonly used in the Peruvian studies was the number of hours post-admission until excretion of the last liquid or semiliquid stool not followed by another liquid or semiliquid stool within 24 hours [162-165]. This was not found to produce a satisfactory endpoint for many children in this study, whose diarrhoea was considered to have stopped by mothers and medical staff, but who either did not have a formed stool, or continued to have occasional semiliquid stools within 24 hours following formed stools. For this reason, a different definition was used in this study, which produced endpoints for diarrhoea in all children by the time of discharge. However, it is likely that this resulted in shorter overall durations being recorded.

The mortality of 6 percent in this study is high compared to the studies cited above, where no children were reported to have died. However, children in this study were relatively much more malnourished, and most of the mortality in this study was in
DISCUSSION

severely malnourished children who were not represented in the other studies. The proportion of treatment failures was also relatively high in this study, probably for the same reason, along with the associated high incidence of systemic infection.

Weight changes in this study over the first 4 days, and at follow up, showed no significant difference between groups. Over the first four days, the individual weight changes (compared to weight on entry to the study) ranged between around -10 to +10%, although with fewer positive gains in the FAD group (see Figure 30, p132). The mean weight changes for the three groups at day 4 were between -0.3 to -1.2%, which corresponds to a change in weight of between -50 and +12 grams, and is similar to that reported in the studies cited in Table 31 (p153). However, these studies used the admission weight before rehydration as their reference, and therefore might have been expected to show greater weight gains.

One initial aim of the study was to determine whether use of the experimental weaning foods would reduce growth faltering. To accomplish this, mothers were asked to keep their children on their study food for the first month after follow-up, in order to detect an effect on weight gain. However, it was not possible to enforce this, and at follow up, between 35 and 70 percent of mothers in the experimental groups did not cite their child's study food as a main food (see Table 28, p143). For this reason, it was not possible to determine whether the study foods had significantly different effects on weight gain after discharge.

4.6 Intestinal Permeability

This study has also investigated the effect of the different dietary treatments on recovery of the intestinal mucosa after acute diarrhoea, by measurement of intestinal permeability, assessed by urinary excretion of lactulose and mannitol following a test dose of these sugars.

The results show that children admitted to hospital with acute diarrhoea had a raised intestinal permeability, as shown by a high L/M ratio (geometric mean 0.85, CI 0.68-1.05), compared with healthy controls (0.14, 0.12-0.17). The Tanzanian controls compare well to healthy UK infants, with a L/M ratio of 0.12 (SD 0.09) [207]. The L/M ratio of study children fell over time, and by the first and second follow ups (approx 17 and 32 days after admission) L/M ratios were close to values found in the control.
children. Previous work has suggested that severely malnourished children, many of whom have diarrhoea, have chronically raised L/M ratios [274].

Because study diets were not regularly continued after discharge, analysis of the effect of dietary treatment on intestinal permeability has concentrated on the fall in L/M ratio by 3 days after admission.

The fall in L/M ratio by day 3 was significantly greater in the FAD group than the other two groups. Comparison of groups on the basis of whether there was a fall in L/M ratio after 3 days exposure to the study food showed a highly significant difference between groups, with more than twice as many patients in the FAD group showing a fall compared to the conventional group, and a similar but less marked effect in the AMD group (75% compared to 44%). It should be noted that the FAD group had a higher initial L/M ratio compared to the other groups. Although this was not statistically significant, it makes the subsequent fall in L/M ratio all the more notable. The analysis of the individual variation of the sugar probes shows that differences between the FAD and the other two groups is mainly explained by changes in the urinary lactulose excretion which showed a large reduction between entry to the study and Day 3.

There are four main mechanisms by which the observed beneficial effects could have occurred. Firstly, there may be a nutritional basis, either due to increased intake of energy and nutrients promoting improvements in systemic nutritional status, and thence mucosal recovery, or because of local delivery of substances important for mucosal cell nutrition and recovery. The former seems less likely here, because the predominant effect on intestinal permeability was in the FAD group, while it was the AMD group who consumed the most energy. Short chain fatty acids (SCFA), which are a normal product of anaerobic fermentation of carbohydrates in the large bowel, have been shown to be important for local mucosal nutrition and growth in the colon in rats [173], and it is possible that similar beneficial fermentation products are present in FAD. Secondly, a microbiological mechanism may be important, since the fermentation process inhibits growth of enteropathogens [122] and production of enterotoxins (personal communication, R.Kingamkono 1995). Thirdly, an immunological mechanism could be involved, since when the mucosal barrier is compromised during diarrhoea, large antigenic molecules are able to traverse it [275]. This may cause immunologically-
mediated local damage, which becomes self-perpetuating. Fermented products, and to a lesser extent amylase-digested foods, contain much simpler carbohydrates which are probably less antigenic. Also fermented products activate macrophages and lymphocytes, which may expedite healing [275]. The fourth mechanism that must be considered is via gut regulatory peptides. There is a massive increase in enteroglucagon during acute diarrhoea in infants, and this probably promotes enterocyte regeneration [276]. Fermented products may exert their effects via this or other peptides. Thus, mucosal recovery following gastroenteritis is a complex process which involves the interplay of many factors, and fermented foods may influence many of them. Fermented foods are widely used in many traditional cultures, and utilization during episodes of diarrhoea in children requires further study, both to determine whether promotion of mucosal recovery is a general property of such foods, and to evaluate possible mechanisms.

The results presented here suggest that a porridge which has been both amylase digested and fermented was more effective than conventional porridge in the treatment of acute diarrhoea, with respect to repair of mucosal damage, through trophic effects on intestinal epithelium. It would be interesting to evaluate the impact of more prolonged feeding with FAD on intestinal permeability in such children. These foods are widely used in traditional diets, although often not perceived as beneficial for children. Factors influencing acceptability, such as taste, temperature, and mothers' perceptions, require consideration when promoting use of such foods in differing cultures, and further research is needed in this area. In the light of work by Lunn et al which suggested that as much as 43% of growth faltering in Gambian children can be explained by chronically raised intestinal permeability [207], the value of culturally acceptable forms of fermented foods may be under-recognised. These alternative food technologies are low cost and available at the household level, and could be widely promoted as a means of combating malnutrition.

It was originally hoped that screening of urinary lactose could be useful in situations where the analysis of lactulose and mannitol would not be possible due to technical constraints. However, the sensitivity and specificity were both low so that lactose screening cannot be recommended.
4.7 Evaluation of amylase-digested foods

On entry to the study, 8 mothers (7%) reported that they knew how to make an amylase-digested porridge, and 7 were actually using such a porridge for child feeding. A larger proportion (17%) reported knowing how to make the fermented feed, and 10 were using FAD for child feeding. Thus, a small proportion of mothers (around 8%) were using AMD and FAD as part of normal child feeding. In addition, the majority of mothers (around 70%) were aware of both ARF and togwa (FAD). This relatively high awareness, and the fact that there already exists a subgroup of mothers using these products for child feeding, is important when considering their promotion within the community.

Mother’s opinions at discharge, after experiencing use of the experimental study foods for feeding their own child, were generally favorable, with between 88 and 95% saying that they liked it. FAD received slightly lower ratings for taste (83%) and AMD for texture (75%). All but three mothers thought that these foods were very useful and suitable for feeding ill children, and a smaller number expressed the same sentiment with respect to feeding healthy children (71% for AMD, and 88% for FAD). No mothers felt there was no place for these foods in the feeding of ill children. Thus, the majority of mothers reacted very positively to the use of the experimental feeds, and there were no special attitudinal problems that would preclude more widespread promotion. However, it is notable that there was a steady reduction in the proportion of mothers using the child’s study food at follow up, particularly for FAD, where 50% were using it at the first follow up, and only 30% at the second visit. The respective figures were 65 and 53% for AMD. This decline in usage may reflect mothers’ beliefs that these foods are best reserved for ill children, or may simply be due to the normal reduction in enthusiasm for any innovation that occurs with time.

The relative costs of the feeds were calculated on the basis of typical prices of grain and flour in Dar es Salaam during the study period. This results in the feeds costing similar amounts on a weight basis, but the experimental feeds were more than 30% cheaper in terms of cost per 100 kcal. Groundnuts may be much more expensive at times, and there were periods prior to the start of the study when they were not available for use on the ward. Thus, there may be considerable variation in relative costs. These costs do not
include preparation and cooking times, and although pounding of groundnuts to produce flour is time consuming, it is likely that preparation of an amylase rich flour is more so, although it can be prepared as a batch and kept for several weeks. 

Mean mean thiocyanate levels were similar in all three groups in the 27 patients tested, all of whom had consumed at least 12 consecutive study meals prior to the urine sample being obtained. This in itself suggests there is unlikely to have been significant effect of the sorghum ARF, which was only present in two of the feeds. The mean urinary thiocyanate levels were all within the range of 14.7 - 17.4 µmol/l, with higher confidence interval limits of 23.8 - 40.0 µmol/l, and the highest measured value being 80.0 µmol/l. This compares with a mean of 757 µmol/l in Zairean population affected with Konzo (due to consumption of cassava cyanogens), 50 in an unaffected Zairean population, and 31 in an unaffected Swedish population [132]. 90 µmol/l is the upper end of the normal range in children under 1 year of age [256]. This provides reassuring data indicating that sorghum grain can be used for manufacture of amylase-rich flour without risk of cyanide toxicity. This is consistent with the laboratory evidence already discussed (see section 1.3.3.1, p56). Further confirmation of this could have been obtained by measuring serum thiocyanates and urinary cyanogenic glycosides, but this was beyond the scope of this study. The other explanation for low urinary thiocyanates is that malnutrition limited sulphur availability for cyanide detoxification. However, this seems unlikely, both because some of these children were not malnourished, and previous work has shown that thiocyanate is produced even when there is a relative lack of sulphur [132].

The pH of FAD was consistently below 4.0 throughout the study period. This is the level below which enteropathogen growth has consistently found to be inhibited [110,122]. This compares with the pH of the other two study feeds of around 6.0.

Although feed osmolality was approximately 3 times higher in AMD, and 15 times higher in FAD, than the conventional feed, this did not have any appreciable effect on severity or duration of diarrhoea during the study. The higher osmolalities in the amylase-digested feeds was as expected, in view of the dextrins and sugars produced by this process. The osmolality of FAD was much higher than AMD. This is probably because of ongoing amylase-digestion during the fermentation process, which further
digests dextrins to simple sugars. By contrast, in the preparation of AMD, reboiling inactivates amylase after less than 5 minutes of activity, when starch digestion is likely to be incomplete.

Although the viscosities of the three porridges were not significantly different when measured at a consistent serving temperature of 60°C, there was a trend for these feeds to have lower viscosities than the conventional feed. FAD feed was found to have a lower mean viscosity than the other two feeds over the whole study period when measured over a range of temperatures. This may have been due to the influence of temperature on viscosity measurements in the conventional and AMD feeds, since a constant-temperature water bath was not used, and it was therefore not possible to maintain the hot feeds at a constant temperature during measurements. Also, the time elapsed since feed preparation varied, and it is possible that during storage in flasks there is some ongoing gelatinisation, even at a relatively constant temperature. The lower viscosity of the FAD feed relative to the AMD feed may indicate an effect of fermentation on viscosity reduction, but is more likely to be due to continuing amylase activity. The difference between feed viscosities is small in terms of the overall viscosity range of weaning foods, and so is unlikely to have had a significant influence on energy intake.

The energy density of the experimental feeds in the this study was relatively low compared to those used in the studies of dietary management cited in Table 30 and Table 31 (pp152-153), and also to that which would be theoretically desirable for a weaning food of 0.7 to 1.0 kcal/g (see 1.2.3.1.4, p36). In view of the fact that the amylase-digested feeds were less viscous than the conventional feed, it is likely that an energy density above 0.7 kcal/g could have been achieved while maintaining iso-viscosity with the conventional feed. This would have further improved energy intake in the amylase-digested groups.

4.8 Optimum feed viscosities for young children

It seems appropriate at this point, before leaving the discussion of the results, to reflect on optimum feed viscosities for young children, and implications for weaning practice in developing countries where low viscosity feeds of high nutrient density are difficult to achieve.
4.8.1 Feed viscosity for healthy children

The recommended viscosity ranges for feeding healthy children of 1000 - 3000 cps [75] and 2000 - 6000 cps [76] appear to have very little published clinical data to support them. It would appear from the contexts that this range is intended to apply to children between 6 months and 2 years of age, but no clear age-range is given. Standard paediatric texts for industrialised countries indicate that children may be expected to eat normal chopped family food by the age 9 - 12 months [277]. There seems no reason why this should not also apply in developing countries. Some single meal dietary intake studies have demonstrated high energy intakes from solid feeds, such as stiff maize ugali (see Table 5, p48). The viscosity ranges given above are likely to be most applicable for foods that are used in the initial phase of weaning, say from 6 to 9 months, but it would seem wise to encourage children onto a modified adult diet as soon as they are able to consume it [63]. For most children, the majority of their nutrient intake could be from such a diet by early in the second year of life, although lower-viscosity foods would supplement this diet to a gradually lessening extent. There remains a need for further work to establish optimum feed viscosities for healthy children at different ages and stages of weaning, particularly in environments where energy density is constrained by dietary bulk. If solid feeds enable similar nutrient intakes to be achieved as those using amylase-digested feeds, then strategies to promote earlier solid feeding could be evaluated.

4.8.2 Feed viscosity for ill children

Although it is assumed that ill children require feeds of low viscosity (less than 6000 cps), there is actually very little published evidence for this. The literature generally refers back to Church's paper in 1979, where a theoretical model was presented for dietary regression during illness, but this had not been tested clinically [69]. Observation of children during illness suggests that such regression occurs. However, children who are more advanced through the weaning process and are normally consuming a modified adult diet are likely to be able consume feeds of higher viscosity during illness than children who are at an earlier stage of weaning. This is particularly relevant when the recovery phase of the illness is reached. For many childhood illnesses, this may be within 2 or 3 days of the onset of symptoms. Most mothers, and experienced nurses, are able to judge when a recovering child is ready for their normal diet, and indeed young children themselves will usually indicate their feed preferences if
given a choice. There is a need to examine what is the optimum feed viscosity for ill children between the ages of 6 and 24 months, both during different phases of illness, and in relation to different stages of weaning, without prior assumptions regarding maximal viscosity. This could concentrate on diarrhoeal illness for the reasons already outlined (see section 1.1).
5 Conclusions

5.1 The importance of the weaning foods used during diarrhoeal illness

Malnutrition is a major global cause of morbidity and mortality. Infection and inadequate dietary intake are together its main immediate causes. The impact of infections is potentiated by malnutrition, and thus a malnutrition-infection cycle exists. Figure 37 (p175) indicates the key role that dietary intake during diarrhoeal illness plays in the immediate causation of malnutrition. This is because diarrhoea has the strongest relationship with malnutrition, both as a cause, and also in terms of its morbidity being increased by malnutrition. Thus, reduced dietary intake related to diarrhoeal illness occupies a fundamentally important position in the genesis of malnutrition.

The main problems with weaning practice in developing countries are weaning foods of low nutrient density and high dietary bulk, which are often contaminated with pathogens, and which are given too infrequently. These problems are even more pertinent when such weaning foods are being used in the dietary management of diarrhoea. This is therefore a key area to target for research to develop effective interventions to prevent or reduce malnutrition at the level of the immediate causes. AMD and FAD both reduce dietary bulk and increase nutrient density, while FAD also addresses the problems of feed contamination and frequency.
5.2 Main findings of the study

This study has shown that AMD, an amylase-digested weaning food produced using inexpensive local ingredients and technologies, increases dietary nutrient intake by 42 percent in the dietary management of acute diarrhoea in malnourished and hospitalised children, compared to a control diet supplemented with groundnuts. This improvement was not associated with any increased morbidity or weight loss during admission, and there was a beneficial effect on intestinal permeability. Although a longer term effect on growth could not be demonstrated within the limits of this study, it is likely that such a significant increase in energy intake during diarrhoeal illness would result in a reduction in growth faltering, particularly if this increase could be sustained over a longer period.
Although FAD had a similar nutrient density and viscosity to AMD, these advantages were offset by reduced consumption, and therefore it did not increase overall nutrient intake compared to the control diet. FAD however has other characteristics which commend its use in the dietary management of diarrhoea. Firstly, it was found to improve intestinal permeability over the first three days of the study. This indicates that FAD hastens mucosal recovery in acute diarrhoea. This was not associated with a significant clinical effect with respect to diarrhoeal symptoms or growth. It is likely that a study of greater power and longer duration than this one would be required in order to detect such an effect, since it would be of relatively small magnitude. Improved intestinal permeability will result in greater nutrient absorption and less damage due to large antigenic molecules traversing the mucosal surface, and should therefore produce an advantageous clinical effect. Since chronically raised intestinal permeability may account for a large proportion of growth faltering [207], use of FAD in diarrhoea may have considerable long term benefits. Secondly, FAD has a low pH of less than 4.0, and this is known to inhibit enteropathogen growth so that storage at room temperature for several hours is safe. This means that fresh feeds do not need to be prepared at every mealtime, and the food can be kept near the child to be offered frequently in small amounts, thus maximising intake. Thus, the use of FAD addresses the problems of feed frequency and contamination. Fermentation should of course not be viewed as a substitute for good hygiene practices in food preparation and storage, but rather as an additional method of achieving food safety, especially in adverse conditions.

AMD and FAD have complementary roles in the dietary management of diarrhoea in malnourished children, and together enable the major problems with current weaning foods to be addressed. It would be logical to use them in combination in order to achieve the greatest benefit: AMD at normal mealtimes to improve energy intake; FAD offered frequently in between meals as a snack food, further increasing energy intake and also improving intestinal permeability. Thus the food technologies of both germination and fermentation, which are applicable in many developing countries, could be promoted to improve the dietary management of paediatric diarrhoea.
5.3 Applicability of study findings to other situations

5.3.1 Applicability to other similar foods

AMD and FAD were chosen to be representative of their respective classes, but an important question is to what extent the advantages demonstrated in this study are generic to each class, or are specific to the particular foods used. Although different amylase-rich-flours may be used in a variety of ways to reduce viscosity, the physical effect on the food is measurable in terms of viscosity and energy density, and is almost certainly the prime factor influencing energy intake. Organoleptic properties (particularly sweetness, due to sugar production) will vary with the particular grain used, and the ratio of ARF to normal flour, but this is likely to be a relatively minor factor in determining intake. Therefore, the property of amylase-digested foods to increase energy intake is likely to be generic to all amylase-digested foods.

For fermented foods, the situation is more complex. Although cereal-based lactic fermentation is practised widely within Tanzania, there is considerable variation in preparation methodology across the country [278]. The main variables are: the cereal(s) used; whether an ARF is used, and if so in what proportion to the total flour; whether fermentation is carried out before or after cooking; and whether it is initiated spontaneously or by a starter culture. These determine which fermenting organisms predominate, the particular mix of organic acids produced, and the presence of other antimicrobial factors in the final product. Since the presence of organic acids (particularly lactic acid) is a general characteristic of all lactic fermentations, all such foods are characterised by low pH (usually 3.4 - 3.8) (see section 1.3.2.2.1, p53). A pH of less than 4.0 inhibits the growth of enteropathogenic bacteria, and therefore all fermented foods should have improved keeping qualities and safety with respect to enteropathogens. The tangy or sour taste that also characterises fermented foods will be influenced by the organic acids present. Thus, organoleptic properties are likely to vary with different fermentation products, and this may influence dietary intake. Although in this study children preferred non-fermented foods to FAD, this may not have been the case had other fermented products been used.
Kingamkono et al. have recently shown that use of a starter culture is more effective in inhibiting reducing pathogen growth than a spontaneous fermentation because of the more rapid reduction in pH [122]. Although spontaneous fermentation was used to prepare FAD in this study, it would therefore seem preferable to use starter culture to achieve an accelerated fermentation. This would not be expected to significantly change other qualities of the food. It should be noted that there are a group of foods which are soured but not actually fermented (for example by the addition of lemon juice), which in some cultures are not distinguished from true fermented foods [279]. These do not inhibit enteropathogen growth since their pH is not significantly reduced. The beneficial effect of the fermented food on intestinal permeability in this study is of great interest, but the mechanism is uncertain. It is therefore not possible to know whether this will prove to be a general characteristic of all lactic-fermented foods, or specific to FAD.

Thus, while improved keeping qualities are likely to be a general characteristic of all lactic-fermented foods, this may not be the case for the organoleptic properties and effect on intestinal permeability.

5.3.2 Applicability to other illnesses

Diarrhoea affects nutrition predominantly by a reduction in both dietary intake and nutrient absorption, and an increase in nutrient utilization to replace damaged enterocytes and stage an acute phase response. In other infections which have been implicated as causes of malnutrition - particularly pneumonia, malaria, and measles - the reduction in nutrient absorption is less pronounced, the acute phase response is usually of greater magnitude, and there is a variable nutrient requirement for the replacement of damaged cells. In all of these situations, the reduction in dietary intake is likely to be a major factor in leading to any growth faltering that occurs. Because the mechanism of anorexia is probably similar in diarrhoea and other infections, being mediated through IL-1 [1], it is likely that the results of this study with respect to improved energy intake from AMD will also apply to children with these other illnesses.

5.3.3 Applicability to healthy children

Most studies of the effect of ARF on dietary intake have been conducted in healthy children, although the majority have lacked scientific rigour. However, a beneficial
effect of ARF has been demonstrated remarkably consistently, and overall these studies have demonstrated increased energy intakes of around 50 to 100 percent. However, some of these studies have suggested that healthy young children can consume feeds of much higher viscosity than has been previously suggested (see section 5.6.1). It can be concluded that for AMD, the findings of this study are likely to apply to healthy as well as ill children, but a valid alternative for increasing nutrient intake may be to promote thicker feeds.

5.4 Safety issues and cautions

The principal safety issue that has been raised is that of cyanide toxicity. This concern is largely confined to foods prepared with a sorghum malt. This study has shown that, in a subset of children who had consumed at least 12 meals of AMD and FAD, there was no increase in urinary thiocyanates. This confirms that sorghum grain can be used for the manufacture of ARF for use in child feeding without risk of cyanide toxicity, providing that normal cooking procedures are followed (although this need not include devegetation). Aflatoxin contamination can be avoided by simply not using mouldy grains in the preparation of ARF. Although the study has confirmed that FAD, and to a lesser extent AMD, have a higher osmolality than the conventional feed, there was no increase in diarrhoea associated with the use of these feeds. Thus, osmotic diarrhoea does not seem to occur in practice.

An area for caution is that anorexia during diarrhoea and other illnesses has been hypothesized to be a protective mechanism for the host, possibly by reducing nutrients that would promote microbial multiplication [1,280]. If true, then strategies to increase dietary intake would be inappropriate. However, the evidence for such an effect is weak, for example being based on animal studies using force feeding techniques [280]. It would seem unwise to force-feed ill children, but rather to ensure that dietary bulk and energy density are not limiting factors for dietary intake during illness. There is a clear consensus of opinion in favour of increasing dietary intake during diarrhoea (see section 1.4.3).

5.5 Promotion of amylase-digested and fermented foods

If any dietary management of diarrhoea regime is introduced, it should recognise cultural beliefs and preferences. There has been little evaluation of the cultural
acceptability of germination and fermentation for child feeding. Available reports suggest great variability in attitudes [77,86]. Fermented foods have traditionally been used for child feeding in many areas, but their popularity has gradually declined in favour of 'modern' alternatives such as tea and fizzy soft drinks, particularly in urban areas [279]. Often this decline has been hastened by western health workers, who have stressed the need for fresh food, and so discouraged fermentation. There have been no major initiatives to promote fermented foods for child feeding.

Although ARF has for generations been used widely throughout Tanzania as a malt for brewing beer, its use for preparation of weaning foods is a relatively new concept, and some reluctance regarding its use for this purpose may stem from this association with alcohol. It is also commonly used to prepare non-alcoholic beverages. Thus, the technology for ARF production is already regularly in use in many homes. This means that use of ARF for child feeding often does not require the teaching of a new technology, but only the transfer of a familiar technology to a novel use. In this study about 70 percent of mothers were aware of ARF, but only half of these knew it could be used for child feeding.

ARF-thinned porridges have been widely promoted in Tanzania, the best known programme being the Joint WHO/UNICEF Nutrition Support Programme (JNSP) in collaboration with the Tanzanian government, which took place in Iringa between 1983 and 1988 [281]. This fostered a "bottom-up" approach to improving household food security and child survival, and encompassed numerous initiatives which largely evolved from within communities. One of these was the promotion of ARF for child feeding. During the project, severe malnutrition (W/A < 60 percent of standard) declined from 5 to under 2 percent, and overall undernutrition (W/A < 80 percent of standard) from 50 to 37 percent. The overall approach was seen as successful, and has since been applied in other parts of the country, but the longterm uptake of ARF for child feeding was disappointing [282]. Constraints for successful promotion that were identified included: difficulty in teaching mothers both the technique of how to use ARF for making thinned porridge and the underlying concepts; the association with alcohol; confusion with other health promotion messages relating to food quality; and mothers not perceiving food quality as being an important issue. The promotion of ARF as "power-flour" may have further confused some mothers, who believed that the flour
itself contained significant amounts of energy and nutrients. Changing cultural patterns of child feeding is inevitably a slow and complex process, but it is even more so when the change involved requires assimilation of several concepts and messages. For healthy children, it may be simpler to promote thicker feeds as a means of increasing nutrient intake [271], and concentrate promotion of ARF and fermented foods for children who are sick or convalescing.

5.6 Recommendations for further research

5.6.1 Optimum feed viscosity for healthy and ill children

Low viscosity feeds are likely to be particularly important for young children when they are ill. However, there is a need for further clarification of optimum viscosity ranges for healthy and ill children at different ages and stages of weaning. In addition, cultural attitudes to feed viscosities during illness and health need to be further explored. In some situations, it may be more appropriate to promote feeds of higher viscosity (particularly for healthy children) than amylase-digested feeds.

5.6.2 Do amylase-digested and fermented foods reduce growth faltering?

Although this study has demonstrated greater energy intake (particularly AMD), and a beneficial effect on intestinal permeability (particularly FAD), no effect on growth was shown. However, children were fed the study foods for too short a period to detect an effect. This was because it was thought unethical to keep children on the ward once they were fit for discharge, and it was logistically difficult to follow children to their homes and ensure continued intake of study foods. However, this remains a crucial question, since unless it can be shown that these foods reduce the growth faltering that occurs after infection, there will always remain some question regarding their widespread promotion in this context. The question would probably be best addressed using a community study, accepting that this makes accurate data collection and standardisation harder.

5.6.3 Effect of fermented foods on intestinal permeability

FAD improved intestinal permeability over a three day period following admission with diarrhoea. Such an effect could clearly be important in the dietary management of diarrhoea, and also for long-term feeding of children at risk of chronically raised intestinal permeability. However, it is necessary to evaluate this effect further. It would
be necessary to determine whether it was limited to the first few days after admission or was more prolonged, whether it also applied to the chronically elevated intestinal permeabilities seen in malnourished children, and whether it occurs with other fermented products. There is also a need to research its mechanism, both through analysis of the biochemical and microbiological composition of FAD, and possibly through animal experiments involving direct exposure of mucosal epithelial cells to different components of FAD, and measuring the effects at the epithelial level.

5.6.4 Combinations of AMD, FAD and other foods in the dietary management of diarrhoea and other illnesses

It would seem logical to combine AMD and FAD and other foods in the dietary management of diarrhoea, as mentioned above (see section 5.2, p175). This regime could be evaluated in a similar study to the current one, to determine whether there may be a synergistic effect. A more varied diet incorporating other protein sources is desirable with regard to improving the overall dietary protein quality, and such variety may be expected to improve dietary intake. AMD is particularly suitable for combination with other available supplementary protein and energy sources, such as groundnuts, fish (e.g. 'dagaa'), beans and oil. Such a varied diet could be tested against AMD, and a combination diet of AMD and FAD. In addition, there is a need to determine whether the findings of this study with respect to the improved energy intake with AMD apply to children with other illnesses and without diarrhoea, especially pneumonia, malaria and measles. It is likely that a combination of AMD and FAD will be effective.

5.6.5 Cultural acceptability of amylase-digested and fermented foods for dietary management of diarrhoea

Dietary regimes using foods like AMD and FAD are likely to be most effectively promoted through their acceptance and use in hospitals and other health facilities. Research into cultural acceptability should therefore initially concentrate on health care personnel. This could identify knowledge, attitudes, practices, and prejudices in relation to these foods, and evaluate education and promotion strategies. Although in this study there was no formal evaluation of the acceptability of AMD and FAD to health care personnel, informal discussions indicated that the foods used were very acceptable.
An industrially-produced amylase may have a place in processing weaning foods for the dietary management of diarrhoea. This may be more acceptable within health service culture, since it might be perceived as a “treatment”, and in addition, the association with beer-brewing would be removed. However, it would be necessary to evaluate this in considerable detail to justify the investment that would be required to make such an option widely available. There would also be a risk of reducing the overall use of ARF in communities in favour of using the “better” industrial amylase. This would then negate one of the important advantages of use of amylase-digestion: that it is a traditional technology available at the household level to any community.

5.7 Practical recommendations for use of amylase-digested and fermented foods in the dietary management of diarrhoea

Research outlined above will take time, and in the meantime there is a need for practical guidelines for the use of amylase-digested and fermented foods in the dietary management of diarrhoea in children between 6 and 24 months of age, based on current knowledge. The following are proposed, based on the results of this thesis, and the literature reviewed within it:

- Breast feeding should be encouraged throughout the illness. Where infants of around 6 months are being exclusively breast fed, this should be continued.
- A varied diet consisting of foods of low viscosity (less than 6000 cps, which corresponds to an easily spoonable liquid or thinner) and high nutrient density (0.7 - 1.0 kcal/g) should be used in the acute phase. Viscosity of feeds should then be progressively increased according to clinical state and appetite of the child, with the intention of achieving a modified adult diet within 2 or 3 days in most children over the age of 9 months.
- Where standard feeding regimes have an energy density of less than 0.7 kcal/g, amylase-digestion and fermentation should be used to enable the feed energy density to be increased, without increasing viscosity. These technologies are particularly useful when other means of achieving such an effect (such as addition of energy-rich foods) are constrained by cost or availability. Addition of supplementary foods rich in energy and/or protein should be be combined with amylase-digestion wherever possible to maximise nutrient density and improve protein quality.
• Fermentation is best used as a means of ensuring that sick children always have food available. Fermented food can be kept near the child, and can be offered frequently between hot meals, without the need for repeated reheating or special refrigeration facilities. Locally available fermented foods which differ significantly from that used in this study may need to be adapted or evaluated for use in the dietary management of diarrhoea. Fermented foods should be produced using starter culture inoculation, rather than spontaneous fermentation, to maximise safety with regard to enteropathogen contamination. Normal good hygiene practices should always be followed when preparing fermented foods.

• Amylase-digested and fermented foods are suitable in the acute phase of diarrhoeal illness, but should also be encouraged during recovery and convalescence as part of a balanced varied diet. They should be especially promoted in this period for young children in an early phase of weaning who are unable to consume solid foods.

5.8 Summary
Amylase-digested and fermented weaning foods do have a place in the dietary management of diarrhoea in developing countries. Amylase-digestion increases energy intake by more than 40 percent compared to a conventional diet, while FAD improves intestinal permeability. These weaning foods are safe, and were acceptable to mothers for child feeding during diarrhoeal illness. The technologies involved are available at the household level, and are traditional to many communities within Tanzania, and many other developing countries. The particular amylase-digested and fermented foods studied here have been shown to have benefits which justify their promotion for hospitalised children with diarrhoea. These benefits are likely to apply widely to other, larger populations, such as children with other illnesses and those ill in the community. They are also likely to apply to other similar foods. There is a need for further research in order to harness the power of these simple technologies to lift the burden of malnutrition that stills weighs so heavily in many developing countries. This thesis holds out the hope that germination and fermentation, which so remarkably transform weaning foods, may also transform lives.
6 References


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REFERENCES


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7 Appendices

Appendix A  Method for Viscosity Measurement

7.1.1 Setting up and initialisation

1. Level the viscometer by referring to the spirit level on the rear of the instrument, and turning the screws on the base of the stand until the bubble is central.

2. Switch power on. The main display will light up, showing “---”, and the spindle display (SPDL) will show EE.

3. Calibrate the instrument using the autozero feature: with no spindle attached, turn the motor on, speed set to 10rpm, and press AUTOZERO. The main display will flash) “0.00” for 10 seconds, and on completion will be set into the Percentage mode at “0.00”. Turn the motor off. Repeat the Autozero feature each time the machine is turned on, and periodically while in use.

4. Choose a suitable spindle (usually No. RV2 or RV3), press SPDL, and enter the spindle number.

5. Press either the % key or the CPS key. In PERCENTAGE mode, the main display reads between 0.00 to 99.9. This is the standard Brookfield display from which all viscosities in centipoise (cps) are calculated. In CPS mode, the main display has a floating point, and indicates the viscosity in cps in conjunction with a power of 10 indicator. The viscosity is calculated from the percentage reading, based on the spindle attached, model of Viscometer, and spindle speed.

7.1.2 Operation

1. Remove the guard leg from the viscometer, and attach the spindle to the lower shaft. Do this carefully to avoid damage to the pivot point and jewel bearing: lift the lower shaft slightly, holding it firmly with one hand while screwing the spindle on with the other (note left hand thread). Avoid putting side-thrust on the shaft.
2. Select the porridge to be tested. Thoroughly mix by stirring thoroughly for 5 seconds. Fill one of the study plastic cups (500 ml) to 3/4 full (up to the top line). Remove any obvious lumps. Record temperature, pH, and hours since porridge prepared on record sheet. Stir thoroughly for 5 seconds, and record time.

3. Lower the spindle into the porridge until the fluid level is at the immersion groove in the spindle’s shaft. Care should be taken not to hit the spindle against the sides of the container.

4. Press the SPDL key, and enter the spindle number. Then press the % or CPS key.

5. To make a viscosity measurement, turn the motor on, and allow time for the display reading to stabilise. Read the viscosity 30 seconds after the end of stirring, at speed 20 rpm, RV2. Return the sample to the jug or flask.

6. If the reading is over-range ("EEE"), under-range ("---") or low-reading (% < 10), repeat using a different spindle to obtain readings within range.

7.1.3 Oscillation tests

These were performed approximately monthly to check the condition of the pivot point and jewel bearing. With the viscometer level, motor off, and no spindle attached, the spindle coupling was turned by hand to deflect the digital display upscale from its zero position to a reading of 5 or 10, and then allowed to swing back under its own power. On each occasion, the coupling swung freely and smoothly, and the display returned to zero, indicating that the pivot point and jewel bearing remained in good condition. A sluggish movement, or lack of return to zero, would have indicated unsatisfactory performance and need for servicing.
Appendix B  Standard weight checks of scales

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<tr>
<th>Scale</th>
<th>Standard weight</th>
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<td>Food 1</td>
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<td>(1-2000g)</td>
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<td>300</td>
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<td></td>
<td>1500g</td>
<td>1499</td>
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<tr>
<td>Food 2</td>
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<tr>
<td>(1-2000g)</td>
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<tr>
<td></td>
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<tr>
<td>Baby scales</td>
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<td></td>
<td>10.00</td>
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</table>

Standard weights were prepared by filling appropriately sized plastic water containers to the required level and sealing with superglue. These were initially checked using standard weights borrowed from the Government Weights and Measures Department.
Appendix C  dBase computer programme used to analyse food records

set talk off
repl all las with 0
go top
num=1
do while num<16
s=trim(str(num))
store 0 to am&s, mea&s
num=num+1
endd
daym=0
do while .not. eof()
keep=keepr
keepm=mealtime
num=0
tot=0
if ngr=2 and restricted=2
    tot=amount
    num=1
end
skip
do while idnum=keepr and mealtime<>keepm and .not. eof()
do case
case num>0 and ngr=2 and restricted=2
    tot=tot+amount
    num=num+1
    case num=0 and ngr=2 and restricted=2
    tot=amount
    num=1
endc
skip
endd
skip -1
daym=daym+1
s=trim(str(daym))
if num>0
    repl ammount with tot/num*5, amountk with amount/weight,
    meain with num
    am&s=amountk
    mea&s=num
    skip
if idnum<>keepr
    skip -1
    daym=0
    num=1
    do while num<16
    s=trim(str(num))
    repl amount&s with am&s, meal&s with mea&s
    am&s=0
    mea&s=0
    num=num+1
endd
    repl las with 1
    skip
endd
endd
set talk on
### Appendix D Programme parameters for CobasFara analyser

<table>
<thead>
<tr>
<th></th>
<th>Mannitol</th>
<th>Lactose</th>
<th>Lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Measurement mode</strong></td>
<td>ABS</td>
<td>ABS</td>
<td>ABS</td>
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<tr>
<td><strong>Reaction mode</strong></td>
<td>P-I-SR1-A</td>
<td>P-I-SR1-A</td>
<td>P-T-A</td>
</tr>
<tr>
<td><strong>Calibration mode</strong></td>
<td>LIN INTER</td>
<td>REAG/DIL</td>
<td>NO BLANK</td>
</tr>
<tr>
<td><strong>Reagent blank</strong></td>
<td>NO BLANK</td>
<td>REAG/DIL</td>
<td>NO BLANK</td>
</tr>
<tr>
<td><strong>Wavelength</strong></td>
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<td>340 nm</td>
<td>340 nm</td>
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<tr>
<td><strong>Temperature</strong></td>
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<td>37.0°C</td>
<td>37.0°C</td>
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<tr>
<td><strong>Decimal position</strong></td>
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<tr>
<td><strong>Unit</strong></td>
<td>mg/l</td>
<td>mg/ml</td>
<td>mg/ml</td>
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</tr>
<tr>
<td><strong>Sample vol.</strong></td>
<td>20.0 ul</td>
<td>20.0 ul</td>
<td>20.0 ul</td>
</tr>
<tr>
<td><strong>Diluent name</strong></td>
<td>H2O</td>
<td>H2O</td>
<td>H2O</td>
</tr>
<tr>
<td><strong>Volume</strong></td>
<td>10.0 ul</td>
<td>10.0 ul</td>
<td>10.0 ul</td>
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<td>OFF</td>
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<td>100 ul</td>
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<td>NO</td>
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<tr>
<td><strong>I Incubation</strong></td>
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<td>30 s</td>
<td>30 s</td>
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<tr>
<td><strong>M</strong></td>
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<td>NO</td>
<td>NO</td>
</tr>
<tr>
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<td><strong>Diluent name</strong></td>
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<tr>
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<td>ON</td>
<td>ON</td>
<td>ON</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td>ON</td>
<td>ON</td>
<td>ON</td>
</tr>
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<td><strong>Range type</strong></td>
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<td>NORM RANGE</td>
<td>NORM RANGE</td>
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<td>NO</td>
<td>NO</td>
</tr>
<tr>
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<td>2000.00 mg/ml</td>
<td>2000.00 mg/ml</td>
<td>2000.00 mg/ml</td>
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<td>ENDPOINT</td>
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<td>1</td>
<td>1</td>
</tr>
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<td>6</td>
</tr>
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<td><strong>CALIBRATION</strong></td>
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</tr>
<tr>
<td><strong>Cal interval</strong></td>
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<td>EACH RUN</td>
<td>EACH RUN</td>
</tr>
<tr>
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<td>5</td>
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<tr>
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<td>56</td>
<td>41</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>STD2: 250.00</strong></td>
<td>0.1250</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>STD3: 500.00</strong></td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td><strong>STD6: 2000.00</strong></td>
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</tr>
</tbody>
</table>
**Dietary Management of Infection Project Questionnaire**

**Part I**

**A: Utafulisho/Introduction**

<table>
<thead>
<tr>
<th>Wilaya/District</th>
<th>Kata/Ward</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shina/Cell</td>
<td>Kijiji/Mtaa Village/Street</td>
</tr>
<tr>
<td>Kabila/Tribe</td>
<td>Baloz1/Cell leader</td>
</tr>
<tr>
<td>Jina la Mama/Mother's Name</td>
<td>Surname  First name</td>
</tr>
</tbody>
</table>

**B: Habori Kubusuni Mototo/Information About the Child**

1. Uzito wa kuzaliwa: [ ] kg 99. Haijulikani
   - Birthweight: [ ] kg
2. Mtoto huyu alizaliwa ukiwa na mima ya miezi mingapi? 
   - When this child was born, how far on was the pregnancy?:
     - [ ] Miezi
     - [ ] Months
3. Mtoto huyu ni pacha? 
   - Is this child a twin?:
     - [ ] Ndiyo/yes
     - [ ] Hapana/no

**C: Habari Kubusuni Ugonjwa Huu/Information About the Current Illness**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Polio (if yes, write number of times in box)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kifua Kikuu (angalia kovu *) (BCG) (look for scar)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kifaduro, pepopanda na donda koo (write in no.of times) (DP)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surua (Measles)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Kama hakuna kovu andika ndiyo hakuna kovu
  - If no scar tick yes and write 'no scar'

   - The researcher should look at the child's clinic card, and if the child has had any of the following vaccinations, should fill them in.

24. Ni dalili gani ziizokuafanya umele moto huyu hospitali? (weka a lam a ya mviringo kwa jibu ukitakalopewa)
   - Which symptoms made you bring your child to the hospital? (Put a circle round each response you are given):
     - [ ] Kukohoa/cough
     - [ ] Homa/fever
     - [ ] Hakua anakula/nyonya vizuri Not eating or breastfeeding properly
     - [ ] Matalizo ya kupumua/Difficulty breathing
     - [ ] Alikuwa hachangamki/amechokachoka Not active/looking tired
     - [ ] Kuharisha/Diarrhoea
     - [ ] Kutapika/Vomiting
     - [ ] Vipele/vidondwa jwili/Skin rash/itching over the body
     - [ ] Vidonda mdomoni/mouth ulcers
     - [ ] Nyungiwezi (taja) Other (name them)

25. Mtoto ameguwa kwa siku ngapi kabla ya kuletwa hapa?
   - How many days was your child ill for before you brought him/her here?

26. Je kinyesi chake kinesuwa cha maji maji zaidi kuliko kwenda yake?
   - Has your child's stool been more watery than usual?:
     - [ ] Ndiyo/yes
     - [ ] Hapana/no

27. Kama ndiyo ni kwa muda gani?
   - If yes, for how long?

---

*Appendix E: Questionnaires*
28. Amepata kinyesri mara ngapi kuanzia jana wakati kama huu? 
How many stools has your child had since this time yesterday?
Mara(No of times)...........................................
99. Hajjulikani/Don't know

29. Kwa kawaida mtoto wako huwa anapata choo mara ngapi?
How many stools per day does your child normally have?
1. Mara/No of times...........................................
2. Kila baada ya siku ngapi/less than once a day............
99. Hajjulikani/Don't know

30. Ametapika mara ngapi kuanzia jana wakati kama huu
How many times has your child vomited since this time yesterday?
Mara/No of times...........................................
0. Hajatapika/Hasn't vomited..................................
99. Hajjulikani/Don't know

31. Mtoto akiwaa akila kiasi gani kuanzia jana wakati kama huu?
How much has your child been eating since this time yesterday?
1. Akiwaa akinyonya/breast feeding..................................
2. Ugali..................................................................
3. Vinginevyo (Taja)/Other (describe)..........................
4. Hakula cho chote/Not eaten anything..........................
99. Hajjulikani/Don't know

32. Kama mtoto akiuvi vizuri, amekua hali vizuri kwa siku ngapi sasa?
If the child is not eating properly, for how many days has he/she not been eating well?
Siku(No. of days)..................................................
99. Hajjulikani/Don't know

33. Niyakula gani mtoto amekua kuanzia jana wakati kama huu? (Weka alama ya mviringo kwa majibu yote yatatakayotolwa)
What type of food has your child been eating since this time yesterday?
1. Ame kua akinyonya/breast feeding..................................
2. Ugali..................................................................
3. Vinginevyo (Taja)/Other (describe)..........................
4. Hakula cho chote/Not eaten anything..........................
99. Hajjulikani/Don't know

34. Umepa kinywaji cho chote kuanzia jana wakati kama huu?
Have you given him/her any sort of drink since this time yesterday?
1. Ndiyo/yes..........................................................
2. Hapana/no (kama hapana nenda sw.37){ If no, go to Qn 37}
99. Hajjulikani/Don't know

35. Kama ndiyo, ni vinhywaji gani?/If yes, what fluids?
1. Kinyonya/breastfeeding..........................................
2. Mazwa ya kopoo/g'ombe Powdered milk/cows milk
3. Dawa ya maji na chumvi(chumvi)(ORS)
4. Maji tu/Just water..............................................
5. Chai/tea................................................................
6. Uji......................................................................

36. Umepa kiasi gani kuanzia jana wakati kama huu?
What amount have you been giving him/her since this time yesterday?
If mother is unable to remember the amount, ask is it more or less than one soda bottle (300mls), and write this in

<table>
<thead>
<tr>
<th>Aina ya kinywaji/Type of drink</th>
<th>Kiisi (volume)</th>
<th>Bajulikani</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mazwa ya kiki/breast milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Mazwa ya kolo/powdered cows' milk</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 3. Maji ya chumvi/salt water(ORS)
| 4. Chai ya ranfi/maziwa / tea with/without milk |
| 5. Baji anwepesi/light uji     |               |            |
| 6. Togwa                      |               |            |
| 7. Maji tu/Just water         |               |            |
| 8. Soda                       |               |            |
| 9. Maji ya matunda/fruit juice |
| 10. Vinginevyo (Taja)/Other (describe) |
| 11. Bakupewa kinywaji cho chote;Has not been given any drink |
| 10. Hajjulikani/Don't know    |               |            |

37. Chakula chake cha kawaida katika kipindi cha wiki moko iliyopita kilikuwa nini?
What was his usual food during the last week?

<table>
<thead>
<tr>
<th>Kinywaji/Type of food</th>
<th>Kiisi (volume)</th>
<th>Bajulikani</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Kinyonya/breastfeeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Mazwa ya kopoo/g'ombe Powdered milk/cows milk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Dawa ya maji na chumvi (ORS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Maji tu/Just water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Chai/tea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Uji</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

38. Mara ya mwisho mtoto ni alitumia linini kabla ya ugonjwa huu wakati huu?
When was the child last sick before this illness?

<table>
<thead>
<tr>
<th>Tarehe nadi ya siku nikiwa nayo</th>
<th>Bajulikani</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

39. Mara ya mwisho mtoto huu alitumia linini kabla ya ugonjwa huu wakati huu?
When was the child last sick before this illness?

<table>
<thead>
<tr>
<th>Tarehe nadi ya siku nikiwa nayo</th>
<th>Bajulikani</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

40. HAIBARI KUHUSU MARADHI YA NYUMA/INFORMATION ABOUT PREVIOUS ILLNESSES
Penguin itakuwa vema pia nikifahumu matatizo ya mtoto huku siku za nyuma.
It would be helpful to know about the child's previous problems.

41. Mara ya mwisho mtoto huu alitumia linini kabla ya ugonjwa huu wakati huu?
When was the child last sick before this illness?

<table>
<thead>
<tr>
<th>Tarehe nadi ya siku nikiwa nayo</th>
<th>Bajulikani</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

42. Mara ya mwisho mtoto huu alitumia linini kabla ya ugonjwa huu wakati huu?
When was the child last sick before this illness?

<table>
<thead>
<tr>
<th>Tarehe nadi ya siku nikiwa nayo</th>
<th>Bajulikani</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
13. Hakuruhusu mwezi wa ziua
Don't know which month

b) Idadi ya siku alizougwa hazijulikani
No. of days not known
a) Lakini a linguza kwa siku . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

But sick for ... days
b) Idadi ya siku alizougwa hazijulikani
No. of days not known

00. Hajaugua. (Kama jibu haajua gwa na sw.41, section E)
Has not been sick yet (go to qn 46 Section E)

39. Aliyuugwa mara ya mwigwa aliakuwa na matatizo gani?
When he/she was last ill, what were the problems?
1. Allikwana na homa Fever
2. Kuvarusha Diarrhoea
3. Kukohoa Cough
4. Surua Measles
5. Makwana anakuwa wizuri Not eating properly
6. Mafua Cold/Flu
7. Mwezi hacampambani/mamechokachoka Not active, tired all the time
8. Vipende/vipenda mwili Rashes over the body
9. Vinginevyo Taja
10. Vinginevyo (Taja) Other (describe)...
99. Hakuruhusu mwezi wa ziua
Don't know

40. Tafadhali niambie magonjwa aliyougua mtoto kabla ya ugonjwa huu, aliugwa kwa siku ngapi na kama alilazwa hospitalini?
Please tell me the illnesses your child has had before the present one, for how long sick, and if the child was admitted to hospital?

Aliyuugwa mara ya siku (month/year)

<table>
<thead>
<tr>
<th>Allikwana na homa</th>
<th>Mwezi/wa wakati</th>
<th>Mawili</th>
<th>Aliyuugwa hospitalini admitted to hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
<tr>
<td>Cough</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
<tr>
<td>Measles</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
<tr>
<td>Not eating properly</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
<tr>
<td>Cold/Flu</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
<tr>
<td>Not active, tired all the time</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
<tr>
<td>Rashes over the body</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
<tr>
<td>Taja</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
<tr>
<td>Other (mention)</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
<tr>
<td>Don't know</td>
<td>Mwezi/wa wakati</td>
<td>Mawili</td>
<td>Aliyuugwa hospitalini admitted to hospital</td>
</tr>
</tbody>
</table>

Don't know (Kama hapa na ndiyo na sw. 48/ If no, go to Qn 48)

43. Kama ndiyo, mara ya mwigwa ulikita tina kimea (Kabla hujalazwa hapa hospitalini)?
When did you last use it (before being hospitalised)?
1. Wiki hihi
2. Wiki lillyopita
3. Meewe huu huu
4. Meewe lipita
5. Vinginevyo (Taja)
6. Other (mention)
7. Hakuruhusu mwezi wa ziua
Don't know

44. Kimea ulichokituma mara ya mwigwa ulikipata wapi?
Where did you get kimea from last time you used it?
1. Nilekifungeneza mwenyewe/Made it myself
2. Nilivepewa na ndugu/rafiki/Was given by relative/friend
3. Nilekuma/Bought it
4. Vinginevyo (Taja)
5. Other (mention)
6. Hakuruhusu mwezi wa ziua
Don't know

45. Unacho kimea sasa hivi huko nyumbani kwako?
Do you have it at home at present?
Don't know

46. Kimea ulikuwa unakufanya nini kama kimea?
What do you normally use kimea for?
1. Pombe Local beer
2. Togwa Togwa
3. Uji Familia (family uji)
4. Uji Chid's uji
5. Other (mention)
6. Hakuruhusu mwezi wa ziua
Don't know

47. Unakufanya kimea huko ni kimea nini kinashahishe?
What do you suppose kimea does when put in uji, togwa, or beer? (let her explain)

48. Unahitaji kimea sasa hivi huko nyumbani kwako?
Do you have it at home at present?
1. Ndiyo/mara moja Yes, once
2. Ndiyo/mara chache Yes, a few times
3. Ndiyo/mara nyingi Yes, many times
4. Hapana Sijawahi (Nenda swali 59) No, never
5. Vinginevyo (Taja)
6. Other (mention)
7. Hakuruhusu mwezi wa ziua
Don't know (go to Qn 59)

49. Kimea ujitoka nini?
Where did you get kimea from last time you used it?
1. Nyumbani/home
2. Kliniki/Clinic
3. Radioni/On the radio
4. Magazetini/vitabu/kwenda vitabu/ Newspaper/books/magazines
5. Ndugu, jirani, rafiki / Family, neighbours and friends
6. Hakuruhusu mwezi wa ziua
Don't know
50. Umeshawahi kuona uji wa kimea ukitayarishwa (Kabla ya kuja hapa)?
Before coming here, had you ever seen how kimea porridge is prepared?
1. Ndiyo mara moja Yes, once
2. Ndiyo mara chache Yes, often
3. Ndiyo mara nyingi Yes, many times
4. Hapana sijawahi (nenda sw. 52) No, never (Go to Qn 52)
5. Vinginevyo taja Other (mention)

9. Haijulikani / Don't know

51. Kama ndiyo uliona wapi?
If yes, where did you see it?
1. Nyumbani/home
2. Jirani/neighbours
3. Kliniki/clinic
4. Kwenyu sumina ya lishe (taja wapi) Nutrition seminar (say where)...
5. Mahali pengine (taja) /Other place (name them)

9. Haijulikani / Don't know

52. Ulyosikia wewe uji wa kimea unaweza ukatumika kufanyia nini?
According to what you heard, what purposed can kimea be used for?
1. Kumlisha mtoto/feeding a child
2. Kuliwa na wakwado tu / for adult consumption only
3. Sikuelewa/sikumbuki /I didn't understand/don't remember
4. Vinginevyo (Taja) other(mention)

9. Haijulikani / Don't know

53. We wewe huwa unatumia uji w a kimea kumlisha mtoto?
Do you use kimea porridge to feed your child?
1. Ndiyo (kama ndiyo, nenda swali 55) Yes (If yes, go to Qn 55)
2. Hapana/no

9. Haijulikani / Don't know

54. Kama hapana kwa mini?
If no, why not?
1. Mto ataharisha/child will get diarrhoea
2. Mume wangu hapendi nimlishe mtoto / My husband doesn't like me to give the child the porridge
3. Mto ataliewa / My child will get drunk/intoxicated
4. Katika Familia yetu hatatumika kimea / We don't use kimea porridge in our family
5. Sina nafaka ya kutayarisha kimea/I don't have cereal to prepare kimea
6. Sitaji jinsi ya kutayarisha kimea / I don't know how to prepare it
7. Siendu tu/I just don't like it
8. Vinginevyo (Taja) Other (describe)
9. Haijulikani / Don't know

Nenda sw 56. Go to No 56

55. Mto wako anampenda?
If yes, does your child like it?
1. Ndiyo/yes

9. Haijulikani / Don't know

56. Wewe unadharani uji wa kimea ni mzuri kutumika kulisha watoto? (toa sababu za jibu lakini)
Do you suppose kimea porridge is good to give to a child? (Give reasons)
1. Ndiyo: Sababu Yes : Reason ....
2. Hapana: No : Reason 

9. Haijulikani / Don't know

57. Umeshawahi kupata matatizo yo yote kwa mtoto wako yote ulipoma uji wa kimea?
Have you ever had any problems with any child of yours when you've given kimea porridge?
1. Ndiyo: Yes
2. Ndiyo/no
3. Hajawahi kumpa mtoto ye yote/have never given it to a child

9. Haijulikani / Don't know

58. Kama ndiyo eleza matatizo uliyopata?
If yes, explain what problems:
F: Matumizi ya Togwa/Use of togwa

59. Unafaham u togwa?
Have you heard of togwa?
1. Ndiyo/yes
2. Hapana/no (kama hapana nenda sw. 67, Part II) (if no, go to Qn 67)

9. Haijulikani / Don't know

60. Kama ndiyo hawo unatumia togwa?
Do you use togwa?
1. Ndiyo/yes
2. Hapana/no (kama hapana nenda sw. 67, Part II) (if no, go to Qn 67)

9. Haijulikani / Don't know

61. Mara ya mwisho ulitumia linii togwa?
When did you last give your child togwa?
1. Wihihihi/this week
2. Wihihihi/last week
3. Mwezi huu huu/This month
4. Mwezi uliopita/last month
5. Vinginevyo Taja Other

9. Haijulikani / Don't know

7. Hapana/no
8. Vinginevyo (Taja) Other (mention)

9. Haijulikani / Don't know
62. Unajua jinsi ya kutengeneza togwa?
Do you know how to make togwa?
1. Ndiyo/yes
2. Hapana/no
9. Haijulikani/Don't know

63. Mara ya mwisho ultengeneza lini togwa
When did you last make togwa?
1. Wiki hii hihi/This week
2. Wiki iliyopita/last week
3. Mwezi huu huu/this month
4. Mwezi uliyopita/last month
5. Vinginevyo taja/other
9. Haijulikani/Don't know

64. Katika kaya yako nani hawa anaruhusiwa kunywaa togwa?
In your family, who is allowed to take togwa?
1. Watu wote katika kaya/all family members
2. Watu wazima/adults
3. Wanaume/men
4. Wanawake/women
5. Watoto/children
6. Waisichana/girls
7. Wavulana/boys
8. Vinginevyo (Taja)Other
9. Haijulikani/Don't know

65. Huyu mtoto wako unaweza kumpa togwa?
Can you give your child togwa?
1. Ndiyo/yes
2. Hapana/no
9. Haijulikani/Don't know

66. Unaweza kumpa togwa mtoto wako kuanzia akawa na umri gani?
At what age can you start giving your child togwa?
Umri wa umri/age in months
99. Haijulikani/Don't know
15. Hauwatumi/togwa/Don't use it

### PART II
To be completed on 2nd or third day after admission

G: Ulishaji wa Mtoto / Feeding of the child

<table>
<thead>
<tr>
<th>Wiki ya kwanza</th>
<th>Mwezi 1 wa kwanza</th>
<th>Mieza 3 ya kwanza</th>
<th>Vyakula vikuu anayokuwa baada ya miezi 3 after 3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>0. Nothing else</td>
<td>0. Nothing else</td>
<td>0. Nothing else</td>
<td>0. Nothing else</td>
</tr>
</tbody>
</table>


Write numbers in from following list:

1. Hazr. ya nq'cmbe/cow's milk
2. Maziwa ya kopo/powdered milk
3. Hâji/water
4. Ujili mwepesi/Tiijht uji
5. Vazi/potatoes
6. Uji
7. Ugali
8. Togwa
9. Ndizi za kupiüa/plantains
10. Chai/tea
11. Soda
12. Maji ya atu tim d a/fruit juice
13. Maji ya kuvi kina/fruit
14. Sigara/cigarettes
15. Mali/rice

69. Ulimisha kwa kutumia chombo gani?
What tool/feeder did you use to feed your child with?
1. Chupa/bottle
2. Kijiko/spoon
3. Kikombe/bakuli cup/bowl
4. Vinginevyo (Taja) Other (describe).................
9. Haijulikani/Don't know
1. Ndiyo / Yes (go to Qn 72) 
2. Hapana/no

99. Haijulikani/Don't know

71. Kama hapana, aliacha akiwa na umri gani? 
If no, at what age did he/she stop? 
Umri ....... .miezi (Age .months) 
99. Haijulikani/Don't know

72. Kwa kipindi cha wiki moja kabla mtoto hajaanza kuugua chakula chake cha kawaida kilikuwa nini? 
In the week before your child became ill, what were the main foods he/she was taking? 
1. Maziwa ya mama/breast milk 
2. Uji 
3. Ugali 
4. Wall/rice 
5. Viazi/potatoes 
6. Ndizi za kupikwa/plantains 
7. Vinginevyo (Taja) Other 

99. Haijulikani/Don't know

73. Hua unachanganya cho chote kwenye vyakula hivi? 
Do you add or mix anything with the following foodstuffs? 

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Uji</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Ugali</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Wall/rice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Viazi/potatoes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Ndiza za kupikwa/plantains</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Vinginevyo (Taja)Other</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

74. Kama ndiyo hua unachanyanga na mini? 
If yes, what do you add? 
Write the number(s) from the list below into the "Ndiyo" box for the appropriate food, in the table above 

1. Mafuta/cooking oil 
2. Mazima/milk 
3. Sukari/sugar 
4. Karanga/groundnuts 
5. Kunde/beans 
6. Chumvi/salt 
7. Nyama/meat 
8. Samaki/fish 
9. Matunda/fruit 
11. Vinginevyo(Taja) other 

99. Haijulikani

75. Umri wa mama/mlezi ni miaka 
Age mother/guardian 

76. Unao watoto wapasi? 
How many children do you have? 

77. Wapasi wameashafariki? 
How many children have died? 

78. Kwa wale waliofariki tafadhali naom baa unieleze umri wa lipofariki na sababu zi izosababisha kifo. 
For those children that have died, please tell me what age they were and the cause of death 

<table>
<thead>
<tr>
<th>Mtoto wa</th>
<th>Umri a lipokupa (miezi)/age</th>
<th>Sababu ya Kifo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mtoto wa 1/ 1st child</td>
<td></td>
</tr>
<tr>
<td>Mtoto wa 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mtoto wa 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mtoto wa 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mtoto wa 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

79. Wehe unaolewa/0a? 
Are you married/have you been married? 
1. Ndiyo/yes 
2. Hapana/no 
3. Mjane/widow 
4. Achika/divorced 
5. Vinginevyo(Taja)Other 
9. Haijulikani/Don't know

80. Unajua kusoma na kuandika? 
Do you know how to read and write? 
81. Umesoma mpaka darasa la ngapi?
What Grade/standard were you educated to?

1. Elimu ya watu wazima (Miaka ............) Adult literacy classes
2. Darasa la 1-4/ Standard 1-4
3. Darasa la 5 / Standard 5
4. Darasa la 6
5. Darasa la 7
6. Darasa la 8
7. Elimu ya Sekondari / Secondary education
8. Zaidi ya elimu ya sekondari / Advanced level (higher than secondary)
9. Sijawahi kusoma darasa lo lote/Never went to school
0. Haijulikani/Don't know

82. Unafanya kazi gani?
What is your work?

1. Mama wa nyumbani/Housewife
2. Nimeajiriwa ofisini/kwenda Office work/civil servant
3. Mfanyabiashara/Business woman
4. Mkuima/farmer
5. Yaya/Governess
6. Vinginevyo (Taja) Other.
9. Haijulikani/Don't know

1. MAKAZI/HOUSING

Nitatenda kizuungumzia juu ya nyumbani unayoishi pia. I'd also like to ask about your house.

83. Nyumba unaoyishi ni ya kwako?
Does the house you're living in belong to you?

1. Yana kuwangu /l's mine
2. Napanga/I rent
3. Yana ndugu yako /It's my relative's
3. Yana ndugu yako /It's my relative's
3. Yana ndugu yako /It's my relative's
9. Haijulikani/Don't know

84. Paa la nyumbani
The roof of the house is made of

1. Bati/corrugated iron
2. Madebe/Tins
3. Bati ya asbestos/asbestos
4. Vigae/clay tiles
5. Makuti/Nyasi /Grass/coconut leaves
6. Zege/Cement
7. Vinginevyo (Taja) Other.
9. Haijulikani/Don't know

85. Sakafu ya nyumbani
The floor of the house is made of

1. Udongo/Sol1/clay
2. Saruji/cement
3. Mbao/wood
4. Vinginevyo (Taja) Other.
9. Haijulikani/Don't know

86. Ukuta ni wako. The walls are made of

1. Udongo/miti soi I/poles
2. Matofali ya saruji/cement bricks
3. Cardboard (Mbao)
4. Mbao/wood
5. Mabati/container/iron roofing
6. Mavinginezo (Taja) Other.
9. Haijulikani/Don't know

87. Familia yako inatumia vyumba vingapi kwa ujumla?
How many rooms does your family occupy?

Vyumbe.................Rooms
9. Haijulikani

88. Kati ya vyumba vingapi mnawitumia kwa ajili ya kula la?
Out of these rooms, how many do you use as sleeping rooms?

Vyumbe.................Rooms
9. Haijulikani

89. Tafadhali nianibie jumla ya wanaume, wanawake na watoto wanaoishi katika kaya yakani ('kaya' here means all those sharing the same pot for meals)

Please tell me the number of men, women, children and young people (age 6-15) who live in your kaya

1. Wanaume wanawako/Min.
2. Wanawake wakubwa/Women.
3. Watoto chini ya miaka 5/Children under 5
4. Watoto miaka 6 kwa 15/Children between 6 and 15 years
5. Watoto miaka 6 kwa 15/Children between 6 and 15 years

90. Mnatumia laa ya alina gani?
What type of lighting do you use?

1. Umeme/Electic
2. Kibatari/Sinal 1 wick lamp
3. Kandiili/Kerosene lamp
4. Karabai/pressure lamp
5. Mavinginezo (Taja) Other.

91. Kupika unatumia jiko la
When cooking, what do you use to cook with?

1. Umeme/electric cooker
2. Gasi/gas cooker
3. Mafuta ya taa/Kerosene stove
4. Kuni/firewood
5. Mavinginezo (Taja) Other.
9. Haijulikani/Don't know

92. Mnatumia choo cha
What latrine do you use

1. Fuvuta kwa maji/european/Asiam
2. Shimo/Pit latrine
3. Vinginevyo (Taja) Other.

93. Choo umachotumia
Is the latrine used by

1. Kinatumika na familia yako peke yake/only your family
2. Ni cha kuchangia/other community members

14
94. Maji mnyotumia yanapatikana kutoka
Where do you get the water you use?
1. Bomba lililopo ndani ya nyumba/Water tap inside
2. Bomba lililopo kwenye eneo la nyumba lakini ni la kuchangia/Community water tap
3. Kisima kilichojengewa/Fortified well
4. Kisima cha wazi/ open well
5. Kununu kwa madebe/ndoo Buying debes/ buckets
6. Mtoni/River
7. Vinginevyo (Taja)/Other......................

95. Mnatupia wapi takataka?
Where do you throw your rubbish
1. Tunafukia chini/Bury it
2. Tunazichoma/Burn it
3. Tunazitupa mahali palipotengwa na mtaa/kijiji /At a specially allocated place by city/town
4. Huwa zinazakusanywa na magari ya site/Collected by city garbage vehicles
5. Popote/ anywhere

Haya ni maswali am bayo nilipenda kufahamu kutoka kwako, ahsante kwa ushirikiano wako.

96. Wewe dinafisi unawezaji kusema nini kuhusu uji wa kimea?
Personally, what is your opinion of the following qualities of kimea?

<table>
<thead>
<tr>
<th>Sifa za uji/ Quality</th>
<th>1. Mzuri/naupenda like it</th>
<th>2. Mzuri kidogo Quite like it</th>
<th>3. Mbaya siupendi kabisa don't like it</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ladha ya uji wake/Flavour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uhalini/uonekae na wachumi/Appears nice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uliani wake/texture</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

97. Unaadhani unafaa kulishwa watoto aina gam?
Which type of child is it useful for?

<table>
<thead>
<tr>
<th>Aina ya watoto/Type of child</th>
<th>Unafaa sana/very useful</th>
<th>Unafaa kidogo/quite useful</th>
<th>Haufa kabisa/No use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watoto wenye afya mzuri/Healthy child</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watoto wenye afya mbaya/ill children</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

98. Una maelezo yo yote zaidi kuhusu uji wa kimea?
Do you have any other comments on uji with kimea?

99. Unawezaji kusema nini kuhusu togwa?
What is your opinion of the following qualities of togwa?

<table>
<thead>
<tr>
<th>Sifa za togwa/Quality of togwa</th>
<th>1. Mzuri/naupenda Good/I like it</th>
<th>2. Mzuri kidogo sana/ Not very good</th>
<th>3. Mbaya siupendi kabisa/Bad, don't like it at all</th>
</tr>
</thead>
<tbody>
<tr>
<td>yludha yake wake/Flavour</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Inavyoonekana machoni/
Appearance
Ulaini
wake/texture

<table>
<thead>
<tr>
<th>Unadhani ni vizuri kumpa togwa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you think togwa is useful to give to?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aina ya watoto/type of child</th>
<th>Inafaa sana/very useful</th>
<th>Inafaa kidogo/quite useful</th>
<th>Haifai kabisa/completely useless</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watoto wenyewe afya mzuri</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watoto wenyewe afya mibaya</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ill children</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>101. Una maelazo yeyote zaidi kuhusu togwa?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you have any other comments about togwa?</td>
</tr>
<tr>
<td>..............................................</td>
</tr>
<tr>
<td>..............................................</td>
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<td>..............................................</td>
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</tbody>
</table>

DMI/SQUESTII
1ST FOLLOW-UP CLINIC FORM

Project Number [ ] Hospital Number [ ]

Surname [ ]

First Name [ ]

Food group [ ]

Diagナル完成者 [ ]

Today's Date [ ]

Date of discharge [ ]

Number of days since discharge [ ]

Length [ ] cm

Weight [ ] kg

Date of next follow up [ ]

Intestinal Permeability Test

1. Diarrhoea
2. Vomiting
3. Rash
4. Other (please describe) [ ]

If no, go to question 6.

6. Has your child still got symptoms? Yes = 1 No = 2

If no, go to question 7.

7. What symptoms are present now?

1. Cough
2. Fever
3. Not feeding
4. Difficulty breathing
5. Lethargy/tiredness
6. Diarrhoea
7. Vomiting
8. Rash
9. Other (please describe) [ ]

If no, go to question 8.

8. Is your child's feeding back to normal now? Yes = 1 No = 2

9. What are the main foods you have been giving your child since discharge?

1. Breast feeding
2. Cooked bananas
3. Plain ugali
4. Ugali/paratha
5. Powdered milk
6. Uyazi
7. Kimea
8. potatoes
9. Fruit
10. Other twist [ ]

If given more than once a day, go to question 12.

11. How often were you giving this?

Usual number of times per day [ ]

If less than once a day, ask how many times per week:

Times per week [ ]

If once a day or less, ask question 12.

12. What is the main reason for not giving this food more often?

1. Child doesn't like it
2. Mother doesn't like it
3. Takes too long to prepare
4. Don't know how to prepare
5. Kimea not available
6. Other (please describe) [ ]

If no, go to question 11.

13. Do you still have any concerns about your child's health?

Yes [ ] No [ ]
13. How many meals did you give your child yesterday? ............

EXAMINATION

General Appearance (1=well 2=mod unwell 3=ill) ...........
Dehydration (1=none/mild 2=mod 3=severe) ............
Temperature ............
Resp rate/min ............
Pulse/min ............

Indicate with tick if any of the following are present:

Fallox

Rash

Rhinitis

Mouth Ulcers

Oral Thrush

Pharyngitis

Otitis Media (Otoscope)

Indrawing of intercostal spaces

Crepitations in chest

Abdominal tenderness

Spleen (cm)

Liver (cm)

Generalized Lymphadenopathy

Other signs/comments:
2ND FOLLOW-UP CLINIC FORM

Project Number:  
Hospital Number:  

Surname:  
First Name:  

Food group:  

Doctor completing form:  

Todays' date:  
Date of discharge:  
Number of days since discharge:  

Length:  cm  
Weight:  kg  
MUAC:  mm  

1. How many days, if any, has your child had diarrhoea for since the last follow up visit?  
   ............days  

2. Has your child still got diarrhoea? yes = 1 no = 2  

3. Has your child been ill at all since last follow up? Yes = 1 No = 2  
   If no, go to question 6  

4. How many days has your child been ill for since the last follow up visit? ............  

5. What have been the main symptoms your child has had since the last visit?  
   1. cough  
   2. fever  
   3. not feeding  
   4. difficulty breathing  
   5. lethargy/tiredness  
   6. vomiting  
   7. rash  
   8. Other (please describe):  
   9. Don't know  

6. Has your child still got symptoms? Yes = 1 No = 2  
   If no, go to question 9  

7. Which symptoms are present now?  
   1. cough  
   2. fever  
   3. not feeding  
   4. difficulty breathing  
   5. lethargy/tiredness  
   6. vomiting  
   7. rash  
   8. Other (please describe):  
   9. Don't know  

8. Is your child's feeding back to normal now? Yes = 1 No = 2  

9. What are the main foods you have been giving your child since the last visit?  
   1. Breast feeding  
   2. Plain jujus  
   3. Ugali/karanga  
   4. Ugali  
   5. Plantain  
   6. Cooked bananas  
   7. Cow's milk  
   8. Powdered milk  
   9. Ogali  
   10. Kimea  
   11. Fruit  
   12. Other  
   13. Don't know  

10. Have you been giving your child any of the food we were giving in hospital?  
   Yes/no:  
   (If no go to question 12)  

11. How often have you been giving this?  
   Usual number of times per day:  
   .........times per day  
   If less than once a day, ask how many times per week:  
   .........times per week  
   If given more than once a day, go to question 13. If once a day or less, ask question 12.  

12. What is the main reason for not giving this food more often?  
   1. Child doesn't like it  
   2. Mother doesn't like it  
   3. Takes too long to prepare  
   4. Don't know how to prepare  
   5. Kimea not available  
   6. Other (Please describe):  

222
10. Don't know

11. How many meals did you give your child yesterday? .........

EXAMINATION

General Appearance (1=well 2=mod unwell 3=ill) .......
Dehydration (1=none/mild 2=mod 3= severe) ....... 
Temperature ............
Respirate/min ............
Pulse/min ............

Indicate with tick if any of the following are present:
Pallor
Rash
Rhinitis
Mouth Ulcers
Oral Thrush
Pharyngitis
Otitis Media (Otoscopy)

Indrawing of intercostal spaces

Crepitations in chest

Abdo tenderness
Spleen (cm)
Liver (cm)

Generalised Lymphadenopathy

Other signs/comments:
Appendix F  Data collection forms

**FLUID INTAKE CHART**

<table>
<thead>
<tr>
<th>Date</th>
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Record when sugar drink given on this chart (day of entry = D1, and D4)
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<tr>
<th>Date</th>
<th>Weight (daily) Kg</th>
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<tr>
<th>Date</th>
<th>Length (cm)</th>
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<tr>
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<th>MUC (mm)</th>
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<thead>
<tr>
<th>Date</th>
<th>Appearance (1 = well, 2 = mod, 3 = unwell)</th>
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<thead>
<tr>
<th>Date</th>
<th>DEHYD (1 = none, 2 = mild, 3 = severe)</th>
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<th>Temperature 0600 hrs</th>
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<th>Stool output (number)</th>
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Indicate with tick if any of the following are present:

- Pallor
- Rash
- Rhinitis
- Mouth Ulcers
- Oral Thrush
- Pharyngitis
- Otitis Media (Otoscopy O/A, then PRN)
- Indrawing of intercostal spaces
- Crepitations in chest
- Abdo tenderness
- Spleen (cm)
- Liver (cm)
- Other

**INVESTIGATIONS** (Write in investigation done in first column, and result under appropriate date):

- Blood slide for malarial parasites
- Rectal swab for cholera
- Blood for acute phase proteins etc
- White cell count
- Intestinal permeability test

**TREATMENT** (Write in name of drug, dose and frequency in first column, and tick for each day given):
**FLUID LOSS CHART (STOOL, URINE, VOMIT)**

**KEY**

- f = formed
- s = semisolid
- p = passed
- w = watery
- u = urine
- b = blood
- v = vomited

<table>
<thead>
<tr>
<th>Name</th>
<th>Hospital Number</th>
<th>Project Number</th>
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</table>

**Total no. stools**

**Total no. times passed urine**

**Total amount in bucket**

- Missed 1st flush amount
- Missed 2nd flush amount
- Missed 3rd flush amount

**Total stool output in last 24hrs**

Stool losses should be indicated on left of dashed line, urine and vomiting on right.
Write food group in top right hand box. If any other food is given, write this in the shaded box for the appropriate meal. Weights are in grams. Both start and finish weights include wt of cup.

<table>
<thead>
<tr>
<th>Meal 1</th>
<th>5am</th>
<th>Start weight (S)</th>
<th>Finish weight (F)</th>
<th>Difference (D=F)</th>
<th>Spilled (P)</th>
<th>Amount taken (D-P)</th>
<th>Tick if vomited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal 2</td>
<td>11am</td>
<td>Start weight (S)</td>
<td>Finish weight (F)</td>
<td>Difference (D=F)</td>
<td>Spilled (P)</td>
<td>Amount taken (D-P)</td>
<td>Tick if vomited</td>
</tr>
<tr>
<td>Meal 3</td>
<td>1pm</td>
<td>Start weight (S)</td>
<td>Finish weight (F)</td>
<td>Difference (D=F)</td>
<td>Spilled (P)</td>
<td>Amount taken (D-P)</td>
<td>Tick if vomited</td>
</tr>
<tr>
<td>Meal 4</td>
<td>7pm</td>
<td>Start weight (S)</td>
<td>Finish weight (F)</td>
<td>Difference (D=F)</td>
<td>Spilled (P)</td>
<td>Amount taken (D-P)</td>
<td>Tick if vomited</td>
</tr>
<tr>
<td>Meal 5</td>
<td>11pm</td>
<td>Start weight (S)</td>
<td>Finish weight (F)</td>
<td>Difference (D=F)</td>
<td>Spilled (P)</td>
<td>Amount taken (D-P)</td>
<td>Tick if vomited</td>
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</table>

Total (g) | Average per meal

If child eats more than 5 meals in one day, use the next column. Write the correct date above each column. If nasogastric tube is used, write 'NGT' in shaded box for every meal given via tube, and give amount prescribed by doctor. Otherwise start weight (including cup) is 350g for each meal.
Summary: Amylase from germinating cereal grains enables the preparation of porridge with a higher energy density than conventional weaning foods. This food can be combined with fermentation, which inhibits pathogen growth. These food technologies are inexpensive, can be implemented at the household level, and are therefore particularly appropriate for use in developing countries. In a controlled clinical trial, 75 children aged 6-25 months admitted to hospital with acute diarrhea were rehydrated and then randomly allocated to three porridge dietary groups: conventional, amylase-digested (AMD), and fermented and amylase-digested (FAD). The study diets were given ad libitum five times daily, and all intakes except breast milk were weighed. Mean daily energy intakes over 4 days in the conventional AMD, and FAD groups, respectively, were 32.4 (95% CI 28.7-36.6), 46.0 (CI 39.6-53.4), and 37.3 (CI 31.8-43.9) kcal/kg/day. The energy intake in the AMD group was 42% higher than the conventional group (p = 0.003). There were no significant differences between the groups for duration of diarrhea, frequency of stooling, or vomiting. Starch digestion using amylase from germination is an effective way of improving energy intake in children with acute diarrhea. Key Words: Diarrhea—Dietary intake—Children—Malnutrition—Amylase—Fermented foods.

Developing countries need better weaning foods in order to combat malnutrition, which is a prevalent cause of morbidity and mortality in children (1-4). Such foods are particularly necessary during and after episodes of diarrheal disease, an important cause of impaired growth and failure to thrive (5-9). The most available weaning foods are often cereal-based porridges with low energy densities due to the water-binding and gelatinization properties of starch, which limit the flour concentration that can be used for a liquid feed (4,10). Energy-rich foods are usually either not available or too expensive for routine use in poor communities.

Amylase can be produced at the household level from germinating cereal grains as an amylase-rich flour (ARF) (11,12). It is very effective in reducing the viscosity of thick cereal porridges by starch digestion (13-15). Amylase synthesized within the grain during germination cleaves amylopectin and amylose within starch granules to produce maltose and low-molecular-weight dextrans. Because these products bind little water and do not gelatinize on cooking, viscosity is markedly reduced. Thus, a feed with a low viscosity suitable for children can contain a much greater concentration of flour, and the nutrient density is more than doubled. Nonalcoholic fermentation improves taste and reduces contamination with pathogenic bacteria (12,16,17), and can be combined with amylase digestion.

A recent World Health Organization (WHO) review called for controlled trials evaluating the ef-
fectiveness of these technologies in improving energy intakes of sick children (18). Subsequently, a randomized study in infants with diarrhea reported improved energy intakes using ARF, but intake measurements were confined to a single daily test meal (19). In this study, we examined 24-h dietary intakes and the course of disease among children admitted to hospital with acute diarrhea who received amylase-digested (AMD) or fermented and amylase-digested food (FAD). AMD is known locally in Tanzania as “kimea” porridge and FAD as “togwa.”

METHODS

Site and Study Population

The study was conducted at the Pediatric Diarrhea Treatment Unit of the Muhimbili Medical Center (MMC), Dar es Salaam, Tanzania between June 30 and December 19, 1992. The MMC is a tertiary referral center. The majority of children with diarrhea (~80%) are referred from other city hospitals and health care facilities; the remainder are self-referrals.

Study Foods

The feeds used in the study are compared in Table 1. The conventional feed was prepared as a thin porridge in the traditional manner; the other two were prepared to a thicker consistency using a higher proportion of flour. After cooling, their viscosity was reduced by the addition of ARF. The ARF was prepared by germinating white sorghum grain for 48 h, sun-drying, and then grinding to a flour. For AMD, the porridge was then reboiled; for FAD, it was allowed to ferment for 24 h at room temperature (27–28°C) and was then used for feeding in the subsequent 18 h. It was served without reheating. Both the conventional and AMD feeds were kept in thermos flasks and served hot.

Feed characteristics are also shown in Table 1. Dry matter and energy density were determined in a representative sample of 32 food specimens by oven-drying to steady weight and performing ballistic bomb calorimetry, according to the method of Miller (20). Protein and fat concentration were calculated from food tables (21). Viscosity at the serving temperature (60°C for conventional and AMD feed; room temperature (28°C) for FAD) was determined in a representative sample of 17 specimens using a Brookfield viscometer, spindle RV2, speed 20 rpm (22). pH was measured using a Whatman PHA 250 portable meter in 112 samples. All three feeds were of a similar viscosity, but the energy densities of the AMD and FAD porridges were ~50% greater than the conventional one.

Study Design

Children between the ages of 6 and 25 months with acute diarrhea were eligible if they had already commenced weaning, did not have any congenital or chronic condition that interfered with food intake, and did not have kwashiorkor (because interpretation of body weight would be difficult due to edema). Acute diarrhea was defined as stools more watery and more frequent than usual for <14 days (parent’s history), severe enough to require admission to the ward. Children who were well enough for discharge the day after admission were ex-

<table>
<thead>
<tr>
<th>TABLE 1. Composition and characteristics of diets</th>
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<tbody>
<tr>
<td>Conventional</td>
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<tr>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Compositiona</td>
</tr>
<tr>
<td>Corn flour</td>
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<tr>
<td>Groundnuts</td>
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<tr>
<td>Amylase-rich flour (ARF)</td>
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<tr>
<td>Water</td>
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<td>Characteristicsb</td>
</tr>
<tr>
<td>Energy density (kcal/100 g)</td>
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<tr>
<td>Protein (g/100 g)c</td>
</tr>
<tr>
<td>Fat (g/100 g)c</td>
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<tr>
<td>Percentage dry matter</td>
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<tr>
<td>Viscosity at serving temperature (cps)</td>
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<tr>
<td>pH</td>
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</table>

a Grams per kg total starting ingredients.
b Plus-minus values are means ± SD.
c These values calculated from food tables.
cluded, as were those who required insertion of a nasogastric feeding tube within 24 h of admission because of very poor appetite or severe systemic illness. Children were entered into the study after rehydration, between 4 and 24 h after admission. Rehydration was performed according to WHO guidelines (23). After obtaining consent from the parent or guardian, children were assigned to the dietary groups by block randomization, using groups of nine sealed envelopes.

The time of each stool passed was recorded. The duration of diarrhea was the number of hours from the time of entry into the study until the earliest of one of the following: (a) passage of a formed stool, or (b) 12 h elapsed without passing any stool, or (c) 24 h with no watery stools and less than four semisolid or formed stools, provided that no watery stools, and less than five semisolid or formed stools, were passed in the subsequent 24 h. The diarrhea was defined as having recurred if more than six stools were passed in any subsequent 24-h period.

Children were followed until discharge. In this paper, we have presented data for the first 4 days after entry, since the majority of children were discharged by the end of the 4th day. The study design allowed children in the AMD and FAD groups to be changed to the conventional food group at the mother's request (subsequent food intake data was not included in the analysis), but not vice versa.

**Dietary Assessment**

The study foods were prepared in the ward kitchen by specially trained staff and served ad libitum in 300-g portions in plastic cups five times per day. The trial was not blind, since temperature and taste differences between the foods could not be disguised. Nearly all mothers were breast-feeding, and this was encouraged, along with use of oral rehydration therapy after watery stools. Children only consumed food that was provided by the diet kitchen, according to the normal ward treatment regime. All intakes of study foods were weighed to 1 g on Soehnle food scales by nine trained nurse enumerators. Special care was taken to avoid food spillage. Breast milk intake was not measured.

**Anthropometry and Other Assessment**

Children were weighed daily on Soehnle baby scales accurate to 10 g; length was measured on admission on a locally made board, accurate to 1 mm; and mid upper arm circumference was measured using a TALC tape (24) accurate to 2 mm. A questionnaire was used to elicit historical information about the illness, and the child's health card was consulted to establish the exact age. A complete physical examination was performed on admission and daily until discharge, and appropriate treatment was provided.

Systemic infection was defined as the presence of pneumonia, meningitis, septicemia, acute otitis media, or urinary tract infection. Pneumonia was defined as any two of the following: cough or difficulty breathing on history, intercostal recession, crepitations, respiratory rate >50 per min, or positive radiograph.

**Statistics and Data Analysis**

Sample size calculations made using the formula according to Kirkwood (25), for a probability of 5% and a power of 80%, estimated that 22 children would be needed in each group in order to show significance for a 20% increase in energy intake in one of the dietary groups.

Data entry was performed using Epilinfo and dBase software programs (26,27). After validation, data was analyzed using Epilinfo and SPSS/PC+ (28) statistical software packages. The food intake and stool output data produced skewed distribution curves; therefore, these data were log-transformed in order to obtain normal distributions. This enabled use of one-way analysis of variance (ANOVA) tests to compare dietary groups. The data presented have been back-transformed, and geometric means are therefore shown. Survival analysis was used to compare duration of diarrhea between the groups.

The research protocol was approved by the ethical committees of the Hospitals for Sick Children, London; the Muhimbili Medical Center; the Tanzania Food and Nutrition Center; and the Tanzanian Commission for Science and Technology.

**RESULTS**

**Exclusions**

The results for 75 patients are presented, after six exclusions. Three of these had dysentery and three did not satisfy the inclusion criteria (too old or nasogastric tube already inserted).
Patient Characteristics on Admission

Table 2 shows the admission characteristics by dietary group. There were no significant differences between the groups for any of the features, except for those receiving antibiotics, where a greater proportion of the AMD and FAD groups had been commenced on antibiotics than the conventional group. As a group, the children were moderately malnourished. Ten patients whose hemoglobin was <5.0 g/dl received a blood transfusion—one (4%) in the conventional group, six (24%) in the AMD group, and three (13%) in the FAD group (no significant difference between groups, p = 0.11). With regard to features of diarrheal illness on admission (Table 3), there were no significant differences between groups on any of the parameters.

Dietary Intake

Table 4 shows a comparison of the geometric mean weights of food consumed in grams per kilogram body weight in each dietary group for the first 4 days of the study. There was no significant difference on any day between the amounts consumed by the conventional and AMD groups; but on days 2 and 4, the FAD group consumed significantly less weight of food than the conventional group. Over the 4 days, the mean amount consumed by the FAD group was 64.4 g/kg body weight, as compared with 85.8 g in the conventional group.

The energy intakes from study foods of the three groups are compared for the 4 days of the study in Table 5 and shown graphically in Fig. 1. The AMD group consumed significantly more energy than the conventional group for each of the first 3 days of the study. Over the 4-day period, the mean daily energy intake was 46.0 kcal/kg/day in the AMD group, as compared to 32.4 kcal/kg/day in the conventional group, which represents an increase of 42% (p = 0.003). The energy intake in the FAD group did not differ significantly from the other two groups on any day, and the overall mean for the days of the study was 37.3 kcal/kg/day.

The following factors were controlled for in the analysis of variance of the 4-day energy intake results by including them as single covariates: age; sex; Z scores for weight/age, height/age, and weight/height; presence or absence of systemic illness; whether received antibiotics or not; temperature during admission; and whether received blood transfusion or not. In each case, the p value for differences in energy intake between the groups remained ≤0.005.

Outcome Features

Time until cessation of diarrhea was compared between groups using survival analysis; results are shown in Fig. 2. Data were used until the 9th day, when all had stopped having diarrhea. There was no difference between the groups (p = 0.54). Other outcome features are shown in Table 6. There were no significant differences between the groups for any of the parameters. One patient in the conventional group (4%), five in the AMD group (20%),

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Conventional (n = 26)</th>
<th>AMD (n = 25)</th>
<th>FAD (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mo)</td>
<td>11.5 ± 4.8</td>
<td>9.6 ± 3.3</td>
<td>11.4 ± 4.3</td>
</tr>
<tr>
<td>Sex (males)</td>
<td>16 (62)</td>
<td>16 (64)</td>
<td>15 (63)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>7.40 ± 1.2</td>
<td>6.94 ± 1.4</td>
<td>7.26 ± 1.5</td>
</tr>
<tr>
<td>Weight for age (Z score)</td>
<td>−2.04 ± 1.1</td>
<td>−2.06 ± 1.4</td>
<td>−2.20 ± 1.4</td>
</tr>
<tr>
<td>Height for age (Z score)</td>
<td>−1.47 ± 0.9</td>
<td>−1.79 ± 1.6</td>
<td>−1.73 ± 1.4</td>
</tr>
<tr>
<td>Weight for height (Z score)</td>
<td>−1.32 ± 0.9</td>
<td>−1.01 ± 0.8</td>
<td>−1.31 ± 1.0</td>
</tr>
<tr>
<td>Mid upper arm circumference (cm)</td>
<td>12.9 ± 1.8</td>
<td>12.5 ± 1.4</td>
<td>12.6 ± 1.6</td>
</tr>
<tr>
<td>Hemoglobin (g/dl [n])</td>
<td>7.0 ± 1.5 [16]</td>
<td>5.8 ± 1.6 [19]</td>
<td>7.1 ± 1.5 [20]</td>
</tr>
<tr>
<td>Temperature ≥38.5°C</td>
<td>4 (15)</td>
<td>5 (20)</td>
<td>8 (33)</td>
</tr>
<tr>
<td>Malaria (any malarial parasites on blood film)</td>
<td>6/19 (32)</td>
<td>8/19 (42)</td>
<td>9/22 (41)</td>
</tr>
<tr>
<td>Systemic infection</td>
<td>6 (23)</td>
<td>11 (44)</td>
<td>6 (25)</td>
</tr>
<tr>
<td>Received antibiotics</td>
<td>4 (15)</td>
<td>12 (48)</td>
<td>10 (42)</td>
</tr>
<tr>
<td>Not breast-feeding</td>
<td>3 (12)</td>
<td>1 (4)</td>
<td>2 (8)</td>
</tr>
</tbody>
</table>

* Plus-minus values are means ± SD; values in parentheses are group percentages.
* Significant difference between groups (p = 0.03); otherwise, there were no significant differences for other parameters.
TABLE 3. Features of diarrheal illness on admission by dietary group

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Characteristic</th>
<th>Conventional (n = 26)</th>
<th>AMD (n = 25)</th>
<th>FAD (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimated degree of dehydration*</td>
<td>None 2/25 (8)</td>
<td>3/23 (13)</td>
<td>3/20 (15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Some 20/25 (80)</td>
<td>15/23 (65)</td>
<td>13/20 (65)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe 3/25 (12)</td>
<td>5/23 (22)</td>
<td>4/20 (20)</td>
</tr>
<tr>
<td></td>
<td>Reported duration of diarrhea (days)</td>
<td>3.5 ± 3.0</td>
<td>3.8 ± 2.6</td>
<td>2.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Reported number of stools in previous 24 h</td>
<td>4.9 ± 2.9</td>
<td>4.6 ± 2.3</td>
<td>6.1 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>History of vomiting in previous 24 h</td>
<td>19/24 (79)</td>
<td>20/23 (87)</td>
<td>15/22 (68)</td>
</tr>
<tr>
<td></td>
<td>Of those vomiting, no. of vomits in previous 24 h</td>
<td>2.3 ± 1.1</td>
<td>2.3 ± 1.1</td>
<td>4.6 ± 2.3</td>
</tr>
</tbody>
</table>

* Plus-minus values are means ± SD; values in parentheses are group percentages. There were no significant differences between the groups.

and three in the FAD group (13%) required further intravenous fluids after entry to the study to correct dehydration (no significant difference between groups, p = 0.21). Four patients left the study because they required nasogastric tube insertion for poor food intake (one in the AMD group and three in the FAD group).

Systemic infection was present in 23 (31%) of the patients. In the conventional group, four had pneumonia, one a urinary tract infection, and one acute otitis media. In the AMD group, five had pneumonia, four septicemia, one a urinary tract infection, and one otitis media. In the FAD group, two patients had pneumonia, one septicemia, one a urinary tract infection, and two otitis media. There were four deaths during admission. Two occurred on the 4th day (one in the AMD, one in the FAD group), and a third on the 5th day (AMD group); each had pneumonia and marasmus (weight for age < -3.3). A fourth nonmalnourished child (AMD group) died on day 8 with continuing diarrhea and a clinical picture of septicemia, although this was not confirmed on blood culture. The overall mortality was 5%.

Median weight changes, as percentage of admission body weight, were between -5.0 and +1.0% for the first 4 days of the study for all groups. There were no significant differences between the groups on any day of the study.

DISCUSSION

These results show that use of an amylase-digested feed (AMD) results in a significantly greater energy intake during acute diarrhea than use of a conventional corn porridge. Weight of food consumed was similar, suggesting that both foods were equally acceptable to each mother-infant pair, but the increased energy density of AMD enabled a much higher energy intake to be achieved (compare Tables 4 and 5 and Fig. 1). Children on FAD consumed a lower mean weight of food per day than the conventional group, which may indicate that it was less acceptable. Possible reasons included temper-
TABLE 5. Energy intake (kcal/kg body weight/day) by dietary group and by day of study

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Days 1-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>27.9 ± 1.3</td>
<td>33.7 ± 1.3</td>
<td>34.1 ± 1.3</td>
<td>39.2 ± 1.2</td>
<td>32.4 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>(24.3–33.0)</td>
<td>(29.4–39.7)</td>
<td>(29.6–39.9)</td>
<td>(30.7–50.0)</td>
<td>(28.7–36.6)</td>
</tr>
<tr>
<td>n</td>
<td>26</td>
<td>26</td>
<td>17</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>AMD</td>
<td>39.4 ± 1.5</td>
<td>46.4 ± 1.4</td>
<td>51.1 ± 1.3</td>
<td>47.8 ± 1.4</td>
<td>46.0 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>(32.6–49.9)</td>
<td>(39.5–56.0)</td>
<td>(43.9–60.3)</td>
<td>(39.1–59.3)</td>
<td>(39.6–53.4)</td>
</tr>
<tr>
<td>n</td>
<td>25</td>
<td>24</td>
<td>19</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>FAD</td>
<td>36.0 ± 1.4</td>
<td>34.9 ± 1.4</td>
<td>42.2 ± 1.3</td>
<td>34.7 ± 1.3</td>
<td>37.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>(30.6–43.8)</td>
<td>(29.1–43.4)</td>
<td>(35.8–50.7)</td>
<td>(26.0–56.5)</td>
<td>(31.8–43.9)</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td>20</td>
<td>18</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Total number in group</td>
<td>72</td>
<td>70</td>
<td>54</td>
<td>28</td>
<td>75</td>
</tr>
<tr>
<td>One-way ANOVA p value</td>
<td>p = 0.016*</td>
<td>p = 0.014*</td>
<td>p = 0.002*</td>
<td>p = 0.109</td>
<td>p = 0.003*</td>
</tr>
</tbody>
</table>

* Significant difference between AMD and conventional groups.

The three porridges were of similar viscosities, and there were no significant differences between measured values. However, the amylase-digested feeds had lower mean viscosities than the conventional feed. Thus, an even higher proportion of dry matter could be used in their preparation, further improving energy intake.

Groundnuts were used in only one of the three groups (the conventional), instead of all three, for several reasons. The normal ward practice is to use a corn feed with groundnuts, so this was used as the control. Addition of groundnuts to FAD would have resulted in a rancid product. Their omission from the amylase-digested feeds allowed comparison of two different methods of improving energy intake (amylase digestion and high-energy supplementary food). When both can be used together, the effect on energy intake would be additive. A further reason for using both is to improve dietary protein quality. Although AMD and FAD contain more protein than the conventional diet (see Table 1), its quality will be poorer since it is derived mainly from the single vegetable source (corn), whereas the pro-

![FIG. 1. Energy intake (kcal/kg body weight/day) by dietary group and by day of study. P values refer to one-way ANOVA tests between groups. The significant p values on days 1–3 are due to differences between the amylase-digested (AMD) and the conventional food groups.](J Pediatr Gastroenterol Nutr, Vol. 21, No. 1, 1995)
Dietary Management of Diarrhea

79

The study design did not allow children in the conventional group to change to the other study foods.

### TABLE 6. Outcome features of diarrheal illness by dietary group

<table>
<thead>
<tr>
<th>Feature</th>
<th>Conventional (n = 26)</th>
<th>AMD (n = 25)</th>
<th>FAD (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time until cessation of diarrhea (h after entry)</td>
<td>47.1 ± 1.8 [26]</td>
<td>46.9 ± 1.9 [22]</td>
<td>54.4 ± 1.1 [22]</td>
</tr>
<tr>
<td>Number of stools day 1</td>
<td>6.6 ± 1.3 [25]</td>
<td>7.7 ± 1.2 [25]</td>
<td>7.1 ± 1.2 [24]</td>
</tr>
<tr>
<td>Number of stools day 2</td>
<td>3.7 ± 1.3 [24]</td>
<td>5.6 ± 1.3 [25]</td>
<td>6.1 ± 1.3 [24]</td>
</tr>
<tr>
<td>Number of stools day 3</td>
<td>4.0 ± 1.2 [15]</td>
<td>5.9 ± 1.3 [19]</td>
<td>4.7 ± 1.3 [18]</td>
</tr>
<tr>
<td>Number of stools day 4</td>
<td>5.9 ± 1.2 [7]</td>
<td>4.5 ± 1.3 [16]</td>
<td>4.3 ± 1.2 [11]</td>
</tr>
<tr>
<td>Vomited during ward study period</td>
<td>14 (54)</td>
<td>17 (68)</td>
<td>13 (54)</td>
</tr>
<tr>
<td>Of those vomiting, percentage of meals vomited</td>
<td>18.8 ± 13.6</td>
<td>16.8 ± 11.5</td>
<td>20.8 ± 15.2</td>
</tr>
<tr>
<td>Meals vomited out of total meals documented in study</td>
<td>51/417 (12.2)</td>
<td>44/439 (10.0)</td>
<td>39/347 (11.2)</td>
</tr>
<tr>
<td>Change of study diet to conventional</td>
<td>2 (8)</td>
<td>1 (4)</td>
<td>5 (24)</td>
</tr>
<tr>
<td>Recurrence of diarrhea</td>
<td>2 (8)</td>
<td>2 (8)</td>
<td>0</td>
</tr>
<tr>
<td>Died during admission</td>
<td>0</td>
<td>3 (12)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

" Plus-minus values are means ± SD; values in parentheses are group percentages; values in brackets are numbers in groups, if less than n. There were no significant differences between groups.

The study design did not allow children in the conventional group to change to the other study foods.
# TABLE 7. Comparison of energy intake (total and from breast milk) during diarrhea and in health in three studies

<table>
<thead>
<tr>
<th>Author, yr. and country of study</th>
<th>Groupings (no. in group)</th>
<th>Age range (mo)</th>
<th>Change in energy intake during diarrhea (% of total)</th>
<th>Mean weight for age Z score</th>
<th>Energy intake (breast milk/total) (kcal/kg/day)</th>
<th>Change in energy intake during diarrhea (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoyle et al., 1980, Bangladesh (29)</td>
<td>No dietary education (15)</td>
<td>6-35</td>
<td>-5</td>
<td>47/75</td>
<td>b</td>
<td>-42</td>
</tr>
<tr>
<td>Healthy controls (11)</td>
<td>6-35</td>
<td>-4</td>
<td>49/61</td>
<td>b</td>
<td>-49</td>
<td></td>
</tr>
<tr>
<td>Dicken et al., 1990, Nigeria (30)</td>
<td>No grouping (45)</td>
<td>5-28</td>
<td>+2</td>
<td>53/85</td>
<td>51/96</td>
<td>+11</td>
</tr>
<tr>
<td>Marquis et al., 1990, Peru (31)</td>
<td>Liquid diet (with amylase) (18)</td>
<td>9-20</td>
<td>0</td>
<td>26/66</td>
<td>26/93</td>
<td>-29</td>
</tr>
<tr>
<td>Semisolid diet (12)</td>
<td>9-20</td>
<td>-4</td>
<td>22/44</td>
<td>18/109</td>
<td>+23</td>
<td></td>
</tr>
</tbody>
</table>

- Weight for age Z score was included in Dicken et al.’s paper but not in the other two papers, and an approximate figure has been derived from available anthropometrical indices.
- Intakes during diarrhea were compared to the control group.

Diabetes of 11–49% is due to reduced calorie intake from weaning foods, whereas there is little change in breast milk intake. Thus, in our study, the increased energy intake in the AMD group is likely to represent a real increase in total energy intake.

If highly nutritious, energy-dense diets involving more expensive ingredients are used, such as industrial amylase and full-fat dried milk, then viscosity reduction yields no advantage (31), because the energy density of the feed given (~1.0 kcal/g) is so high that bulk properties become irrelevant. However, most families in developing countries are not able to buy such diets. Non-breast milk energy intakes in our nonmodified feed group were similar to those in the studies of children with diarrhea managed with basic, locally available foods (8, 29, 30). The benefits of amylase addition in our study suggest that this technology might be of value in other communities. These low-cost methods for improving weaning foods can often be adapted for child feeding from those already in use for the production of local foods and beverages (12).

New or modified weaning foods are likely to be more easily accepted if they are similar in viscosity to the normal weaning foods already in regular use. It seems advisable in Tanzania to promote low-viscosity foods (~1000 cps) of high energy density (>0.7 kcal/g). These can best be produced by a combination of high-energy supplementary foods, in combination with some form of easily available amylase. A cheap industrial amylase is technically feasible, but it is unclear how sustainable such an option would be. Promotion of an amylase-rich flour derived from germination, prepared in the home or on the ward, would seem a much better option, in view of its simplicity and the fact that the technology already exists in Tanzania. AMD is now in regular use on the diarrhea treatment unit, without special promotion.

This study has demonstrated the effectiveness of a locally available product, AMD, which uses germination to reduce dietary bulk and thus enables production of feeds of higher energy density. This technology could also be used for dietary management of infections other than diarrhea and among malnourished children. Once prepared, fermented food can be kept safely at room temperature and served without reheating. FAD could therefore be promoted as a snack food for sick children that could be given frequently in between hot AMD porridge meals. Thus, both these food technologies, which are applicable in many developing countries, could be promoted to improve the dietary management of pediatric diarrhea.

Acknowledgment: We thank the following people for all their help: the nursing and dietary staff of the Diarrhea Treatment Unit for their hard work as enumerators and with food preparation; Mrs. H. Peter and Mrs. B. Abel from the Tanzania Food and Nutrition Center (TFNC) for help with questionnaires and viscosity measurements; Prof. A. Swai at Muhimbili Medical Center (MMC) for help with computing; all the staff of the Centre for International Child Health for encouragement, ideas, and technical help. Finally, we thank the Overseas Development Administration for funding this study.
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Dietary Management of Acute Diarrhoea in Children: Effect of Fermented and Amylase-Digested Weaning Foods on Intestinal Permeability

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*Centre for International Child Health, Institute of Child Health, London, UK; †Department of Paediatrics, Muhimbili Medical Centre; and ‡Tanzania Food and Nutrition Centre, Dar es Salaam, Tanzania

Abstract:
Background: There is a strong relationship between diarrhoea, malnutrition, and intestinal integrity. To investigate the effect of different dietary treatment on intestinal permeability during acute diarrhoea, 87 Tanzanian children aged 6-25 months were recruited to this study when admitted to hospital.

Methods: Children with acute diarrhoea were rehydrated and then randomly assigned to one of three dietary treatment groups: a conventional low-energy density porridge, a high-energy density amylase digested porridge (AMD), or a high-energy density amylase digested and then fermented porridge (FAD). Lactulose/mannitol permeability tests were performed on admission, at 3 days, and at follow-up 2 and 4 weeks after discharge. The lactulose/mannitol (L/M) ratios were compared between dietary treatment groups and to a group of age-matched, healthy control subjects.

Results: Children with diarrhoea had higher L/M ratios (geometric mean 0.85, 95% CI 0.68–1.05) compared with control subjects (0.14, 0.12–0.17) on admission. There was a significant difference in the change in L/M ratio between admission and 3 days between dietary treatment groups in favour of the FAD group (p < 0.05).

Conclusions: Dietary treatment and intestinal damage at admission explain 13.5% of the variation in L/M ratio, but when age at admission and age at weaning are included as covariants, 21.9% is explained. FAD porridge seems to be more effective in the treatment of intestinal permeability than AMD or conventional porridge. Urinary lactose concentrations in spot urine samples taken prior to the permeability test were also measured. There was a significant correlation with the L/M ratio (correlation coefficient = 0.62, p < 0.001). JP G N 24:235-241, 1997.

Key Words: Diarrhoea—Intestinal permeability—Lactulose; mannitol ratio—Children—Amylase—Fermented foods—Lactose. © 1997 Lippincott-Raven Publishers

Diarrhoea is one of the major causes of childhood mortality in developing countries (1–3) and when associated with malnutrition is the commonest cause of morbidity in children. There is a strong relationship between diarrhoea, malnutrition, and intestinal integrity (4–6). Intestinal permeability is markedly increased during acute diarrhoea in otherwise healthy children, but returns to normal within a few weeks (7,8). In malnourished children permeability is chronically raised, and it is suggested that mucosal damage may persist for a prolonged period because the normal repair response to villous atrophy is impaired (9). Diarrhoea and mucosal damage result in growth faltering due to reduced energy intake and absorption, and increased metabolic demands during infection (1,10).

Absorption across healthy intestinal mucosa is highly selective and demonstrates marked discrimination with respect to the molecular dimension of inert molecules, which becomes impaired in disease states associated with mucosal damage (11). In conditions associated with villous atrophy, there is a reduction in mucosal surface area and a reduction in the number of cells. This results in reduced transcellular transport of small molecules and an increase in the paracellular transport of larger molecules (12).

The differential sugar absorption test is a noninvasive test that has been validated, by small bowel biopsy studies, as a useful indicator of mucosal damage in children with diarrhoea (5) and accepted as an indirect technique for monitoring intestinal mucosal changes (7). It utilizes sugars that are not enzymatically digested in the gut and are excreted intact by the kidneys. The specificity and
sensitivity of the test for severe villous atrophy are 98 and 95%, respectively (13). The test uses lactulose (radius = 0.54 nm) and mannitol (r = 0.4 nm), which are thought to be absorbed para- and trans-cellularly, respectively, and the ratio of these sugars will change as mucosal damage is repaired. The ratio of lactose to mannitol (L/M ratio) recovered in the urine after a standardised test dose will therefore give an indication of intestinal permeability and overcomes the difficulty of complete urine specimen collection (5). Under pathological conditions, intestinal permeability to larger sugars increases and that to smaller molecules stays the same or decreases, resulting in a higher L/M ratio. It has also been suggested by Behrens (5) that lactose, which is normally digested in the intestinal lumen before transport across the mucosa, may cross the mucosal surface intact in conditions of villous damage.

Intestinal permeability is expected to decrease during recovery from diarrhoea, but in view of the trophic effects of intraluminal nutrients, the type of food used during refeeding may influence the rate of intestinal recovery. Traditional food technologies already in use in some parts of Tanzania include amylase digestion and fermentation of weaning foods. These porridges are different from the conventional bulky, low-energy density maize porridge widely used in weaning in less developed countries.

Amylase is produced from the germination of cereal grains as an amylase-rich flour (ARF) (14,15). The addition of small quantities of ARF is effective in reducing the viscosity of thick cereal porridge by starch digestion and so allows the energy density of a porridge of given viscosity to be doubled (14,16). ARF also increases the availability of proteins, amino acids and vitamins while reducing the concentration of trypsin inhibitors and phytic acid (17,18). Non-alcoholic carbohydrate fermentation of porridge improves the taste, reduces contamination with enteropathogenic bacteria (19), and improves protein digestibility and iron availability (20). The locally prepared fermented food also uses amylase digestion and so combines the benefits of both types of food. Locally germinated and fermented foods are well accepted and are extensively used by communities in Tanzania (21).

This study investigated whether dietary treatment of acute diarrhoea with amylase digested (AMD) or fermented and amylase digested (FAD) porridge, as opposed to the conventional porridge, improved intestinal permeability and repair of mucosal damage, as assessed by the urinary L/M ratio. It also investigated whether the concentration of lactose found in urine samples taken before the differential sugar test correlated with the L/M ratio, and whether the measurement of undigested lactose in spot urine samples might give an indication of intestinal damage for future screening of children with diarrhoea.

METHODS

Study Population and Food Preparation

The study was conducted at the Paediatric Diarrhoea Treatment Unit of the Muhimbili Medical Centre, Dar es Salaam, between June 30 and December 19, 1992. Patients and study foods have been described elsewhere (22). Seventy-five of the 87 patients included in this study were analysed in the previous paper, owing to recording errors for food intake data in some initial subjects, who were then considered part of the pilot study with respect to dietary intake. The 87 patients reported here were not significantly different from the 75 with respect to clinical parameters, duration of diarrhoea, and nutritional status.

Study Design

Children between the ages of 6 and 25 months with acute diarrhoea were eligible if they had already commenced complementary feeding, did not have any congenital or chronic condition interfering with food intake, and did not have kwashiorkor. Acute diarrhoea was defined as stools that were more watery and more frequent than usual for <14 days (parent's history), severe enough to require admission to the ward. Children who were well enough for discharge the day after admission were excluded, as were those who required insertion of a nasogastric feeding tube within 24 h of admission because of very poor appetite or severe systemic illness. Children were entered into the study after rehydration, between 4 and 24 h after admission. Rehydration was performed according to WHO guidelines (23). Clinical diagnosis was made on symptoms at admission, and in some children this was later confirmed with laboratory tests. Malaria was defined as the presence of any malarial parasites on a blood film, and systemic infection as the presence of pneumonia, meningitis, septicaemia, acute otitis media or urinary tract infection. After obtaining consent from the parent or guardian, children were assigned to one of the three dietary groups by block randomisation, using groups of nine sealed envelopes.

Intestinal permeability tests were carried out at entry into the study, ~3 days after admission, and at follow-up 2 and 4 weeks after discharge. Children were given 20 ml of water containing 5 g lactulose and 1 g mannitol. Urine samples were collected into a bag containing one drop of 20% wt/vol chlorhexidine gluconate (to prevent bacterial degradation of the sugars) before and for 5 h after the administration of the dual sugar solution. Intestinal permeability tests were also carried out on 30 healthy children of the same age as the patients, at a local well child clinic. Urine sample volumes were recorded, and aliquots of urine were stored at ~20°C.

The study diets (22) were prepared in the hospital and given ad libitum five times a day. Breast-feeding and the
use of oral rehydration therapy after watery stools was encouraged. Energy intakes in hospital, anthropometric data, and the duration of diarrhoea were recorded for each child.

The research protocol was approved by the ethical committees of the Hospitals for Sick Children, London; the Muhimbili Medical Centre; the Tanzania Food and Nutrition Centre; and the Tanzanian Commission for Science and Technology.

**Laboratory Analysis**

**Mannitol**

The mannitol assay uses Blood's method (24), which is based on the method of Lunn et al. (25), using a CobasFara centrifugal analyzer (Roche). The assay is based on the oxidation of mannitol to fructose by NAD-specific D-mannitol dehydrogenase from Leuconostoc mesenteroides (Biocatalysts Ltd.). The change in absorbance at 340 nm is measured to indicate the rate of reduction of NAD to NADH. Hexokinase is added to the coenzyme reagent together with Mg and ATP to abolish the inhibitory effect of fructose.

**Lactulose and Lactose**

The lactulose and lactose assays use the method of Northrop et al. (26) using the CobasFara centrifugal analyzer (Roche). The assay is based on the same principle as the lactose assay, without the second step involving PGI. In this way, it aims to measure only the free glucose and glucose from lactose. The lactose concentration was measured in spot urine samples taken before the administration of the differential sugar absorption test solution in order to test the hypothesis that this could be used as a simple alternative test of intestinal permeability.

**Statistics and Data Analysis**

Data entry was performed using Epilnfo and dBase software programmes (27,28). After validation, data was analyzed using Epilinfo, SPSS/PC+ (29), and SAS (30) statistical software packages. The L/M ratio for each dietary treatment group and controls produced skewed distribution curves, and therefore these data were log transformed in order to obtain normal distributions. The data presented here have been back-transformed, and geometric means with 95% CI are shown. Standard statistical methods including analysis of variance (ANOVA), analysis of covariance, and regression were used and were considered significant at the 5% probability level.

**RESULTS**

The details of the patient characteristics in each dietary group are shown in Table 1. The mean age of the children with diarrhoea (n = 86) was 10.7 months on admission. There were 64.4% (n = 56) males. It was found that 47.6% (n = 41) had a weight for age Z score below -2, 19.8% (n = 17) a weight for height Z score below -2, and 30.2% (n = 26) a height for age Z score below -2. As for weaning, 64.0% (n = 55) had been given food other than breast milk within the first week of life (hitherto referred to as age at weaning). Clinical diagnosis on admission showed that 44.2% (n = 38) had diarrhoea only, whereas the remainder were thought to have either a systemic infection, malaria, or both, complicating their diarrhoeal illness. In those children whose clinical diagnosis was confirmed by laboratory analysis, 42.0% (29/69) had uncomplicated diarrhoea, whereas the remainder had systemic infection, or malaria, or both. There was no significant difference between groups (x^2 = 2.34, p = 0.31). Four children died during admission (3 from the AMD group and 1 from the FAD group). There was no significant difference between the treatment groups for any features shown in Table 1. With regard to features of diarrhoeal illness on admission, there were no significant differences between groups in time to cessation of diarrhoea, number of stools per day or vomiting (22).

The 30 control children had a mean L/M ratio of 0.14 (0.12-0.17) compared with the 87 cases with a mean of 0.85 (0.68-1.05) on admission. Table 2 shows the geometric mean L/M ratio (95% CI) for each dietary treatment group on admission, after 3 days, and at the first and second follow-up examinations, carried out -17 and 32 days after admission. Analysis of variance showed a significant difference in L/M ratio between cases and...
TABLE 1. Patient characteristics of treatment groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Porridge (n = 33)</th>
<th>AMD (n = 28)</th>
<th>FAD (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean months ± SD</td>
<td>10.9 ± 4.5</td>
<td>9.7 ± 4.2</td>
<td>11.6 ± 4.1</td>
</tr>
<tr>
<td>Sex, male</td>
<td>63.6 (21)</td>
<td>67.9 (19)</td>
<td>64.0 (16)</td>
</tr>
<tr>
<td>Weight-for-age &lt; 2&quot;</td>
<td>54.5 (18)</td>
<td>35.7 (10)</td>
<td>52.0 (13)</td>
</tr>
<tr>
<td>Weight-for-height &lt; 2&quot;</td>
<td>21.2 (7)</td>
<td>10.7 (3)</td>
<td>28.0 (7)</td>
</tr>
<tr>
<td>Height-for-age &lt; 2&quot;</td>
<td>27.3 (9)</td>
<td>35.7 (10)</td>
<td>28.0 (7)</td>
</tr>
<tr>
<td>Clinical diagnosis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea only</td>
<td>50 (18)</td>
<td>42.9 (12)</td>
<td>32.0 (8)</td>
</tr>
<tr>
<td>Confirmed diagnosis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea only</td>
<td>50 (13/26)</td>
<td>27.3 (6/22)</td>
<td>45.5 (10/22)</td>
</tr>
<tr>
<td>Weaned at &lt; 1 wk</td>
<td>66.7 (20)</td>
<td>74.1 (20)</td>
<td>65.2 (15)</td>
</tr>
<tr>
<td>&lt; 1 mo</td>
<td>3.3 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3 mo</td>
<td>20.0 (6)</td>
<td>7.4 (2)</td>
<td>13.1 (3)</td>
</tr>
<tr>
<td>&gt; 3 mo</td>
<td>10.0 (3)</td>
<td>18.5 (5)</td>
<td>21.7 (5)</td>
</tr>
</tbody>
</table>

AMD, high-energy density amylase digested porridge; FAD, high-energy density amylase digested and fermented porridge.

TABLE 2. Geometric mean L/M ratio for each dietary treatment group during the study

<table>
<thead>
<tr>
<th>Diet</th>
<th>L/M ratio on admission (95% CI)</th>
<th>L/M ratio at 3 days (95% CI)</th>
<th>L/M ratio at first followup (95% CI)</th>
<th>L/M ratio at second followup (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porridge</td>
<td>0.78 (0.56-1.10)</td>
<td>0.60 (0.42-0.86)</td>
<td>0.17 (0.10-0.29)</td>
<td>0.17 (0.09-0.30)</td>
</tr>
<tr>
<td>AMD</td>
<td>0.74 (0.53-1.05)</td>
<td>0.60 (0.41-0.88)</td>
<td>0.23 (0.16-0.33)</td>
<td>0.20 (0.11-0.44)</td>
</tr>
<tr>
<td>FAD</td>
<td>1.10 (0.75-1.64)</td>
<td>0.38 (0.21-0.67)</td>
<td>0.31 (0.18-0.55)</td>
<td>0.16 (0.08-0.33)</td>
</tr>
<tr>
<td>Controls</td>
<td>0.14 (0.05-0.37)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

L/M, lactose/mannitol; AMD, high-energy density amylase digested porridge; FAD, high-energy density amylase digested and fermented porridge.

control subjects at admission (p < 0.001) but no significant difference between the treatment groups at any point during the study.

Figure 1 shows the fall in mean L/M ratio in each dietary treatment group during the study. There was no significant difference between groups on admission, although the FAD group appeared to have a higher mean L/M ratio. Comparison of the L/M ratios for each patient on Day 0 and Day 3 showed that there had been a fall in the L/M ratio in 11/25 (44%) of the conventional group, 15/20 (75%) of the AMD group, and 17/19 (89%) of the FAD group (χ² = 10.9, p = 0.004).

When the variations of lactulose and mannitol were analysed separately, the geometric mean urinary concentrations of lactulose were found to be 161, 184, and 403 mg/L on Day 0 and 124, 187, and 116 mg/L on Day 3 in the conventional, AMD, and FAD groups, respectively. The respective values for mannitol were 189, 231, and 363 mg/L on Day 0 and 190, 309, and 265 mg/L on Day 3. The values for control subjects were 115 mg/L for lactulose and 794 mg/L for mannitol.

When the change in L/M ratio between admission and day 3 was analysed, there was a significant difference between treatment groups (p = 0.037). This had been lost by the first and second follow-up visits. Since the L/M ratios at the first and second follow-up visits are near to the control levels, and as there was no means of ensuring that the children were still being fed the treatment diet after discharge from the hospital, these have not been analysed further.

Analysis of variance also showed there to be no significant difference in L/M ratio for each clinical diagnosis group on admission and no significant difference attributable to age at weaning.

The ANOVA for L/M ratio at day 3 was repeated, including as covariants factors known to be causes of variation and increase in the error term. Factors such as sex and anthropometric data did not significantly affect the L/M ratio at day 3. Table 3 shows the results of the analysis of L/M ratio at 3 days, using initial L/M ratio, age on admission, and age at which foods other than breast milk were introduced as covariants.

Dietary treatment and initial L/M ratio significantly influenced L/M ratio at day 3 (p < 0.05). Age on admission and age at introduction of foods other than breast milk were not significant, but both contributed to the overall model when added sequentially.

FAD was shown to be significantly different from both conventional porridge (p = 0.009) and AMD (p = 0.006) by the t test on means, which had been adjusted for the covariants by a least squares technique (30). Intestinal permeability 3 days after admission was influenced by both the
The results show that children admitted to hospital with acute diarrhoea had a raised intestinal permeability, as shown by a high L/M ratio (0.85, 0.68–1.05), compared with healthy control subjects (0.14, 0.12–0.17). The Tanzanian control subjects compare well with healthy UK infants, with a L/M ratio of 0.12 (SD 0.09) (6). The L/M ratio of study children fell over time, and by the first and second follow-up visits (~17 and 32 days after admission), L/M ratios were close to values found in the control children. Previous work has suggested that severely malnourished children, many of whom have diarrhoea, have chronically raised L/M ratios (31). It would be interesting to evaluate the impact of more prolonged feeding with FAD on intestinal permeability in such children.

During the time the study children spent in hospital, they were randomly allocated to one of three dietary treatment groups, and although mothers were encouraged to continue the assigned diet after discharge, this was not enforced. For this reason, analysis of the effect of dietary treatment on intestinal permeability has concentrated on the fall in L/M ratio by 3 days after admission.

**DISCUSSION**

This study investigated the effect of different dietary treatments on recovery of the intestinal mucosa after acute diarrhoea, by measurement of intestinal permeability, assessed by urinary excretion of lactulose and mannitol following a test dose of these sugars.
The fall in L/M ratio by day 3 was significantly greater in the FAD group than the other two groups. Comparison of groups on the basis of whether there was a fall in L/M ratio after 3 days of exposure to the study food showed a highly significant difference between groups, with more than twice as many patients in the FAD group showing a fall than in the conventional group, and a similar but less marked effect in the AMD group (75% vs. 44%). It should be noted that the FAD group had a higher initial L/M ratio than the other groups. Although this was not statistically significant, it makes the subsequent fall in L/M ratio all the more notable. The analysis of the individual variation of the sugar probes shows that differences between the FAD group and the other two groups is mainly explained by changes in the urinary lactulose excretion, which showed a large reduction between entry to the study and Day 3.

We postulate four main mechanisms by which the observed beneficial effects could have occurred. First, there may be a nutritional basis, either due to increased intake of energy and nutrients promoting improvements in systemic nutritional status, and hence mucosal recovery, or because of local delivery of substances important for mucosal cell nutrition and recovery. The former seems less likely here, because the predominant effect on intestinal permeability was in the FAD group, while it was children in the AMD group who consumed the most energy (22). Short-chain fatty acids (SCFA), which are a normal product of anaerobic fermentation of carbohydrates in the large bowel, have been shown to be important for local mucosal nutrition and growth in the colon in rats (32), and it is possible that similar beneficial fermentation products are present in FAD. Second, a microbiological mechanism may be important, since the fermentation process inhibits growth of enteropathogens (19) and production of enterotoxins (personal communication, R. Kingamoko 1995). Third, an immunological mechanism could be involved, since when the mucosal barrier is compromised during diarrhoea, large antigenic molecules are able to traverse it (33). This may cause immunologically mediated local damage, which becomes self-perpetuating. Fermented products, and to a lesser extent amylase-digested foods, contain much simpler carbohydrates, which are probably less antigenic. Also, fermented products activate macrophages and lymphocytes, which may expedite healing (34). The fourth mechanism that must be considered is one involving gut regulatory peptides. There is a massive increase in enteroendocagoc in small infants with diarrhoea in infants, and this probably promotes enterocyte regeneration (35). Fermented products may exert their effects via this or other peptides. Thus, mucosal recovery following gastroenteritis is a complex process, which involves the interplay of many factors, and fermented foods may influence many of them. Fermented foods are widely used in many traditional cultures (36), and utilization during episodes of diarrhoea in children requires further study, both to determine whether promotion of mucosal recovery is a general property of such foods and to evaluate possible mechanisms.

The results presented here suggest that a porridge that had been both amylase digested and fermented was more effective than conventional porridge in the treatment of acute diarrhoea, with respect to repair of mucosal damage, through trophic effects on intestinal epithelium. These foods are widely used in traditional diets, although they are often not perceived as beneficial for children. Factors influencing acceptability to children (22), such as taste, temperature, and mothers' perceptions, require consideration when such foods are promoted in differing cultures, and further research is needed in this area. In the light of work by Lunn et al., which suggested that as much as 43% of growth faltering in Gambian children can be explained by chronically raised intestinal permeability (6), the value of culturally acceptable forms of fermented foods may be under-recognised. These alternative food technologies cost little, are available at the household level, and could be widely promoted as a means of combating malnutrition.

We had hoped that screening of urinary lactose could be useful in situations where the analysis of lactulose and manitol would not be possible because of technical constraints. However, the sensitivity and specificity were both so low that lactose screening cannot be recommended.

Acknowledgment: We would like to thank the following people for all their help: the nursing and dietary staff of the Paediatric Diarrhoea Treatment Unit, Muhimbili Medical Centre, for their hard work as enumerators and with food preparation; Mrs. H. Peter and Mrs. B. Abel from the Tanzania Food and Nutrition Centre (TFNC) for help with questionnaire design; Professor A. Swai at Muhimbili Medical Centre for help and advice with computing; Ron Behrens from the Hospital for Tropical Diseases for technical advice; all the staff of the Centre for International Child Health for encouragement, ideas, and technical help, especially Suzanne Felteau. Finally, we would like to thank the Overseas Development Administration for funding this study.

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Dietary Management of Diarrhoea

241

Weaning food technology

There is a need for better weaning foods in developing countries. At the household level, food technologies of germination and fermentation are relatively inexpensive ways of improving locally available cereal porridges. This article discusses the main problems with weaning practice, and shows how these technologies can be used to address them.

Malnutrition affects millions of children in the world today, and is a major cause of morbidity and mortality. At the level of the individual child, it is the product of inadequate food quality and quantity, and the effect of illness, usually due to infection.

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Use of ARF enables the preparation of cereal feeds with a much higher energy and nutrient density, because the starting flour concentration can even be tripled without increasing the final feed viscosity. The cost of this increase in energy density is mainly that of the extra flour used, and is a much cheaper way of providing extra calories than use of energy rich supplements. A variety of other foods can be added in order to ensure a balanced nutrient intake. The production of ARF is labour efficient, in that only a small amount is required per feed (approximately 10 per cent of the total flour used), and a batch will keep for several weeks if stored in a dry, airtight container. The procedure for producing ARF is familiar to many communities in Tanzania, since it is traditionally used in the production of local beverages. Feeds produced using ARF have a sweeter taste, due to the sugars produced from starch digestion, and this may improve their appeal to children. The process of germination has other beneficial effects, such as improving bioavailability of iron and zinc, due to reduction of phytate.

The theoretical possibilities of osmotic diarrhoea, due to increased sugars, and cyanide toxicity when sorghum ARF is used, have been investigated and not found to occur in practice. Normal cooking practices, effectively remove cyanide precursors. After the addition of ARF, porridge should be reboiled to ensure this is the case, as well as to complete cooking and kill any bacteria that may contaminate the ARF. Mouldy grain should not be used to prepare ARF, because of the risk of aflatoxins from some moulds. Regular washing of the grains during germination prevents mould formation.

### TABLE I

**GOOD WEANING PRACTICE**

<table>
<thead>
<tr>
<th>Good weaning practice consists of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>* exclusive breast feeding until the age of 4-6 months</td>
</tr>
<tr>
<td>* gradual introduction of optimum weaning foods</td>
</tr>
<tr>
<td>- medium to high energy density (&gt;0.5 kcal/g)</td>
</tr>
<tr>
<td>- appropriate micronutrients and protein quality</td>
</tr>
<tr>
<td>- microbiologically safe</td>
</tr>
<tr>
<td>- low viscosity (semi-liquid or soft) for easy consumption</td>
</tr>
<tr>
<td>* high feed frequency (5-6 times per day) in addition to breast feeds</td>
</tr>
<tr>
<td>* breast feeding encouraged throughout the first two years</td>
</tr>
<tr>
<td>* avoidance of bottle feeding with its hazard of infection</td>
</tr>
</tbody>
</table>

### TABLE II

**PROBLEMS WITH WEANING**

**Low energy density**

Most weaning foods are made by cooking starchy staple flour with water to form a porridge.

Starch granules bind water, swell, and gelatinise on cooking, to give a thick voluminous food.

In order to produce a food of a consistency thin enough for child feeding, large amounts of water must be used to dilute the flour, resulting in a low nutrient and energy density.

Addition of energy rich foods is often too expensive.

**Microbiological contamination**

Studies show high levels of contamination with pathogenic bacteria in traditional local weaning foods.

The weanling's dilemma is the difficult choice between:

* prolonged exclusive breast feeding - microbiologically safe, but eventually inadequate for growth
* early commencement of weaning foods, with the associated hazard of infection

**Infrequent child feeding**

Weaning children require frequent feeds because the small stomach capacity limits the intake per feed.

In practice, feed frequency is usually only two to three times per day.

Reasons for low feed frequency:

* food preparation consumes time and fuel - both may be scarce
* feeding the child is also time consuming
* storage of prepared food is difficult
TABLE III

MAKING AND USING AMYLASE RICH FLOUR

1. Select whole, unbroken grains of sorghum, millet, maize or wheat, clean to get rid of debris, and wash.
2. Add water (about three times the volume of the grains), cover and leave to soak overnight (6-12 hours).
3. Pour off the water, and spread the grains out on a clean wet cloth or banana leaves, cover (e.g., by folding the cloth over), and leave to germinate for 48-72 hours in damp, dark conditions at 25-30°C. Keep moist by sprinkling with water three to four times per day. Do not use if mould forms (regular washing with water during germination prevents this).
4. Dry the germinated grains completely, either in bright sunshine for eight hours, or by light-roasting over a low flame. Devegetate the grains by rubbing in a sieve (devegetation is not necessary if the final porridge is reboiled as in Step 8).
5. Mill to flour (hand pounding or electric grinder), and store in a dry, airtight container. This is ARF.
6. Make up child feed as normal (for example maize porridge) using ordinary flour (not ARF), but use two to three times as much flour, so that the porridge is very thick (a spoon will stand up in it).
7. Allow the porridge to cool for about an hour until it is warm, not hot (about 40°C), and then add a small amount of the ARF (approximately 10 per cent of the weight of flour used to make the porridge), and stir until the porridge becomes thin (three to four minutes).
8. Bring the porridge back to the boil, then serve as normal. Note that other foods such as groundnut flour, beans, egg, fish etc can be added during preparation of this porridge.

Fermentation - a way of reducing pathogens and increasing feed frequency

Here, complex carbohydrates, such as starches, are partially broken down by bacterial action, with the production of acids, and possibly antimicrobial substances. This technology has been used for food preparation and preservation across the world since ancient times, and examples of its use occur in almost every culture. The main fermenting organisms used are yeasts (for bread and alcoholic beverages), moulds (for cheese production) and bacteria. Lactic-acid producing bacteria are responsible for most fermented cereal porridges and drinks and it is these that are most often suitable for use as weaning foods. Maize is the most commonly used cereal and in Africa there are at least 20 different fermented maize products. Fermentation may be carried out either before or after cooking, or both, and may be allowed to occur spontaneously or initiated by inoculation with a starter culture.

In Tanzania, fermentation is usually combined with the use of ARF, and is initiated either by microbes contained within this flour,
PRACTICAL POINTS

1. Improving weaning practice is an important way of reducing malnutrition and its consequences.

2. The weaning food technologies of germination and fermentation contribute to good weaning practice by improving energy density of feeds, reducing contamination with pathogens, and facilitating more frequent child feeding.

3. These technologies are generally cheap, and can be performed at the household level.

or by addition of a starter culture. During fermentation, the pH drops, and this is associated with the production of a characteristic tangy taste. After 12-24 hours at room temperature (25-30°C), the pH of the porridge falls below 4, a level at which studies have shown that the growth of enteropathogenic bacteria is inhibited, and the porridge is suitable for consumption over at least the next 12 hours without refrigeration or reheating. Fermentation is therefore a method of improving microbiological safety of weaning foods, and allows the child to be fed more frequently because a fresh meal does not need to be prepared every time.

Germination and fermentation can be used together. Foods produced in this way have the combined advantages of viscosity reduction and improved microbiological safety. Recent research suggests that these foods are of particular value in improving energy intake in sick children, especially those with acute diarrhoea.

Conclusion

Germination and fermentation are traditional household-level food technologies which can be used to improve weaning practices in developing countries. Amylase rich flour produced by germination is particularly effective in improving feed energy density, while fermentation reduces contamination of feeds with pathogens, and facilitates more frequent child feeding.

References