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Full Length Research paper

Investigation of the potential of fortified instant Matooke flour (ITF) in rehabilitation of malnourished children (Part II): Testing potential of ITF as a vehicle food for malnutrition intervention

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This study was done to establish potential of instant *Tooke* (cooking banana) flour (ITF) as a vehicle food for malnutrition intervention. Hypotheses generated were: (1) There is a significant difference in response with respect to weight gain between malnourished subjects rehabilitated using fortified IFT and *Mwanamugimu* Dietary Regime (MDR); (2) There is a significant difference in response with respect to serum albumin, ferritin and retinol levels between fortified ITF and MDR. To test the hypotheses, 100 malnourished children admitted at *Mwanamugimu* Nutrition Unit in Uganda, were randomly allocated to a test (IFT diet from part I of study) or control (MDR diet) group. Growth rates and food intake were measured daily, while blood samples were withdrawn during recruitment and before discharge for testing serum albumin, ferritin and retinol levels. Means were compared using SPSS version 11. The test and control diets were adequate in protein but relatively lower for energy against RDA. Protein and energy intake in the test group was significantly ($P < 0.05$) higher for porridge, while protein intake in the control group was significantly ($P < 0.05$) higher for *kitoobero.* Weight gain in the test was significantly ($P < 0.05$) higher than the control group. However, both groups showed no significant difference in the bio-chemical indicators. The superiority of fortified ITF formulation over the MDR was proved with respect to weight gain but not for the biochemical indicators. Hypothesis one was accepted but two was rejected.

Key words: Instant *tooke* flour (ITF), *matooke*, malnourished, rehabilitation

INTRODUCTION

Bananas are an important staple food for more than 70% of Uganda's population. Cooking bananas also known as '*matooke*' (East African highland cooking bananas) are grown by 72% of farmers on an average garden size of one hectare, which is 12.3% of the world total (Karugaba and Kimaru, 1999). The high levels of production and consumption of *matooke* notwithstanding, there are still high levels of malnutrition in Uganda, with 50% of children fewer than five years in rural areas either stunted or underweight (UBOS and ORC Macro, 2006).

Malnutrition is a condition resulting from an inadequate (under-nutrition) or excessive intake (over-nutrition) or impaired utilization of one or more nutrients in the body. There are two types of under nutrition, macro and micronutrient malnutrition. Macro nutrient malnutrition in children occurs as protein energy malnutrition (PEM) presenting clinically as kwashiorkor, marasmus or marasmic-kwashiorkor (Waterloo, 1993). Symptoms of kwashiorkor

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may include oedema, sparse brown hair, rashes, misery and apathy, while those of marasmas include retardation, under weight, absence of subcutaneous fat and wasting of muscles (Cameron et al., 1983; Latham, 1989). Marasmic-kwashiorkor is a combination of kwashiorkor and marasmas symptoms (Cameron et al., 1983).

It has been observed that PEM is often accompanied by vitamin and mineral deficiencies especially Vitamin A and Iron deficiencies. The underlying factors for malnutrition have been identified to include low energy density of weaning foods and lack of ready to prepare formulations, which lead to infrequent feeding due to the heavy workload of the caregivers especially among the rural poor (Kikafunda, 1996).

In response to the need for energy and protein dense foods, the national children's rehabilitation centre (Mwanamugimu) at Mulago, the main referral Hospital in Kampala, Uganda, developed a series of multi-mix recipes also called *kitoobero* from locally available foods. The recipes incorporate local starchy staples mainly *matooke or rice.* The Mwanamugimu has developed a standard dietary regime which is code named Mwanamugimu dietary regime (MDR) comprising a total of seven meals: one from porridge (with maize cereal as energy base) lunch and supper from *kitoobero* and in between four meals of a high energy milk (HEM) with energy enhancement from oil. The porridge and HEM diets are sweetened and supplemented by a complex mineral vitamin (CMV). The process of preparing maize porridge and *kitoobero* are, however, laborious and time consuming, therefore their rate of adoption by the caregivers, after discharge is limited.

Advances in processing of *matooke* have succeeded in reducing the relative bulk of *matooke* starch by pre-gelatinization (Muranga, 1998 and Muranga et al., 2007). Muranga (1998) reported that *matooke* has high starch content (^{dB}: Dry basis 81.8 - 85.7%) and subsequently has a high potential as a calorie resource base. Muranga et al. (2007) comprehensively reported on the effect of processing techniques on the bioavailability of *matooke* starch. Further in the same study using mice, Muranga et al. (2007) demonstrated the significance of available carbohydrate in the diet at the weanling stage of mice. The authors subsequently concluded that solubilisation of *matooke* starch through heat processing improved its bioavailability and enhanced availability of other nutrients in the diet. The pregelatinised cooked form of *matooke* has since been branded as Instant *Tooke* Flour (ITF). Additionally, pilot studies revealed relatively high Protein Efficiency Ratios (PER), when ITF fortified with soybean and sesame was fed to weanling rats (Muranga et al., 2003). The above studies recommended testing of the pregelatinised flours in combination with high protein mixes in malnourished children in a clinical setting prior to adoption for application as a weaning food. In our first paper we established the optimal level of fortification of ITF with soy and sesame as 65:16:19. The objective of this study therefore was to establish whether the pregel form of *matooke*, as ITF brand, fortified at the above ratio with soy and sesame and micronutrients can serve as a vehicle food for malnutrition intervention using human subjects. Two hypotheses were thereby tested in this part of the study namely:

(1) There is a significant difference in response with respect to weight gain between subjects rehabilitated using fortified IFT and *Mwanamugimu* dietary regime (MDR).
(2) There is a significant difference in response with respect to serum albumin, ferritin and retinol levels between fortified ITF and MDR.

METHODS

Study subjects

A baseline of the age, sex, heights and weights was taken before commencing the study. Table 1 shows the general characteristics of the study population. The study was carried out at the national children's rehabilitation centre (*Mwanamugimu* nutrition unit) in Mulago hospital, Kampala Uganda, where 100 severely malnourished children aged 6 -59 months were randomly allocated to either a test or control group of equal size using an open labeled randomized trial design. Sample size was determined using a formula reported by Kirkwood, (1988). Generally age range was between 6-59 months of which 50% had kwashiorkor, 31% marasmus and 19% marasmic kwashiorkor. 55% were otherwise free from secondary infection while the rest suffered malaria or other secondary infections during the study. A blood sample was also taken from subjects during recruitment and before discharge.

The test group was fed on fortified Instant *Tooke* Flour (ITF) diet, while the control group was fed on the *Mwanamugimu* dietary regime (MDR). Both diets were fortified with a therapeutic complex mineral vitamin (CMV) manufactured by Nutriset Malaunay, France. The diets consisted of three definite menu categories, porridge or Uji, High Energy Milk (HEM) and *Kitoobero* (main menu) for both the test and control groups. The test porridge consisted of ITF supplemented with soy and sesame (65:16:19), sugar and milk and fortified with CMV. The control was similar in formulation but had the ITF composite replaced by maize flour. The High Energy Milk (HEM) diet basically consisted of milk, sugar and CMV with the energy enrichment coming from IFT for the test diet and from oil for the control. The *Kitoobero* test diet (a multi-mix recipe cooked together in one pan) consisted of ITF-soy-sesame formulation, salt and water while the control consisted of a triple mix of rice, fish powder and groundnut paste. The test and control groups were fed daily on their respective diet regime to provide calories ranging from 150 kcal to 220 kcal per day, with 150 kcal being the minimum. The calorie (energy) requirement given was calculated basing on the body weight of the subject, and this increased as the weight and appetite of the patient increased. According to WHO (2003), a vigorous approach is required to achieve very high intakes and rapid weight gain of >10 g gain/kg/d. The formula used in determining the quantity of feeds per meal for each subject is as shown below. Each subject was given seven meals per day.

$$\text{Amount of food served/meal} = \frac{150\ \text{Kcal} \times \text{body weight}}{7} \quad (1)$$

The feeding regime therefore required administration of porridge at 6 am, HEM four times at 9 am, 3 pm , 9 pm and 12 midnight) where as *Kitoobero* diets were administered for both lunch and supper at 12 noon and 6 pm respectively. Meal times were observed by the

Table 1. General characteristics of the study population.

Characteristic	Test group (%)	Control group (%)	Overall (%)
Sex of child			
Male	28	20	48
Female	22	30	52
Age (months)			
6 - 12	13	14	27
13 – 24	34	25	59
25 – 36	4	7	11
37 – 59	0	3	3
Malnutrition type			
Kwashiorkor	23	27	50
Marasmus	18	13	31
Marasmic-kwashiorkor	10	9	19
Secondary infection			
Malaria	22	17	39
Diarrhea	3	2	5
Septic wound	0	1	1
No infection	24	31	55
Occurrence of infection			
Week 1	12	5	17
Week 2	17	17	34
Week 3	1	4	5
Length of hospital stay			
1 week	15	17	32
2 weeks	21	24	45
3 weeks	14	9	23
Outcome			
Discharged (blood withdrawn)	43	47	90
Runaway	6	0	6
Discharged (blood not withdrawn)	1	3	4

study nurse or investigator to avoid sharing of meals.

Every meal given to the subjects was weighed before feeding and the leftover food was also weighed to determine the actual intake of food by difference. The actual food intake by difference was calculated by subtracting the leftover food from the food served to each child and was used to calculate the protein (P) and energy (E) intakes per meal follows:

$$\text{Protein intake per meal} = \text{Food intake} \times \sum \text{Protein content per diet} \quad (2)$$

The energy intake was also calculated as in equation 2 while Table 2 shows a summary of the calculations.

The adequacy of the meal was benchmarked against the Recommended Daily Allowance (RDA) for the ages of the children used in the study. The adequacy was compared with respect to the percentage increase in protein and energy requirements over the standard RDA. The increase was calculated as shown thus:

$$\%\ \text{increase in RDA for Protein} = \frac{\text{Actual Protein intake (g/day)} - \text{RDA of age group for Protein} \times 100}{\text{Protein RDA of age group}} \quad (3)$$

Percentage increase in RDA for energy was also calculated as in equation (3) but replacing energy for protein.

The subjects were fed for a period ranging between 1 and 3 weeks depending on how long they took to attain the median weight or at least 85% of the median weight. This was determined by the initial weight, and secondary infections suffered, such as diarrhea and malaria. The Discharge criteria included meeting the weight gain requirement as stated above and being on the study for at least two weeks (If the child had met the weight requirement and had been on the study for two weeks, they were discharged to avoid congestion due to limited space in the ward). However, the ability of the caretaker to adopt and maintain behavior change

Table 2. Calculation of the energy and protein contents of the diets.

Group	Diet	Energy (Kcal)	Calculation of energy intake for each child	Protein (g)	Calculation of protein intake for each child
Test	Porridge (ITF + sesame +soybean + milk + sugar)	898.1	Intake*898.1/800	26.3	Intake*26.3/800
	Kitoobero (ITF + sesame + soybean)	1004.2	Intake*1004.2/700	22.2	Intake*22.2/700
	High energy milk (HEM) (milk + ITF + sugar)	1002.02	Intake*1002.02/1000	43.09	Intake*43.09/1000
Control	Porridge (maize flour + milk + sugar)	837.0	Intake*837/1000	28. 5	Intake*28. 5/1000
	Kitoobero (rice + g/nuts + fish powder)	1178.4	Intake*1178.4/780	39.78	Intake*39.78/780
	High Energy Milk (HEM) (milk + oil + sugar)	1006.8	Intake*1006.8/1000	38	Intake*38/1000

Figure 1. Comparison in food intake per meal between the test and control group.

learning how to feed the child) was taken into consideration in order to avoid a relapse of malnutrition.

Serum albumin, ferritin and retinol analyses

The serum albumin content was determined using Bromo Cresol Purple (BCP) method a WHO standard method while, ferritin con-tent was determined using a photometric method by a procedure as reported by Tietz (1987). Retinol content was determined using Colorimetric method based on Carr-Price Reactions using Trichoro Acetic Acid as reported by Underwood and Stekel (1984).

DATA ANALYSES

All the data was analyzed using SPSS version 11. The analysis for each response variable was based on a three factor analysis of variance model in which the groups were one of the factors. For each type of meal there was a three factor ANOVA which included group (test versus control), age and time (length of stay on study). The response variables included protein and energy intake, weight gain, serum albumin, ferritin and retinol levels.

RESULTS

Food intake

Figure 1 shows the comparison in food intake per meal between the test and control groups. The tests here for ITF include ITF-soy-sesame vs. maize flour in porridge, ITF vs. oil in HEM, and ITF-soy-sesame vs. rice and groundnut in the *kitoobero* diets. There was no significant diff-errence ($p > 0.05$) between the test and the control groups in the intake of porridge, *kitoobero* and HEM. However, in all cases, the control group took in more food compared to the test group.

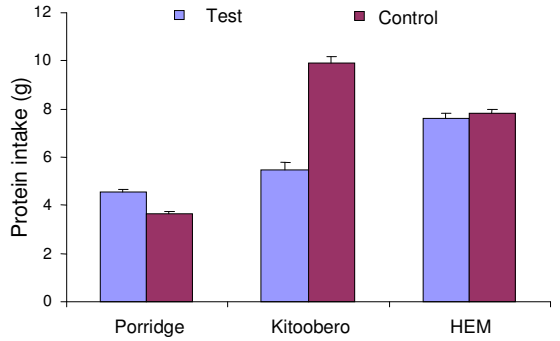

Figure 2. Comparison in protein Intake per meal between the test and control group.

Protein intake

Figure 2 shows the comparison in protein intake per meal between the test and control groups. There was a significant difference ($p<0.05$) between the test and control groups in the protein

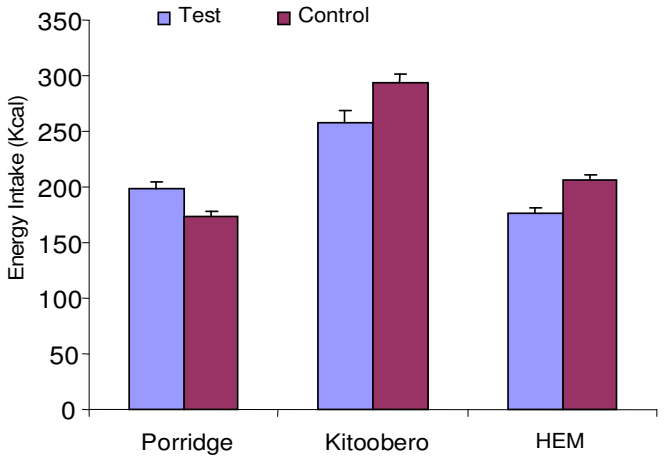

Figure 3. Comparison in energy intake per meal between the test and control groups.

Table 3. Comparison of adequacy of Test (ITF) and Control (MDR) diets for protein and energy with recommended dietary intake (RDA) for the respective subjects in the study.

Age GP	Protein (g/day)				Energy (Kcal/day)			
	Test GP	Increase in RDA (%)	Control GP	Increase in RDA (%)	Test GP	Increase in RDA (%)	Control GP	Increase in RDA (%)
6 - 11	16.41	49.18	16.98	54.36	596	-13.96	535.86	-22.64
12 - 23	17.15	31.92	20.2	55.38	616.14	-39.94	630.79	-38.51
24 - 35	19.16	47.38	21.07	62	684.54	-33.28	659.99	-35.67
36 - 59	-		30.31	78.29	-		960.02	-34.01

intake for porridge. There was indeed a significant difference ($p < 0.05$) between Control and Test groups in the protein intake for *kitoobero* but no significant difference ($p > 0.05$) in the protein intake for HEM.

Energy intake

Figure 3 shows the comparison in energy intake per meal between the test and control groups. There was a significant difference ($p < 0.05$) between the test and control groups in the energy intake for porridge but no significant difference ($p > 0.05$) in the energy intake for *kitoobero* and HEM. The test porridge was therefore better than the control porridge with respect to energy, which credits the IFT formulation, but the energy intake for *kitoobero* and HEM for the two groups was comparable. Table 3 shows the relative adequacy of the food intake for the different subject groups against standard recommended daily allowance for normal subjects (National Academies Press, 2005). The adequacy was compared with respect to the percentage increase in protein and energy requirements over the standard RDA. There was no significant ($p > 0.05$) difference between the test and the control groups within the different age groups. Except for age 12 - 23 months The food intake for both the test and control diets was

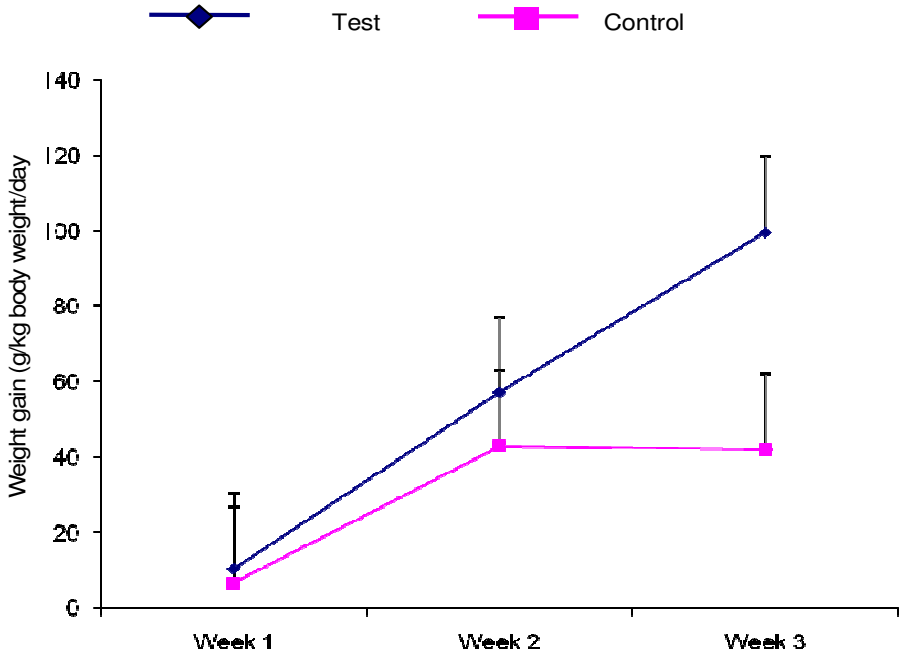

Figure 4. Comparison in weight gain between the test and control groups over study period.

adequate for protein since it was close or well above the 50% recommended increase for subjects under rehabilitation. Energy intake was however, low for both the test and control groups since all the percentages were negative and therefore far below the 10% recommended energy increase for such subjects. Given the overall adequacy of the protein as per Table 2 against the RDA values for the respective age groups, focus of the test is therefore on the performance of the energy sources in the test vs. the Control diet.

Weight gain

Figure 4 shows the comparison in weight gain between test and control group over the period of study. There was a significant difference ($p < 0.05$) in the weight gain between the test and control groups over the period of study. Both the test and control groups had a progressive gain in weight in the first two weeks. However, there was a slight decrease in weight gain for the control group in the third week. The difference in weight gain between the test and control group was particularly significant in the third week as it shot up for the test group but decreased for the control group.

Figure 5 shows the comparison in weight gain between the test and control groups by age groups over the period of study. There was no significant difference ($p > 0.05$) in weight gain by age group over the period of study between the test and control groups. However, the test group in the 6 - 11 months and the 24 – 35 months age groups, had a higher weight gain compared to the control group in week 1, 2 and 3. The 12 - 23 months age group had a comparable weight gain for both groups in week 1, in week 2, the control group had a higher weight gain, while in week 3, the test group had a higher weight gain.

The test group also had a higher weight gain in the 36 - 59 months age group in week 1 and 2.

Blood serum levels

Serum albumin: There was an increase in the serum albumin levels for both groups. The test group had an increase from 31.095 - 36.137 g/l while the control group had an increase from 27.953 - 34.148 g/l. However, there was no significant ($p < 0.05$) difference between the test and control groups in increase in the serum albumin levels over the period of study, implying that the two groups did not respond differently from each other.

Serum ferritin: The test group had an increase in the serum ferritin level from 13.447 - 16.056 µmol/l while the control group instead had a drop in the serum ferritin level from 15.169 - 14.704 µmol/l. However, there was no significant difference ($p > 0.05$) between the test and control groups over the period of study implying that the two groups did not respond differently from each other.

Serum retinol: The test group had an increase in the

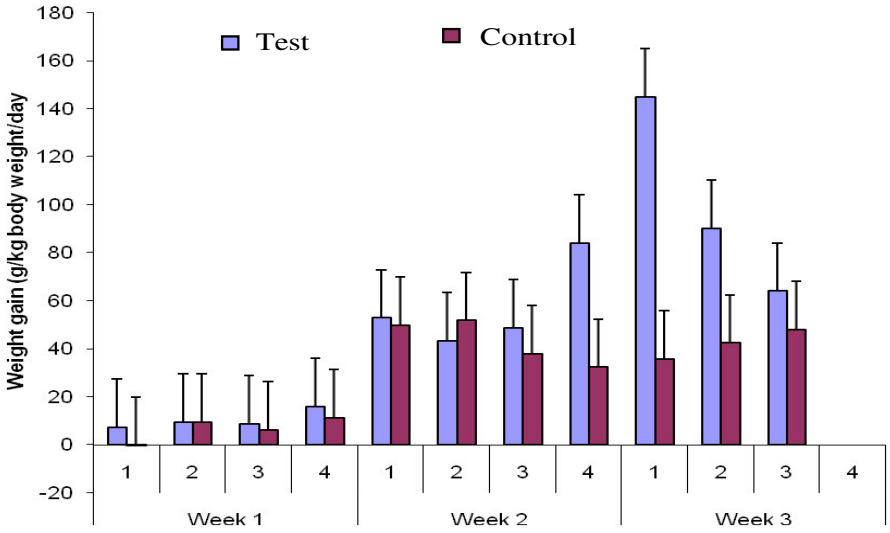

Figure 5. Comparison in weight gain between the test and control groups by age groups over study period without outliers. Key: 1 = 6-11 months, 2 = 12-23 months, 3 = 24 – 35 months and 4 = 36 – 59 months.

serum retinol level from 5.197 - 7.421 µg/dl, while the control group had a slight drop in serum retinol level from 6.320 - 6.299 µg/dl. However, there was no significant difference ($p < 0.05$) between the test and control group over the period of study, implying that the two groups did not respond differently from each other over the period of study.

DISCUSSION

The data revealed the relative energy inadequacy of both the test and control diets. Further the fact that the food intake for the test group though not statistically significant was less compared to the control group is unfortunate for the instant formulation since it is indicative of a significant barrier to energy emanating from inherent bulk in the ITF. In Muranga et al. *(*2009) the end viscosity of the ITF-soy-sesame optimal formulation was set at 191 RVU compared to that 423RVU for pure ITF which was approximately 50% reduction in viscosity due to incorporation of the protein mixes. Therefore whereas pre gelatinisation of the matooke served the reduction of the bulk to a certain extent further reduction in bulk requires to be effected to enable children in this disadvantaged health state to consume enough amounts to meet their high energy requirements. To this end Muranga et al. (2007) proposed extrusion cooking, which yields an instant flour that is virtually soluble in warm water. The above observations are however, consistent with a previous study reported by Brown et al. (1995) and Bennet et al. (1999) where poor food intake was attributed to the low energy density of the control diet (Brown et al., 1995; Bennet et al., 1999). Generally lower glycaemic and the correspondingly high satiety responses are associated with high amylose starches (FAO, 1988). Matooke starch, however, is a low amylose starch, but has been associated with heat mediated transformations that could affect the endotherm values (gelatinization transition temperature) and therefore the pregelatinisation process (Muranga, 1998) particularly given that the method of gelatinization was a slow steam operation.

That both diet regimes met the RDAs for protein is a fact that can be in part attributable to the milk supplementation in both diet regimes but particularly in the control.

The differences in the protein intake between the test and control group for the *Kitoobero* meals underscores the significance of animal protein in the diet. The control group consumed animal protein from the dried silver fish also known as 'mukene', whereas the test group only consumed plant protein (soybean and sesame) as shown in Tables 2 and 3. On the contrary lack of significant differences in the protein and energy intake for HEM after substitution of oil for ITF is significant for the ITF as a vehicle for protein/energy supplementation since oil is a more expensive ingredient.

That diets for both the test and control groups did not meet the Recommended Daily Allowance (RDA) for energy is consequent to the carbohydrate bulk barrier in the control MDR from the maize porridge and the rice *Kitoobero*. The reason for the IFT regime is already high lighted above under nutrient adequacy. The differences observed between the test and control in energy intake with respect to porridge is therefore attributed to the relatively higher energy density and the low viscosity of the IFT (Kanyago et al., 2009). Capdevila et al. (1998) reported volume intake and energy density to be one of

main factors influencing energy intake. It is, however, important to note that the RDA is calculated for a normal population and the study population was an abnormal one for which management was being done in order to restore them to normalcy. The calculation for energy requirements per meal was based on the weight of the individual subjects and this increased as the weight of the subject increased.

The short fall in energy intake notwithstanding, our results served to demonstrate that the IFT formulation was a superior to the MDR as vehicle for malnutrition intervention which is evidence of a superior bioavailability of energy from the IFT formulation in contrast to the control. Muranga *et. al.* (2007) indeed reported on this availability after testing growth responses of mice on matooke flour formulations. Indeed the progressive gain in weight for the test group was commensurate with other studies conducted to rehabilitate severely malnourished children. These studies generally report a higher weight gain for the children receiving high-energy-density porridge compared to those receiving low-energy-density porridge (Donmen et al., 1996; Bennet et al., 1999).

Catch-up growth represents accelerated tissue gain beyond that expected for chronological age; it occurs after a period of slowing or cessation of growth. Protein and energy may influence both normal and catch-up growth (Tanner, 1989). The test diet regime was therefore able to sustain both the normal and catch growth for the subjects throughout the period of study. This is consistent with what was reported by Uauy and Alvear (1991), that weight loss is usually fully reversible, since weight gain is mainly affected by energy supply in relation with energy expenditure. Nonetheless, the excellent performance in weight gain for the test group in only in the third week is indicative of a requirement for a more available source of carbohydrate at the initial rehabilitation phase in malnutrition similar to that in the weaning stage of animals as reported by Muranga et al. (2007). The control diet was able to some extent in the first week to serve a lower catch up growth but in the second week it fell under negative energy balance and hence negative catch up growth which is unfortunate given the intensity of labour and good will associated with its administration to date.

The change in blood serum levels for albumin, ferritin and retinol for both the test and control groups was not significant. Albumin is a hepatically synthesized protein frequently used to estimate overall nutrition status (Marinella and Markert, 1998), while serum ferritin and retinol are used to determine iron and Vitamin A status respectively. Serum albumin for both groups was within the normal ranges at the end of the study. The normal levels range from 35 - 50 g/l (Latham, 1997). The initial serum ferritin levels for both groups were within the normal range, which is between 9.0 - 30.0 µmol/l (Tiez, 1987). This could be attributed to infection, inflammation, liver disease, and states of increased RBC turnover (e.g. ineffective erythropoiesis and hemolysis), which can falsely elevate these levels (Lanzkowsky, 2000). Also there were instances of hemolysis due to resistance from the study subjects during withdrawal blood samples. Despite the high reliability of low concentrations of ferritin in plasma or serum as an indicator of iron deficiency, concentrations in the normal range in iron deficiency are not uncommon (Garibay et al., 2001). The same author also reported iron deficient subjects to be having plasma ferritin concentrations within the normal range.

The drop in serum ferritin level for the control group is not easy to explain and is an area for further study. The serum retinol results for both groups were below the normal levels. Blood-serum retinol concentrations of below 20 µg/dl signify marginal deficiency and that below 10 µg/dl signify definite deficiency (Lindblad et al., 1998; Saskia and Dary, 2002). The control group also had a drop in the serum retinol levels like in the serum ferritin. This also is an area for further study. Serum retinol concentration reflects an individual's vitamin A status, particularly when the body's reserves of vitamin A are limited, because serum retinol concentration is homeostatically controlled and will not drop until body stores are significantly compromised (Olson, 1994). However, serum retinol concentration is also affected by factors that affect release of holo-RBP from the liver, infection, protein status, adequacy of other nutrients and organ disease. In general, these factors lower serum retinol concentration (Olson, 1994).

Overall the results of the biochemical indicators in this study showed that they were a less reliable benchmark for monitoring rehabilitation of malnourished children.

The major limitation to this study was time and financial constraints. However, the cost effectiveness issues are being covered in the follow-up study. However, the ITF preparation is a simple technology in which the government of Uganda has invested to ensure effective technology transfer to all *Matooke* growing communities in the country.

SUMMARY

Our results demonstrated that there is a significant difference in response with respect to weight gain between subjects rehabilitated using fortified ITF and Mwanamugimu dietary regime (MDR) so hypothesis one was accepted. Further we also evidenced that there was no significant difference in response with respect to serum albumin, ferritin and retinol levels between subjects rehabilitated on fortified ITF and those on MDR and so hypothesis two was not accepted. The results further justified the significance of energy density over protein intake in protein energy malnutrition rehabilitation. We therefore recommend exploring technology for further solubilisation of *matooke* flours in order to optimize energy density.

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