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Production and evaluation of weaning foods based on sorghum and legumes

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Abstract. Five weaning formulations (F1-F5) based on sorghum, groundnuts, sesame seeds, chickpeas, and skim milk powder were processed by a twin-roller drum dryer and evaluated for composition, functional properties (bulk density, water absorption capacity, and apparent viscosity), in vitro indices (protein digestibility and available lysine), protein quality (PER, NPR, and NPU) and effects of feeding on rat livers. Composition and properties of the five formulations were compared to those of Cerelac. The results indicated that F3 (60% sorghum, 20% chickpeas, 5% sesame, 8.5% skim milk powder, 5% sugar, and 1.5% vitamins and minerals) and F2 (55% sorghum, 15% chickpeas, 5% groundnuts, 10% sesame, 8.5% skim milk powder, 5% sugar, and 1.5% vitamins and minerals) formulations had compositions and properties comparable to those of Cerelac and hence have a good potential for use as weaning foods.

Key words: Functional properties, Legumes, Liver histopathology, Nutritional qualities, Proximate composition, Sorghum, Weaning foods

Introduction

The problem of providing nutritious, low-cost protein supplements to the diets of young children whose intakes consist mainly of cereals and tubers has been engaging the attention of nutrition workers in several countries and international organizations. Few low-income countries have, to date, managed to develop comprehensive weaning food (WF) policies and programs. Experience has shown that these programs must be based on local foods that are culturally acceptable, easily prepared, and affordable by the majority.

The prevailing weaning foods and practices in Sudan are inadequate. At present, there are no weaning foods manufactured in Sudan from locally-available food resources. Only a small sector of the community uses imported baby foods. There is, therefore, an urgent need to conduct studies that help in production of weaning foods based on locally-available materials.

Table 1. Selected weaning food formulations

Ingredient	Formulations (%)				
	F1	F2	F3	F4	F5
Sorghum	60	55	60	70	60
Chickpea	10	15	20	15	20
Groundnut cake	5	5	–	–	10
Sesame	10	10	5	–	3.5
Skim milk powder	8.5	8.5	8.5	8.5	–
Sugar	5	5	5	5	5
Vitamins and minerals mixture*	1.5	1.5	1.5	1.5	1.5
Total	100	100	100	100	100

* Composition/100 g of mixture: Ca: 0.89 g; P: 0.6 g; Fe: 10 mg; Vitamin A: 1500 IU; Vitamin D: 300 IU; Vitamin B1: 0.5 mg; Vitamin B2: 0.6 mg; Niacin 5 mg; Vitamin C: 30 mg.

Various workers [1–3] have developed protein-enriched, low-cost weaning foods containing cereals and legumes, but the information on biological, functional and nutritional qualities of these weaning foods is scarce. Based on these facts, a study was undertaken to formulate weaning foods based on food materials locally-available in Sudan and to evaluate their composition and properties.

Materials and methods

Raw materials. The raw materials selected for formulation of weaning foods (WFs) included sorghum (*Sorghum vulgare*), groundnut seeds (*Arachis hypogaea*), sesame seeds (*Sesamum indicum* L.), chickpeas (*Cicer arietinum*), and commercial grade skim milk powder (SMP). Preliminary processing (dehusking of sorghum, chickpeas and sesame to reduce their fiber content) and production of edible groundnut cake, to lower the fat content, to improve nutritional value and suitability for weaning foods was carried out.

WF formulation. Five formulas for WFs based on the method of Jansen & Harper [4] were chosen. Selection of the formulas was based on (a) a reasonably high amino acid score (>65%), (b) a protein content within the specified values for weaning foods [5, 6], and (c) the use of variable combinations and percentages of raw materials. Composition of the five formulas (F1 to F5) is given in Table 1.

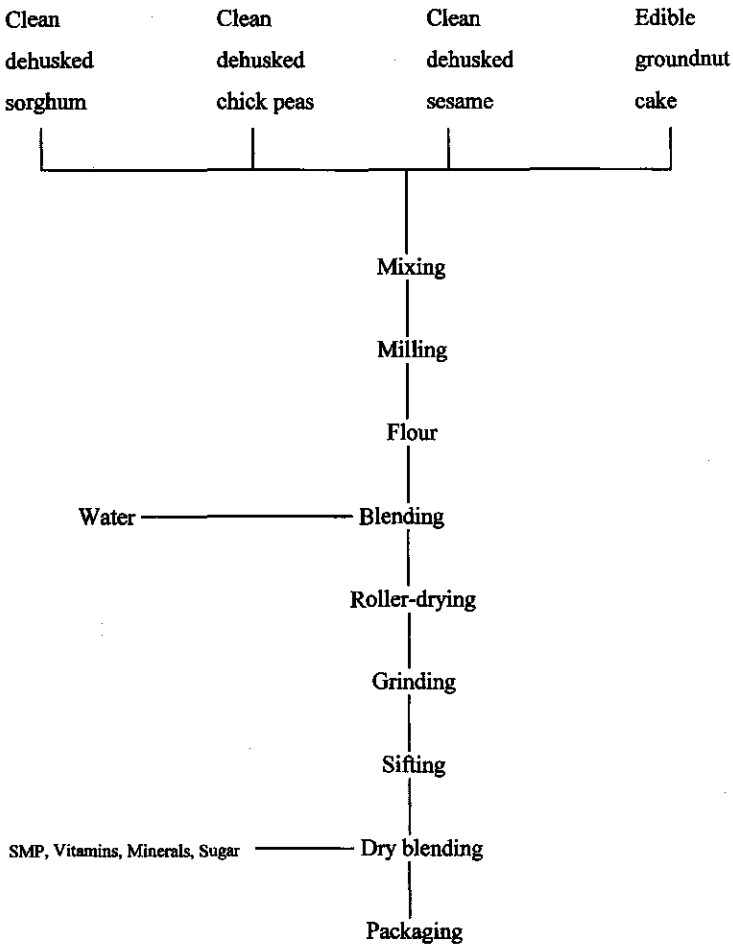

Figure 1. Process flour sheet for roller-dried weaning foods.

Processing of the WFs. Figure 1 shows the steps followed in the processing of the five formulations. The specified amounts of sorghum, chickpeas, groundnut cake and sesame for each formulation (Table 1) were weighed using a triple-beam Ohaus Balance and manually mixed together thoroughly. The mixture was milled in a plate mill (Rajan Trading Co., India) to a fine (0.1 mm particle size) flour. Water was added to the flour in the ratio of 1:1. The slurry was first hand mixed and then passed through a Fryma Mill (Rajan Trading Co., India) for homogenization. A twin-roll drum dryer was used for processing. The resulting flakes were milled to a fine powder using an Apex Mill (Rajan Trading Co., India) fitted with a B 164 sieve. This was followed by sifting to obtain a homogeneous product. Skim milk powder, sugar, vitamins

and minerals were then dry blended with the resulting flour. The processed formulations (in powder form) were stored in tightly-closed containers and kept in a cold room (7 ± 2 °C) until taken for analyses.

Evaluation of WFs quality. The selected formulations were evaluated for proximate composition, functional properties and in vitro indices. Cerelac, a commercial weaning food, was also evaluated for comparison.

Proximate composition. AOAC [7] methods were followed in the determination of moisture (7.003), fat (7.048), crude (non-digestible) fiber (7.057) and total ash (14.006) contents. Protein content was determined using the Buchi apparatus which consisted of a Buchi 425 digester and a Buchi 321 distillation unit. About 100 mg of sample were placed in a digestion flask. A small amount of the digestion mixture was added and 10 ml concentrated H_2SO_4 were used for digestion which was allowed to proceed for 2–3 hours. The cooled clear digest was diluted to 250 ml in a volumetric flask and 10 ml aliquots were used for distillation. Distillation was carried out, after adding 40 ml of 32% NaOH and 10 ml water, for two minutes. Distilled ammonia was received in 10 ml of 2% boric acid to which two drops of a universal indicator (methyl red + bromocresol green) were added. Titration was carried out against N/70 HCl, the acid factor (AF) of which had been determined using $(NH_4)_2SO_4$. Protein % was calculated using the following equation:

$$\text{Protein } \% = t \times df \times 100 \times 6.25/AF \times w \times 1000,$$

where t = titer; df = dilution factor; AF = acid factor; w = weight of sample.

Digestible carbohydrate content was obtained by difference. The total energy (kcal) was calculated by multiplying the digestible carbohydrate and protein contents by four and the fat content by nine.

Functional properties. Bulk density (BD) was determined using the method of Wang & Kinsella [8]. Ten g of the test material were placed in a 25 ml graduated cylinder and packed by gentle tapping of the cylinder on a bench top ten times from a height of 5–8 cm. The final volume of the test material was recorded and expressed as g/ml. The method described by Cegla et al. [9] was used for determination of water absorption capacity (WAC). Six g of material were weighed in a 100 ml beaker. A known volume of water was pipetted into the beaker. The wet material was carefully stirred and allowed to equilibrate for one hour at 26 °C. After complete water absorption, the sample was further treated with 0.01 ml water portions with 10 minute interval before visual observation. The volume that gave a complete absorption of

water (no visible free water) was recorded. WAC was calculated as the ratio of maximum volume of water in g absorbed by 100 g dry material. Apparent viscosity (AV) was determined by the method described by Quinn & Beuchat [10]. Cold water slurries containing 20% sample solids were heated in a boiling water bath with constant stirring until boiling which was continued for three more minutes. They were cooled to room temperature and their viscosity was measured with a Brookfield Synchro-electric Viscometer using RVT Spindle No. 4 at a constant speed of 100 rpm. Conversion into cps units was done using the specific factor for Spindle 4.

In vitro indices. In vitro protein digestibility (pepsin-pancreatin digest) was carried out according to Saunders et al. [11]. The method of Carpenter [12] modified by Booth [13] and described by Pellet & Young [14] was followed in the determination of available lysine.

Protein quality evaluation by animal feeding experiments. Eight different diets (Table 2) were prepared and used for animal feeding experiments. Weanling male albino rats of the Wistar strain (21–23 days old) weighing 36-39 g were distributed into 8 groups with 8 rats per group using the randomized block design method. Rats were housed individually in cages with mesh bottoms. Protein efficiency ratio (PER) was determined following the method of AOAC [7] and the Indian Standards Institute [15]. Net protein ratio (NPR) was determined by the method of Bender & Doell [16]. Net protein utilization (NPU) was determined by the direct carcass analysis technique developed by Miller & Bender [17].

Studies on the livers of rats fed the WF formulations. Livers of rats used in the PER test were studied to investigate any histopathological changes and/or abnormalities (Figure 2). The livers studied were those of rats fed diets used in the PER determination (except the basal diet), in addition to three extra groups: one fed Cerelac with 15.5% protein, a second fed F2 with 20% protein and a third fed F3 with 16.7% protein. The purpose of including the three extra groups was to see the effect of protein level on the rats' livers. Livers were weighed individually and a small section, of known weight, of the main liver lobe was taken for histopathological studies. These sections were kept in 10% formalin before further treatment for histopathological analysis. The remaining portions of livers for each group of rats were pooled separately and were directly freeze-dried. Analysis for percent moisture, protein and fat were carried out on the freeze-dried liver [7].

Statistical analysis. Data were analyzed using Analysis of Variance (ANOVA) in accordance with standard methods of statistical analysis [18]. Tests

Table 2. Composition of diets used in animal experiments

Diet	Test material (g/100 g of diet)	Refined groundnut oil (g/100 g of diet)	Sugar (g/ 100 g of diet)	Salt mixture* (g/100 g of diet)	Vitamin mixture** (g/100 g of diet)	Corn starch (g/100 g of diet)
F1 (at 10% protein)	50	10	10	2	2	26
F2 (at 10% protein)	50	10	10	2	2	26
F3 (at 10% protein)	60	10	10	2	2	16
F4 (at 10% protein)	53	10	10	2	2	23
F5 (at 10% protein)	51	10	10	2	2	25
Cerelac (at 10% protein)	65	10	10	2	2	11
Casein (at 10% protein)	12.5	10	10	2	2	63.5
Basal (N-free)	–	10	10	2	2	76
Cerelac (at 15.5% protein)	100	–	–	–	–	–
F2 (at 20% protein)	100	–	–	–	–	–
F3 (at 16.7% protein)	100	–	–	–	–	–

* Salt mixture contains (g/kg): $CaCO_3$ (543); $MgCO_3$ (25); $MgSO_4$ (16); NaCl (69); KCl (112); H_2PO_4 (212); $FePO_4.4H_2O$ (20.5); KI (0.08); $MnSO_4$ (0.035); NaF (1); $Al_2(SO_4)2KSO_4$ (0.17); $CuSO_4$ (0.9); $ZnCO_3$ (1).

** Vitamin mixture contains (/kg): A (200000 IU); D3 (100000 IU); E (10 g); K (0.5 g); B1 (0.5 g); B2 (1 g); B6 (0.4 g); B12 (2 mg); Folic Acid (0.2 g); Niacin (4 g); Biotin (0.02 g); Inositol (25 g); Choline chloride (200 g); PABA (10 g); Starch (to make up to 1 kg).

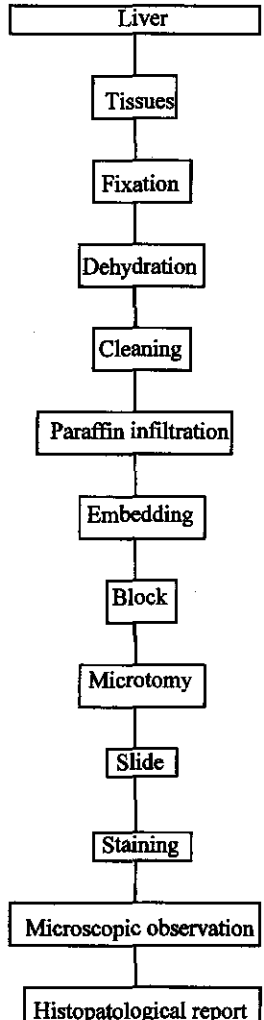

Figure 2. Flow diagram for preparation of rat liver tissues for histopathological investigation.

of significance were carried out using Duncan's multiple range tests [19]. Significance was accepted at $p < 0.01$ level.

Results and discussion

Proximate composition. The moisture (6.4 to 7.7 g), protein (16.7 to 20.3 g), crude fiber (3.0 to 4.0 g), ash (1.4 to 1.7 g) and caloric (359 to 374

Table 3. Proximate composition* of weaning food formulations and Cerelac

Component	F1	F2	F3	F4	F5	Cerelac
Moisture (g/100 g)	6.4 ± 0.2	6.8 ± 0.2	7.7 ± 0.1	7.2 ± 0.2	7.2 ± 0.1	2.5 ± 0.1
Protein (g/100 g)	20.3 ± 1.2	20.0 ± 1.8	16.7 ± 1.4	18.8 ± 1.2	19.6 ± 1.4	15.5 ± 1.1
Ether extract (g/100 g)	4.4 ± 0.1	4.0 ± 0.1	1.8 ± 0.1	1.1 ± 0.1	2.4 ± 0.1	9.0 ± 0.2
Crude fiber (g/100 g)	1.7 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
Ash (g/100 g)	4.0 ± 0.2	3.5 ± 0.1	3.1 ± 0.1	3.0 ± 0.2	3.0 ± 0.1	2.7 ± 0.1
CHO (g/100 g)	63.2	64.2	69.0	68.5	66.3	68.9
Energy (kcal)	374	373	359	359	365	419
% protein calories[a]	21.7	21.4	18.6	20.9	21.4	14.8

* Values are means ± SD of three independent determinations.
[a] = Protein % X 4/total energy of formulation.

Table 4. Functional properties* of the weaning food formulations and Cerelac

Property	F1	F2	F3	F4	F5	Cerelac
Bulk density (g/ml)	0.59 ± 0.02	0.53 ± 0.01	0.53 ± 0.01	0.50 ± 0.02	0.49 ± 0.02	0.50 ± 0.02
Water absorption capacity (g/100 g)	340 ± 3.2	400 ± 3.5	400 ± 3.4	390 ± 2.9	410 ± 3.6	150 ± 2.5
Apparent viscosity (cps) (at 20% w/w gruel concentration)	4150 ± 11.2	5125 ± 9.7	5400 ± 10.1	5100 ± 8.9	5100 ± 9.5	2250 ± 9.0

* Values are means ± SD of three independent determinations.

kcal) contents per 100 g of formula (Table 3) were similar to the specifications for weaning foods [5, 20]. Fat content (1.1 to 4.4 g), however, was low when compared to the specifications. The percent of protein-derived kcals ranged between 18.6 and 21.7; that of Cerelac was 14.8. According to the Indian Council of Medical Research [21], the recommended optimal protein-calorie requirement for pre-schoolers is 7.1% in total mixed diets. The results indicated that all five formulations were adequate in protein for weaning purposes.

Functional properties. Results related to the three functional properties studied are summarized in Table 4. The BD's of F2, F3, F4, F5 and Cerelac (0.53, 0.53, 0.50, 0.49, and 0.50 g/ml, respectively) were not significantly ($p > 0.01$) different. The BD of F1 was significantly ($p < 0.01$) different from the BD's of the other four formulas and that of Cerelac. The WACs (which indicate the volume of water needed to form a gruel with a suitable thickness for child feeding) of the five formulations were high (340–410 g/100 g) compared to that of Cerelac (150 g/100 g). The WACs for F2, F3, F4 and F5 were

Table 5. In vitro protein digestibility* of the weaning food formulations compared to casein

Formulation	Pepsin-pancreatin digestibility (%)
F1	74 ± 1.7
F2	82 ± 2.1
F3	85 ± 1.9
F4	78 ± 1.8
F5	72 ± 1.7
Cerelac	87 ± 2.3
Casein	97 ± 1.8

* Values are means ± SD of three independent determinations.

not significantly ($p > 0.01$) different from one another, whereas the WAC of Cerelac was significantly ($p < 0.01$) different from all the formulations. The WAC of F1 was significantly ($p < 0.01$) different from the WAC's of the other formulations and that of Cerelac. AV values (measured at 20% w/w gruel consistency) of the formulation were also high (4150–5400 cps) in comparison to Cerelac (2250 cps). The AV value of F2, F3, F4 and F5 were not significantly ($p > 0.01$) different from each other. Cerelac was less viscous than the other treatments and its AV was significantly ($p < 0.01$) different from the AV's of all of the formulations. The AV of F1 was lower than the other four formulations, but was higher than that of Cerelac, and was significantly ($p < 0.01$) different from the AVs of all tested formulations including Cerelac. Ideally a weaning food should have a high BD, a low WAC and a low AV. The five formulations followed the same trend (high values) with respect to the three parameters. WAC and AV need to be lowered in order to produce a more nutritious and suitable weaning food. This could be achieved by reducing the viscosity of the starchy components by malting [22, 23]. A low-viscosity (less bulky) food contains a higher nutrient content since the volume of the food is low.

In vitro indices. The pepsin-pancreatin in vitro protein digestibility of the five formulations is shown in Table 5. The highest digestibility among the formulations was that of F3 (85%) followed by F2 (82%) with no significant ($p > 0.01$) difference between them. Protein digestibility of F3 was not significantly ($p > 0.01$) different from that of Cerelac. On the other hand, digest-

Table 6. Available lysine of the weaning food (WF) formulations compared to calculated total lysine

Parameter	WFs formulations					
	F1	F2	F3	F4	F5	Cerelac
Calculated total lysine (g/16 g N)	4.01	4.18	4.57	4.07	3.74	–
Experimental available lysine (g/16 g N)*	2.13 ± 0.10	2.79 ± 0.13	3.24 ± 0.13	2.24 ± 0.09	2.01 ± 0.07	3.92 ± 0.12
Lysine lost during processing (% of calculated total lysine)	47.00	33.00	29.00	45.00	46.00	–
Available lysine as % of FAO pattern	38.70	50.70	58.90	40.70	36.50	71.30

* Experimental available lysine values are mean ± SD of three independent determinations.

ibility of all weaning foods, including Cerelac, were significantly (p <0.01) lower than that of casein.

The results of the effects of processing on available lysine of the formulations are given in Table 6, which also shows the values of total lysine. During the processing of protein foods, lysine residues of the protein react with reducing sugars to form enzyme-resistant linkages, thus becoming biologically unavailable. This reaction leads to lowering of lysine content of the heat-processed foods. Since lysine is an essential amino acid and because many vegetable proteins are known to be deficient in lysine, any processing which decreases available lysine content is likely to affect the nutritional quality of the protein and consequently the food. It is, therefore, necessary to evaluate available lysine after heat processing. Considering the calculated values of total lysine as truly representing the amounts originally present in the raw material before processing, the effect of processing was found to be negative. Percentage of lysine lost ranged between 29 (in F3) and 47 (in F1). In terms of g/16 g N experimental available, lysine values ranged between 3.24 (in F3) and 2.01 (in F5) compared to that of Cerelac (3.92). Taken as % of FAO pattern, available lysine of all five formulations was low (35.56–58.90), and significantly (p <0.01) lower than that of Cerelac (71.30). However, even

Table 7. PER*[1], NPR*[2], RNPR[3], and NPU*[4] of the various weaning food formulations

Formulation	Protein quality parameter			
	PER	NPR	RNPR	NPU
F1 (at 10% protein)	1.70 ± 0.1	3.51 ± 0.2	0.85	36 ± 2
F2 (at 10% protein)	2.00 ± 0.2	3.92 ± 0.1	0.95	43 ± 3
F3 (at 10% protein)	2.07 ± 0.2	3.81 ± 0.2	0.92	55 ± 2
F4 (at 10% protein)	1.67 ± 0.1	3.57 ± 0.1	0.87	38 ± 1
F5 (at 10% protein)	1.10 ± 0.1	2.99 ± 0.1	0.73	47 ± 1
Cerelac (at 10% protein)	2.14 ± 0.2	3.93 ± 0.2	0.95	66 ± 1
Casein (at 10% protein)	2.50 ± 0.2	4.12 ± 0.2	1.00	86 ± 3

*Values are means ± SD of three independent determinations.
[1] PER = Protein Efficiency Ratio.
[2] NPR = Net Protein Ratio.
[3] RNPR = Relative Net Protein Ratio.
[4] NPU = Net Protein Utilization.

Cerelac did not contain adequate levels of available lysine, which meant it should not be used as the sole source of lysine in weaning mixes.

Protein quality evaluation using animal experiments. Table 7 data summarize the results of animal experiments for the determination or protein quality of the WFs formulations, in addition to Cerelac and casein. The protein quality was related to increase in body weight and represented by PER, NPR NPU. Two of the five formulations (F3 and F2) had PERs suitable for weaning foods (2.07 and 2.00, respectively) compared to the PERs of casein (2.50) and Cerelac (2.14). The PERs of F3 and Cerelac were not significantly ($p > 0.01$) different from each other; that of casein was significantly ($p < 0.01$) higher than all WFs including Cerelac. PERs of F1, F4 and F5 were low and consequently these formulations would not be suitable for use as weaning foods. Weaning mixes with PER's lower than 2.00 would not be regarded as suitable for weaning foods. A similar trend was noted with the other protein quality parameters (NPR, RNPR, and NPU), with F3 then F2 ranking high, indicating their potential for use as weaning foods.

Effects of WFs on the livers of rats. Compositional aspects. Table 8 data show the results of the composition of livers of rats fed the diets used in protein quality evaluations. Average liver weights (g/100 g body weight) ranged between 4.03 and 4.46. The average weight of livers of rats fed casein (3.82) was significantly ($p < 0.01$) lower than weights of livers of rats fed each of the weaning formulations, except for F5 which was not significantly ($p > 0.01$)

Table 8. Effect of feeding diets based on weaning food formulations on rat liver components*

Diet	Rat liver				
	Weight		Moisture	Protein	Fat
	(g)	% of body weight	(%)	(%)	(%)
F1 (at 10% protein)	2.86 ± 0.1	4.46	67.1 ± 2.0	13.2 ± 0.9	18.5 ± 1.2
F2 (at 10% protein)	2.91 ± 0.2	4.30	70.2 ± 2.2	14.0 ± 1.1	15.3 ± 1.0
F3 (at 10% protein)	2.73 ± 0.1	4.03	68.8 ± 2.2	13.4 ± 1.0	16.8 ± 0.9
F4 (at 10% protein)	2.40 ± 0.1	4.16	69.6 ± 2.4	15.7 ± 1.0	13.7 ± 1.0
F5 (at 10% protein)	1.95 ± 0.1	3.89	69.9 ± 2.1	12.1 ± 0.9	16.2 ± 1.2
Cerelac (at 10% protein)	7.45 ± 0.3	4.43	68.2 ± 1.9	18.1 ± 1.0	11.1 ± 1.0
Casein (at 10% protein)	3.17 ± 0.2	3.82	69.1 ± 2.0	17.9 ± 1.0	7.2 ± 0.8

* Values are means ± SD of three independent determinations.

different from casein. Average protein and fat contents of the rat livers were variable depending on the diet composition. An inverse relationship between the fat and protein contents of the livers of each group was noted. Taking Cerelac as the reference, it could be said that a weaning formula should aim at producing low fat and high protein in the livers of animals fed these formulas.

Histopathological profiles. Liver sections from each group of rats were studied for histopathological features. Five specimens were taken from each group randomly. The following observations were reported:

- Diet 1 (F1 at 10% protein): In all five specimens marked generalized cytoplasmic granulation and vacuolation were evident.
- Diet 2 (F2 at 10% protein): Same as in diet 1, but in four specimens.
- Diet 3 (F3 at 10% protein): Same as in diet 1, but in three specimens.
- Diet 4 (F4 at 10% protein): Same as in diet 1, but in four specimens.
- Diet 5 (F5 at 10% protein): Same as in diet 1, but in four specimens.
- Diet 6 (Cerelac at 10% protein): Same as in diet 1, but in two specimens.
- Diet 7 (Casein at 10% protein): Mild periportal cytoplasmic granulation and vacuolation observed in two specimens.
- Diet 9 (Cerelac at 15.5% protein): Normal livers in all specimens.
- Diet 10 (F2 at 20% protein): Normal livers in all specimens.
- Diet 11 (F3 at 16.7% protein): Very mild periportal cytoplasmic granulation and vacuolation observed in two specimens.

These observations indicated that, among the diets fed to rats, only Cerelac and F2 (at 15.5% and 20% protein, respectively) resulted in normal livers.

This meant that they were balanced diets with regard to essential nutrients. F3 (at 16.7% protein) was rated next, because nearly normal livers were noted. At the 10% protein level, all the livers of the rats fed the diets showed abnormalities to different extents. The diet based on casein resulted in fewest abnormalities, followed by Cerelac and F3. The abnormalities might be attributed to low protein level.

From these observations, it may be concluded that low protein quantity and quality will induce abnormalities in mammalian livers. These abnormalities may be in the form of fatty infiltration, which under the microscope appears as vacuoles due to the removal of fat during preparation of tissues for histopathological investigations.

Conclusion

The weaning formulations in the present study are based on commonly consumed, low-cost food materials locally-available in Sudan. Two of the formulations (F3 and F2) showed compositional, functional and nutritional properties, which are comparable to the commercial formula, Cerelac. They will be potentially suitable for use as weaning foods, both at the home and commercial levels. The fact that these formulas are inexpensive, easily available and nutritious could make them effective in solving some of the nutrition problems facing infants and children.

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