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Studies on the Physiological & Nutritional Effects of Newly Developed Low Cost Supplementary Formula Containing Less Familiar Food Stuffs on Experimental Animals

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Abstract: *An attempt had been made to develop a low cost Ready to UseSupplementary Formula (RUSF) using kitchen waste such as jackfruit seed, watermelon seed, drumstick leaves and fish bone for lower income group of people in India. The objective of this research work is to check the physiological effect of RUSF on albino rats. Eighteen male Wister albino rats having body weight from 70-80 gm were divided into control and treated group. Control group was fed on stock diet and treated group was given RUSF for 28 days. Overall growth pattern, serum glucose, protein, calcium, liver glycogen and liver protein levels were almost similar in both groups without any significant difference. No significant difference was found in serum SGPT, SGOT and ALP levels, indicating normal liver function in both groups. Hemoglobin level of treated groupwas significantly higher than control group. Serum triglyceride and VLDL-C levels were found significantly lower in treated group. This finding indicates cardio-protective effect of RUSF on treated rats. Overall RUSF showed comparable results in different parameters with stock diet and did not show any harmful effect on rat. Thereforeit can be assumed that the newly developed formula will be safe for human consumption and useful for most Indians to fill the nutrition gap with least expense.*

Keywords: *Albino rat, Cardio-protective effect, Kitchen waste, Low cost, Supplementary formula.*

I. Introduction

India is the second most populous country in the world where 269.3 million people are living below poverty line[1]. Extremely limited purchase capacity and excessive price hike of food stuffs compelling people to suffer silent hunger. Food supplements are required to meet the nutrition gap. But commercial supplements are very expensive and not accessible to the people who need them most. Therefore utilization of nutritionally potential food waste to develop low cost food supplements can be an alternative measure. The large amount of food waste like seeds, skins of fruits and vegetables, non conventional fruits and vegetables, underutilized leaves etc may be converted into functional food and included into daily diet. Jackfruit seeds, watermelon seeds, drumstick leaves and fish bones are such example which are generally considered as kitchen waste or rarely used in cooking. But research throughout the world has shown that these underutilized food parts are highly rich in nutrition. Jackfruit seeds contain 81.64% of carbohydrate and 11.02% of protein[2] which had been used to prepare breakfast cereal[3], and other bakery products. On the other hand watermelon seeds are very rich in protein and fat. Watermelon seeds contain 35.66% of protein, 50.10% of fat[4] and can be utilized as substitute of nuts. Beside these, 100 gm of drumstick leaves powder contain 3405 mg of calcium and 24 mg of iron[5], possessing tremendous potential for combating macro and micro-nutrient deficiencies. Fish bones are another food part which has been regarded as waste. Due to high mineral content, fish bones can be well suitable as a natural source of calcium. An attempt had been made to develop supplementary food utilizing these kitchen wastes. After cleaning and need based processing with different cooking methods, each ingredient had been mixed thoroughly to develop a supplementary formula. All the macro-nutrient and major micro-nutrient content of newly developed supplementary formula are almost similar with other available commercial supplements. The new formula is few times cheaper compared to other supplements available in the market which makes the product usable for most Indians with least expense. But food supplements are not safe for human intakeuntil their physiological and biochemical effects are being studied after consumption for a specific time frame. Therefore the objective of the present work is to study the physiological and biochemical effect of the newly developed ready to use supplementary formula on experimental animals.

II. Materials and Methods

1.1. Selection of animals

Eighteen young male Wister albino rats having body weight ranging from 70-80gm were selected for the present experimental study. Animals were purchased from a registered supplier in Kolkata, India. The

animals were acclimatized in the laboratory for one week before initiating the experiment. They were fed on standard stock diet and water ad libitum.

1.2. Preparation of stock diet

The stock diet was prepared using wheat flour, skimmed milk powder, wheat bran, roasted Bengal gram flour, groundnut oil and multivitamin drop in the laboratory. All the ingredients were purchased from local market were mixed thoroughly and packed in an airtight container. The diet was prepared weekly and kept in a cool dry place. The composition and nutritional profile has been given in Table 1 and 3.

1.3. Preparation of Ready to Use Supplementary Formula (RUSF)

Jackfruit seeds, watermelon seeds, drumstick leaves, roasted Bengal gram flour, wheat flour and fish bones of katla were used to develop the supplementary formula. Among which watermelon seeds and fish bones of katla were collected from kitchen waste. Drumstick leave were collected from local tree. Rice flakes, jackfruit seeds and roasted Bengal gram flour were purchased from the local market. Different need based cooking and preservation methods such as cleaning, shade drying, boiling, roasting, grinding, sieving were applied to process each ingredient and obtain powder. Ready to Use Supplementary Formula (RUSF) was developed by mixing all ingredients thoroughly and packed in an airtight container. The formula was prepared weekly and kept in a cool dry place. Table 2 and 3 shows the detail of composition and proximate analysis of the supplementary formula.

Table 1: Composition and Nutritive value of Stock diet

Ingredient	Amount (per 100 g)
Wheat flour (g)	53
Skimmed milk powder (g)	29
Roasted Bengal gram flour (g)	10
Wheat bran (g)	3
Ground nut oil (g)	5
Multivitamin drop (ml)	0.2

Table 2: Composition and Nutritive value of Experimental diet (RUSF)

Ingredient	Amount (per 100 g)
Jackfruit seed powder (g)	22.5
Watermelon seed powder (g)	20
Drumstick leave powder (g)	5
Fish bone dust of Katla (g)	2.5
Rice flakes powder (g)	30
Roasted Bengal gram powder (g)	20

Table 3: Nutritive value of stock diet and experimental diets (per 100 g)

Proximate composition	Stock diet	Experimental diet
Energy (Kcal)	376.64	410.42
Carbohydrate (g)	59.24	61.33
Protein (g)	20.16	20.89
Fat (g)	6.56	8.53
Fiber (g)	2.39	4.82
Calcium (mg)	430.73	1192.5
Iron (mg)	4.25	6.82

1.4. Experimental design

Wister albino male rats were randomly divided into two groups namely control and treated group. Each group had nine rats. The rats were kept in individual cages under normal environmental conditions. During experiment control group was fed on stock diet whereas treated group was given supplementary formula for 28 days.

1.5. Collection of samples

All the rats were sacrificed on 29^{th} day under light anesthetic ether. Animals were dissected and blood was taken by cardiac puncture of each rat. Serum was separated by usual methods.

1.6. Parameters measured

1.6.1. Growth pattern

Daily Food intake was recorded. Weight of each rat was measured once in a week to understand the growth rate. Feed conservation efficiency (FCE) and protein efficiency ratio (PER) was also calculated.

1.6.2. Biochemical parameters

For the evaluation of hepato-protective activity of stock and experimental diet, liver weight, serum glutamic oxaloacetate transeaminase (SGOT), serum glutamic Pyruvate transeaminase (SGPT) and Alkaline phosphatase (ALP) level were measured. Beside this serum was taken for estimation of protein, glucose,calcium, triglyceride (TG), total cholesterol (TC), and high density lipoprotein (HDL).The level of VLDL and LDL were also measured. Liver tissues were taken for determination of glycogen and protein content. Hemoglobin was measured from collected blood samples.

1.7. Method used for biochemical parameters

Diagnostic kits were used for estimation of ALP, SGOT, SGPT, serum calcium, TC, TG, and HDL level. ALP was estimated by Kind and King's method [6]. SGOT and SGPT were analysed by Reitman and Frankol method[7]. O-CresolphthaleinComplexone, End Point Assay[8] and GOD-POD method[9] was used to determine serum calcium and glucose level respectively. Haemoglobin was estimated by cyanmethaemoglobin method[10]. For the quantitative estimation of TG in serum, GPO-POD method with TBHA as Chromogen[11] was applied. TC and HDL cholesterol was measured by CHOD-PAP and PEG-CHOD-PAP,End Point Assay with Lipid Clearing Factor(LCF) respectively[12]. LDL[12] and VLDL[11] cholesterol level were also determined.Biuret method[14] was used to estimate serum protein and liver protein. Liver glycogen was measured by hydrolysis followed by standard method of estimation of glucose[13].

1.8. Statistical Analysis of Results: Student t test was applied for statistical analysis[13].

III. Result and Discussion

The results of growth pattern have been given in Table 4. Mean total as well as daily food intake were little higher in treated group. This might have occurred due to comparatively high fat content and presence of fish bone dust in RUSF resulting better palatability of diet for treated group. However statistical analysis has shown that there was no significant difference in the intake of both diet. This finding is similar with a study done by Addass et al in 2010 on the effect of type and levels of animal protein supplements on the growth rate of rats where supplementation with 2.5% of fish meal shows highest feed consumption in rats[14].

Table 4: Growth pattern of experimental animals

Indicator	Control Group Mean ± SEM (n=9)	Treated Group Mean ± SEM (n=9)	P Value
Mean initial weight (g)	94.44±1.5	95±1.17	NS
Mean final weight (g)	126.11±1.11	132.22±2.06	< 0.05
Mean total weight gain (g)	32.77±1.60	37.22±1.4	NS
Mean daily weight gain (g)	1.16±0.06	1.32±0.05	NS
Mean total food intake (g)	279.38±4.59	282.89±4.00	NS
Mean daily food intake (g)	9.97±0.16	10.09±0.14	NS
Feed conservation efficiency(FCE) (feed intake/gm weight gain)	8.71±0.50	7.68±0.31	NS
Mean total protein intake (g)	56.09±0.92	59.09±0.83	< 0.05
Protein efficiency ratio (PER) (weight gain/gm protein intake)	0.57±0.03	0.62±0.02	NS

NS- not-significant

The final weight of treated group was significantly higher than control group though no significant difference was found in total weight gain. Stock diet and RUSF contain 20.16 gm and 20.89 gm of protein respectively. Higher feed intake might be the reason for greater weight gain for treated group even after having almost same protein content in both diets. No significant difference was found comparing FCE and PER data between control and treated groups.No significant difference was found in overall growth pattern of control and treated group. It is important to note that animal protein like skimmed milk powder along with roasted Bengal gram powder were major protein source in stock diet but supplementary formula was made protein rich using only plant protein like watermelon seed powder and roasted Bengal gram powder. Watermelon seed powder had been reported to have 87.91% of in vitro protein digestibility and 2.62 as PER value. Watermelon seed powder contains higher levels of leucine, tryptophan and lower levels of lysine and isoleucine as compared with the FAO/WHO reference pattern[4]. On the other hand though jackfruit seed powder contain comparatively low amount of protein but the seed powder contains eight out of the nine essential amino acids. The amino acid profile of jackfruit seed cake showed that it was highest in glutamate followed by aspartate and lowest in methionine and tryptophan. The indispensable amino acids present in jackfruit seed powder are leucine, isoleucine, valine, lysine, threonine, phenylalanine and histidine[15]. Therefore, when watermelon and jackfruit seed powder were mixed along with roasted Bengal gram powder the limiting amino acids were complemented

which improve the protein quality of the overall formula and become comparable with skimmed milk powder used in stock diet.

Table 5: Liver weight and Liver function tests

Enzyme	Control	Treated	P Value
Liver weight (g/100g body wt)	2.81±0.16	2.93±0.13	NS
SGPT (IU/L)	107.06±2.51	114.91±3.33	NS
SGOT (IU/L)	398.76±1.85	403.46±1.64	NS
ALP (KA Unit)	86.02±3.06	90.67±3.66	NS

NS- not-significant

The liver weight and liver function test results are showed in Table 5. The mean liver weight of control and treated group were 2.81 and 2.93 gm/100g of body weight respectively. SGOT and SGPT are released in higher level in blood when the liver or heart is damaged. On the other hand, ALP is formed in the liver. Abnormally high values of ALP in blood may indicate disease in liver. Table 5 shows treated group is having slightly higher level of SGPT, SGOT and ALP than control group. However statistical analysis has shown that there was no significant difference among the values obtained from both the groups. The ingredients used to develop supplementary formula such as seeds, leaves and fish bone dust generally contain different anti nutritional factors. Therefore special effort was given to process each ingredient by using different cooking method during preparation of the supplement. This made the formula ready to use by eliminating anti nutritional factors. Therefore after analyzing the obtained values it may be said that supplementary formula have not modified or altered liver enzyme activity indicating normal functioning of liver.

Table 6: Other biochemical parameters observed

Parameters	Control	Treated	P Value
Serum glucose(mg/dl)	77.43±2.69	74.1±0.45	NS
Liver glycogen (mg/100mg)	1.71±0.01	1.74±0.01	NS
Serum protein (mg/dl)	6.1±0.19	6.39±0.10	NS
Liver protein (gm/100mg)	0.036±0.0016	0.040±0.0017	NS
Serum calcium(mg/dl)	9.74±0.14	10.29±0.21	NS
Haemoglobin (gm/dl)	15.02±0.25	16.29±0.27	< 0.05

NS- not-significant

Table 6 represent glucose, glycogen, protein, calcium and hemoglobin level of both groups after 28 days of intervention. Blood glucose is an index for the diagnosis of diabetes mellitus. In this study, serum glucose level of control and treated group found to be 77.43 and 74.1 mg/dl respectively. Though control group had comparatively higher glucose level and lower liver glycogen level, the results were not statistically significant. Comparatively low serum glucose level and higher liver glycogen content were found in treated group might be due to presence of 22.5 % of jackfruit seed as one of the major carbohydrate source. Presence of compound such as α- D- Galactose specific lectin in jackfruit seed has the capacity to bind mono and oligosaccharide yielding low glycemic index by either reducing glucose absorption or by slowing the process of digestion[16]. Beside this,drumstick parts are being used in folk medicine for the treatment of diabetes[17]. To verify this property, Ndong et al. in 2007 conducted a study by giving of 2g of glucose/kg of body weight by oral gavage, without or with 200 mg/kg-body weight drumstick leaf powderto rats. Treatment with drumstick leaf powder resulted in a lower glycemic response in rats[18]. Treated group found to have higher serum protein and liver protein level than control group though the data were not statistically significant. Data in Table 6 illustrated that treated group having higher hemoglobin (16.29gm/dl) and serum calcium level (10.53 mg/dl) than the control group. The result was statistically significant. Administration of 1gm/kg of drumstick leave crude extract significantly increased hemoglobin level of albino rat[19]. Therefore incorporation of 5% of drumstick leave powder could be the reason for higher heamoglobin level in treated group. On the other hand, 2.5 % of fish bone dust of katla and 29% of skimmed milk powder was used to prepare the supplementary formula and the stock diet respectively which were prime calcium source for both diets. Since bioavailability of skimmed milk powder and fish bone dust are quite appreciable[20], both the group have almost same level of calcium in serum.

Table 7: The serum lipid profile of experimental animals

Parameters	Control	Treated	P Value
Triglyceride (mg/dl)	87.58±1.78	65.08±1.68	<0.001
Total cholesterol(mg/dl)	63.03±1.07	59.0±1.38	NS
HDL-C (mg/dl)	25.69±1.95	27.88±0.78	NS
LDL-C (mg/dl)	19.82±1.01	18.09±1.09	NS
VLDL-C (mg/dl)	17.51±0.35	13.01±0.33	0.001

NS- not-significant

Table 7 illustrates the serum lipid profile of albino rats fed on stock diet and supplementary formula. Significantly lower triglyceride and VLDL level was recorded for the treated group. Control group was having triglyceride level 87.58 mg/dl whereas for treated group the value was 65.08 mg/dl. Similarly VLDL level was estimated 17.51 mg/dl for control group but for treated group had a level of 13.01mg/dl. The level of total cholesterol and LDLwas found to be higher in control group though the data were not statistically significant. In contrast, HDL level in control group were found to be lower than the treated rats. HDL level was reported 25.69mg/dl for control and for treated group the data was 27.88mg/dl. However statistical analysis showed that there was no significant difference.The finding of the lipid profile i.e significantly lower triglyceride level and lower VLDLis a positive finding as VLDLis often referred as "bad cholesterol" and comparatively higher HDL, the "good cholesterol" of treated group might considered as positive development. Total cholesterol and LDLwas found to be little lower in treated group compared to control rats. Groundnut oil was used in the stock diet whereas watermelon seed was the prime source of fat in supplementary formula. Watermelon seed kernel contain 78.35% of total unsaturated fatty acids which consisted mainly of linoleic (59.64 %) followed by oleic and palmitoleic acid. The presence of high amounts of the essential fatty acid suggests the oil to be highly nutritious. The oil contains 59.99% PUFA and 18.36% MUFA. The major saturated fatty acids in seed kernels of watermelon were palmitic (11.30%) and stearic acids (10.24%)[4]. Beside this supplementation with drumstick leave helped to reduce plasma cholesterol level[21].

IV. Conclusions

The supplementary formula developed by utilizing kitchen waste has a potential of being incorporated in daily diet. The formula showed positive effect on experimental animals after feeding for a period of continuous 28 days. It was found that the treated rats consumed good amount of experimental diet and as a result gained appreciable weight during the experimental period. Hemoglobin level in treated group was found to be significantly higher compared to control group. Hepato-protective activity of enzymes showed no significant adverse effect on the treated rats; the formula might therefore be safe for human consumption. Beside these it significantly lowered serum triglyceride and VLDL level indicating cardio protective property of supplementary formula. Therefore it can be said that the low cost newly developed formula is safe for human consumption and useful for most Indians to fill the nutrition gap with least expense.

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