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WEANING FOODS AND PLASMA BIOCHEMICAL PERFORMANCE IN WISTARS RATS

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ABSTRACT

The aim of this study was to find out the in vivo impact of yam composite flours made by Digbeu et al.[1] usage as potential weaning diets and show the relationship between blood levels of urea and creatinine with blood glucose levels, cholesterol and triglycerides in young experimental wistar albino growing rats. Potential weaning diets formulated from available plants (yam *Dioscorea alata* variety Bètèbètè and *Dioscorea cayenensis* variety Lokpa, soybean and cassava) and animal (rats wistars) sources were evaluated for their nutritional efficiency in a comparative study. A total of thirty six (36) males wistar albino rats were divided equally into 6 groups of 6 animals each, housed in stainless steel cages and fed ad-libitum on 6 different types of weaning formulae. Six different diets: RTC, RCC, FBBF, FBBNF, FLOF and FLONF were prepared and fed to weaning rats for a period of 28 days of which one group served as the control (RTC, a commercial weaning food); one served as a reference protein (RCC) and the rest as test groups were used. The experimental design for the animal feeding trial was a single factor, Completely Randomized Design (CRD). Animal experimentation lasted 28 days among which four days of adaptation with a commercial diet of standard type. Results were recorded as values of mean± S.E unit (range of values): At the end of the experiment, the animals were anesthetized and killed, and blood was drawn immediately for analysis of the biochemical profile: total cholesterol, triglycerides, glucose, creatinine concentration and urea concentrations. Results showed a no significant disturbance of plasma biochemical parameters throughout the experiment. Therefore, the liver and the kidneys do not present dysfunction due the fact those carbohydrate metabolisms were quickly hydrolyzed.

KEYWORDS: Yam, *Dioscorea alata*, *Dioscorea cayenensis*, Composite Flours, Weaning Food.

INTRODUCTION

In developing countries, traditional weaning foods are low in protein content and also deficient in vital nutrients that are required for normal child growth and development.[2] After 6 months, the maternal milk isn't any more enough for covering the needs for the child. This is the way mothers have turned has food of weaning. However, many studies have been carried out on the processing of traditional weaning foods with a view to preventing and/or alleviating protein energy malnutrition in infants.[3,4]

Complementary feeding, also known as weaning mixed feeding or introduction of solid foods, should begin for infants by six months of age. Generally, in developing countries, weaning foods are usually made with mixture cereal-legume. But yams, although little used in the food formulae, seems sensible as source of energy for the elaboration of a food of complement. Furthermore, yams besides being accessible would contain nutritional characteristics which would deserve to be exploited in association with the soybean (a very rich legume).

Yam tubers are the most important staple food in West Africa, after cereals.[5,6] It's consumed in different forms such as foutou (yam boiled in water and crushed), foufou (yam boiled in water and crushed in mixture with palm oil), yam mush (ngbô), roasted yam, yam stew (yam cooked in a sauce accompanied by vegetables), fried, baca (yam crushed after cooking and moistened) and yam couscous (wassawassa).[7,8] Yam is also known for its high starch content of about 84 %. However, Yam is relatively poor in protein (2.1%) and other essential nutrients. To improve its nutritional value, data showed that other crops like cowpea (25% protein), soybean (40% protein) and groundnut (25% protein) richer in protein and minerals can be added to yam. Legumes in weaning formula are found to improve nutrient density of food and improve nutrient intake, which result in the prevention of protein energy malnutrition.[9] In this study, we complement respectively yam (60%) with soya bean (30%) and cassava starter (10%) proportions.[10]

We have used the fermentation because it improves the nutritional quality of food while reducing the antinutritional factors; what improves the digestibility of proteins.

The relevance of using albino rats in nutritional studies, for the purpose of evaluating the nutritional quality of diets is founded on the fact that wistar albino rats have a dietary requirement for the same essential amino acids as human infants.[11]

The purpose of the present study was to evaluate some biochemical performances of young rats fed with various formulated diets.

MATERIAL ET METHODS

Animal Experiment

The animal used for the study of the blood nutritional, biometric and biochemical parameters was constituted by young rats of stump wistar old from 45 to 65 days and weighing on average 60 ± 2g. The animal experiment lasted 28 days of which four days of adaptation with a commercial standard diet. The total quantity of animals used was among 36. They arised from the Laboratory of Nutrition and Pharmacology in Department Biosciences of the University of Cocody-Abidjan. The wistar albino rats were divided into six groups of 6 animals each and were randomly distributed in metabolic cages and fed on normal diets for acclimatization to the environment before starting the experiments. After this period, animals were fed ad libitum on six different types of weaning diets [commercial weaning food (RTC), reference control diet (RCC), two unfermented composite flour diets (FBBNF and FLONF) and two fermented composite flour diets (FBBF and FLOF)].

Plasma biochemical analyses

At the end of the experiment, food was withdrawn 12-14 hours before decapitation of animals. Animals were anesthetized with sodium pentobarbital (150mg/kg) and killed. Then blood was obtained from the jugular vein at the level of the carotid,[12] into dry tubes containing heparin (20 units/ml). The blood was therefore centrifuged at 3000 g for three minutes to collect the serum which was taken in tubes Eppendorf and preserved in a cold room in – 20°C before analyses.

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under peroxidase activity, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye, an indicator that was recorded at 500 nm.[13,14]

The cholesterol was determined in a routine diagnostic analyzer CHOP PAD with cholesterol oxidase-peroxidase enzymatic colorimetric assay.[13]

The triglycerides were determined in GPO-PAP, glycerol-3-phosphate oxidase enzymatic colorimetric assay.[13]

Urea was measured based on the reaction of salicylate and hypochlorite with the ammonium ions to form a green complex (2.2 dicarboxylindophenol) which can be read at 600 nm.[15]

Estimation of creatinine were determined according to colorimetric method at 492 nm.[16]

All these determinations were carried out using standard commercial test kits (RANDOX Laboratories Ltd, Co. Antrim, UK). The manufacturer's instructions on the assay procedures were strictly followed.

The lean body mass (liver and kidneys) were recorded.

Statistical analysis

Statistical analyses were carried out in triplicate. The results were processed by the software STATISTICA 7 (Stat soft Inc, Tulsa-USA, Head quarters). Thus, results were expressed as means ± standard deviation. The statistical differences among the means of data were calculated using one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Differences at $P<0.05$ were considered significant.

RESULTS

Plasmatic glucose

There was no significant difference in plasmatic glucose concentration between the groups of rats fed to the various experimental diets and those submitted to the reference (RCC); excluding the rats fed with the control diet RTC which were significant different (Table1).

The glucose plasmatic concentration of rats fed with the control diet RTC was 0. 86 ±0.05g / l where as that of the rats consumed the diet RCC borders 0.71 ± 0.02 g/l. As for the rats of the other diets, their glycemias varied of 0.69 ± 0.03g/ l to ±0.78 0.08 g/l. The lowest glycemia was obtained with the animals of the diet FLOF (0.69±0.03 g / l) while the strongest glycemia was observed with their congener feed with the diet RTC (0.86 ± 0.05g / l).

Table 1: Biochemical indices: glycemia, triglyceride and cholesterol on rats consumed the test and reference weaning formulate.

Feeding regimes	Glycemia concentrations (g/l)	Triglyceride concentrations (g/l)	Cholesterol concentrations (g/l)
RTC	0.86 ±0.05 [a]	0.82 ± 0.30 [a]	0.89 ± 0.09 [a]
RCC	0.71 ±0.02 [c]	0.60 ± 0.01 [a]	0.96 ± 0.06 [b]
FBBF	0.73 ±0.04 [b c]	0.71 ± 0.05 [a]	0.76 ± 0.14 [a]
FLOF	0.69 ±0,03 [c]	0.71 ± 0.04 [a]	0.78 ± 0.07 [a]
FBBNF	0.78 ±0,08 [c]	0.87 ± 0.50 [a]	0.86 ± 0.10 [a]
FLONF	0.72 ±0,04 [bc]	0.83 ± 0.05 [a]	0.84 ± 0.04 [a]

Results are expressed as mean ± standard error (S.E) (unit) (n = 6). Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

RTC (control diet cérélac), RCC (Control Diet Casein), FBBF (Yam Bètèbètè Flour Fermented), FLOF (Yam Lopka Flour Fermented), FBBNF (Yam Bètèbètè Flour Unfermented), FLONF (Yam Lopka Flour Unfermented).

Plasmatic cholesterol

The plasmatic cholesterol level and triglycerides mean values investigated this study are presented in Table 1. The results obtained showed that there were any significant (P < 0.05) differences due in plasmatic cholesterol values except those fed with the control diet RTC.

Plasmatic triglycerides

The triglyceridemie of animals fed with the various diets was between 0.60 ± 0.01 g/l and 0.87 ±0.50 g/l. There was no significant difference concerning the values of the triglyceridemie (table1).

Nitrogen Metabolism

Plasmatic urea and creatinine

As shown in Table 2, no significant variation of the urea concentration in all the rats subjected with various diets was observed comparatively with control groups. Simultaneously, it was the same as for the concentration of creatinine which varied from 5±0.89 (FBBNF) to 6,.4 ±1.02 (FLOF).

Table 2: Plasmatic urea and creatinine.

Feeding regimes	Urea (g/l)	Creatinine (mg/l)
RTC	0.15 ±0.02 [a]	5.2 ±0.40 [b]
RCC	0.15 ±0.01 [a]	5.2 ±0.75 [b]
FBBF	0.16 ±0.02 [a]	5.4 ±0.49 [b]
FLOF	0.14 ±0.03 [a]	6.4 ±1.02 [b]
FBBNF	0.14±0.02 [a]	5.0 ±0.89 [b]
FLONF	0.13 ±0.01 [a]	5.4 ±1.02 [b]

Results are expressed as mean ± standard error (S.E) (unit) (n = 6). Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

RTC (control diet cérélac), RCC (Control Diet Casein), FBBF (Yam Bètèbètè Flour Fermented), FLOF (Yam Lopka Flour Fermented), FBBNF (Yam Bètèbètè Flour Unfermented), FLONF (Yam Lopka Flour Unfermented).

Liver and kidneys weight

The liver of a wistar albino rat fed with various diets was recorded a relative weight of liver going from 2.70 ± 0.19 to 3.65 ± 0.18, as shown in the table 3. Rats subjected to the diet check RCC presented relative weights of liver significantly lower than the others.

Relative weights of kidneys

The results of relative weight of kidneys proved to the experimental animals are shown in table 3. The relative weight of kidneys subjected to different diets varied from 1.13 ± 0.08 (RTC) to 2.20 ± 0.09 (FBBNF). This result were not significantly different (p≤0.05) between them except those fed with the control diet (RTC)

Table 3: Liver and kidneys weight on rats consumed the test and reference weaning formulate.

Feeding Régimes	Relative liver weight	Relative weights of kidneys
RTC	3.65 ± 0.18 [a]	1.13 ± 0.08 [a]
RCC	2.70 ± 0.19 [b]	1.84 ± 0.02 [b]
FBBF	3.43 ± 0.37 [a]	2.00 ± 0.07 [b]
FLOF	3.55 ± 0.47 [a]	1.60 ± 0.06 [b]
FBBNF	3.20 ± 0.63[a]	2.20 ± 0.09 [b]
FLONF	3.15 ± 0.41 [a]	1.77 ± 0.05 [b]

Results are expressed as mean ± standard error (S.E) (unit) (n = 6). Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

RTC (control diet cérélac), RCC (Control Diet Casein), FBBF (Yam Bètèbètè Flour Fermented), FLOF (Yam Lopka Flour Fermented), FBBNF (Yam Bètèbètè Flour Unfermented), FLONF (Yam Lopka Flour Unfermented).

DISCUSSION

The dosage of the biochemical parameters in the studies of nutrition was established well. The excess or the deficit of production of a substance synthesized by an organ was revealing of a disfonctionnement of this one.

It was at the same time, a way of indirect exploration of said organs regulators of the metabolism and a means of appreciation the nutriments metabolism.

According to Durimel and Mannoni,[17] the normal glycemia of rats varied from 0. 7 to 1.2 g/l. That of rats submitted to the control diets (RTC, RCC) varying from 0. 71± 0.02 to 0. 86±0.05g/l, was situated in the range of the normal. Consequently, the rats used in this study didn't present anomaly as regards of the metabolism of carbohydrates.

As for rats submitted to the various regimes with yam, whatever is the diet (FF or FNF), the glycemia was also situated in the normal range, what didn't pull disturbance of the carbohydrate metabolism by these diets.

Similar results were reported by Bouafou et *al*.[18] with rats fed mixture of fish meals and dried worm's diet.

Diets used in the present study didn't pull glycogenic overload because these molecules of glucose which come from some starch were quickly hydrolyzed.

The values of the uremia obtained from various diets line up between 0.13±0.01 and 0.16±0.02g / l. Authors such as Mogensen et *al*.[19] explains that the plasma level of urea , is in touch with the functioning of kidneys. The urea comes from the proteins destruction, and its filtration takes place at the level of the glomerule. However, the values of uremia reported in the present study were not significantly difference between them, but were lower than those obtained by Méité.[20] Furthermore, the urea of rats fed with the control diets (RTC, RCC) was similar to those fed to other formulated diets. It gaves evidence of a good renal physiology.[19]

The blood level of the creatinine was a better scorer of the renal function. There is no significant difference ($p<0.05$) between the creatininemies of rats, fed with the control diets (RTC and RCC) and those of the rats subjected to the diets FF and FNF.

As recommended by Seronie et *al*.,[21] the dosage of the serum creatinine was used for the routine evaluation of the renal function. Indeed, this biological constituent presents several advantages on the physiopathological plan. Its production was relatively constant at an individual's and depends only on its mass. There was no significant difference ($p<0.05$) between the creatininemies of rats, feed with the control diets (RTC and RCC) and those submitted to the over diets FF and FNF. It could translate a renal smooth running of the young rats ingesting these diets.[22]

The level of cholesterol recorded with animals subjected to the control diet (RTC) were not significantly different from those obtained with rats fed with the diets FF and FNF. These results were similar to those presented by Brou;[23] with rats fed with food containing variable proportions of legumes, cereal and plantains. However, according to Zulet et *al*.,[24] the inclusion of saturated fatty acids in the food was at the origin of a hypercholesterol level to rats.

Besides, given that the values recorded in this study are nearby of those of animals fed with the control diets, we conclude that the formulae diets (FBBF, FBBNF, FLOF, FLONF) didn't entail major disturbances of the cholesterol metabolism.[25]

As regards the triglyceridemie, the result showed that there wasn't significant differences in the rats fed with different diets. Similar observations were brought back by Bouafou,[18] Meité[20] and the values which they brought back were comparable to those obtained in this study. Indeed, the cholesterol level and the triglyceridemie, regulated by the liver were modified only in case of hepatic diseases.[26] These observations on the triglyceridemie abound in the same sense as those who were made on the cholesterol level, while confirming the absence of negative impact of the diets studied on the lipids metabolism.

Concerning the biometrics organs, the relative weight of organs is fundamental for the diagnosis of these last.[27] The liver, the kidney and many other organs such as the heart, the spleen and the lungs are primary organs affected by the metabolic reactions caused by toxic matter.[27] The liver is known to be a self-regenerating organ that plays important roles in the body. It is also the main organ of purge of the compound foreigners in the body.[28] Splenic enlargement and liver are indirect indicators of infections and the invasion of bacteria. However, there are no significant weight variations between the relative weight of the liver of animals subjected to the experimental diets and those of the control (RTC).

As for the weight of the kidney, the experimental diets didn't make undergo of significant weight variations between them, while the weight of the kidney of the latter is significantly higher than that of the control diet RTC. Other authors have observed biochemical benefits with use of weaning food. Zannou,[29] found that rats fed with diets containing mixtures of cassava flours with the soya had their kidney weights superior to those of the control (fed with the casein).

According to Adrian et *al*.,[30] the weight increase can be attributed to an activity of substances with difficulty metabolisables or by necessity of the secretion, filtration and excretion of an excess of waste of the metabolism but also in the wealth of the diets in lipids. Indeed, further to the ingestion of a too fat diet, a coat (layer) of adipose tissue forms and recovers the kidney. The weight of the kidney increased without the organ was hypertrophied. Consequently an increase in weight at the organ level didn't still result from an increase in weight at the viscera level.

CONCLUSION

The present study highlights the importance of composite flours made with yams during the period of weaning. Especially the fermented yam composite flours just like food not ferments compared favorably with the reference diets (Cérélac and casein) in all the parameters investigated. They induces good and consistent biological responses in experimental animals. The biochemical data obtained showed that the mean, blood glucose, urea, creatinine, triglyceride and total cholesterol values were favorably compared with that reported in the available literature for juvenile wistar rats. Therefore the liver and the kidney didn't present functional anomaly. The composite flours obtained could constitute good food formulas for infants and young children, pregnant and lactating women which are among the most vulnerable people in developing countries.

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