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EFFECT OF CARBOHYDRATE AND PROTEIN SUBSTITUTION WITH *IRVINGIA GABONENSIS* FLOUR ON BIOCHEMICAL PARAMETERS IN A RAT GROWTH DIET

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ABSTRACT

Irvingia gabonensis, a non-timber forest product (NTFP) widely used in tropical Africa for its nutritional properties, was the subject of this study to evaluate the effect of substituting its flour for carbohydrates and proteins in a rat growth diet. Fifteen growing albino rats were divided into three groups of five: a control group on a standard diet, a group with carbohydrate substitution using *Irvingia gabonensis* flour, and a group with protein substitution. The experiment lasted 21 days. Blood glucose levels decreased by 3.77% in rats whose diet consisted of *Irvingia gabonensis* flour as a carbohydrate substitute and by 16.03% in rats whose diet consisted of *Irvingia gabonensis* flour as a protein substitute. Urea and creatinine concentrations decreased slightly by 10.25% (urea) and 16.19% (creatinine) in rats whose diet was substituted for carbohydrates with *Irvingia gabonensis* meal, and by 15.38% (urea) and 17.81% (creatinine) in rats whose diet was substituted for proteins with *Irvingia gabonensis* meal, indicating good renal tolerance. A non-significant increase in AST concentration of 5.74% and 5.17% was observed in rats whose diet was substituted for carbohydrates with *Irvingia gabonensis* meal and for proteins with *Irvingia gabonensis* meal, respectively. A non-significant decrease in ALT concentration of 6.17% and 2.46% was observed in rats whose diet was substituted for carbohydrates with *Irvingia gabonensis* meal and for proteins with *Irvingia gabonensis* meal, respectively. No major changes in total cholesterol were observed, but a non-significant decrease in total protein concentration was noted. This study highlights the nutritional value of *Irvingia gabonensis* flour, which could serve as a local dietary alternative for the partial replacement of carbohydrates and proteins, while ensuring satisfactory growth and good metabolic balance in rats.

Keywords: *Irvingia gabonensis*, Flour, Carbohydrates, Proteins, Replacement, Biochemical Parameters, Rat.

1 INTRODUCTION

Growth is a critical period in the development of the organism and is characterized by increased nutritional needs. Nutrition plays an important role in the development of growth and the maintenance of physiological balance in living organisms. In this sense, micronutrients and macronutrients are essential elements. Indeed, macronutrients represent the majority of caloric intake and constitute the body's main source of energy. They consist primarily of carbohydrates, proteins, and lipids [1; 2]. Carbohydrates are the main source of energy for living organisms. Their consumption promotes the absorption of glucose by tissues and its storage as glycogen. Fiber, a type of indigestible carbohydrate, promotes satiety, improves gastrointestinal function, and reduces cholesterol levels [3]. As for proteins, they are molecules composed of several amino acids linked by peptide bonds. Their primary function is to provide amino acids, which are used for mechanical and structural purposes and contribute to the synthesis of enzymes, hormones, antibodies, cytokines, transporters, and neurotransmitters [3; 4]. An unbalanced or insufficient intake of these macronutrients during the growth phase can have lasting repercussions on the development and biochemical balance of the organism. However, access to protein and carbohydrate sources remains limited by economic, seasonal, and logistical constraints, exposing vulnerable populations to unbalanced diets. This situation justifies the search for local, available, and inexpensive food resources that could constitute viable alternatives to conventional carbohydrate and protein sources. For example, *Irvingia gabonensis*, known as the African wild mango, is a plant species whose seeds are used as a thickener for sauces and as an energy ingredient in various local dishes [5]. Furthermore, these seeds are also used in traditional medicine for the treatment of certain metabolic and digestive disorders [6; 7]. Furthermore, several studies have reported either the importance or the beneficial biological effects associated with the consumption of preparations made from *Irvingia gabonensis*, notably an improvement in the glycemic and lipid profile in adult or overweight subjects [8; 9]. These promising results open the way to other potential uses of this plant, particularly the processing of the seeds into flour for incorporation into various diets, as a partial substitute for conventional sources of carbohydrates and proteins of cereal or animal origin [10]. Such a substitution of a food (carbohydrates or proteins), which could thus modify the intestinal absorption of nutrients during the growth phase, remains poorly documented.

It is within this context that the present study is situated, aiming to evaluate, in rats on a growth diet, the effect of substituting dietary carbohydrates and proteins with *Irvingia gabonensis* flour on the main biochemical parameters. More specifically, it is a matter of determining whether this substitution is compatible with normal metabolic development, or whether it induces significant, favorable or deleterious, changes in the biochemical parameters studied.

2 MATERIAL AND METHODS

2.1 Study Type and Period

This was an experimental study conducted over 21 days at the Central Laboratory of the Polytechnic University of Man (UPM), located in the city of Man, Côte d'Ivoire.

2.2 Plant Material

The plant material consisted of fine flour made from *Irvingia gabonensis*, commonly called Pklé in the Yacouba language (an ethnic group in western Côte d'Ivoire). The seeds of *Irvingia gabonensis* were harvested, cleaned, dried, and ground into a fine flour. This flour was used as a partial substitute for carbohydrates and proteins in the experimental diets (Figure 1).

2.3 Animal Material

The animal material consisted of laboratory rats of the species *Rattus norvegicus* (Wistar albino rats). These rats were young, growing, with an initial weight between 50 and 100 g (Figure 2). They were raised under standard laboratory conditions, with free access to water and food. The rats were divided into different groups, including a control group receiving a standard diet and two experimental groups receiving diets containing *Irvingia gabonensis* meal. The experiments conducted on the rats complied with ethical standards as defined by international bodies, taking into account the welfare of laboratory animals [11]. The various experimental protocols were followed in accordance with the European Council's guidelines on the protection of laboratory animals (Legislation 2012/707) [12].

2.4 Method for Substituting Carbohydrates and Proteins with *Irvingia gabonensis* Flour in a Growth Diet

This test is described as in [13]. Fifteen (15) rats were used and divided into three (3) equal groups: one control group and two experimental groups. All rats were fed according to the following method:

- Control group rats: standard diet consisting of maize (55g), fish (22g), lipids (4g), mineral salts (6g), and water (13g).
- Experimental group 1 rats: same diet as the control rats except that the carbohydrates (maize) were replaced with *Irvingia gabonensis* in the same quantity as in the standard diet.
- Experimental group 2 rats: same diet as the control rats except that the proteins (fish) were replaced with *Irvingia gabonensis* in the same quantity as in the standard diet.

2.4.1 Blood Sampling

After three (3) weeks of experimentation, a blood sample was taken. The animals were anesthetized with ether, and the rats' blood was collected in dry collection tubes.

2.4.2 Biochemical Parameters

The blood collected in the red or dry tubes allowed for the determination of blood glucose, renal parameters (urea and creatinine), liver parameters (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), total cholesterol (TC), and total protein. An automated analyzer (RAYTO CHEMRAY 120) was used to determine these biochemical parameters. The biochemical analyses were performed using the enzymatic and colorimetric method according to the work of [14]. The percentage increase or decrease was calculated as follows:

$$\% \text{ réduction ou d'augmentation} = \frac{\mathrm{A} - \mathrm{B}}{\mathrm{A}} \text{ X } 100$$

- A: measurement of the parameter in the control rats.
- B: Measurement of the parameter in rats treated with *Irvingia gabonensis* meal.

2.5 Statistical Analyses

The statistical analysis of the data from this study was performed using GraphPad Prism 8.01 software (San Diego, California, USA). The results are presented as the mean followed by the standard error of the mean (M ± SEM). One-way analysis of variance (ANOVA) was used for multiple comparisons with the Turkey-Kramer test at a significance level of $p < 0.05$.

3 RESULTS

3.1 Effect of *Irvingia gabonensis* flour on serum biochemical parameters

Effect of *Irvingia gabonensis* flour on blood glucose

During the experimental period, a decrease in blood glucose was observed in rats fed diets containing *Irvingia gabonensis* flour compared to the control group. The blood glucose level of the rats in the control group was 1.06 ± 0.05 g/L. In the experimental groups where carbohydrates were replaced with *Irvingia gabonensis* flour and those where proteins were replaced with *Irvingia gabonensis* flour, the values obtained were 1.02 ± 0.08 g/L and 0.89 ± 0.04 g/L, respectively. This decrease in blood glucose corresponds to a decrease of 3.77% and 16.03%, respectively, in the group where carbohydrates were replaced by *Irvingia gabonensis* flour and the group where proteins were replaced by *Irvingia gabonensis* flour. Thus, the rats in the experimental groups exhibited lower blood glucose levels than the control group, with a more pronounced decrease in rats in group 2 (Table 1).

3.2 Effect of *Irvingia gabonensis* flour on renal markers

Table 1: Effect of *Irvingia gabonensis* flour on blood glucose, urea and creatinine

Parameters	**Control group N = 5**	**Group with carbohydrates replaced by *Irvingia gabonensis* flourN = 5**	**Group with proteins replaced by *Irvingia gabonensis* flour N = 5**
	M ± ESM	**M ± ESM (%)**	**M ± ESM (%)**
Blood glucose (g/L) / Percentage reduction (%)	1.06 ± 0.05	1.02 ± 0.08 (-3.77%)	0.89 ± 0.04 (-16.03%)

$p > 0.05$: No significant difference vs. control group; ($p > 0.05$): No significant difference between the groups in which **Irvingia gabonensis** substituted for proteins and carbohydrates. n = 5, M ± SEM.

The renal marker values of the rats subjected to the different diets are presented in Table 2. The results show a decrease in mean urea levels in the rats supplemented with *Irvingia gabonensis* flour compared to the control group. Indeed, the urea value decreased from 0.39 ± 0.09 g/L in the control group to 0.35 ± 0.04 g/L and 0.33 ± 0.04 g/L in the group where carbohydrates were replaced with *Irvingia gabonensis* flour and the group where proteins were replaced with *Irvingia gabonensis* flour, respectively. Similarly, a reduction in creatinine levels was observed in the rats that received *Irvingia gabonensis* flour. The values decreased from 2.47 ± 0.66 mg/dL in the control group to 2.07 ± 0.22 mg/dL and 2.03 ± 0.32 mg/dL, respectively, in the group where carbohydrates were replaced with *Irvingia gabonensis* flour and the group where proteins were replaced with *Irvingia gabonensis* flour. This decrease in urea corresponds to a reduction of 10.25% and 15.38% in the experimental groups compared to the control group. As for creatinine, a decrease of 16.19% and 17.81% was observed in the group where carbohydrates were replaced with *Irvingia gabonensis* flour and the group where proteins were replaced with *Irvingia gabonensis* flour, respectively, compared to the control group.

Table 2: Effect of *Irvingia gabonensis* meal on kidney parameters

Parameters	**Control group N = 5**	**Group with carbohydrates replaced by *Irvingia gabonensis* flour N = 5**	**Group with proteins replaced by *Irvingia gabonensis* flour N = 5**
	M ± ESM	**M ± ESM (%)**	**M ± ESM (%)**
Urea (g/L) / Percentage reduction (%)	0.39 ± 0.09	0.35 ± 0.04 (-10.25%)	0.33 ± 0.04 (-15.38%)
Creatinine (mg/dL) / Percentage reduction (%)	2.47 ± 0.66	2.07 ± 0.22 (-16.19)	2.03 ± 0.32 (-17.81%)

$p > 0.05$: No significant difference vs. control group; ($p > 0.05$): No significant difference between the groups in which **Irvingia gabonensis** substituted for proteins and carbohydrates. n = 5, M ± SEM.

3.3 Effect of *Irvingia gabonensis* meal on liver enzymes

The liver enzyme values of rats fed the different diets are presented in Table 3. The results show an increase in AST levels in rats from both experimental groups compared to the control group. Specifically, the mean values increased from 134 ± 22.1 g/L in the control group to 184 ± 20.1 g/L and 183 ± 14.3 g/L in the group where carbohydrates were replaced with *Irvingia gabonensis* and those in the group where proteins were replaced with *Irvingia gabonensis*, respectively. Furthermore, a decrease in ALT levels was observed in the rats fed *Irvingia gabonensis* meal. The mean values decreased from 162.0 ± 12.1 mg/dL for the control group to 161 ± 69.0 mg/dL and 158.0 ± 41.5 mg/dL for the rats in the group where carbohydrates were replaced with *Irvingia gabonensis* and those in the group where proteins were replaced with *Irvingia gabonensis*, respectively.

3.4 Effect of *Irvingia gabonensis* flour on total cholesterol and total protein

The values of total cholesterol and total protein for rats fed the different diets are presented in Table 4. The results show that the mean values of total cholesterol remained generally stable between the different rat groups. Indeed, the value observed in the control group was 0.67 ± 0.08 g/L, while the values for rats in the group where carbohydrates were replaced with *Irvingia gabonensis* and those in the group where proteins were replaced with *Irvingia gabonensis* were 0.68 ± 0.02 g/L and 0.67 ± 0.01 g/L, respectively. These results indicate no significant variation in total cholesterol in the rats of the two experimental groups. Furthermore, the mean values of total protein decreased in the rats fed *Irvingia gabonensis* meal. The value decreased from 86.3 ± 4.81 g/l in the control group to 78.3 ± 0.67 g/l and 78.0 ± 2.08 g/l respectively in the rats in the group where carbohydrates are substituted by *Irvingia gabonensis* and those in the group where proteins are substituted by *Irvingia gabonensis*.

Table 3: Effect of *Irvingia gabonensis* flour on liver markers

Parameters	**Control group N = 5**	**Group with carbohydrates replaced by *Irvingia gabonensis* flour N = 5**	**Group with proteins replaced by *Irvingia gabonensis* flour N = 5**
	M ± ESM	**M ± ESM(%)**	**M ± ESM(%)**
AST (g/L) / Percentage Increase (%)	134 ± 22.1	184 ± 20.1 (5.74%)	183 ± 14.3 (5.17%)
ALT (mg/dL) / Percentage Reduction (%)	162.0 ± 12.1	161 ± 69.0 (-6.17%)	(158 ± 41.5) (-2.46%)

$p > 0.05$: No significant difference vs. control group; ($p > 0.05$): No significant difference between the groups in which **Irvingia gabonensis** substituted for proteins and carbohydrates. n = 5, M ± SEM..

Table 4: Effect of *Irvingia gabonensis* meal on total cholesterol and total protein

Parameters	Control group N = 5	Group with carbohydrates replaced by *Irvingia gabonensis* flour N = 5	Group with proteins replaced by *Irvingia gabonensis* flour N = 5
	M ± ESM	**M ± ESM(%)**	**M ± ESM(%)**
Total Cholesterol (g/L) / Percentage Increase (%)	0.67 ± 0.08	0.68 ± 0.02 (1.49%)	0.67 ± 0.01 (0%)
Total Protein (g/L) / Percentage Reduction (%)	86.3 ± 4.81	78.3 ± 0.67 (-9.26%)	78.0 ± 2.08 (-9.61%)

p > 0.05: No significant difference vs. control group; (p > 0.05): No significant difference between the groups in which *Irvingia gabonensis* substituted for proteins and carbohydrates. n = 5, M ± SEM.

4 DISCUSSION

During the rat growth diet, replacing carbohydrates and proteins with *Irvingia gabonensis* flour led to a decrease in blood glucose levels in the experimental groups compared to the control group. This decrease was more pronounced in the protein-substituted group, indicating a greater hypoglycemic effect. Indeed, the reduction in blood glucose could be explained by the presence of fiber and bioactive compounds that promote blood glucose regulation. Thus, *Irvingia gabonensis* flour could improve glucose utilization or slow its intestinal absorption. These results highlight the potential of this flour in regulating carbohydrate metabolism. In fact, according to [15], biochemical parameters, particularly blood glucose, are indicators of metabolic function in rats. Furthermore, several studies have shown that *Irvingia gabonensis* seeds possess hypoglycemic properties linked to their high soluble fiber and active compound content [16; 17]. These observations confirm the beneficial effect of this flour on blood glucose levels, depending on the level of substitution.

Regarding urea and creatinine, the results show a decrease in urea and creatinine concentrations in rats in the group where carbohydrates were substituted and those in the group where proteins were substituted with *Irvingia gabonensis* flour, compared to the control group. This decrease is more pronounced (-15.38%) and (-17.81%) in the group where proteins were substituted. Indeed, the reduction in urea and creatinine could reflect improved kidney function and better elimination of nitrogenous waste. Thus, *Irvingia gabonensis* flour could have a protective effect on the kidneys. These results highlight the potential of this flour in regulating renal metabolism. According to [15], biochemical parameters such as urea and creatinine are indicators of kidney function in rats. Furthermore, several studies have shown that foods rich in bioactive compounds can improve kidney function by reducing metabolic waste [18]. These observations confirm the beneficial effect of *Irvingia gabonensis* flour on renal markers, depending on the level of substitution.

Regarding AST and ALT levels, the results show that carbohydrate and protein substitution with *Irvingia gabonensis* flour leads to a non-significant increase (p > 0.5) in AST levels in rats in both the carbohydrate-substituted and protein-substituted groups compared to the control group. In addition, a decrease in ALT levels is observed in rats in the experimental groups compared to the control group. The decrease in mean ALT levels could indicate proper liver function or liver adaptation to diets consisting of *Irvingia gabonensis* flour. These results highlight the effect of *Irvingia gabonensis* flour on hepatic metabolism. Indeed, according to [15], liver enzymes are indicators of liver function and integrity in rats. Furthermore, several studies have shown that certain dietary modifications can lead to an increase in transaminases, linked to metabolic adaptations or dose-dependent toxic effects [18]. These observations suggest that *Irvingia gabonensis* flour influences liver activity, and that its effect is independent of the level of dietary incorporation.

Analysis of total cholesterol results shows that protein and carbohydrate substitution with *Irvingia gabonensis* flour does not result in a significant variation in total cholesterol between rats in the experimental and control groups. The values remain generally stable between the control group (0.67 ± 0.08 g/L) and the experimental groups (0.68 ± 0.02 g/L; 0.67 ± 0.01 g/L). This stability in total cholesterol suggests that the flour had no major effect on overall lipid metabolism. Thus, the tested diets did not significantly alter the lipid profile of the animals. However, a decrease in total protein was observed in rats supplemented with *Irvingia gabonensis* flour compared to the control group. This decrease could reflect a change in protein metabolism related to the composition of the diet. These results highlight the limited effect of *Irvingia gabonensis* flour on certain lipid parameters. Indeed, according to [15], biochemical parameters such as cholesterol and total protein reflect the overall metabolic state in rats. Furthermore, several studies indicate that dietary modifications can influence lipid and protein metabolism without necessarily altering total cholesterol [18]. These observations suggest that *Irvingia gabonensis* flour has a moderate effect on lipid parameters, with a more pronounced effect on protein than lipid metabolism.

5 CONCLUSION

This study highlighted the nutritional and biological potential of this flour. Regarding carbohydrate substitution, the results showed improved blood glucose levels, as well as stable total cholesterol. The presence of dietary fiber in the flour could promote better regulation of carbohydrate and lipid metabolism. As for protein substitution, *Irvingia gabonensis* flour maintained relatively satisfactory growth in rats while preserving acceptable levels of total protein. It also showed an influence on renal and hepatic markers, indicating good tolerance of the body to diets consisting of *Irvingia gabonensis* flour. In summary, this study shows that *Irvingia gabonensis* flour is a valuable food resource that can be used as a partial substitute for carbohydrates and proteins in the diets of undernourished or malnourished populations.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

“The authors declare no conflicts of interest regarding this manuscript”.

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