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The Effect of Fortified Bread with Defatted Cake of *Citrullus lanatus* on Blood Biochemical Parameters in Rat

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Abstract This study aims at determining biochemical parameters of rats fed with bread in which the wheat flour has been substituted by *Citrullus lanatus* defatted cake at different proportions (0 %, 5 %, 10 %, 15 % and 20 %). Thirty rats (30) teamed up into five per group were fed during 14 days with the six different diets. Control diet made with casein like protein source (RTC); diet containing classic bread based on wheat flour (RPC); and diets based on bread in which the wheat flour has been substituted by defatted cake of *Citrullus lanatus* with different proportions (RPFd). At the end of the experimental period, animals were sacrificed and blood sample were collected for glucose, urea, creatinine, total-cholesterol, triglyceride, calcium and phosphorus analyses. Glycaemia of rats fed with diets RTC (1.24±0.13 g/l) and RPC 1.09±0.07 g/l) were significantly superior ($p \leq 0.05$) to that of rats fed with diets RPFd (0.24±0.11 g/l to 0.28±0.06 g/l). Uraemia of rats fed with diet RPFd were significantly inferior to that of rats fed with diets RTC (0.39±0.15 g/l and RPC (0.47±0.11 g/l). As for creatinineamia, it was not significant between values obtained on rats fed with diets RTC and RPC and that obtained on rats fed with diets RPFd. Cholesterolemia of rats fed with diets RTC and RPC were significantly superior ($p \leq 0.05$) to that of rats fed with diets RPFd. There was no significant difference between triglyceride of rats fed with the different diets which were constituted. The ratio calcium/phosphorus of rats fed with the different diets were between 1.26±0.12 and 2.45±1.47 and did not show any significant difference. This study showed that wheat flour bread fortification with defatted cake of *Citrullus lanatus* modifies blood biochemical parameters. Complementary studies will be useful to determine if these modifications could provoke some effects on physiological functionalities of nutritional regulating organ.

Keywords: *blood biochemical parameters, food fortification, wheat flour, Citrullus lanatus*

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1. Introduction

Malnutrition in developing countries is very worrying than that observed in others regions in the world. In fact, according to the FAO [1] in a malnourished population of 842 million, there are 798 million in developing countries.

In Africa, malnutrition due to protein deficiency and lack of energizing substances is the first nutritional problem [2]. Côte d'Ivoire, with 22 % of person suffering, is not spare of this curse [3]. Among precaution use to avoid malnutrition, food fortification takes an important place. In fact, Serna-Saldivar *et al.* [4] show that in developing countries, cereal-based food fortification with substances rich in protein can enhance nutritional status for population especially of children after weaning.

Studies made by Méïté *et al.* [5,6] show that the substitution of wheat flour by defatted cake of *Citrullus lanatus* increases protein content of bread which has been fortified and enhanced zootechnic parameters of rats which consumed this bread. Neverveless, these results do not concern others blood metabolic parameters which give some information about the functionalities of some regulating organ. This is why this study is made in order to determine biochemical parameters of rats fed with bread obtained in substituting a part of wheat flour by defatted cake of *Citrullus lanatus*.

2. Material and Methods

2.1. Animal Material

30 young *albinos wistar* rats weighing (65±5 g) were used. They were 45 to 65 days of age. They were bred in

the animal house of Unity of formation and research (UFR) Biosciences of the University Félix HOUPHOUËT-BOIGNY of Abidjan, Côte d'Ivoire.

2.2. Food Composition in the Experimentation

Six kinds of diets were prepared according to a technical sheet offered by the Analysis Official Methods [7] with some modifications:

- control diet was based on casein like protein reference (RTC);
- second diet was made only with wheat flour (RPC);
- four diets was based on bread in which a part of wheat flour were substituted by defatted cake of *Citrullus lanatus* (RPFd) in a proportion 5 % (RPFd5), 10 % (RPFd10), 15 % (RPFd15) and 20 % (RPFd20).

All the diets were equilibrated in vitamin and minerals content. Energizing adjustment were performed using maize oil and maize starch « Maizena» found in the trade. Sugar was used to make the diets appealing.

2.3. Animal Feeding

Animals were teamed up on homogeneous groups (five per group) according to their weight. Each group was put individually in a metabolic cage and maintained under standard laboratory conditions (temperature 25±2°C) with dark and light cycle (12h/24h).

The experimentation was conducted during 14 days. Before the beginning of the real experimentation two days was taken to acclimatize the animals to this condition with aswitchboard ailment call FACI (food factory in Côte d'Ivoire). During the 14 days of experimentation, each group of rats were fed *ad libitum* with the diets. After the 14 days of experimentation, animals were sacrificed and blood sample were collected.

2.4. Blood Sample Collected and Blood Parameters Analyzed

2.4.1. Blood Sample Collected

At the end of the experimentation blood sample was collected at the vena cava level of all animal and put in individually vacuum valve. The blood samples were used for analyses. Samples for biochemical parameters were centrifuged at 3000 trs/min for three minutes to havest the plasma which was used for the various analyses.

2.4.2. Measurement of Biochemical Parameters

Biochemical analysis of serum samples was performed using an automatic chemistry analyzer (Hitachi model 902, Roche). Biochemical parameters measured were glucose, urea, creatinine, total cholesterol, triglyceride and two mayors mineral which are calcium and phosphorus.

2.5. Statistical Methods

The experimental results were expressed mean±SEM. Data were assessed by the method of analysis of ANOVA and Newman Keuls test thanks to STATISTICA 6.0 Software. The level ($p \leq 0.05$) was considered as the cut-off value for significance. Histogram were made using Graph Pad Prism 5.0 Software.

3. Results

3.1. Parameter of Carbohydrate Metabolism: Glycaemia

The mean glycaemia of rats fed with diet RTC was 1.24±0.13 g/l. this glycaemia was higher ($p \leq 0.05$) than that of rats fed with diets based on bread.

Glycaemia of animals fed with diets RPFd vary from 0.74±0.12 g/l to 0.88±0.13 g/l. All these glycaemia do not show significant difference ($p \geq 0.05$) compare to each other. They are significantly low ($p \leq 0.05$) than that of rats fed with diet RPC which is 1.09±0.07 g/l. (Figure 1)

3.2. Parameters of Nitrogen Metabolism

3.2.1. Uraemia

Rats fed with diets RTC and RPC have got uraemia content of 0.39±0.15 g/l; 0.47±0.11 g/l respectively. These values do not show significant difference ($p \geq 0.05$) when compared to each other. These values are on the other hand higher ($p \leq 0.05$) than that obtain on rats fed with diets RPFd which are between 0.24±0.11 g/l and 0.28±0.06 g/l. Values of rats fed with diets RPFd do not show any significant difference ($p \geq 0.05$) between each other. (Table 1)

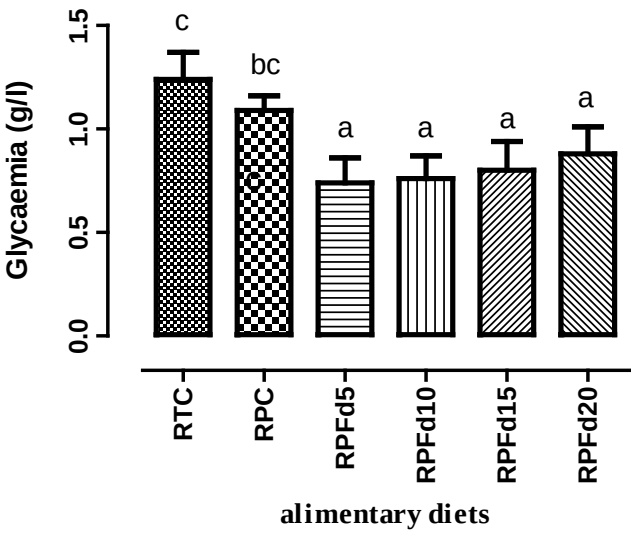

Figure 1. Glycaemia of rats fed with the different diets

Each value is the mean ± SEM of five rats
a, b, c There is no significant difference ($p \geq 0.05$) between the two values in the histogram when designer by the same letter
RTC: Diet made with casein like protein source,
RPC: Diet containing classic bread based on wheat flour,
RPFd: Diets based on bread in which a part of the wheat flour has been substituted by defatted cake of *Citrullus lanatus* at different proportion 5 % (RPFd5); 10 % (RPFd10); 15 % (RPFd15); 20 % (RPFd20).

The higher glycaemia is that obtained on rats fed with diet RTC (1.24±0.13 g/l) followed by that obtained on rats fed with diet RPC (1.09±0.07 g/l). Both glycaemia are significantly higher ($p \leq 0.05$) than that obtained on animals fed with diets RPFd which vary from 0.74±0.12 g/l to 0.88±0.13 g/l.

3.2.2. Measurement of Blood Creatinine

Blood creatinine of rats fed with diets RTC and RPC were 0.82±0.27 mg/l and 0,85±0,31 mg/l respectively.

Rats fed with diets RPFd have blood creatinine concentration which vary between 0.43±0.40 mg/l to 1.14±1.08 mg/l. Statistical analyzed show that all these values are not significantly different ($p \geq 0.05$) (Table 1).

3.3. Parameters of Lipid Metabolism

3.3.1. Blood Cholesterol

Blood cholesterol of rats fed with diets RPFd were 0.71±0.09 g/l; 0.78±0.13 g/l; 0.84±0.10 g/l and 0,83±0,12 g/l for RPFd5; RPFd10; RPFd15 and RPFd20 respectively. Statistical analyzed show that these blood cholesterol are not significantly different ($p \geq 0.05$) to each other. These analyzed show on the other hand that these blood cholesterol are lower ($p \leq 0.05$) than that of rats fed with diets RTC and RPC which values are 1.28±0.22 g/l and 1.07±0.06 g/l respectively. Blood cholesterol values of rats fed with diets RTC and RPC do not show any significant difference ($p \geq 0.05$). (Table 1)

3.3.2. Measurement of Blood Triglyceride

Blood triglyceride of rats fed with diets RTC and RPC were respectively 0.81±0.65 g/l and 0.52±0.45 g/l respectively. Statistical analyzed show that these values are not significantly different ($p \geq 0.05$) to that obtained with diets RPFd which vary between 0.63±0.13 g/l and 0.97±0.88 g/l. (Table 2)

3.4. Parameter of Mineral Metabolism: Blood Calcium/Blood Phosphorus Ratio

The ratio blood calcium/blood phosphorus of rats fed with diet RTC was 1.71±0.86 and that of rats fed with diet RPC was 2.45±1.71. Concerning rats fed with diets RPFd, the ratio were 1.36±0.55; 1.80±0.61; 1.36±0.40 and 1.26±0.12 for RPFd5; RPFd10; RPFd15 and RPFd20 respectively (Figure 2).

Table 1. Uraemia[1] and blood creatinine[1] of rats fed with the different diets

Diets	Uraemia (g/l)	Creatinine (mg/l)
RTC	0.39±0.15^{ab}	0.82±0.27^{a}
RPC	0.47±0.11^{ab}	0.85±0.31^{a}
RPFd5	0.28±0.06^{a}	0.66±0.52^{a}
RPFd10	0.24±0.11^{a}	0.43±0.40^{a}
RPFd15	0.25±0.04^{a}	0.54±0.23^{a}
RPFd20	0.27±0.06^{a}	1.14±1.08^{a}

Each value is the mean ± SEM of five rats
a,b,c There is no significant difference ($p \geq 0.05$) between the two values in the histogram when designer by the same letter
RTC: Diet made with casein like protein source,
RPC: Diet containing classic bread based on wheat flour,
RPFd: Diets based on bread in which a part of the wheat flour has been substituted by defatted cake of *Citrullus lanatus* at different proportion 5 % (RPFd5); 10 % (RPFd10); 15 % (RPFd15); 20 % (RPFd20).

Uraemia of rats fed with diets RPFd are between 0.24±0.11 g/l and 0.28±0.06 g/l. These values do not show any significant different ($p \geq 0.05$) between each other. These values are inferior to that found with diets RTC and RPC which are 0.39±0.15 g/l and 0.47±0.11 g/l respectively.

Statistical analyzed do not show any difference ($p \geq 0.05$) with the values of craetinine of all the animals fed with the differents diets. These values are between 0.43±0.40 and 1.14±1.08 mg/l.

Table 2. Blood cholesterol[1] and blood triglyceride[1] of rats fed with the different diets

Diets	Blood cholesterol (g/l)	Blood triglycaeride (g/l)
RTC	1.22±0.22^{b}	0.81±0.65^{a}
RPC	1.07±0.06^{b}	0.52±0.45^{a}
RPFd5	0.71±0.09^{a}	0.85±0.35^{a}
RPFd10	0.78±0.13^{a}	0.86±0.54^{a}
RPFd15	0.84±0.10^{a}	0.97±0.88^{a}
RPFd20	0.83±0.12^{a}	0.63±0.13^{a}

Each value is the mean ± SEM of five rats
a,b,c There is no significant difference ($p \geq 0.05$) between the two values in the histogram
when designer by the same letter
RTC: Diet made with casein like protein source,
RPC: Diet containing classic bread based on wheat flour,
RPFd: Diets based on bread in which a part of the wheat flour has been substituted by defatted cake of *Citrullus lanatus* at different proportion 5 % (RPFd5); 10 % (RPFd10); 15 % (RPFd15); 20 % (RPFd20).

Total cholesterol of rats fed with diets RTC is 1.28±0.22 g/l. A value of 1.07±0.06 g/l was obtained on rats fed with diets RPC. These two values of total cholesterol do not show any significant different ($p \geq 0.05$) between each other. These values are higher ($p \leq 0.05$) to that found with diets RPFd which vary from 0.71±0.09 g/l to 0.84±0.10 g/l.

Rats fed with diet RTC have a total triglyceride of 0.81±0.65 g/l and that fed with diet RPC have a total trigleceride of 0.52±0.45 g/l. The total triglyceride of rats fed with diets RPFd are between 0.63±0.13 g/l and 0.97±0.88 g/l. Statistical analyzed show that there is any significant difference ($p \geq 0.05$) of triglyceride in animals whatever the diet consumed.

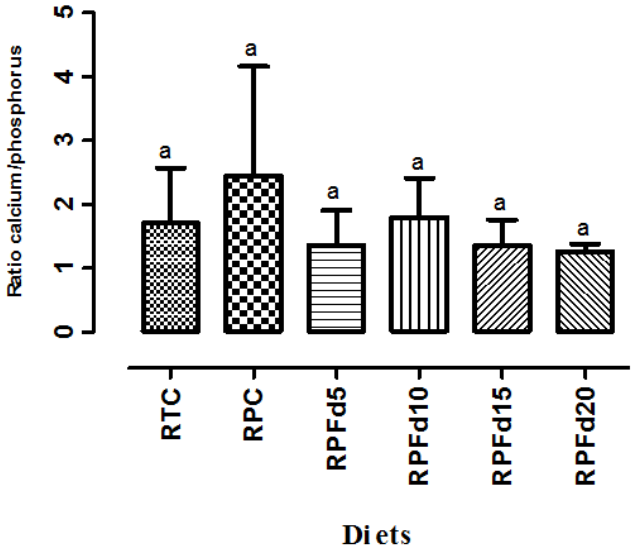

Figure 2. Ratio calcium/phosphorus of rats fed with the different diets

Each value is the mean ± SEM of five rats
a,b,c There is no significant difference ($p \geq 0.05$) between the two values in the histogram
when designer by the same letter
RTC: Diet made with casein like protein source,
RPC: Diet containing classic bread based on wheat flour,
RPFd: Diets based on bread in which a part of the wheat flour has been substituted by defatted cake of *Citrullus lanatus* at different proportion 5 % (RPFd5); 10 % (RPFd10); 15 % (RPFd15); 20 % (RPFd20).

The ratio calcium/phosphorus of rats fed with the different diets are 1.71±0.86; 2.45±1.71; 1.36±0.55; 1.80±0.61; 1.36±0.40 and 1.26±0.12 for diet RTC; RPC; RPFd5; RPFd10; RPFd15 and RPFd20 respectively. Statistical analyzed show that all these values are not significantly different ($p \geq 0.05$).

4. Discussion

Significant variation ($p \leq 0.05$) of rats glycaemia fed with the different diets has been observed. A high glycaemia was obtained on rats fed with RTC diet.

Variations which have been noticed can be explained by the fact that glycaemia is influenced by many factors such as equilibrium between energetic nutriment substances and non-glucidic substances (lipids, proteins, *etc*), individual metabolism, the feeding time and the hour of glycaemia sample [8]. Nevertheless, glycaemia of rats fed with the different diets which have been studied is between 0.74±0.12 and 1.24±0.13 g/l may be normal because according to Durimel *et al*. [9] normal glycaemia on rat are between 0.7 and 1.2 g/l. Then these diets do not provoke mayor disruption on carbohydrate metabolism. Moreover, diets RPFd aim to reduce glycaemia. The reduction of glycaemia could be a good thing for person who suffers hypoglycaemia attact.

Values obtain in this study go along with that reported by Zannou [10] when talking about rats fed with diets made with proportion of cassava flour, soyabeen flour, maize starch, casein and fish powder. These values are still go along with that reported by Dally *et al.* [11] in their work made on rats fed with three Ivorian food. The decrease of uraemia on rats fed with diets RPFd and that of rats fed with diets RTC and RPC could be explained by abnormality on the functionning of kidney. The fact that there is no significant difference ($p \geq 0.05$) on blood creatine of rats fed with diets RTC and RPC when compared to that obtain on rats fed with diets RPFd could testify the good functioning of kidney on rats fed with the different diets [12,13]. This contrary assertion could be explained by the fact that blood urea can be influence by external factors of kidney functionality. These factors could be protein in diet, fever which increase protein catabolism on tissue and diuresis. However, blood creatinine, a constituent of muscle protein, which is only eliminated by kidney, is not concerned by the external factor. Its production is relatively constant and depends on individual body muscle. Blood creatine is then the best indicator of kidney functionality. Blood cholesterol found on animals fed with diets RPFd was less than that obtained on rats fed with diets RTC and RPC. This shows that these diets do not provoke high disturbance of cholesterol metabolism [14].

Diets used in this study do not show significant variation ($p \geq 0.05$) of blood triglyceride. Zannou [10] and Bouafou *et al.* [15] obtained comparable result with ours. The result of Zannou [10] and Bouafou *et al.* [15] testify that our results in the assertion has no negative effect on lipid metabolism.

No significant difference ($p \geq 0.05$) was observed on the ratio Ca/P of rats fed with diets RTC and RPC with that of rats fed with diets RPFd. The ratio obtained neighbouring 2. This is the proof that calcium and phosphorus are well metabolized by parathyroid gland (parathyroid hormon, calcitonin) and the control of blood calcium and blood phosphorus by nephron of kidney is well done [14,16].

5. Conclusion

According to this study, the fortification of bread with defatted cake of *Citrullus lanatus* are provoke change on blood biochemical parameters such as glycaemia, uraemia, blood cholesterol of animals which consumed these diets. On the other hand, others parameters such as blood creatinine, blood triglyceride and the ratio calcium/phosphorus do not show change when compared to that obtained on rats which consumed the classic diet (RTC) and the classic bread (RPC).

Complementary studies will be useful to determine wether the change observed have biometric and histopathological consequences on the nutritional regulating organ of metabolism.

Statement of Competing Interests

The authors have no competing interests.

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