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Blood biochemical parameters of rats fed with powder from the flesh of the *Limicolaria flammea* snail

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Abstract

With a view to the nutritional value of the *Limicolaria flamea* snail and the contribution to the fight against protein deficiencies, our study consisted of determining the blood biochemical parameters, blood mineral levels and serum enzyme activity in rats fed the different diets (ESC, RTC and RPP), in particular the diet containing powder derived from the flesh of the *Limicolaria flammea* snail. After the rats had been fed the diets on a daily basis for 15 days, the protein intake of the diets was used to measure the effect of consuming powder derived from the flesh of the *Limicolaria flammea* snail on the biochemical and mineral indicators of the rats fed the different diets. The results show that the urea content of rats fed the RTC, ESC and RPP diets respectively was 0.26 ± 0.06 g/L, 0.3 ± 0.15 g/L and 0.29 ± 0.01 respectively. The creatinine values in the blood of rats on their respective diets (RTC, ESC and RPP) were 3.25 ± 0.5 g/L, 3.25 ± 0.5 g/L and 3.5 ± 0.57 g/L respectively. Thus, the serum enzyme values of the rats on their respective diets showed no significant difference at the 5 % threshold, particularly for serum enzymes such as Aspartane Amino Transferase (ASAT) and Alanine Amino Transferase (ALAT).

Keywords: Blood biochemical parameters; *Limicolaria flammea*; Snail; Serum enzymes

1. Introduction

The consumption of snail meat is growing in many West African countries, particularly in Côte d'Ivoire, where it is a delicacy of choice for tourists and restaurateurs. The biochemical and nutritional composition of snail meat offers a range of nutritional and technological advantages that make this animal species a moderate alternative to traditional sources of protein such as meat and fish. Thus, the multiple nutritional potential offered by snail consumption makes it possible to popularise the various snail species [1]. In addition, the assessment of biochemical blood parameters following snail consumption is important in human and animal nutrition because it removes any ambiguity about the relative or potential dangers of consuming a food [2,3]. It is an indirect way of exploring the state of function of the organs which regulate nutrient metabolism [4]. For example, urea and creatinine are markers of kidney function. Their increase in the blood indicates kidney dysfunction to a greater or lesser extent [5, 6]. However, all the toxicological studies on snail consumption provide very little data on the biochemical blood parameters of rats fed snail meat powder,

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in particular *Limicolaria flammea*. The aim of this study was to assess the biochemical blood parameters of rats fed powder derived from the flesh of the snail *Limicolaria flammea*.

2. Materials and methods

2.1. Biological material and technique

The biological material consisted of mature *Limicolaria flammea* snails and wistar ratus norvegicus rats aged between 45 and 60 days and weighing between 45 and 55 g. The snails were collected from gardens (often enclosed areas used for growing plants) and scrubland (sparse vegetation consisting of tall grasses interspersed with few trees). Taxonomic identification of the species was carried out at the Entomology Laboratory of the University Félix Houphouët-Boigny, Côte d'Ivoire. The experimental rats were supplied by the pharmacology and nutrition laboratory of the Biosciences UFR of the Université Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire). Herring fish powder (*Clupea harengus*) was also used. he technical equipment used consists of a large double-sided experimental cage comprising three rows of ten individual mini-cages for each rat. Each mini-cage has a grid floor for collecting the rest of the food and faeces, in addition to the other facilities required for feeding the rats.

2.2. Methods

2.2.1. Preparation of Limicolaria flammea snail powder

The snails collected were left to fast for three days in order to release their escrements. The shells were removed and washed thoroughly before being dried in an oven at 80 °C for 24 hours. After drying, they were ground in a Moulinex-type blender (LM 240, IDF, France, Lille). The fine powder obtained was sieved on a 150 µm mesh sieve and constituted our study sample.

2.2.2. Formulation of diets

The diets were formulated according to the indications in the [7] data sheets on the dietary requirements of rats. Three diets were formulated for the rats. A control diet (RTC) based on herring fish powder (*Clupea harengus*), a diet based on *Limicolaria flammea* snail powder (ESC) and a diet based on maize meal containing no protein source (RPP) to monitor the effect of protein sources on the rats. Each diet was cooked for fifteen minutes in a cooker containing 125 mL of distilled water. The diets thus formulated are given in Table 1.

Table 1 Composition of formulated diets

Ingredients (g / kg)	Control diet (RTC)	Protein-free diet (RPP)	Snail diet (ESC)
Fish flour	36	0,00	-
Snail powder	0,00	0,00	36
Merck starch	146,25	182,25	189
Fats	18	18	-
Mineral supplements	15,75	15,75	-
Vitamin supplement	2,25	2,25	-
Cellulose and agar-agar	6,75	6,75	-
Test sample	100	100	100
Total dry matter	225	225	225

RTC: Control diet (herring fish), ESC: Powder diet snail (Limicolariaflamma); RPP: Protein-deprived diet

2.2.3. Determination of blood biochemical parameters

Determination in the automated system

Once in the laboratory, serum was obtained by centrifuging whole blood at 5000 rpm for 20 minutes. The serum was collected in cryotubes (eppendorfs) and stored at -10 °C until the following biochemical parameters were determined: glucose, urea, creatinine, total cholesterol, triglycerides, total proteins, HDL lipoproteins, LDL lipoproteins and

electrolytes (calcium and phosphorus). Serum metabolites were assayed using a HITACHI 704 R (Roche Diag-nostics, Meylan, France).

Blood sampling

At the end of the 15-day experiment, the rats were fasted for 16 h before blood sampling. After this fasting period, the rats in each batch were anaesthetised with ether. Blood sampling followed the experimental protocol described by [8].

Determination of serum glucose

Blood glucose was determined using the enzymo-colorimetric test of [9].

According to this method, in the presence of glucose oxidase, glucose is oxidised to gluconic acid and hydrogen peroxide. The hydrogen peroxide resulting from this reaction is then oxidised by the peroxidase into a coloured product.

Creatinine assay

Creatinine was determined using the colorimetric method of [10] coupled to the automated system. According to this method, creatinine forms a red complex with yellow-orange picric acid in an alkaline medium.

Urea assay

Urea in plasma is assayed using the enzymatic-colorimetric method described by [11]. In this method, urea is hydrolysed in the presence of urease to give ammonia and carbon dioxide (CO_2). The ammonia released during the hydrolysis reaction then gives a blue-green coloration in the presence of a mixture of salicylate, hypochloride and nitroprusside. The intensity of the coloration measured at 578 nm by the automaton is proportional to the urea concentration expressed in mg / dL.

Determination of triglycerides

Triglycerides were determined using the enzymatic and colorimetric method of [12] coupled to the automated system. According to this method, in the presence of lipase, the triglycerides present in the serum were hydrolysed into glycerol and free fatty acids. The liberated glycerol was then converted to glycerol-3-phosphate and then to hydrogen peroxide in the presence of glycerol kinase and glycerol-3-phosphate oxidase.

Total cholesterol levels

The plasma cholesterol content was determined using the enzymatic and colorimetric method of [9] coupled to the automated system. According to this method, in the presence of cholesterol esterase, cholesterol esters are hydrolysed into free cholesterol and fatty acids.

Determination of HDL cholesterol (High Density Lipoprotein)

HDL cholesterol was determined using the colorimetric method described by [13] coupled to the automat. According to this method, Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in serum are precipitated by phosphotungsten in the presence of magnesium ions.

LDL cholesterol (Low Density Lipoprotein)

LDL cholesterol was determined using the method of [14] coupled to the automated system. According to this method, a first detergent solubilised only particles that were not LDL lipoprotein.

Determination of serum calcium

Calcaemia was determined using a colorimetric test, arsenazo III mono reagent [15]. According to this method, calcium reacted with arsenazo III (1,8-dihydroxy-3,6-disulfo-2,7-naphthalene-bis (azo)-dibenzene arsonic acid) at neutral pH, to produce a blue complex whose intensity, measured at 520 nm with the spectrophotometer integrated into the automaton, was proportional to the concentration of calcium in the assay medium.

Determination of serum phosphorus

The level of phosphorus in the blood is determined by the colorimetric method [15, 16] coupled to the automated system. The inorganic phosphate present in the serum reacted with sodium molybdate to form phosphomolybdate.

Phosphomolybdate was then converted by reduction with 1,2-phenyldiamine into blue colloidal molybdenum, which was determined with the spectrophotometer integrated into the automaton at 340 nm.

Measurement of enzymatic activities

Aspartate amino transferase and alanine amino transferase were determined using the method [17], while alkaline phosphatase was determined using the method [12]. To 100 µL of substrates of each enzyme (p-nitrophenylphospate, L-alanine and aspartate) were added 50 µL of serum and the mixture was incubated at 37 °C for 15 min. Staining intensity was determined using a spectrophotometer at 405 nm.

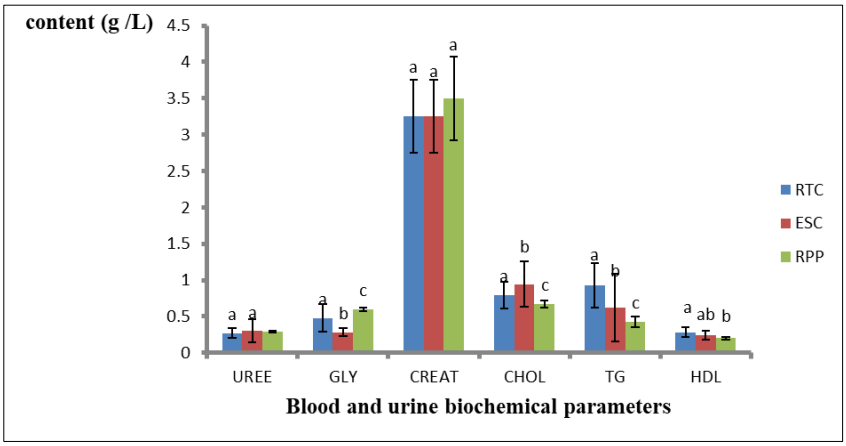
3. Results

3.1. Biochemical blood parameters of rats fed the different diets

The urea content in the urine of rats fed the RTC, ESC and RPP diets was 0.26 ± 0.06 g/L, 0.3 ± 0.15 g/L and 0.29 ± 0.01 respectively. These urea values are statistically identical at the 5 % threshold.

Creatinine levels in the blood of rats fed the different diets were 3.25 ± 0.5 g/L, 3.25 ± 0.5 g/L and 3.5 ± 0.57 g/L for RTC, ESC and RPP respectively. These levels were not significantly different at the 5 % threshold for rats fed the RTC and ESC diets. In contrast, blood glucose levels in rats fed the RTC, ESC and RPP diets were 0.47 ± 0.19 g / L, 0.28 ± 0.05 g / L and 0.59 ± 0.02 g / L respectively. These glucose levels were significantly different at the 5 % threshold from one diet to another. Rats fed the RPP diet had high blood glucose levels (0.597 g / L) while those fed the ESC diet had low glucose levels (0.28 g / L).

The serum cholesterol concentration of the rats was 0.79 ± 0.14 g / L for RTC, 0.95 ± 0.31 g / L for ESC and 0.67 ± 0.04 g / L for the RPP diet. Triglyceride levels were 0.92 ± 0.30 g / L, 0.62 ± 0.46 g / L and 0.42 ± 0.07 g / L for the RTC, ESC and RPP diets respectively. Both triglyceride and cholesterol levels in rat blood differed significantly between rat batches at the 5 % level. The high-density lipoprotein (HDL) content determined in the blood of rats fed the RTC, ESC and RPP diets was 0.28 ± 0.04 g / L; 0.24 ± 0.06 g / L and 0.2 ± 0.01 g / L respectively. There was a significant difference at the 5% level in the concentration of HDL in the blood of the rats (Figure 1)



RTC: Control diet (herring fish), ESC: Snail powder diet (*Limicolaria flammea*); RPP: Protein deprived diet. Gly: Blood glucose; CREAT: Creatinine; CHOL: Cholesterol; TG: Triglycerides; HDL: High-density lipoprotein.

Figure 1 Biochemical parameters in the blood of rats fed different diets

3.2. Blood lipid indices of rats fed the different diets

The artherogenicity index (Ia1) reflecting the ratio of total cholesterol to HDL cholesterol in rats fed the RTC, ESC and RPP diets was 2.82, 2.5 and 3.5 respectively, while the Ia2 index reflecting the ratio of LDL cholesterol to HDL cholesterol in rats was 0.64 for the RTC diet, 0.70 for the ESC diet and 0.80 for the RPP diet. These indices were statistically identical at the 5 % threshold in rats fed the RTC and ESC diets. However, the Ia1 of rats fed the RPP diet was higher than the Ia1 of rats fed the RTC and ESC diets respectively. Furthermore, the Ia2 of rats fed the RPP diet was lower than the Ia2 of rats fed the RTC and ESC diets respectively. The LDL-cholesterol content of rats fed the RTC, ESC and RPP diets was 0.26 ± 0.06 g / L; 0.17 ± 0.03 g / L and 0.16 ± 0.05 g / L respectively. There was no significant difference in the LDL cholesterol levels of rats fed the ESC and RPP diets at the 5 % level (Table 2).

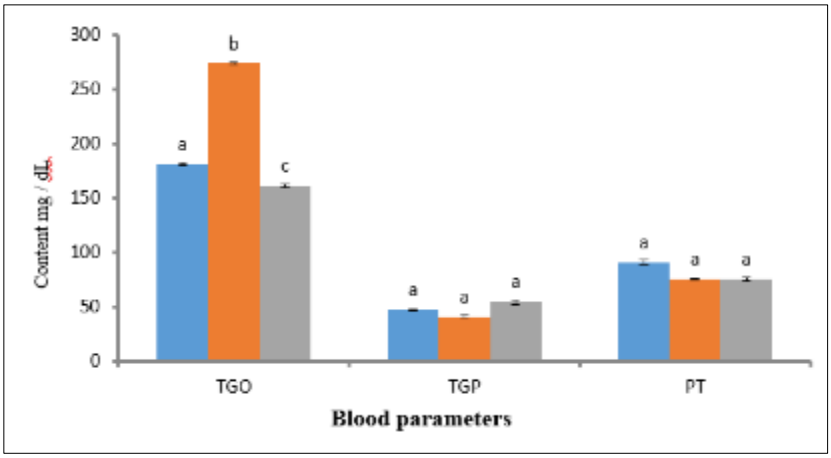
Table 2 Blood lipid indices of rats fed the different diets

Paramètres (g /L)					
	CholT	**HDL-C**	**LDL-C**	**CholT / HDL (Ia_1)**	**LDL-C / HDL-C (Ia_2)**
RTC	0,79 ± 0,24[a]	0,28 ± 0,04[a]	0,26 ± 0,06[a]	2,82[a]	0,64[a]
ESC	0,60 ± 0,31[b]	0,24 ± 0,06[a]	0,17 ± 0,03[b]	2,5[a]	0,70[a]
RPP	0,67 ± 0,04[a]	0,20 ± 0,01[b]	0,16 ± 0,05[b]	3,5[b]	0,80[b]

Mean values marked with the same letter do not show statistically significant differences ($p < 0.05$) within the same column. RTC: Control diet (herring fish), ESC: Snail powder diet (*Limicolaria flammea*); RPP: Protein deprived diet; CholT: Total cholesterol; HDL-C: High-density lipoprotein linked to cholesterol; LDL-C: Low-density lipoprotein linked to cholesterol; Ia1: Index of artherogenicity corresponding to the CholT / HDL ratio; Ia2: Index of artherogenicity corresponding to the LDL-C / HDL-C ratio.

3.3. Glutamo-oxaloacetic transaminase, Glutamopyruvic transaminase and total protein levels

Glutamo-oxaloacetic transaminase (GOT) levels in the blood of rats fed the RTC, ESC and RPP diets were 185.25 ± 0.95 mg / dL; 274 ± 1.63 mg / dL and 161 ± 1.4 mg / dL respectively and that of Glutamo-Pyruvic Transaminases (GPT) was 47 ± 0.81 mg / dL for the RTC diet, 40.75 ± 0.95 mg / dL for ESC and 54.25 ± 2.06 mg / dL for RPP. Total protein (TP) levels were 91 ± 1 mg / dL for the RTC diet, 75.25 ± 0.95 mg / dL for ESC and 75 ± 1.63 mg / dL for RPP (Fig 2).



RTC: Control diet (herring fish); ESC: Snail powder diet (*Limicolaria flammea*); RPP: Protein deprived diet; TGO: Glutamo-oxaloacetic transaminase; TGP: Glutamo-pyruvic transaminase; PT: Total protein.

Figure 2 Glutamo-oxaloacetic transaminase, Glutamopyruvic transaminase and Total protein in rats fed different diets

3.4. Serum mineral content and ratio

The calcium content in the blood of rats fed the RTC, ESC and RPP diets was 98 ± 1.5 mg / L; 99.5 ± 1.15 mg / L and 97.25 ± 2.21 mg / L respectively and that of phosphorus was 80.25 ± 2.62 mg / L for RTC, 80.25 ± 2.78 mg / L for RTC and 77.25 ± 6.29 mg / L for RPP. The ratio of calcium to phosphorus in the blood of rats fed the RTC, ESC and RPP diets was 1.22 ± 0.04;1.23 ± 0.18 and 1.25 ± 0.10 respectively. There was no significant difference between the values calculated at the 5% threshold (Table 3).

Table 3 Activity of some serum enzymes in rats fed different diets

		Diets	
Enzymes (UI / L)	**RTC**	**ESC**	**RPP**
ASAT	266,92 ± 30,7[a]	257,35 ± 21,37[a]	261,42 ± 23,41[a]
ALAT	37,47 ± 1,54[a]	36,12 ± 0,8[a]	39,85 ± 3,78[a]
PAL	164,45 ± 24,18[a]	158,29 ± 23,79[b]	145,36 ± 10,54[c]

The mean values of three replicates followed by their standard deviations, assigned the same letter (a) do not show statistically significant differences ($p < 0.05$) on the same line. RTC: Control diet (herring fish), ESC: Snail powder diet (*Limicolaria flammea*); RPP: Protein deprived diet; ASAT: Aspartane Amino Transferase; ALAT: Alamine amino-transferase; PAL: Alkaline phosphatase.

3.5. Activity of some serum enzymes in rats fed the different diets

Serum ASAT (Aspartane Amino Transferase), ALAT (Alanine Amino Transferase) and PAL (Alkaline Phosphatase) levels are reported in Table IV. The results show that serum ASAT levels in the blood of rats fed the RTC diet were 266.92 ± 30.7 IU/L, 257.35 ± 21.37 IU/L for ESC and 261.42 ± 23.41 IU/L for RPP. There was no significant difference between these values at the 5% level.

The highest serum ALT concentration was found in rats fed the RPP diet (39.85 ± 3.78 IU/L). On the other hand, the serum values of this same enzyme in the blood of rats fed the RTC and RPP diets were 37.47 ± 1.54 IU / L and 36.12 ± 0.8 IU / L respectively. There was no significant difference between these values ($p \geq 0.05$).

In terms of PAL content, the table shows that rats fed the RPP diet had the lowest concentration (145.36 ± 10.54 IU / L), while those fed the RTC and ESC diets had concentrations of 164.45 ± 24.18 IU / L and 158.29 ± 23.79 IU / L respectively. Statistical analysis showed that these serum PAL concentration values differed significantly ($p < 0.05$).

4. Discussion

The assessment of blood biochemical parameters is important in human and animal nutrition because it removes any ambiguity about the relative or potential hazards of consuming a food [2, 3]. It is an indirect way of exploring the state of functioning of the organs that regulate nutrient metabolism [4]. Urea and creatinine are markers of kidney function. Their increase in the blood indicates kidney dysfunction [5, 6]. However, an increase in plasma urea concentration does not necessarily indicate renal damage, as it depends not only on renal function but also on diuresis, dietary nitrogen intake and endogenous protein catabolism [5]. Creatine currently remains the most critical clinical factor in assessing renal function, as it is predominantly eliminated renally through glomerular filtration [18]. In this work, the serum creatinine concentration of rats fed the ESC diet was 3 ± 0.5 g / L. This level is similar to that of rats fed the control diet (of 3.25 ± 0.5 g / L). This could suggest normal renal function in the rats. The blood glucose levels of rats fed the ESC diet were lower than those fed the RTC and RPP diets, but remained within the range (50 - 135 mg/dL) recommended for normal rats [19]. This situation could be explained by the high nitrogen intake in the ESC diet. However, the values obtained are higher than those reported by [20], who demonstrated the effect of farmed and caught fish on rat growth. He concludes that the development of these animals is due to the significant contribution of this protein source to the proper functioning of their organs.

Serum protein levels are a parameter that is strongly influenced by the protein content of the diet [21]. Their importance stems from the beneficial effect they have on health by maintaining osmotic pressure, transporting molecules, purifying plasma, boosting the immune system and coagulating blood [22]. Serum protein levels in rats fed the ESC (75.25 ± 0.95 g / L) and RTC (91 ± 2 g / L) diets are high but remain within the range of values found in normal rats, i.e. between 65 and 95 g / L [23]. These high levels could be explained by the high protein content of the food consumed by the rats [24]. These values are in line with those found by [25], who estimated that the addition of soya to roasted maize meal is a source of protein capable of optimising the functioning of the rat body without causing renal dysfunction. Determining the cholesterol and triglyceride profile also makes it possible to assess the lipidic influence of food consumption on the state of health of the rats fed. These lipid compounds fulfil several physiological functions in the body (vitamins, steroids, bile acids, etc.) [26]. Rats fed the ESC and RPP diets had cholesterol levels that were not significantly different at the 5% level. Rats fed the RTC diet had the highest cholesterol levels. However, the relative concentrations of plasma cholesterol do not necessarily reflect a cholesterolemia that is favourable or unfavourable for the animal. Rather, this would be linked to its distribution in the blood, which is reflected by the index of artherogenicity (Ia), which is the main factor in the discussion of possible cholesterolaemia in animals [27]. Thus, the HDL cholesterol index of rats fed the ESC diet (2.5) and that of rats fed the RTC control diet (2.8) did not differ significantly at the 5% threshold. These index values are less than 3 and indicate a good distribution of cholesterol in the blood of the rats fed the diet. According to [28], cholesterolaemia is induced when the distribution of cholesterol in the blood is three times greater than the unit index. The Ia1 and Ia2 indices in this work are in the assessment range (1-3), suggesting a good distribution of cholesterol in the blood of rats.

Electrolytes (calcium, phosphorus, magnesium and sodium) are important for the functioning of the body. Phosphorus and calcium are involved in the formation and maintenance of healthy bones and teeth [29]. However, excessive serum phosphorus levels disrupt the hormonal regulation of calcium, which in turn upsets the physiological balance, leading to bone demineralisation and the risk of fractures [30]. The results of this study show that the ESC and RPP diets do not cause major variations in serum calcium and phosphorus levels compared with the RTC control.

Transaminases are enzymes with significant metabolic activity within cells. They are involved in certain energy reactions. An increase in their serum level reflects cellular damage, particularly in the liver and certain cardiac cells [31], resulting in an accumulation of hepatitis proteins, which may contribute to the formation of hepatomegaly. In this study, the transaminases investigated were alanine amino transferase (ALAT) and aspartate amino transferase (ASAT).

ALAT is a cytoplasmic enzyme which catalyses a reversible reaction that deaminates alanine to form pyruvate. Pyruvate can enter the neoglucogenesis pathway or the Krebs cycle [32]. The serum ALT concentration of young rats fed the ESC diet was statistically identical to that of rats fed the RTC reference diet. Furthermore, this value is within the 37 - 38 IU / L range defined for properly fed Wistar rats [33]. This suggests that there would be no risk of liver damage with the consumption of *Limicolaria flammea* snail meat. Our values are in line with those of [25], who note that soy protein intake in roasted maize contributes to a healthy regulation of transaminase concentration. However, these results are still lower than those of [34] who observed ALAT concentration values in the range 151 - 156 IU / L enabling them to rule out the hepatotoxicity of the total aqueous extract of *Chrysophyllum perpulchrum* (ETACp) with an LD50 greater than 5000. The other enzyme frequently used to aid diagnosis of neuromuscular diseases is aspartate amino-transferase (ASAT). This is a cytoplasmic and mitochondrial enzyme belonging to the amino-transferases or transaminases [35]. It therefore catalyses a reversible reaction which, like ALAT, enables the transfer of an amino group from an amino acid to an acetonic acid, the products of the reaction being a new amino acid and a new keto acid. In the case of ASAT, the amine group of aspartate is transferred to give oxaloacetate, which can enter the Krebs cycle [32]. In this study, the ASAT concentration of rats fed the ESC diet was statically identical to that of rats fed the RTC diet. However, the latter remained above the range (106 - 113 IU / L) defined by [33] but below the critical threshold, which is three times the value of the reference concentration. This concentration may be justified by the non-local specificity of this enzyme, as it is found in large quantities in various organs: striated skeletal and cardiac muscles, liver, kidney, brain and other tissues, particularly erythrocytes [35]. Our values are in line with those of [34], who observed ASAT concentration values in the range 256 - 263 IU / L, enabling them to rule out possible liver damage due to ingestion of the total aqueous extract of *Chrysophyllum perpulchrum* (ETACp).

Alkaline phosphatases (ALPs) are enzymes with phosphatase activity in an alkaline medium. (PAL) are enzymes present in the body, but especially in the liver, bones, intestine, kidneys and white blood cells. Damage to these organs causes the release of alkaline phosphates. An increase in ALP activity is always significant in animals [36]. However, our study shows that the serum ALP concentration of rats fed the ESC diet belongs to the range (136 – 165 IU/L) in Wistar rats and remains lower than that of rats fed the RTC diet. Indeed, according to [37], the differential diagnosis of an increase in the concentration of ALP involves hemolysis, hepatopathy or extrahepatic pathology without however incriminating the normal growth of the animals. However, it turns out that our animals are particularly growing. This would therefore be a production of PAL linked to the growth of young rats. Our values are lower than those of [34] who limit them in the case of normal rats to around 254 IU/L.

5. Conclusion

Consumption of the flesh of the *Limicolariaflamma* snail presents in rats fed with the diet (ESC) a positive serum effect which is translated by the normal functioning of the organs and this through the concentration values of blood parameters (urea, blood sugar, creatinine, cholesterol and triglycerides), lipid indices, enzymatic activity, serum mineral contents which remain particularly consistent with the standards indicating the functioning of the organs. The flesh of the *Limicolaria flammea* snail remains a complementary and convincing alternative in the availability of protein sources without great health risk for less well-off populations in terms of food supplements.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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