American Journal of Food Science and Technology, 2021, Vol. 9, No. 2, 43-52
Available online at http://pubs.sciepub.com/ajfst/9/2/3
Published by Science and Education Publishing
DOI:10.12691/ajfst-9-2-3

Changes During Nitrogen Balance of Biochemical Nutritional Parameters of Rats (*Rattus Norvegicus*) Fed with Different Food Formulations Containing *Moringa Oleifera*

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Received April 21, 2021; Revised May 27, 2021; Accepted June 06, 2021

Abstract Blood chemistry parameters are an efficient and reliable way to assess nutritional status. Serum contains many substances, such as proteins, enzymes, lipids and minerals. These substances which constitute the biochemical blood parameters provide information about the state of tissues and organs in the body as well as the metabolic state of the individual. Understanding the effects of a food formulation on these biochemical parameters is necessary for the vulgarisation of that formulation. The aim of this study is to explore variations in biochemical parameters in rats (*Rattus norvegicus*) fed with different food formulas containing *Moringa oleifera* during a nitrogen assessment. Rats of wistar strain were fed for 15 days with five food formulations in which *Moringa oleifera* leaf powder has been incorporated respectively at 0, 25, 50, 75 and 100% in partial or total substitution to soybean meal and codified L3P, $L3P_{25}$, $L3P_{50}$, $L3P_{75}$ and $L3P_{100}$. Blood samples were taken just before the experiment and two weeks of individual feeding in dry and gray tubes for the determination of blood biochemical parameters. The results indicated that only albumin and albumin/globulin ratio were significantly increased in the L3P50 rats. Regarding lipid parameters, High density lipoprotein (HDL) cholesterol showed a significant increase in all formulated foods. Aspartate Aminotransferase (ASAT) levels decreased significantly in all formulations, while the other biochemical blood nutritional parameters showed no significant difference. Our formulations based on Moringa increase blood levels of certain protein and lipid parameters, do not alter mineral levels and cause a decrease in the level of ASAT in rat.

Keywords: *Moringa oleifera, food formulations, biochemical parameters, rat (Ratus norvegicus)*

Cite This Article: Mathieu Nahounou BLEYERE, Baudouin Angoua KOKORE, Adama Koffi AMARA, and Paul Angoué YAPO, "Changes During Nitrogen Balance of Biochemical Nutritional Parameters of Rats (*Rattus Norvegicus*) Fed with Different Food Formulations Containing *Moringa Oleifera*." *American Journal of Food Science and Technology*, vol. 9, no. 2 (2021): 43-52. doi: 10.12691/ajfst-9-2-3.

1. Introduction

Due to the high cost of animal protein sources in developing countries and in Côte d'Ivoire in particular, consumption of protein from plant sources has increased. Moringa oleifera, a plant species of Asian origin of the Moringaceae family, is widespread in tropical and subtropical regions, particularly in sub-Saharan Africa. Due to its traditional therapeutic use and its high nutritional value, it is increasingly used in food formulations [1,2]. Moringa leaves remain the most used parts of the plant because they are much more accessible. Their incorporation into the diet of livestock such as laboratory rats is necessary. A prerequisite is therefore to guarantee the safety of the various indications of the leaves, which are increasingly sold as a food supplement in West Africa, through scientific studies [3]. In addition, Haematological and biochemical parameters are an effective and reliable means of assessing nutritional status [4]. Serum contains many substances such as proteins, enzymes, lipids, hormones. These substances, which constitute the biochemical blood parameters, provide information on the state of tissues and organs in the body and the metabolic status of the individual. Their rate in the body indicates the health status of the individual [5]. These biochemical serum values are of great importance as they highlight the influence of the use of available food resources in West Africa on the health of livestock and guide the choice of these food resources for the production of alternative foods [6]. What is the influence of the incorporation of moringa in the diet on the biological blood parameters of the rat (*Rattus norvegicus*)? The objective of this study is to explore variations in blood biochemical parameters in rats (*Rattus norvegicus*) fed

different feed formulas containing *Moringa oleifera* during a nitrogen assessment.

2. Methodology

2.1. Animals

Wistar strain rats (*Rattus norvegicus*) were used for the nitrogen balance phase of the study. For this purpose, six groups were formed with 6 animals per group with mean average weights ranged from 98.37 ± 5.30 g to 112.41 ± 13.62 g. The different distributions were made according to the food formulations to be administered containing *Moringa oleifera*. Control groups fed only the feed without *Moringa oleifera* and a reference group where the rats received the therapeutic feed, Plumpy nut(Tpn). After an adaptation period of 11 days, they were subjected to different foods and did not receive any medication during the study.

2.2. Food Formulations

The method used is that described by [7]. Leafy branches of *Moringa oleifera* were dried for five days at a temperature of 18-20°C until they became crispy, brittle and crunchy. These dried leaves were finely pulverized with a RETSH electric mill, type SM 100 (Haan, Germany). The powder obtained was packed in small bags of about 5 kg to be used for food preparation. From the various ingredients, five diets have been formulated. These were diets L3P, $L3P_{25}$, $L3P_{50}$, $L3P_{75}$ and $L3P_{100}$ in which *Moringa oleifera* leaf powder was incorporated respectively at 0, 25, 50, 75 and 100% as a partial or total substitution to soybean meal according to the composition indicated in Table 1. Distilled water was added at a rate of 640 mL/kg of compound feed to form a more or less rounded, homogeneous, malleable paste (Figure 1). Feed distribution was carried out once a day between 7:30 and 8:30 in the morning. Water was served in bottles in the morning during the feed distribution and renewed every three days. As for the Plumpy nut, it was served at will.

2.3. Nitrogen Balance and Determination of Blood Parameters

The individual feeding experiment was also carried out with growing rats and covered 15 days of feeding according to the method of [8]. This phase took place in the animal house of the vivarium of the Higher Normal School (HNS) in Abidjan. Blood sampling was carried out just before the experiment and two weeks after individual feeding of the animals according to [9] and modified by [10]. Blood was drawn by puncture from the retroorbital sinus in rats. It is a technique that has respected all recommended health and ethical conditions for laboratory animals. Pasteur pipettes were used depending on the quantity to be sampled and the operation was performed under anaesthesia. The volume of blood collected is 0.5 to 2 ml depending on the weight and age of the animal in dry tubes for the determination of biochemical parameters by a spectrophotometer (RAYTO RT 9200).

2.4. Statistical Analysis

The values of the different haematological parameters were expressed by means associated with their standard error on the mean (SEM). To assess the impact of individual feeding on biological parameters, an analysis of variance (Anova 1) was used. To better appreciate the interaction between the selected nitrogen balance periods and the different foods, another two-factor analysis of variance (Anova 2) was used. All these analyses were combined with Dunett as the post hoc test. This statistical analysis used the computer program Graph Pad Prism 5.01 (San Diego California, USA). Statistical significance was set at $p < 0.05$ for expression of results.

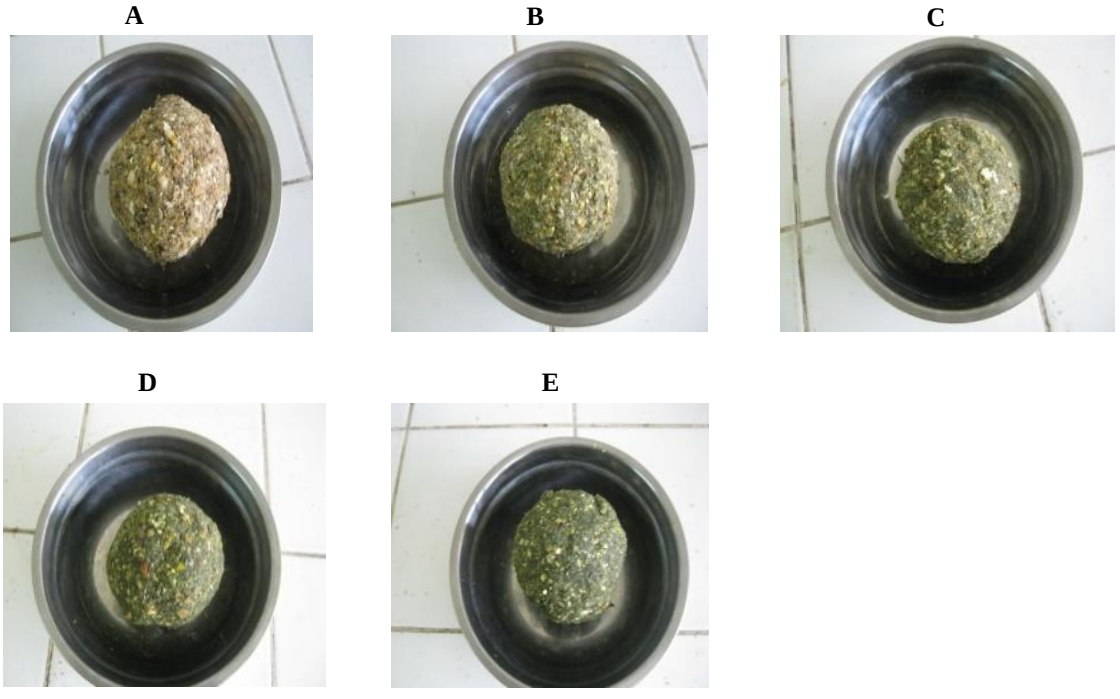

Figure 1. Different photographs food formulations, **A** : L3P Food ; **B** : $L3P_{25}$ Food ; **C** : $L3P_{50}$ Food ; **D** : $L3P_{75}$ Food ; E : $L3P_{100}$ Food

Table 1. Composition of the different diets of the study

Table 1a. Ingredient constitution of food formulations

	Different food formulations				
Ingredients (g)	L3P	$L3P_{25}$	$L3P_{50}$	$L3P_{75}$	$L3P_{100}$
Bread powder	44.5	44.5	44.5	44.5	44.5
Cracked corn	25	25	25	25	25
Fish powder	16	16	16	16	16
Soy powder	14	10.5	7	3.5	0
Moringa powder	0	3.5	7	10.5	14
Salt	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Table 1b. Nutritional value of Plumpy nut

Nutrients	Par sachet de 92 g	Nutrients	In pack of 92 g
Energy	500 kcal	Vitamin A	840 µg
Proteins	12.5 g (13.59%)	Vitamin D	15 µg
Lipids	32.86 g	Vitamin E	18.4 mg
Calcium	276 mg	Vitamin C	49 mg
Phosphorus	276 mg	Vitamin B1	0.55 mg
Potassium	1 022 mg	Vitamin B2	1.66 mg
Magnesium	84.6 mg	Vitamin B6	0.55 mg
Zinc	12.9 mg	Vitamin B12	1.7 µg
Copper	1.6 mg	Vitamin K	19.3 µg
Iron	10.6 mg	Biotin	60 µg
Iodine	92 µg	Folic acid	193 µg
Selenium	27.6 µg	Pantothenic acid	2.85 mg
Sodium	< 267 mg	Niacin	4.88 mg

2.5. Ethics

Experimental procedures and protocols used in this study were approved by ethical committee of Health Sciences, University Nangui Abogoua (Abidjan/Côte d'Ivoire). These guide lines were in accordance with the internationally accepted principles for laboratory use and care. Then, this study was approved by the Ministry of Animal Production and Fishery Resources in the Republic of Côte d'Ivoire.

3. Results

3.1. Variation in Nutritional Indicators

Mean values of the biochemical parameters of the study just before the nitrogen balance are reported in Table 2. Analysis of the results showed no significant differences between the biochemical parameters studied in rats fed with 0% moringa leaf powder compared to other rats fed with 25%, 50%, 75%, 100%, respectively, in substitution for the soybean in the feed formulation. Similarly, no significant differences between the biochemical parameters studied in control food-fed rats (Plumpy'nut) compared to other rats fed at (0%, 25%, 50%, 75%, 100%, respectively) in substitution for the soybean in the formulation of the codified food (L3P, $L3P_{25}$, $L3P_{50}$), $L3P_{75}$, $L3P_{100}$) was not detected. During the experiment, a comparison with the control feed L3P showed only significant differences ($P < 0.05$) in albumin and albumin/globulin ratio in rats in $L3P_{50}$. These parameters showed an increase for $L3P_{50}$. Conversely, no other blood protein parameters reported a significant variation ($P > 0.05$) between different feeds. Very significant differences were observed compared to the therapeutic food (Tpn) for HDL cholesterol. For this lipid blood parameter, an increase has been revealed in all the feeds formulated in our laboratory. The increase was more significant for $L3P_{50}$ feed. However, lipid indices have decreased. This decrease was more significant for $L3P_{75}$ (Total cholesterol/HDL cholesterol) and $L3P_{50}$ (low-density lipoprotein (LDL) cholesterol/HDL cholesterol). In addition, ASAT showed significant changes to varying degrees. A very highly significant ($P < 0.001$) decrease for $L3P_{50}$ and $L3P_{75}$ feeds, highly significant ($P < 0.01$) for $L3P_{25}$ and $L3P_{100}$, and simply significant ($P < 0.05$) decrease for L3P. Other biochemical blood nutritional parameters showed no significant differences ($P > 0.05$) comparatively to the Tpn.

Table 2. Distribution of biochemical blood parameters before nitrogen balance

Biochemical parameters	Control foods		Experimental foods				P
	TPn	L3P	$L3P_{25}$	$L3P_{50}$	$L3P_{75}$	$L3P_{100}$	
Total proteins (g/l)	85.58 ± 4.18	72.20 ± 5.11	78.83 ± 5.43	71.25 ± 4.00	68.89 ± 3.71	75.00 ± 3.81	> 0.05
Albumin (g/l)	31.91 ± 2.10	27.09 ± 1.62	34.81 ± 2.07	25.37 ± 1.20	34.50 ± 2.30	30.14 ± 1.99	> 0.05
Globulin (g/l)	53.67 ± 2.71	49.84 ± 5.77	52.36 ± 1.00	40.49 ± 3.91	38.59 ± 1.50	41.59 ± 8.403	> 0.05
A/G ratio	0.60 ± 0.04	0.57 ± 0.07	0.67 ± 0.05	0.65 ± 0.08	0.89 ± 0.03	0.81 ± 0.21	> 0.05
Blood sugar (g/l)	0.90 ± 0.08	1.11 ± 0.08	1.00 ± 0.08	0.89 ± 0.08	0.85 ± 0.06	0.93 ± 0.09	> 0.05
Triglycerides (g/l)	0.89 ± 0.09	1.05 ± 0.05	1.06 ± 0.04	0.94 ± 0.02	0.99 ± 0.06	1.05 ± 0.06	> 0.05
Total cholesterol (g/l)	0.86 ± 0.04	0.93 ± 0.07	0.98 ± 0.07	1.08 ± 0.10	0.99 ± 0.05	0.93 ± 0.06	> 0.05
HDL (g/l)	0.64 ± 0.07	0.89 ± 0.05	0.79 ± 0.04	0.69 ± 0.15	0.84 ± 0.12	0.68 ± 0.08	> 0.05
LDL (g/l)	0.12 ± 0.02	0.25 ± 0.04	0.18 ± 0.06	0.33 ± 0.12	0.29 ± 0.09	0.25 ± 0.14	
Atherogenicity indices							
ChT/HDL	1.40 ± 0.13	1.06 ± 0.09	1.27 ± 0.14	2.46 ± 0.98	1.53 ± 0.54	1.36 ± 0.12	> 0.05
LDL/ HDL	0.20 ± 0.03	0.27 ± 0.036	0.22 ± 0.07	1.18 ± 0.81	0.59 ± 0.38	0.15 ± 0.05	> 0.05
ASAT (UI/L)	151.73 ± 27.43	211.83 ± 14.73	172.17 ± 15.42	205.50 ± 17.02	164.67 ± 10.23	160.50 ± 9.37	> 0.05
ALAT (UI/L)	56.32 ± 2.12	42.74 ± 6.83	46.25 ± 3.47	43.00 ± 2.01	39.87 ± 2.55	42.01 ± 6.49	> 0.05
Creatinin (mg/l)	8.55 ± 0.90	7.50 ± 0.85	7.33 ± 0.49	8.33 ± 0.76	6.33 ± 0.49	6.20 ± 0.37	> 0.05
Urea (g/l)	0.37 ± 0.03	0.26 ± 0.04	0.27 ± 0.06	0.24 ± 0.02	0.24 ± 0.02	0.26 ± 0.04	> 0.05
Calcium (mg/dL)	56.72 ± 4.32	57.14 ± 4.09	51.10 ± 6.19	62.40 ± 1.52	59.90 ± 2.80	55.02 ± 3.52	> 0.05
Chlorine (mEq/L)	108.90 ± 5.14	132.27 ± 7.46	128.57 ± 4.80	132.66 ± 6.25	118.62 ± 6.22	128.41 ± 8.06	> 0.05
Sodium (mEq/L)	141.41 ± 7.37	154.64 ± 4.27	164.81 ± 5.99	164.68 ± 4.50	166.86 ± 6.28	160.68 ± 11.34	> 0.05
Potassium (mEq/L)	4.46 ± 0.28	6.09 ± 0.55	5.23 ± 0.35	6.12 ± 0.46	5.53 ± 0.13	5.10 ± 0.62	> 0.05
Sodium/Potassium	32.15 ± 2.35	25.77 ± 1.76	32.06 ± 1.81	27.97 ± 1.81	30.65 ± 0.68	35.17 ± 6.77	> 0.05

A/G: albumin/globulin; HDL; High Density Lipoprotein; LDL: Low density lipoprotein; ChT: Cholesterol Total; ASAT: Aspartate Aminotransferase; ALAT:alanine aminotransferases.

3.2. Interaction between Different Foods and Selected Periods of Nitrogen Balance on Blood Biochemical Parameters

The administration of the different feeds resulted in significant changes in blood glucose, total protein, albumin, globulin and albumin/globulin (A/G) ratio during the nitrogen balance period (Figure 2).

Thus, apart from the L3P feed, blood glucose levels in all rats fed to other feeds increased on day 14 compared to day 0 (Figure 2-A). This was significant ($P < 0.05$) in the Tpn control and highly significant ($P < 0.01$) in $L3P_{75}$. For total protein, the Tpn control feed showed a highly significant decrease ($P < 0.01$) at day 14 compared to day 0 (Figure 2-B), whereas feed containing Moringa leaf powder showed an increase with a very significant difference ($P < 0.01$) at the $L3P_{75}$ lot on day 14 compared to the Day 0 (Figure 2-B).

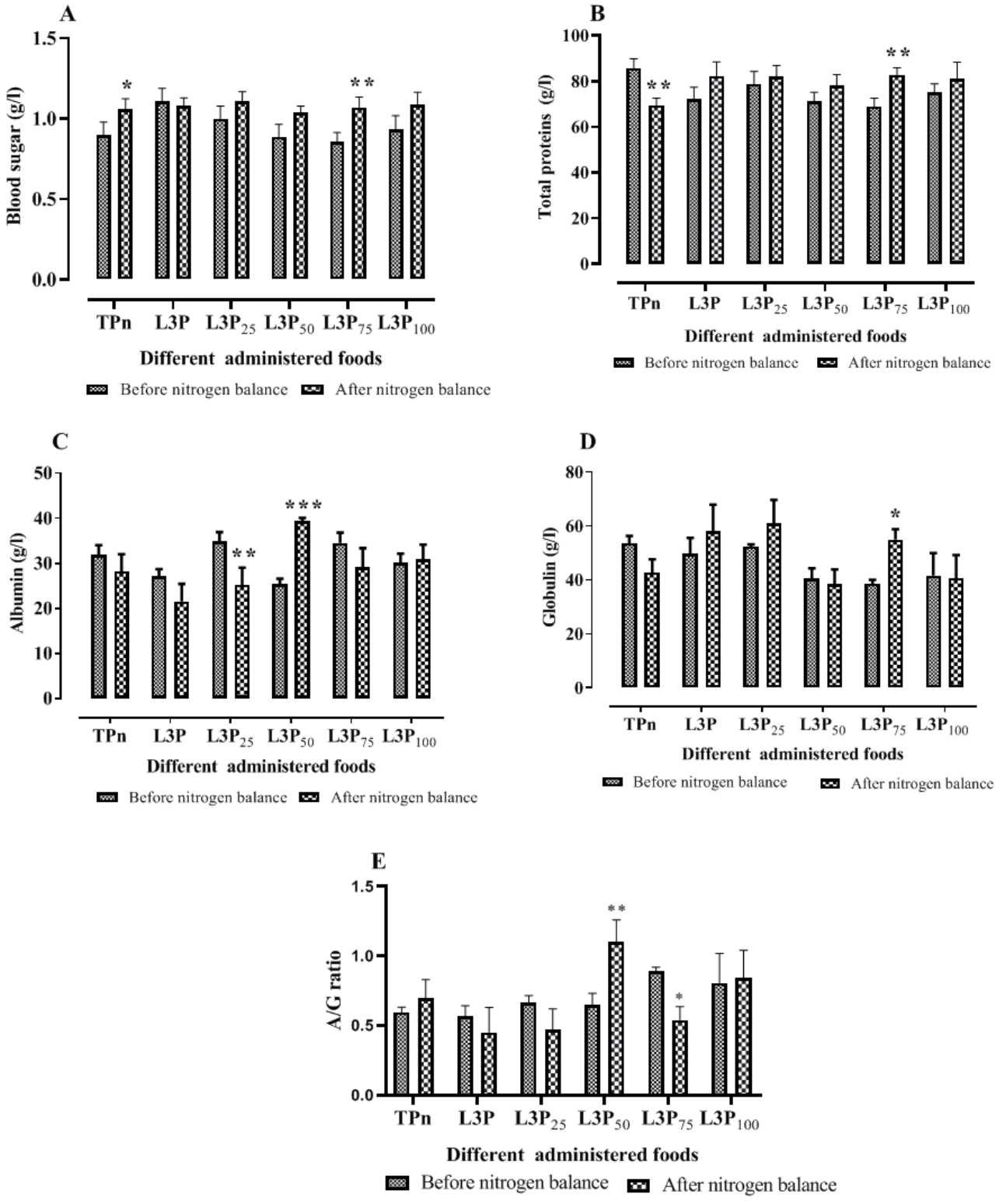

Figure 2. Evolution of glucose and some blood proteins according to the types of food during the nitrogen balance **A** : Blood sugar ; **B** : Total proteins ; **C** : Albumin, **D** : Globulin; **E** : A/G ratio

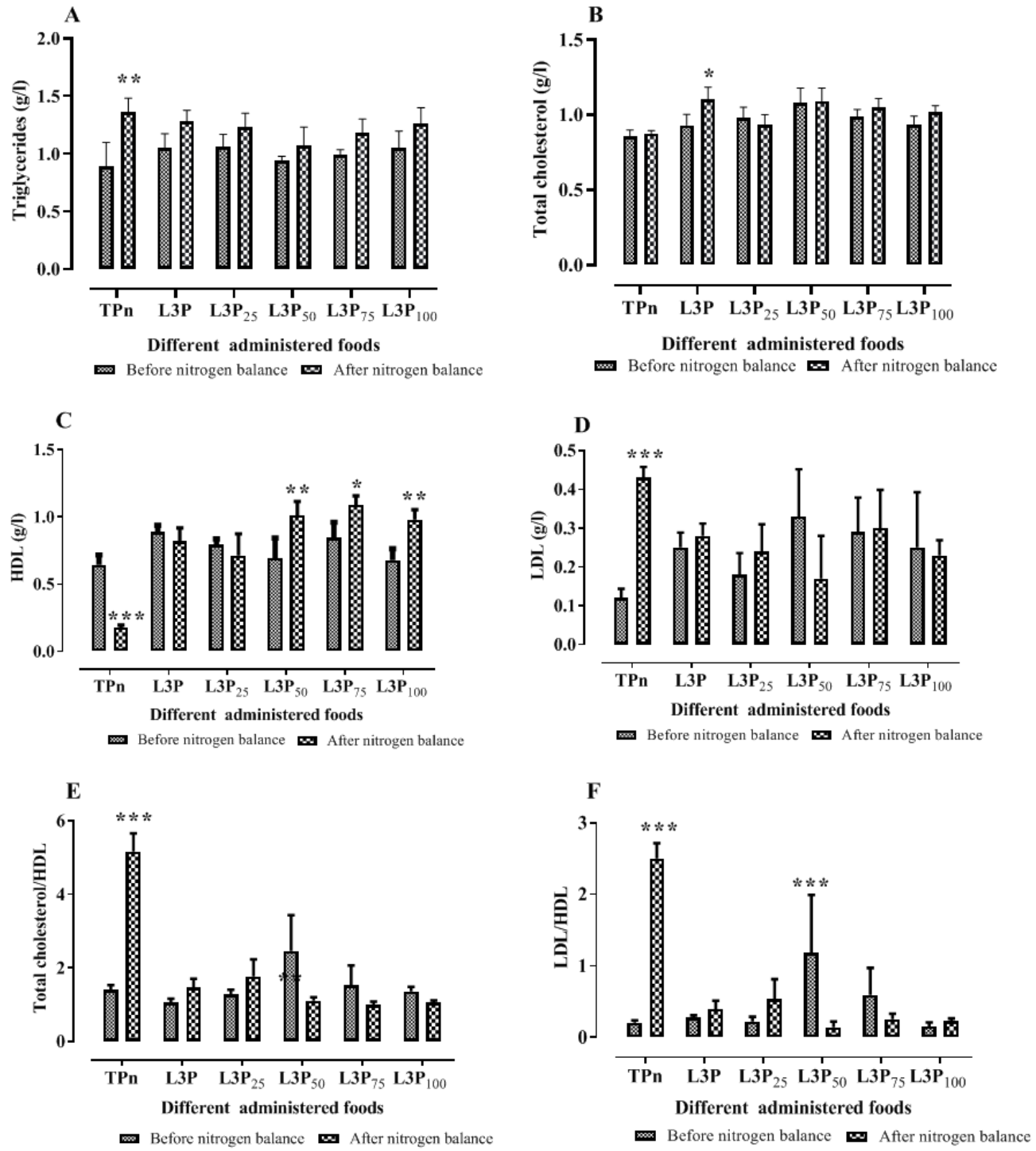

Figure 3. Variation in blood lipids depending on the type of food during the nitrogen balance **A** : Triglycerides ; **B** : Total Cholesterol ; **C** : HDL cholesterol, **D** : LDL cholesterol ; **E** : Total Cholesterol / HDL cholesterol; **F**: LDL cholesterol/ HDL cholesterol

During the same treatment period, apart from the $L3P_{50}$ lot, which had a highly increased albumin level ($P < 0.001$), all other lots had a very significant decrease in albumin level ($P < 0.01$) on the fourteenth day compared to the first day of sampling (Figure 2-C). The globulin level decreased from the first day to the fourteenth day of treatment in the Tpn controls and the $L3P_{50}$ group, whereas the other groups experienced an increase in this rate with a significant difference ($P < 0.05$) in the $L3P_{75}$ group (Figure 2-D). The control feed lot Tpn showed an increased A/G rate as the $L3P_{50}$ with a very significant difference ($P < 0.01$) at the $L3P_{50}$ lot level. In contrast, the A/G ratio was decreased in the other lots with a significant difference ($P < 0.05$) observed at the $L3P_{75}$ lot level (Figure 2-E). At the lipid level, when rats were treated with the different feeds of the experiment, significant changes in triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol, and atherogenicity indices assessed on LDL/HDL and cholesterol/HDL ratios from day 0 to day 14 were revealed (Figure 3).

During this period, triglyceride levels increased in all rats with a very significant difference in the control lot Tpn (Figure 3-A). In the same vein, an increase in total cholesterol, with the exception of $L3P_{25}$, was recorded with a significant difference ($P < 0.05$) in L3P (Figure 3-B).

For HDL cholesterol, rats fed with Tpn, L3P and $L3P_{25}$ diets showed a decrease in levels, with a highly significant difference ($P < 0.001$) observed in the Tpn diet from day 0 to day 14. In contrast, rats that consumed other feeds had their rate increased with highly significant difference ($P < 0.01$) in $L3P_{50}$, $L3P_{100}$, and with significant difference ($P < 0.05$) in $L3P_{75}$ from day 0 to 14 (Figure 3-C). Then, during this phase only the rats fed $L3P_{50}$ and $L3P_{100}$ showed low LDL cholesterol levels, unlike other rats that showed a high level with a highly significant difference ($P< 0.001$) (Figure 3-D). Finally, the atherogenicity indices (Cholesterol Total ChT/HDL and LDL/HDL) showed an almost identical evolution. Thus, an increase in rates was recorded in rats that consumed Tpn, $L3P_{25}$, and $L3P_{50}$ with a very significant difference ($P < 0.01$) in the Tpn lot. In addition, the other rat batches ($L3P_{50}$, $L3P_{75}$, and $L3P_{100}$) showed a rate decrease with highly significant difference ($P< 0.001$) in the L3P50 batch (Figure 3-E; Figure 3-F).

From day 0 to day 14 of treatment, the different rates of ASAT, alanine aminotransferases (ALAT), creatinine and urea showed changes (Figure 4). In fact, outside the control lot Tpn which has experienced a highly significant increase ($P < 0,001$) at the level of the ASAT; ASAT and ALAT levels in all other rat groups decreased. This decrease is highly significant ($P < 0.001$) in $L3P_{50}$ lots, at the ASAT level, and Tpn control at the ALAT level, respectively, and very significant ($P < 0.01$) in L3P lot, at the ASAT level and at the ALAT level, respectively (Figures 4-A and 4-B). In addition, when rats were subjected to different diets, the level of urea showed a highly significant increase ($P < 0.001$) in all rats that used moringa leaf powder diets, whereas there was no real change in those who consumed the control diets Tpn from day 0 to day 14 (Figure 4-D).

During the same period, the creatinine level was significantly reduced ($P < 0.05$) in the control feed Tpn and then no significant difference ($P > 0.05$) in the $L3P_{50}$ food (Figure 4-C). In contrast, in L3P, $L3P_{25}$ $L3P_{75}$ and $L3P_{100}$ lots, an increase was observed with a highly significant difference ($P < 0.001$) in $L3P_{75}$ and $L3P_{100}$ lots on day 14 compared to day 0 (Figure 4-C).

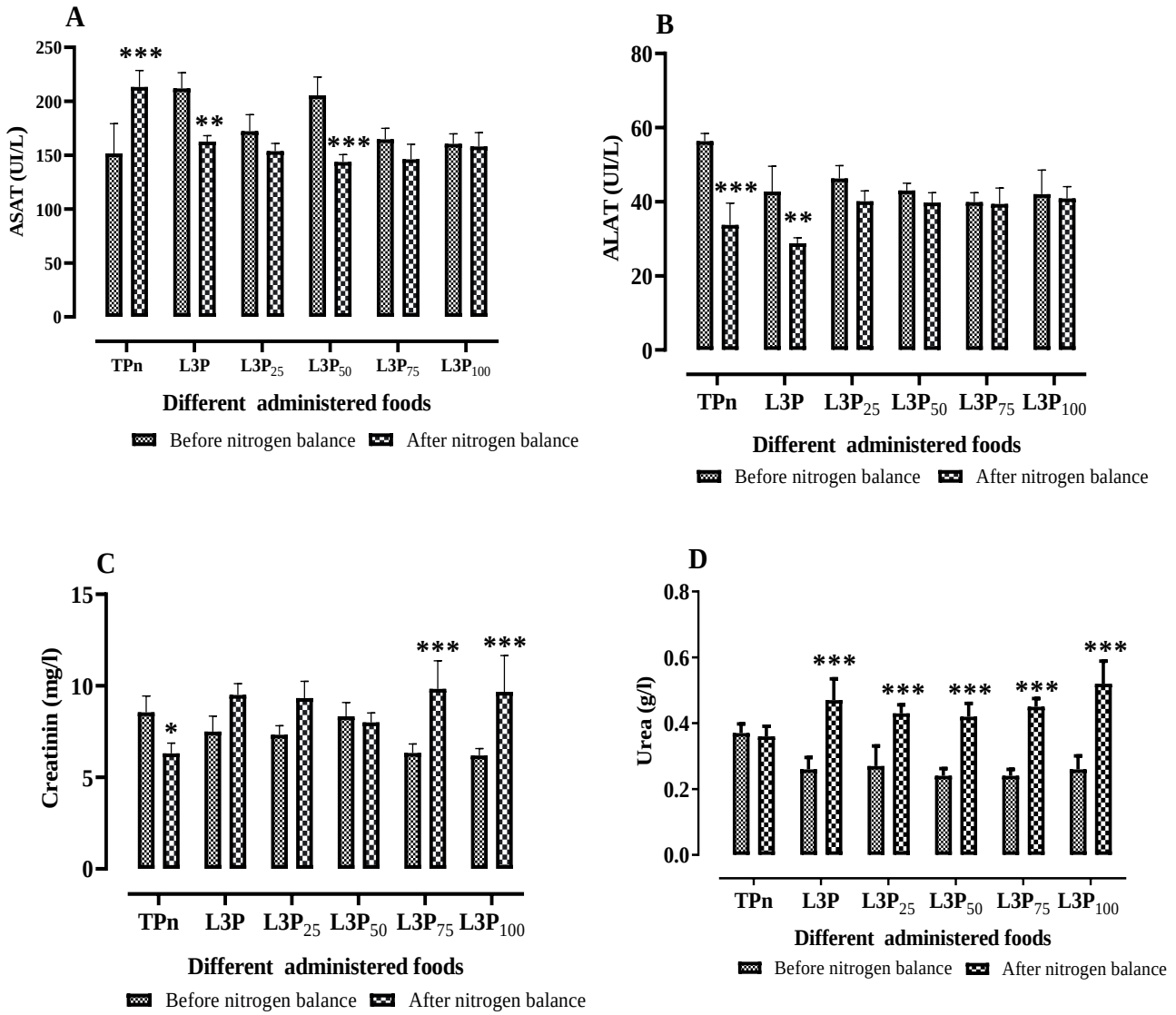

Figure 4. Distribution of some hepatic and renal blood parameters according to the types of food during the nitrogen balance **A** : ASAT; **B** : ALAT ; **C** : Creatinin, **D** :Urea

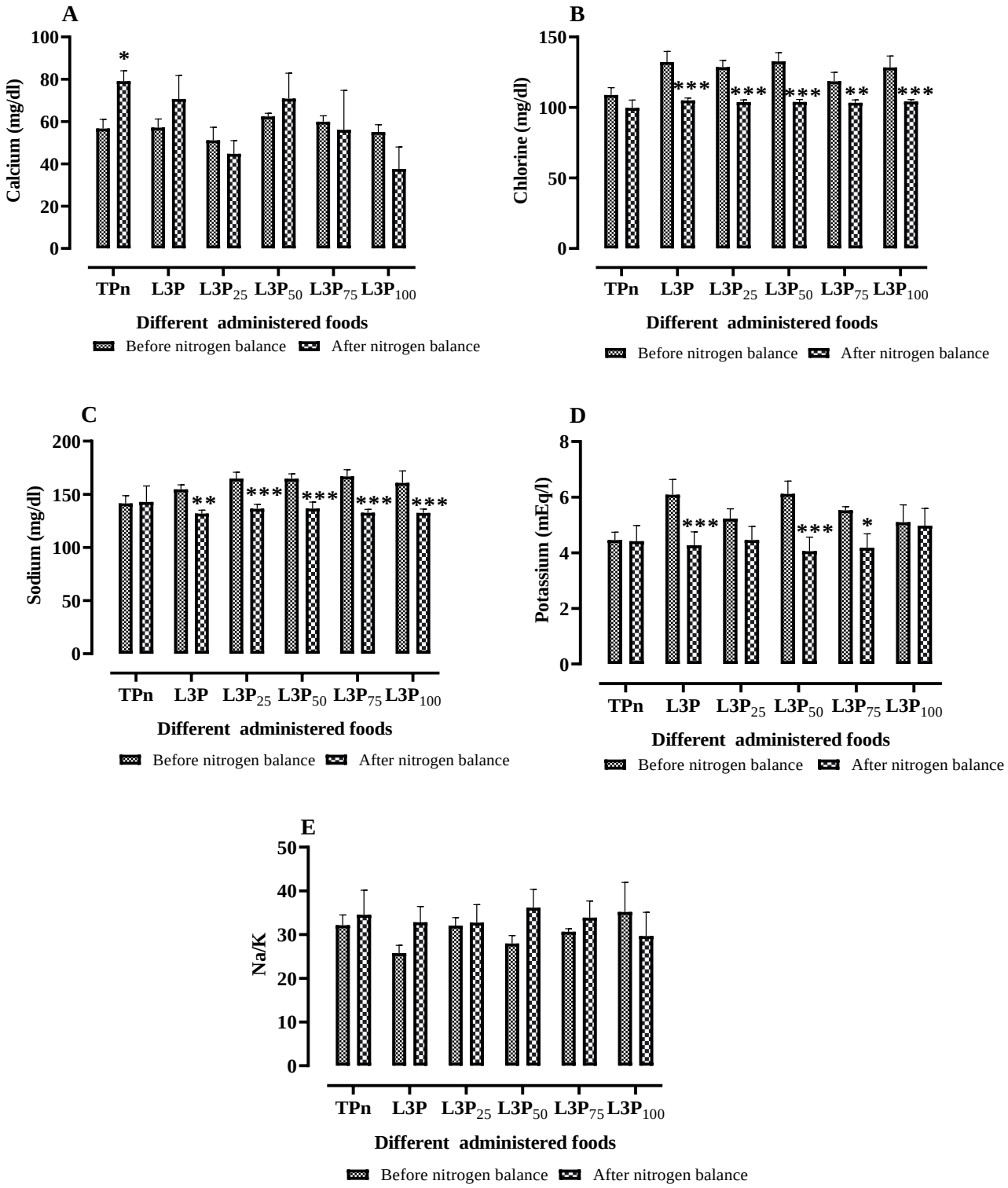

Figure 5. Distribution of some blood ions according to the types of food during the nitrogen balance **A** : Calcium; **B** : Chlorine ; **C** : Sodium, **D** : Potassium **; E** : Na/K

From day 0 to the fourteenth day of treatment, the different serum levels of electrolytes including calcium, potassium, sodium, chlorine and Na/K ratio were modified (Figure 5). Chlorine, potassium, and sodium levels decreased with highly significant differences ($P < 0.001$) in all food-fed rats with different levels of moringa leaf powder (Figure 5-B), while those fed with the control feed from day 0 to day 14 showed a decrease in chlorine and sodium levels.

For potassium, this decrease is highly significant ($P < 0.001$) in L3P and $L3P_{50}$ batches, then significant ($P < 0.05$) in $L3P_{75}$ and not significant ($P > 0.05$) with Tpn controls (Figure 5-D).

No significant differences were found in the Na/K ratio from day 0 to day 14. However, an increase was observed in all rats outside the $L3P_{100}$ lot (Figure 5-D).

With calcium, control (Tpn), L3P, and L3P50 feed showed a rate increase with significant difference ($P < 0.05$) in Tpn controls and a non-significant decrease ($P > 0.05$) with other groups from day 0 to day 14 of treatment (Figure 5-A).

3.3. Proportions of Changes Evolution in Blood Biochemical Parameters

From day 1 to the end of the nitrogen balance, the administration of the various feeds to rats reduced ALAT, chlorine (although not significantly at P > 0.05) and potassium levels in all rats in the experiment. This reduction is less than 50%. With the greatest reductions observed, in the control Tpn at the level of the ALAT, in the $L3P_{50}$ at the level of chlorine and in the $L3P_{50}$ at the level of potassium. In addition, only triglycerides showed a true increase in all rats in the experiment with the highest rate of 52.81% observed in the control Tpn lot and the lowest in the L3P50 lot of 13.83%. Furthermore, with the exception of total protein levels in control lot Tpn, blood glucose levels at the $L3P_{50}$ lot, total cholesterol levels at the $L3P_{25}$ lot, urea levels at the Tpn and Na/K levels at the $L3P_{100}$ lot, all other rat lots experienced increases in these parameters during nitrogen balance (Table 4). At the ASAT level outside the control lot Tpn all other lots experienced a rate decrease, the most significant being observed in the $L3P_{50}$ lot.

Table 3. Distribution of biochemical blood parameters after nitrogen balance

Biochemical parameters	Control foods		Experimental foods			
	Tpn	L3P	$L3P_{25}$	$L3P_{50}$	$L3P_{75}$	$L3P_{100}$
Total proteins (g/l)	69.47 ± 3.14	82.27 ± 6.14	82.03 ± 4.81	77.95 ± 4.96	82.74 ± 3.15	81.08 ± 7.15
Albumin (g/l)	28.21 ± 3.79	21.52 ± 3.88	25.19 ± 3.78	39.40 ± 0.64**	29.07 ± 4.28	30.88 ± 3.25
Globulin (g/l)	42.80 ± 4.940	58.19 ± 9.75	60.87 ± 8.82	38.55 ± 5.44	55.03 ± 3.85	40.65 ± 8.64
A/G ratio	0.70 ± 0.13	0.45 ± 0.17	0.47 ± 0.15	1.09 ± 0.16*	0.54 ± 0.10	0.84 ± 0.20
Blood sugar (g/l)	1.06 ± 0.06	1.08 ± 0.05	1.11 ± 0.06	1.04 ± 0.04	1.07 ± 0.07	1.09 ± 0.07
Triglycerides (g/l)	1.36 ± 0.12	1.28 ± 0.10	1.23 ± 0.12	1.07 ± 0.16	1.18 ± 0.12	1.26 ± 0.14
Total cholesterol (g/l)	0.87 ± 0.02	1.10 ± 0.08	0.93 ± 0.07	1.09 ± 0.09	1.05 ± 0.06	1.02 ± 0.04
HDL (g/l)	0.18 ± 0.02	0.82 ± 0.10###	0.71 ± 0.16###	1.01 ± 0.11###	1.09 ± 0.07###	0.98 ± 0.07###
LDL (g/l)	0.43 ±0.03	0.28 ± 0.03	0.24 ± 0.07	0.17 ± 0.11	0.30 ± 0.10	0.23 ± 0.04
Atherogenicity indices						
ChT/HDL	5.17 ± 0.49	1.46 ± 0.24###	1.77 ± 0.46###	1.11 ± 0.09###	0.99 ± 0.09###	1.06 ± 0.06###
LDL/ HDL	2.50 ± 0.21	0.40 ± 0.12###	0.53 ± 0.28###	0.14 ± 0.08###	0.25 ± 0.078###	0.23 ± 0.04###
ASAT (UI/L)	213.30 ± 15.22	162.64 ± 5.46#	153.72 ± 7.10##	143.76 ± 6.81###	146.24 ± 13.98###	158.02 ± 12.83##
ALAT (UI/L)	33.75 ± 5.84	28.79 ± 1.50	40.13 ± 2.77	39.76 ± 2.68	39.37 ± 4.30	40.88 ± 3.20
Creatinin (mg/l)	6.31 ± 0.56	9.50 ± 0.62	9.33 ± 0.92	8.00 ± 0.52	9.83 ± 1.54	9.67 ± 1.98
Urea (g/l)	0.36 ± 0.03	0.47 ± 0.06	0.43 ± 0.03	0.42 ± 0.04	0.45 ± 0.02	0.52 ± 0.07
Calcium (mg/dL)	79.14 ± 4.89	70.68 ± 11.14	44.70 ± 6.29	70.81 ± 12.09	56.12 ± 18.63	37.55 ± 10.40
Chlorine (mEq/L)	99.70 ± 5.52	105.02 ± 1.66	103.82 ± 1.56	103.90 ± 1.82	103.35 ± 1.97	104.24 ± 1.32
Sodium (mEq/L)	142.89 ± 15.01	131.95 ± 3.18	136.46 ± 4.11	136.60 ± 6.01	132.68 ± 3.13	132.51 ± 3.68
Potassium (mEq/L)	4.42 ± 0.56	4.27 ± 0.48	4.46 ± 0.49	4.06 ± 0.51	4.18 ± 0.50	4.98 ± 0.62
Sodium/Potassium	34.53 ± 5.60	32.81 ± 3.60	32.76 ± 4.07	36.17 ± 4.15	33.90 ± 3.79	29.68 ± 5.41

Comparison between L3P and (L3P25, L3P50, L3P75 and L3P100): *: significant, **: highly significant, ***: very highly significant
Comparison between Plumpy'Nut control food (TPn) and (L3P, $L3P_{25}$, $L3P_{50}$, $L3P_{75}$ and $L3P_{100}$): #: significant, ##: very significant, ###: highly significant; A/G: albumin/globulin; HDL; High Density Lipoprotein; LDL: Low density lipoprotein; ChT: Cholesterol Total; ASAT: Aspartate Aminotransferase; ALAT:alanine aminotransferases.

Table 4. Proportions of variation evolution of biochemical blood parameters during the nitrogen balance

Biochemical parameters	Control foods		Experimental foods				P
	Tpn	L3P	$L3P_{25}$	$L3P_{50}$	$L3P_{75}$	$L3P_{100}$	
Total proteins (g/l)	-18.82	13.95	4.06	9.40	20.10	8.11	< 0.01
Albumin (g/l)	-11.59	-20.56	-27.64	55.30	-15.74	2.45	< 0.001
Globulin (g/l)	-20.25	16.75	16.25	-4.79	42.60	-2.26	< 0.001
A/G ratio	16.67	-21.05	-29.85	67.69	-39.33	3.70	< 0.001
Blood sugar (g/l)	17.78	-2.70	11.00	16.85	25.88	17.20	< 0.001
Triglycerides (g/l)	52.81	21.90	16.04	13.83	19.19	20.00	< 0.001
Total cholesterol (g/l)	1.16	18.28	-5.10	0.926	6.06	9.68	< 0.001
HDL (g/l)	-71.87	-7.86	-10.13	46.38	29.76	44.12	< 0.001
LDL (g/l)	258.33	12.00	33.33	-48.48	3.45	-8.00	< 0.001
Atherogenicity indices							
ChT/HDL	269.29	37.73	39.37	-54.88	-35.29	-22.06	< 0.001
LDL/ HDL	1150.00	48.15	140.91	-88.14	-57.63	53.33	< 0.001
ASAT (UI/L)	40.58	-23.22	-10.72	-30.04	-11.19	-1.54	< 0.001
ALAT (UI/L)	-40.07	-32.64	-13.23	-7.53	-1.25	-2.69	< 0.001
Creatinin (mg/l)	-26.20	26.67	27.28	-3.96	55.29	55.97	< 0.001
Urea (g/l)	-2.70	80.77	59.26	75.00	87.50	100.00	< 0.001
Calcium (mg/dL)	39.53	23.70	-12.52	13.48	-6.31	-31.75	< 0.001
Chlorine (mEq/L)	-8.45	-20.60	-19.25	-21.68	-12.87	-18.82	> 0.05
Sodium (mEq/L)	1.05	-14.67	-17.20	-17.05	-20.48	-17.53	< 0.001
Potassium (mEq/L)	-0.90	-29.88	-14.72	-33.66	-24.41	-2.35	< 0.001
Sodium/Potassium	7.40	27.32	2.18	29.32	10.60	-15.61	< 0.001

A/G: albumin/globulin; HDL; High Density Lipoprotein; LDL: Low density lipoprotein; ChT: Cholesterol Total ; ASAT: Aspartate Aminotransferase; ALAT:alanine aminotransferases.

Parameters such as albumin, globulin, creatinine, calcium, LDL, HDL, LDL/HDL ratios, A/G ratio, and ChT/HDL showed mixed variation during this growth phase (Table 4). However, at the level of HDL cholesterol the lots: Control Tpn, L3P and L3P25 showed a reduction in contrast to the L3P50, L3P75 and L3P100 lots. In contrast, at the level of ChT/HDL the lots: Control Tpn, L3P and L3P25 recorded an increase in contrast to the batches: L3P50, L3P75, and L3P100. On both sides, this decrease or increase is above 50% (Table 4).

4. Discussion

At the beginning of the experiment, no differences were observed between the biochemical parameters of all rat lots. A change in these parameters during the experiment would be due to the addition of Moringa leaf powder to food preparations. Apart from the albumin level and the albumin/globulin ratio, no other biochemical parameter shows any difference between the L3P feed and the feeds formulated from Moringa. Moringa leaves could therefore be used as a substitute in the preparation of laboratory rat feed following the example of L3P feed which has been studied satisfactorily from a nutritional point of view [11].

Albumin levels and albumin/globulin ratios increased for L3P50 food, indicating an improvement in nutritional status through the use of moringa as reported by [12]. This increase in albumin is stimulated by the addition of proteins, amino acids, mineral, vitamins and other compounds contained in the leaf powder of Moringa oleifera [13,14]. Compared to the therapeutic food (TPn) a significant increase in HDL cholesterol is observed.

HDL is considered the 'good' cholesterol as it has the ability to capture excess cholesterol and transport it to the liver for elimination [15]. There is clinical evidence that increasing HDL levels is highly beneficial to health as it reduces the risk of cardiovascular disease [16]. This increase is highlighted in studies by [16,17,18] made from Moringa extract. However, all other lipid indices and atherogenicity indices decreased.

In general, the literature reports the Moringa cholesterol-lowering effect [19,20,21] as in our study.

Aspartate aminotransferase showed a significant decrease in different feed rations compared to TPn. This decrease is reported in the work of [18] from aqueous extracts of Moringa leaves. The evolution of blood biochemical parameters during the experiment indicates an often-significant variation depending on the type of parameters and composition of the feed formulation. Among our formulations, $L3P_{75}$ feed causes a significant increase in glucose levels, total proteins, globulins and a significant decrease in A/G ratio. This increase in blood glucose within normal values in rats is indicative of glucose intake from the food formulation. Also, the increase in total protein reflects a good nutritional status of animals fed $L3P_{75}$. On the other hand, the decrease in A/G ratio supports the increase in globulin levels. This feed formation ($L3P_{75}$) would be the best for the evolution of glucose and blood proteins.

5. Conclusion

At the end of this work, we can conclude that the dietary formulations based on Moringa leaf powder used in our experiment showed a positive effect on the biochemical blood parameters of rats. They have similar effects to the L3P formulation which is a reference formulation in our Laboratory. Moringa leaf powder is therefore a substitute to be explored in the formulation of laboratory rat feed especially in Sub-Saharan Africa.

Conflict of Interest Statement

The authors declare no conflict of interest.

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