

Research Article

Effect of Cassava (*Manihot Esculenta* Crantz) Leaves Infected with Mosaic Virus on the Growth and Organs of Mice

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Abstract

In the Central African Republic (CAR), cassava leaves infected with the African cassava mosaic virus (ACMV) are one of the most consumed vegetables. Several studies have been carried out on cassava leaves but none of these studies have focused on the impact of consuming cassava leaves infected with CMV on the growth and organs of a mammal. The objective of this study is to verify the effects of feeding cassava leaves on the growth of mice. 35 mice were selected for the experiment. The presence of ACMV in cassava leaves was determined by PCR test. During the first month mice were feed with 25% cassava leaves and 75% corn cake. At the second month the diet consisted of an equal proportion of cassava leaves and corn cake. Finally at the third month 75% of cassava leaves and 25% of corn cake were used to formulate the diet of mice. Regular weight gain was performed to evaluate the growth of the mice. The mortality rate of the mice was determined during the three months of observation. Reference enzymes such as Aspartate Amino Transferase (ASAT) and Alanine Amino Transferase (ALAT) were assayed and creatinine content of mice was determined to evaluate liver and kidney functions. The results showed that cassava leaves collected with different levels of ACMV symptoms (severity symptom index ranging from 1 to 5) were positive for the presence of begomoviruses. There was a weight gain in mice fed with the diet consisting of 25% of cassava leaves. Inclusion of 50% cassava leaves did not result in a weight gain of the animals greater than 10%. The inclusion of 75% cassava leaves resulted in a decrease in animal weight. ASAT/ALAT enzymes and creatinine levels were normal. Consumption of cassava leaves infected with mosaic virus is not a health risk. Cassava leaves infected with mosaic virus can be used for food and feed.

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Keywords

Cassava Leaf, African Cassava Mosaic, Feed Intake, Growth

1. Introduction

Cassava is a multiannual plant cultivated in the tropical regions of most African, Asian and Latin American countries. The ease of cultivation of this plant, its ability to adapt to different types of soil and its tolerance to drought have contributed to the expansion of its cultivation [10]. This plant is cultivated for its starchy tubers that provide food for more than 800 million people worldwide and particularly in tropical countries [12]. Cassava leaves, prepared according to various culinary recipes, are one of the most consumed vegetable dishes in human nutrition in Central Africa [7].

In the Central African Republic, cassava leaves are regularly consumed in both rural and urban households. Cassava leaves that have been yellowed by the effects of the CMV are valued in the various uses of cassava leaves. The average protein content in leaves with mosaic symptoms is 22% [13]. The presence of viral particles in the leaves leads to an increase in protein content at the expense of chlorophyll content. This leads to a more or less important deformation of the infected leaves, classified as symptom severity index (SSI) on a scale of 1 to 5 [12]. According to the results of surveys conducted in rural and urban areas in the CAR, cassava leaves infected by the mosaic virus have no effect on the health of 80% of consumers, while 17% of consumers claim that cassava leaves infected by mosaic have a direct effect on their health [9]. Research needs to be conducted to verify whether cassava leaves infected with a phytovirus can have a beneficial effect, especially with regard to organoleptic and nutritional qualities due to the content of proteins and other secondary metabolites [10]. On the other hand, the consumption of leaves infected by the ACMV could be a source of toxicity for an animal organism. Indeed, some varieties of cassava have leaves that contain hydrocyanic acid, which gives the leaves a bitter taste and is responsible for some thyroid gland pathologies. The effects of cassava leaf consumption on other organs such as the liver and kidneys are not yet sufficiently documented. The objective of this work is to study the effects of food formulated with cassava leaves infected or not by mosaic on the growth and organs (liver, kidneys) of mice.

2. Materials and Methods

2.1. Mice and Cassava Leaf Collection

The mice (*Mus musculus*), aged (21-42 days) weighing an average of 12 ± 0.3 g were selected at the animal house of the Pasteur Institute of Bangui. They were then transported in cages to the Laboratory of Biological and Agronomical Sciences for Development of the University of Bangui. The experiment was conducted over a period of three months.

Cassava leaves were collected in the cassava plot of the Higher Institute of Rural Development, M'Baïki (Latitude 17.98.28 138; Longitude 3.86.65. 018). Cassava leaves were collected from healthy plants of the Togo or TMS66 variety resistant to the African Cassava Mosaic Virus (ACMV) and from plants showing symptoms of ACMV of the M66033 variety aged three months. Cassava leaf samples were placed in envelopes for molecular analysis to confirm the presence of the virus. Some of the leaf samples were also packed in coolers for the preparations of the mice's food ration. The samples collected were divided according to the degree of severity of the ACMV symptoms. A total of five (6) samples of cassava leaves were taken: one (1) sample of severity 0 and the other four (5) of severity ranging from 1 to 5. The leaves were subjected to blanching or steaming and then dried at room temperature. This pre-treatment allows the softening of cassava leaves by the degradation of portions of amylopectin and starch and the release of cyanogenetic acids.

2.2. Molecular Analysis for the Determination of the ACMV in the Cassava Leaves

Total DNA extraction was done from fresh young cassava leaf samples using the cetyltrimethylammonium bromide (CTAB) method, and the protocol described by the research [6]. A weight of 0.3 g of leaves was ground in CTAB extraction buffer (2% hexadecyl trimethyl ammonium bromide, 1.4 mM NaCl, 0.2% mercaptoethanol, 20 mM, EDTA, 100 mM Tris-HCL, pH 8.0) and heated at 65°C for 5 min with stirring. After centrifugation at 6000g an equal volume of chloroform and isoamyl alcohol mixture (24:1) was added. This addition clears the mixture of debris suspended in the aqueous phase. The aqueous phase was then transferred to a new tube and 2/3 isopropanol was added to allow precipitation of

the nucleic acids. The nucleic acids after rinsing with 500 µl of ethanol (70%) for at least 20 min were collected in a volume of 50 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.4) according to the mass of pellet obtained.

Polymerase Chain Reaction (PCR) amplification was conducted according to the protocols [3]. The universal primer pair for the detection of begomoviruses in cassava leaf samples was used with the following sequence:

VD1863/C12D2391 (TCRTCAATGACGTTGTAC-CA/TTTCCAYCCVAACATTTCARGG).

The PCR mix was composed of HPLC water, MgCl₂ (25 mM), buffer, Taq polymerase, DNTPs and primers. DNA amplification was programmed at 4 minutes at 94°C, 35 cycles of 1 minute at 72°C and 10 minutes at 72°C and then stored at -4°C [6].

The agarose gel (Sigma grade Molecular biology RNase free) at 1% (w/v) was prepared in TAE buffer (100 mM Trizma base, 100 mM acetic acid, 2 mM EDTA) while adding Ethidium Bromide (BET) and then poured onto the gel holder for electrophoresis. The electrophoresis vessel was filled with TAE buffer (1%) until the gel was fully immersed. A quantity of 10 µl of PCR amplicon was deposited in the wells of the 1% agarose gel. Electrophoretic migration was performed at 120 volts for 27 min and visualization of the gel was performed under a UV lamp.

2.3. Formulation of Feeds Based on Cassava Leaves and Corn Cakes

Each batch of leaves, depending on the severity level, was ground and mixed at 25%, 50% and 75% with corn meal. Formulations were coded according to the SSI that characterize the severity of cassava leaf MAM as described by Zinga. [12].

2.4. Sampling and Feeding of Mice

The mice divided into 7 batches of 5 mice (2 males and 3 females in each batch). The first batch was fed only with corn cake (control batch or batch 1). The second batch (batch 2) was fed with the feed formulation described above and with healthy leaves (with an SSI = 0). The third batch (batch 3) was fed the above described feed formulation and with leaves collected with very mild symptoms characterized by the appearance of yellow spots covering less than one third of the leaf surface (SSI = 1). For the fourth lot (lot 4) the leaves used had yellow spot covering more than two-thirds of the leaf area (SSI = 2). For the fifth batch (batch 5) the leaves used had yellow stain covering more than two-thirds of the leaf area with visible deformation in some areas of the leaves (SSI = 3). For the sixth batch (batch 6) the leaves used were totally deformed (SSI = 4). Finally, for the seventh batch (batch 7), the leaves used were totally stunted and

desiccated (SSI = 5) by the ACMV.

After mixing according to the food formulation, 30 g of the food was weighed and distributed every four days to the mice according to a gradual logic of the proportion of leaves in the food ration. Thus, at the first month, the ration is composed of 25% of cassava leaves. During the second month, the proportion of cassava leaves in the ration was increased to 50%, then to 75% in the third month.

2.5. Consumption Index and Determination of Weight Gain

The formulated food was weighed (30g) and distributed to the mice every four (04) days. The uneaten food was weighed again, which made it possible to determine the individual Consumption Index (CI) according to the following formula:

$$CI (g/d) = (AFD (\text{period}) - AFR (\text{period})) / (\text{Period length} \times \text{Number of subjects})$$

Where AFD = Amount of food distributed and AFR = Amount of food refused.

The weight of the mice was measured with a scale during the 3-month (90 days) observation period. This observation period was divided into 4 intervals: (day 0 to day 7), (day 7 to day 30), (day 30 to day 60) and (day 60 to day 90). The weight gain of mice was determined according to the following formula:

$$\text{Weight gain (\%)} = 100 - (Pi * 100) / Pa$$

Where: Pi is the initial weight of the mouse at day 0 and Pa is the weight acquired by the mouse at a given period.

2.6. Mortality Rate

A check was made each day to see if there were any dead animals in the batch. The mortality rate is determined according to the formula below (every 30 days):

$$\text{Mortality Rate (\%)} = (\text{Number of deaths in 30 days} * 100) / \text{Total number of animals.}$$

2.7. Determination of Hepatic and Renal Toxicity Indicators

The blood of the mice was collected at the National Laboratory of Clinical Biology and Public of Bangui. The collected blood was poured into a dry tube and then centrifuged at 3000 rpm. The serum collected was used for analysis. Aspartate Amino Transferase (ASAT) or Glutamo-Oxaloacetate Transaminase (GOT) and Alanine Amino

Transferase (ALAT) assays were performed according to the method [1].

The transamination of alanine to pyruvate is carried out in the presence of Alanine Amino Transferase (ALAT=GPT, Glutamo-Pyruvic Transferase). The pyruvate obtained is reduced to lactate in the presence of the coenzyme NADH^{H^+} and lactate dehydrogenase. The rate of NADH reduction is proportional to the amount of pyruvate formed in the medium and thus to the activity of alanine. The control solution (control serum) was added with 50 μl of each serum sample to be assayed in an automaton 250 μl of R1 reagents and 250 μl of R2 of the transaminase kit are then taken and poured into a tube and made homogeneous with a vortex. The calibration of the automaton and the dosage of the sera were then programmed via a microcomputer integrated to the system. The different readings of the absorbance of NADH^{H^+} were realized thanks to a spectrophotometer integrated to the automaton.

Creatinine reacts with picric acid in alkaline medium (NaOH) to give a red-orange complex whose optical density is proportional to the quantity of creatinine. Creatinine was determined using the kit 500 μl of reagent R1 (Picric acid 17.5 mmol/l) was mixed with equal volume of reagent R2 (Alkaline reagent Sodium hydroxide 0.29 mol/l). The mixture of reagents was introduced into the automaton presented above. The control solution (Creatinine Standard) was added to the automaton along with 50 μl of the serum samples to be assayed. The optical density of the complex formed during the reaction was measured by the automaton. Then, the concentration of creatinine in the sera was deduced.

2.8. Statistical Analysis

A generalized linear model (GLM) with a Poisson family error distribution was fitted to the data to compare data from

the SSI 3 leaf treatment (the most productive) and the Control with the other treatments. All these analyses were done with R software (version 3.6.1) and the probability threshold for a significant difference was set at 0.05.

3. Resultats and Discussions

3.1. Presence of Begomoviruses in Leaves

Cassava leaf samples with ACMV symptoms of severity 1 to 5 were tested positive for begomoviruses after PCR amplification except for leaves without symptoms (SSI 0; Figure 1). This shows that begomovirus DNA is undetectable in leaves collected without symptoms.

3.2. Consumption Indexes (CI)

The results shown in Table 1 present the values of the CI determined in each batch of mice. The average CI value for mice in Lot 1 (control lot fed only with corn meal) is 2.01g/d in the first month. It can be seen that at the first month, the CI of the mice in batch 2 (fed with leaves without ACMV symptoms) and batches 3 and 4 (fed with leaves with mild ACMV symptoms) is relatively low (1.35 - 1.42 g/d) compared to the CI (1.72 - 1.85 g/d) of the mice in batches 5, 6 and 7 fed with the leaves with more pronounced ACMV symptoms. Overall, the CI of the mixed feed (corn cake + leaves) was significantly lower than that of the corn cake only feed (Control). This trend is also observed in the second and third month with a progressive decrease of the CI of the mixed feed, while the CI of the corn cake only feed remains relatively stable.

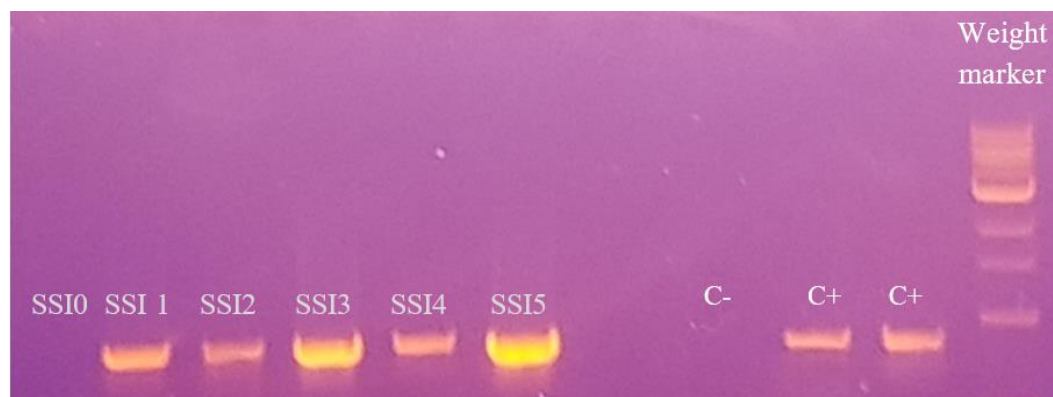


Figure 1. 1% agarose gel migration of PCR amplicons from cassava leaf samples without ACMV symptoms (SSI 0) and with different levels of symptoms (SSI 1-5). C- and C+ are negative and positive controls, respectively.

Table 1. Individual Food Consumption Index (values with different letters are significantly different, $P < 0.05$).

First month	Control	SSI ₀	SSI ₁	SSI ₂	SSI ₃	SSI ₄	SSI ₅
Number N=35	n=5	n=5	n=5	n=5	n=5	n=5	n=5
CAI g/d	2.01±1.5 ^a	1.35±1 ^b	1.42±0.7 ^b	1.38±0.5 ^b	1.85±1.5 ^a	1.75±1 ^a	1.72±1 ^a
Batch	Batch 1	Batch 2	Batch3	Batch 4	Batch 5	Batch 6	Batch7

Month 2	Control	SSI ₀	SSI ₁	SSI ₂	SSI ₃	SSI ₄	SSI ₅
Number N=30	n=5	n=4	n=4	n=4	n=5	n=4	n=4
CAI en g/d	2,05±1 ^a	1,07±0.5 ^b	1.10±0.5 ^b	1,12±1 ^b	1,65±1 ^{a b}	1,20±0.5 ^b	1,24±0.7 ^b
Batch	Batch1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7

Month 3	Control	SSI ₀	SSI ₁	SSI ₂	SSI ₃	SSI ₄	SSI ₅
Control N=27	n=5	n=4	n=4	n=4	n=4	n=3	n=3
CAI en g/d	2,03±1.3 ^a	1,03±0.8 ^b	0,98±0.5 ^b	0,91±0.3 ^b	1,15±1 ^b	0,93±0.3 ^b	0,86±0.5 ^b
Batch	Batch 1	Batch2	Batch 3	Batch 4	Batch 5	Batch 6	Batch7

3.3. Mortality Rate

Table 2 shows the results of the mortality rate of mice at the second and third months of observation. No cases of mouse deaths were recorded in the first month. The mortality rate

increased from 14% in the second month to 22.6% in the third month. This shows that the increase in the proportion of cassava leaves from 50 and 75% in the mice's diet could be a factor that promotes the death of mice. It could be argued that the high mortality rate in the second and third months may be due to the refusal of the mice to consume cassava leaves.

Table 2. Mortality of animals at the second and third month.

Mortality at second month	Control	SSI ₀	SSI ₁	SSI ₂	SSI ₃	SSI ₄	SSI ₅
Initial number of animals	5	4	4	4	4	4	4
Number of dead animals	0	1	1	1	1	1	1
Mortality rate (%)	0	25	25	25	25	25	25
Batch	Batch1	Batch 2	Batch 3	Batch4	Batch 5	Batch 6	Batch7

Mortality in the third month	Control	SSI ₀	SSI ₁	SSI ₂	SSI ₃	SSI ₄	SSI ₅
Initial number of animals	5	4	4	4	4	3	3
Number of dead animals	0	1	1	1	1	2	2
Mortality rate (%)	0	25	25	25	25	40	40
Batch	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7

3.4. Growth of Mice in the First Month, Second Month and After 90 Days

At the first month, the mice were fed 25% of the cassava leaves (plus 75% of corn cake). The best weight gain of the mice was obtained in the case of infected cassava leaves with the SSI 3 (mice from batch 5), with a gain of more than 27% on the initial weight after 30 days (Figure 2). The weight

gain was about 20% for the diet with leaves infected with SSI 4 (batch 6) and SSI (batch 7) and with leaves without ACMV symptoms (batch 2) after 30 days (Figure 2). The lowest weight gains (16%) were recorded in mice from the batch fed only with corn meal (mice from batch 1), and mice from batches 3 (SSI1) and 4 (SSI2) fed with leaves that presented mild ACMV symptoms (12.5 and 9.5% respectively).

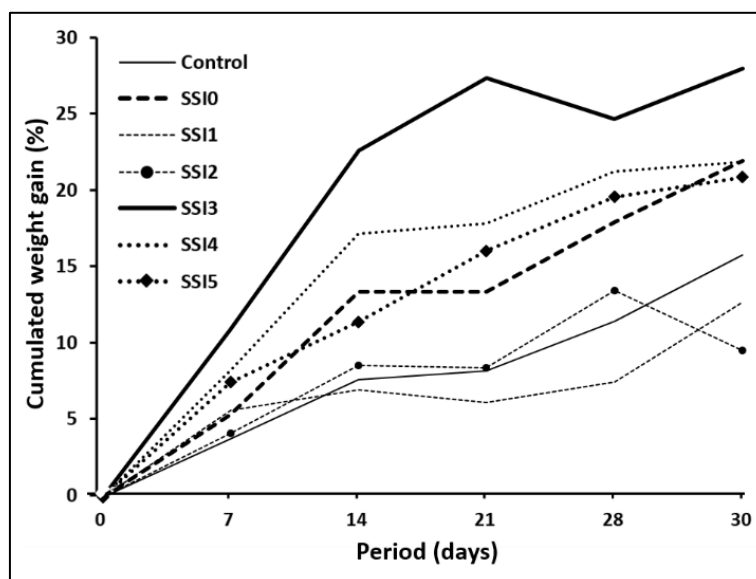


Figure 2. Assessment of body mass of mice fed with a mixture of corn cake over the month 1. Control = lot Batch (Batch 1) fed exclusively with corn cake; SSI 0 = Batch 2 fed with 25% of cassava leaves without symptoms; SSI 1 = lot 3 fed with 25% of cassava leaves with very mild symptoms; SSI 2 = lot 4 fed with 25% of cassava leaves with mild symptoms; SSI 3 = batch 5 fed with 25% of cassava leaves with fairly severe symptoms; SSI 4 = batch 6 fed with 25% of cassava leaves with severe symptoms; SSI 5 = batch 7 fed with 25% of cassava leaves with very severe symptoms.

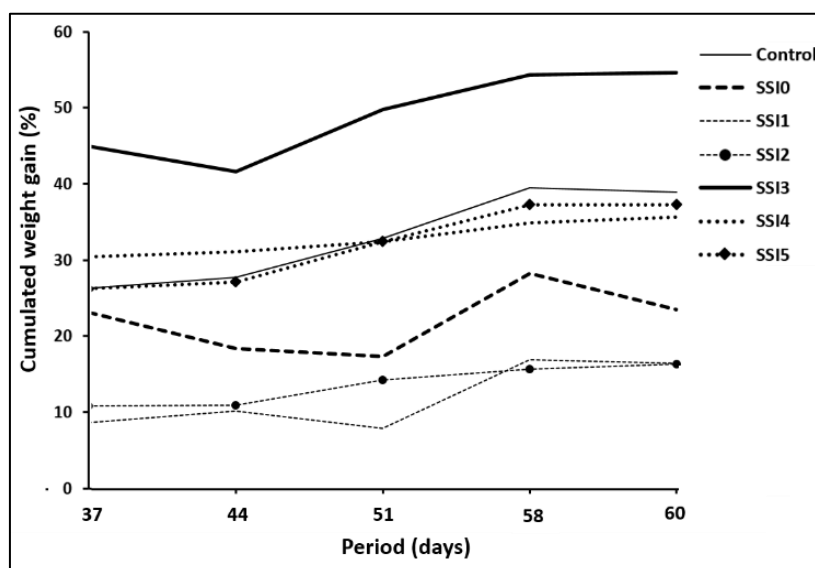


Figure 3. Assessment of body mass versus time at month 2. See legend of Figure 2 for details.

At the second month, mice were fed equal proportions of corn meal and cassava leaves. Weight gain in mice in batch 5 increased from 27% to 45% in the first week of the second month and then to 50% by the end of the second month (Fig-

ure 3). Batches 1 (control), 6 (SSI 4), and 7 (SSI 5) had a weight gain around 30% at the end of the second month. No interesting variation was recorded in batches 2 (SSI 0), 3 (SSI 1) and 4 (SSI 2) during the second month (Figure 3).

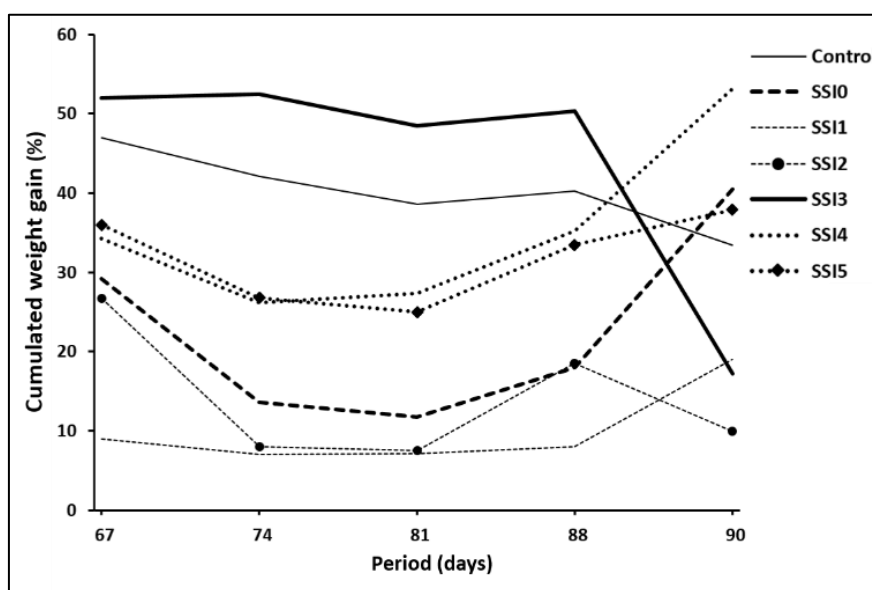


Figure 4. Assessment of body mass versus time at month 3. See legend of Figure 2 for details.

At the third month, the mice were fed 25% corn cake and 75% cassava leaves. The results (Figure 3) show that there was a decrease in the weight of the mice in the batches fed with leaves presenting SSI 0, SSI 1, SSI 2, SSI 4, SSI 5. Only mice from batch 3 (SSI 3) maintained their weight but followed by a sudden decrease beyond 88 days. The food ration distributed has a negative effect on the growth of mice. These results showed that the feed ration supplemented with 75% cassava leaves is not effective in promoting weight gain.

3.5. Liver Disease Indicator

The amounts of GPT (Glutamo-Pyruvic Transferase) and GOT (Glutamo-Oxaloacetate Transaminase) in the sera of animals of lot 6 and lot 7 are 82 ± 66 and 86 ± 18 IU/l, respectively (Table 3). Decrease in GPT value was observed in animals of lot 4 and lot 5 (18 ± 1 and 19 ± 8 IU/l, respectively). Despite these variations, the amounts of ASAT/GOT and ALAT/GPT measured in the mice did not exceed the threshold of the usual value (20-90 IU/l). This may indicate that cassava leaves infected with mosaic virus are not a source of liver toxicity in mice.

Table 3. ASAT/ALAT content (values with different letters are significantly different, $P < 0.05$).

Treatment							
Number N =27	Control n= 5	SSI0 n=4	SSI1 n= 4	SSI2 n=4	SSI3 n=4	SSI4 n=3	SSI5 n=3
ASAT /GOT (UI /L)	24 ± 1^a	28 ± 8.5^c	19 ± 7.5^b	21 ± 1^{ab}	22 ± 5^{ab}	80 ± 58^d	83 ± 15^d
ALAT/GPT (UI/L)	22 ± 5.8^a	25 ± 6.5^{ac}	22 ± 6.5^{ab}	18 ± 1^b	19 ± 8^b	82 ± 66^d	86 ± 18^d
Usual Value (IU/l) (UI/l)	20-90	20-90	20-90	20-90	20-90	20-90	20-90
Batch	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7

3.6. Indicators of Renal Disease

The results in Table 4 show the obtained values of creatinine concentration in the sera of the animals compared to the usual values in rodents. The amounts of creatinine are all between

0.8 and 1 mg/dl (usual value 0.5 - 1.5 mg/dl). As a result, the treatments given to the mice have no effect on kidney function. Therefore, cassava leaves infected with ACMV do not show any effect on the kidney function of the animals.

Table 4. Creatinine content.

Treatment							
Number N=27	Control n=5	DSI0 n=4	DSI1 n=4	DSI2 n=4	DSI 3 n=4	DSI4 n=3	DSI5 n=3
Creatinine (mg/dl)	0,82	0,87	0,80	0,82	0,95	0,86	0,98
Usual Value (mg/dl)	0,5-1,5	0,5-1,5	0,5-1,5	0,5-1,5	0,5-1,5	0,5-1,5	0,5-1,5
Batch	Batch 1	Batch2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7

Cassava is a staple food for more than one billion people in the world. However, it plays an important role in human and animal nutrition. It should be noted that cassava leaves play an important role in the food intake of the Central African population.

A PCR test was carried out on cassava leaf samples to reveal the presence of ACMV. The results showed that cassava leaves infected with SSI1, SSI2, SSI3, SSI4, SSI5 were found to be positive for the virus. These results confirm those of (Zinga et al., 2013) who show that cassava leaf samples collected from several localities in Central Africa were tested positive for cassava mosaic virus.

According to these results cassava leaves showing the symptom of mosaic virus severity (SSI) 0 to 5 were supplemented with corn cake at 25, 50 and 75%. It was found that the diet formulated with 25% of mosaic-infected cassava leaves promoted weight gain of the animals over an initial weight after 30 days, with a gain of more than 27% for the SSI3 ration. These results are similar to those of [2], which shows that feed concentrates containing 40% cassava leaves instead of maize give excellent results in these animal species.

The incorporation of cassava leaves from mosaic virus-infected plants into the diet of mice allowed for nutrient enrichment of the formulated feed.

The results showed that diets formulated with 50% cassava leaves and 50% maize meal resulted in growth in batch 5 at SSI3. The growth of mice treated with SSI3 was statistically significant compared to batches treated with SSI0, SSI1, SSI2 and the control (GLM, $P < 0.0001$), which explains that the SSI3 diet provided more nutrients that contribute to the weight gain of the animals, after 60 days compared to the other treatments. These results are contrary to

those of [2], which shows that the incorporation of 5 to 10% of cassava leaf meal in pig and poultry rations is the best compromise between ration cost and animal performance. However, we introduced 50% of cassava leaves in the ration of mice, which did not result in a significant weight gain except for the SSI3 treatment.

The results also showed that there was a decrease in the weight, of mice whose ration consisted of 75% of cassava leaves and 25% of corn cake. The GML model revealed a highly significant difference, compared to the weights of mice treated only with corn cake (GLM, $P < 0.0001$). This explains why the feed composed of 75% cassava leaves and 25% corn cake cannot be recommended in the diet of mice. Furthermore [8] have shown that the inclusion of cassava leaf meal in the diet of guinea pigs did not affect the pre and postpartum weight development of breeding females.

Consumption Index or CI was 2.01 g/d at the first month in mice fed only with corn meal. While this CI decreases with the content of cassava leaves in the mice's diet. It is also noted that the CI varies from 1.35 to 1.85 g/d in the first month in mice fed a ration composed of 25% of cassava leaves. Although this CI is low, there was a weight gain of 10%. According to studies conducted [5], the CI of mice varies between 5-8g/d, which translates that the weight gain obtained by mice on a ration composed of 25% of cassava leaves and 75% of corn cake, could be due to a balanced intake of nutrients.

The results of biochemical analysis of ASAT/ALAT enzymes show a slight increase in the level of GTP enzymes in the batch treated with SSI4, SSI5. These results are similar to the work of [4] whose studies showed that Wistar rats exposed to 200 mg/L of cadmium sulfate had elevated transam-

inases. While, in the batches treated with leaves presenting SSI3 and SSI4, it was found that there was a decrease in the GTP level in the serum of the animals, this decrease in GTP content may be related to a deficit to vitamins in cassava leaves. According to the results of the work [11] which show that the chronic alcoholization with Koutoukou (ACK) involves a fall of the rates of ASAT/ALAT as well as that of the ratio ASAT/ALAT in rats (*Rattus norvegicus*).

Moreover, the results of the analysis of the creatinine level show that all the animals presented a normal creatinine level, which allowed us to affirm that the food consumed does not have an impact on the kidney function. These results are in agreement with that of Tehoua. L et al. [11] who showed that the creatinine did not vary significantly in rats consuming the alcoholic beverage.

The experimental study conducted showed that after 30 days there were no cases of death in the batches, but after 60 days there were 25% of deaths in batches 2-7. We note, in the third month the mortality rate increased by 40% in batches 6 and 7, which allowed us to assert that the increase in mortality rate could be related to the loss of weight of animals stimulated by the refusal of food whose content of cassava leaves was increased from 50% to 75% in the feed ration. Therefore, we can deduce that the incorporation of cassava leaves at 50% and 75% is not recommended in the diet of mice. These results verify the work [8] which shows that the incorporation of 10% of cassava leaf meal resulted in the highest pre-weaning mortality rate in piglets.

4. Conclusion

Cassava leaves occupy an important place in the diet of many communities. The potential of cassava leaves has not been fully explored. Through this study three feeding formulas including cassava leaves infected with mosaic virus of severity 0 to 5 as well as corn meal in variable proportion in the diet of mice. This research explored cassava leaves infected with mosaic virus as an available food resource and can be valorised in several areas.

Conflicts of Interest

The authors declare no conflicts of interest.

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