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Research Article

Food-to-Food Fortification of a Traditional Pearl Millet Gruel with a Natural Source of β-Carotene (Sweet Potato) Improves the Bioaccessibility of Iron and Zinc

Saliou Mawouma,[1] **Emmanuel Awoudamkine**,[1] **Roger Ponka**,[2] **Yaya Verlai Ndjidda**,[1] **and William Dzusuo Tedom**[3]

[1]*Department of Biological Sciences, Faculty of Science, The University of Maroua, P.O. Box 814, Maroua, Cameroon*
[2]*Department of Agriculture, Livestock and By-Products, National Advanced School of Engineering of Maroua, The University of Maroua, P.O. Box 46, Maroua, Cameroon*
[3]*Department of Food Sciences and Nutrition, National School of Agro-Industrial Sciences, The University of Ngaoundere, P.O. Box 455, Ngaoundere, Cameroon*

Correspondence should be addressed to Saliou Mawouma; mawouma2001@yahoo.fr

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Iron and zinc deficiencies are still a major public health concern in the Far North Region of Cameroon where staple foods are mainly mineral rich cereals which equally contain inhibitors of their bioaccessibility. The effect of food-to-food fortification of a traditional pearl millet gruel with a natural source of β-carotene on the bioaccessibility of iron and zinc was assessed. A sensory evaluation of gruels fortified at 20, 30, and 40% with mashed sweet potato was carried out. The samples were analysed for carotenoids, phytates, polyphenols, iron, and zinc contents. Bioaccessible iron and zinc were evaluated using *in vitro* digestion method. The gruel fortified at 20% with mashed sweet potato had better scores ($P < 0.05$) of taste (3.93), colour (3.36), and overall acceptability (3.80) compared to the control. Carotenoid, polyphenol, and phytate contents were higher in fortified gruels ($P < 0.05$) compared to the control, while iron and zinc contents were lower. A significant increase ($P < 0.05$) in bioaccessibility of 8.08% and 26.96% for iron and 53.79% and 62.92% for zinc was observed at 20 and 30% incorporation level, respectively. However, at 40% incorporation level, the increase in bioaccessible iron was less important and bioaccessible zinc decreased. Mashed sweet potato can be used as a fortificant to improve the bioaccessibility of iron and zinc contents of local pearl millet gruel, if added moderately.

1. Introduction

Micronutrient deficiencies also called "hidden hunger" represent one of the major health concerns across the globe [1]. Muthayya et al. [2] stated that micronutrient deficiencies affect approximately one third of the world's population, with tropical regions of Africa being particularly affected. Among these deficiencies, those concerning iron and zinc are the most critical and the most widespread [3]. In 2018, the National Institute of Statistics of Cameroon (INS) revealed that in the Far North Region, 43.2% of women of childbearing age and 60.2% of children aged between 6 and 59 months had a deficient iron status [4]. Few years before, a study conducted by Engle-Stone et al. [5] in the same region showed that 89% of women of 15 to 49 years and children aged 12 to 49 months had low plasmatic zinc concentration. Iron deficiency is one of the main causes of anemia in Africa (Mwangi et al. [6]). It seriously affects the cognitive development of young children, thus altering their learning abilities [7, 8]. Physical performance of adults is also affected [9, 10]. On the other hand, several studies have highlighted the impact of zinc deficiency in growth delay and

high risk of infectious diseases due to the impaired immune system [11, 12].

One of the main causes of iron and zinc deficiencies in Sahelian regions are diets based on cereals which though contain appreciable amounts of minerals equally contain high levels of antinutritional factors [13, 14]. These antinutritional factors form insoluble, nonabsorbable complexes with iron and zinc, thereby reducing their bioaccessibility [15]. Household processing methods such as soaking, germination, and fermentation are known to reduce the levels of antinutritional factors in cereals and legumes, thus increasing the bioaccessibility of minerals [16, 17]. However, current culinary practices in the Far North Region do not include these processing methods that involve additional workload for housewives [18]. Also, animal tissues (meat, fish, and poultry) known as activators of nonheme iron found in cereals are not accessible to all social classes due to their high cost. Food-to-food fortification is an encouraged solution to micronutrient deficiencies in low-income countries of the globe [21], Ohanenye et al. [19]). This approach uses a food called fortificant rich in a nutrient that is added to another food commonly consumed by the target population. The commonly used fortificants are fruits, vegetables, and even tubers [21–22]. It is therefore possible to use a fortifier containing an activator of the bioaccessibility of iron and zinc, in food products rich in these trace elements, such as pearl millet [23]. β-carotene is an activator of the bioaccessibility of iron and zinc [24] with the advantage of being efficient at high pH and in the presence of significant amounts of antinutritional factors [25, 26]. Moreover, using β-carotene food source as fortifier may contribute to solving problems link to vitamin A deficiency, which is another public health concern in the Far North Region of Cameroon [27].

The objective of this study was to investigate the effect of fortifying a local pearl millet gruel with a natural source of β-carotene on the bioaccessibility of iron and zinc.

2. Materials and Methods

2.1. Materials. The biological material used in this study was purchased at the "Abattoir" market in the city of Maroua, the regional capital of the Far North Region of Cameroon. Pearl millet grains have been identified by the IRAD (Institute of Agricultural Research for Development) as *Pennisetum glaucum* species. Sweet potatoes (*Ipomea babatas*) used was of the yellow variety (TiB 1).

2.2. Preparation of Mashed Sweet Potato. Sweet potato was washed with tap water and then cooked in boiling water for 30 minutes. Once cooled, it was peeled and cut into small pieces. To 1 kg of sweet potato, 1.5 L of water was added, followed by grinding in a domestic blender (SONASH, Dubai, UAE) until a smooth mash was obtained. A part of mashed sweet potato was used for food-to-food fortification while the remaining part was kept under 0°C for phytochemical and mineral analyses.

2.3. Preparation of the Gruel. The pearl millet grains were washed with tap water and dried in the sun. After drying, they were sorted and then crushed using an IMEX brand mill, type Y132S1-2. Two hundred grams of this flour and 400 g of peanut paste were mixed in 1 L of water and the homogeneous mixture obtained was sieved using a 250 μm diameter mesh sieve. The mixture was cooked for 20 min while homogenising and 220 g of sugar was added at the end of cooking. The gruel obtained was used in the food-to-food fortification process.

2.4. Food-to-Food Fortification. The gruel was fortified with mashed sweet potato at the incorporation level of 20, 30, and 40% (volume/volume). A part of fortified gruels was used for sensory evaluation and the other one kept under 0°C for further analyses.

2.5. Sensory Evaluation of Fortified Gruels. A sensory test was carried out with a panel of 30 housewives who were regular consumers of local cereal gruels. Each panellist had to rate the texture, colour, odour, taste, and overall acceptability of the samples using a five-point hedonic scale, where 1 = very unpleasant, 2 = unpleasant, 3 = fair, 4 = pleasant, and 5 = very pleasant [28].

2.5.1. Sensory Panel. Informed consent was obtained from each subject prior to their participation in the sensory test.

2.6. Chemical Analyses of Mashed Sweet Potato and Fortified Gruels

2.6.1. Determination of Carotenoids. Carotenoid contents were determined using the AOAC [29] standard method. In brief, 30 mL of a hexane-acetone 30/70 (v/v) solution were added to 1 g of sample dried at 45°C for 24 hours. The mixture was then heated for one hour and filtered after cooling. The filtrate was washed with distilled water in a separatory funnel; the lipid phase was transferred to a 25 mL volumetric flask and the volume was adjusted to the mark with hexane. The obtained solution was diluted 10 times with hexane and maximum absorbance was read between 430 and 450 nm using a spectrophotometer.

2.6.2. Determination of Phytates. The phytate contents were evaluated according to the method of Gao et al. [30]. In a centrifuge tube, 0.5 g of sample was introduced and mixed with 2.4% HCl to 10 mL. After shaking at 200 rpm for 16 h, the mixture was centrifuged at 1000 rpm at 10°C for 20 min. The crude extract was transferred to tubes containing a pinch of NaCl, shaked at 350 rpm for 20 minutes to dissolve the salt, and then incubated at 4°C for 60 min. The mixture was centrifuged at 1000 rpm at 10°C for 20 min. One milliliter of the clear supernatant was diluted 25 times with distilled water. To 3 mL of diluted sample, 1 mL of Wade's reagent (0.03% $FeCl_3.6H_2O$ + 0.3% sulfosalicylic acid) was then added, before mixing vigorously and centrifuging at 1000 rpm at 10°C for 10 min. Absorbance was read at 500 nm.

2.6.3. Determination of Polyphenols. Polyphenols were determined using the method described by Makkar et al. [31]. Dry samples (0.1 g) were placed in a 25 ml beaker, and 10 ml of 70% acetone were added. The mixture was placed in a boiling water bath and stirred for 20 minutes. The contents of the beaker were centrifuged for 10 min at 3000 g and 4°C in a tube, 200 μL of each supernatant was made up to 500 μL with distilled water. 250 μL of Folin–Ciocalteu reagent and 1.25 mL of 20% sodium carbonate solution were added to each tube. The extracts were shaked and stored in the dark for 40 min. The absorbance was read at 725 nm.

2.6.4. Determination of Total Ash, Total Iron, and Total Zinc Contents. The total ash content of the gruels was quantified using the method proposed by [32]. Total iron and total zinc contents were determined using atomic absorption spectrophotometry [33]. In brief, concentrated HNO_3 acid was added to 0.5 g of finely ground sample, followed by digest for 4 hours on a block digester. The digestate r was allowed to cool down and was diluted to 50 mL with deionised water. The supernatant was carefully transferred into centrifuged tubes for analysis on the Flame Atomic Absorption Spectrophotometry (Buck Scientific 200 Serie AA, East Norwalk, Connecticut, USA).

2.6.5. Determination of Iron and Zinc Bioaccessibility

(1) Preparation of Simulated Digestive Fluids. The simulated digestive fluids (SSF: simulated salivary fluid, SGF: simulated gastric fluid, and SIF: simulated intestinal fluid) are prepared as shown in Table 1, using Minekus et al.'s [34] method, with slight modifications.

(2) Oral Digestion. Five grams of gruel was mixed with 5 mL of salivary α-amylase solution (150 U.mL^{-1}) obtained by mixing 1 g of α-amylase (86250 Sigma-Aldrich Chemie Gmbh) with 0.25 mL of 0.3 M $CaCl_2$ and SSF electrolyte solution to reach a final volume of 10 mL. The mixture was homogenised for 2 minutes at 37°C.

(3) Gastric Digestion. Ten milliliters of oral digestate was mixed with 7.5 mL of SGF electrolyte solution, 1.6 mL pepsin solution of 25 000 U.mL^{-1} made up by mixing 0.1 g of pepsin (P7125 Sigma-Aldrich Chemie Gmbh) with 5 μL of 0.3 M $CaCl_2$, and 0.695 mL of distilled water. The mixture was acidified with HCl 1 M to reach pH 3.0 and incubated during 2 hours at 37°C under gentle regular shaking.

(4) Intestinal Digestion. Twenty milliliters of gastric digestate was mixed with 11 mL of SIF electrolyte solution, 5.0 mL of a pancreatin solution 800 U.mL^{-1} obtained by mixing 0.42 g pancreatin (P7545 Sigma-Aldrich Chemie Gmbh), 1.25 g bile extract (B8631 Sigma-Aldrich Chemie Gmbh), 40 μL of 0.3 M $CaCl_2$ and 50 mL of SIF. One molar NaOH solution was added to reach pH 7.0, and the mixture was incubated for 2 hours at 37°C under gentle regular shaking.

The final digestates obtained were placed in ice until cooling to stop enzyme activities, and then filtered using Macherey–Nagel MN 640d filter paper.

One milliliter of each filtrate was then used as starting material to quantify iron and zinc using flame atomic absorption spectrophotometry as described in Section 2.6.4.

(5) Bioaccessibility. Bioaccessibility of iron and zinc was determined using the following formula:

$$\text{bioaccessibility}\,(\%) = \frac{\text{quantity of mineral in the filtrate}}{\text{quantity of mineral in the sample}} \text{x } 100. \quad (1)$$

2.6.6. Statistical Analyses. The results obtained were subjected to one-way analysis of variance (ANOVA) using IBM SPSS Statistics software version 19.0.1. Differences between means were tested by Duncan's multiple range comparison with a significance level of 5%.

3. Results and Discussion

3.1. Sensory Evaluation. The technique of food-to-food fortification usually modifies the organoleptic properties of target foods and hence their acceptability [21, 35]. Table 2 presents the results of the sensory evaluation of pearl millet gruels fortified with mashed sweet potato. The general acceptability scores of fortified gruels range from 3.36 to 3.80. The taste of the gruel fortified at 20% was significantly more appreciated ($P < 0.05$) compared to that of the control. The observed scores could be explained by the additional sweet taste brought by mashed sweet potato. However, at 30% incorporation level, we noticed a decrease in taste and overall acceptability scores. This result suggests that mashed sweet potato, incorporated at 30%, would have considerably altered the original flavour of the control pearl millet gruel, resulting in an uncommon taste less appreciated by the panel. At 40% incorporation level, the increase in taste and overall acceptability scores seems paradoxal but could be explained by the dominance of more appreciated organoleptic properties of mashed sweet potato.

3.2. Phytochemical Content of Mashed Sweet Potato and Fortified Gruels. The ideal food-to-food fortification process is the one in which the plant-based fortificant has notably higher levels of the desired bioavailability enhancers and/or lower levels of antinutrients, compared to the unfortified food product [21]. The carotenoid, polyphenol, and phytate contents of fortified gruels proportionally increased with the percentage of incorporation of mashed sweet potato (Table 3). The carotenoid contents vary from 1.22 to 2.05 mg/100 g dry matter. These values were significantly higher ($P < 0.05$) than those of the control. This result could be explained by the high carotenoid content in smashed sweet potato used as fortificant (70.35 mg/100 g dry matter). Previous studies revealed sweet potato tubers to be rich in carotenoids, especially β-carotene [36–38].

Table 1: Composition of simulated digestive fluids.

Constituents	Concentration (mmol.L^{-1})	SSF pH 7 Volume in the fluid (mL)	SGF pH 3 Volume in the fluid (mL)	SIF pH 7 Volume in the fluid (mL)
KCl	0.5	30.2	20.7	13.6
KH_2PO_4	0.5	7.4	2.7	1.6
$NaHCO_3$	1	13.6	37.5	85
NaCl	2	-	35.4	19.2
Mg_2SO_4	0.15	1	1.2	2.2
$(NH_4)_2SO_4$	0.5	0.12	1.5	-

SSF: simulated salivary fluid; SGF: simulated gastric fluid; SIF: simulated intestinal fluid.

Table 2: Organoleptic characteristics of the fortified pearl millet gruels.

Gruel	Taste	Odour	Colour	Texture	Overall acceptability
G0	3.46 ± 1.04^{bc}	3.20 ± 1.18^{a}	2.50 ± 1.07^{a}	3.33 ± 1.34^{ab}	3.50 ± 0.90^{ab}
GP1	3.93 ± 1.01^{d}	3.33 ± 1.29^{a}	3.36 ± 1.15^{b}	3.73 ± 1.22^{b}	3.80 ± 1.03^{b}
GP2	3.26 ± 1.08^{ab}	3.16 ± 1.14^{a}	3.50 ± 1.00^{b}	3.13 ± 1.27^{ab}	3.36 ± 0.96^{a}
GP3	3.70 ± 0.79^{cd}	3.20 ± 1.27^{a}	3.30 ± 1.05^{b}	2.80 ± 1.37^{a}	3.73 ± 0.82^{b}

G0: control (unfortified gruel); GP1: gruel fortified with mashed sweet potato at 20%; GP2: gruel fortified with mashed sweet potato at 30%; GP3: gruel fortified with mashed sweet potato at 40%. Mean values in the same column with different superscript letters are significantly different ($P < 0.05$).

Table 3: Phytochemical composition of mashed sweet potato and fortified gruels.

Gruel	Carotenoids (mg/100 g DM)	Phytates (mg/100 g DM)	Polyphenols (mg/100 g DM)
P	70.35 ± 2.14^{d}	43.60 ± 0.07^{c}	0.44 ± 0.03^{a}
G0	0.57 ± 0.06^{a}	33.92 ± 0.22^{a}	0.40 ± 0.05^{a}
GP1	1.22 ± 0.09^{b}	35.14 ± 0.03^{b}	0.79 ± 0.02^{b}
GP2	1.23 ± 0.49^{b}	45.32 ± 0.07^{c}	0.87 ± 0.02^{b}
GP3	2.05 ± 0.35^{c}	47.61 ± 0.08^{d}	0.90 ± 0.07^{c}

DM: dry matter; P: mashed sweet potato; G0: control (unfortified gruel); GP1: gruel fortified with mashed sweet potato at 20%; GP2: gruel fortified with mashed sweet potato at 30%; GP3: gruel fortified with mashed sweet potato at 40%. Mean values in the same column with different superscript letters are significantly different ($P < 0.05$).

Polyphenol and phytate contents vary from 0.79 to 0.90 mg/100 g dry matter and from 35.14 to 47.61 mg/100 g dry matter, respectively, and were significantly higher ($P < 0.05$) compared to the control. The results observed could be explained by the abundant presence of polyphenols and phytates in sweet potato tubers as shown by previous studies [37, 39–42]. However, the amounts of phytates and polyphenols in mashed sweet potato used in our study were not very high, compared to those of the unfortified gruels. In some case, the amounts of phytates and polyphenols in mashed sweet potato were inferior to the amounts observed in fortified gruels. This paradoxal findings could be attributed to storage conditions of mashed sweet potato, which would have favoured biochemical processes (such as fermentation), resulting in the degradation of phytochemicals.

The inhibitory effect of polyphenols and phytates on the bioaccessibility of iron and zinc could therefore compete with the activating effect of carotenoids [43–46].

3.3. Mineral Contents of Mashed Sweet Potato and Fortified Gruels. We investigated the effect of fortifying a pearl millet gruel with mashed sweet potato on mineral contents. Table 4 shows the ash, total iron, and total zinc contents of the fortificant and various fortified gruels. The ash contents of fortified gruels were all significantly lower ($P < 0.05$) than that of the control. This may be due to the diluting effect of added mashed sweet potato, which had low ash content compared to the unfortified gruel. Previous studies revealed relatively poor mineral content of sweet potato tubers [42, 47, 48]. Also, leaching of minerals through diffusion in boiling water would have occurred during the cooking step of the preparation of mashed sweet potato.

The iron and zinc contents of the fortified gruels decreased from 4.53 to 3.65 mg/100 g dry matter and from 2.19 to 2.06 mg/100 g dry matter, respectively. These values are significantly lower ($P < 0.05$) than that of the control. This could be explained by the low iron and zinc content of mashed sweet potato compared to the unfortified pearl millet gruel. A comparison of results obtained by Hurrell and Egli [49], Mohanraj and Sivasankar [38], and Eke-Ejiofor and Onyeso [48] revealed the superiority of pear millet grains on sweet potato tubers as far as iron and zinc contents are concerned. The incorporation of mashed sweet potato into the control consequently reduced the iron and zinc contents of fortified gruels. These contents were lower than those found in millet porridges fortified with a carrot-

Table 4: Ash, total iron, and total zinc contents of mashed sweet potato and fortified gruels.

Gruel	Ash (g/100 g DM)	Total iron (mg/100 g DM)	Total zinc (mg/100 g DM)
P	1.50 ± 0.50^{a}	3.15 ± 0.60^{a}	0.71 ± 0.04^{a}
G0	4.4 ± 0.01^{d}	5.42 ± 1.86^{d}	2.44 ± 0.07^{c}
GP1	2.5 ± 0.01^{b}	4.53 ± 0.12^{c}	2.08 ± 0.04^{b}
GP2	3.0 ± 0.10^{c}	3.93 ± 1.73^{b}	2.19 ± 0.10^{b}
GP3	3.0 ± 0.01^{c}	3.65 ± 0.07^{a}	2.05 ± 1.77^{b}

DM: dry matter; P: mashed sweet potato; G0: control (unfortified gruel); GP1: gruel fortified with mashed sweet potato at 20%; GP2: gruel fortified with mashed sweet potato at 30%; GP3: gruel fortified with mashed sweet potato at 40%. Mean values in the same column with different superscript letters are significantly different ($P < 0.05$).

Table 5: Bioaccessibility of iron and zinc.

Gruel	Bioaccessible iron (%)	Increase in bioaccessible iron (%)	Bioaccessible zinc (%)	Increase in bioaccessible zinc (%)
G0	31.49 ± 3.17^{a}	-	29.17 ± 3.55^{a}	-
GP1	39.58 ± 5.41^{a}	8.08	82.96 ± 3.25^{b}	53.79
GP2	58.45 ± 0.82^{b}	26.96	92.07 ± 4.73^{b}	62.92
GP3	50.34 ± 0.6^{b}	18.85	21.24 ± 1.08^{a}	−7.93

G0: control (unfortified gruel); GP1: gruel fortified with mashed sweet potato at 20%; GP2: gruel fortified with mashed sweet potato at 30%; GP3: gruel fortified with mashed sweet potato at 40%. Mean values in the same column with different superscript letters are significantly different ($P < 0.05$).

mango mixture. The difference observed could be explained by the intrinsic mineral content of the various fortificants used in each study [21].

It is important to note that the quantities of minerals in foods are not an indicative tool to predict their bioaccessibility. A previous study showed that adding moringa powder with high iron content to a millet-based food decreased the percentage of bioaccessible iron and zinc [21].

3.4. Bioaccessibility of Iron and Zinc in Fortified Gruels. A simulated *in vitro* digestion method was carried out to evaluate the effect of using mashed sweet potato as fortifier on mineral bioaccessibility of fortified pear millet gruels. Table 5 shows the percentages of bioaccessible iron and zinc in the different gruels. There is an increase in bioaccessibility of iron with the level of incorporation except at 40%. These results could be due to the activating effect of carotenoids and more specifically β-carotene on the bioaccessibility of iron [24, 49]. β-carotene would form a soluble complex with iron in the gut, which would promote the absorption of iron even in the presence of antinutritional factors [24]. A study also showed that β-carotene overcomes the inhibitory effects of phytates or tannic acid depending on their concentrations [50]. The percentages of bioaccessible iron and zinc in fortified gruels are all higher, compared to millet porridge fortified with a carrot-mango mixture [21].

The percentage of bioaccessible zinc in the gruels fortified at 20 and 30% is significantly higher ($P < 0.05$) than that of the control. These results could be due to the stimulatory effect of β-carotene on the bioaccessibility of zinc [50]. These results are superior to those obtained in a sorghum gruel achieved by Mouquet-Rivier et al. [51]. The decrease in mineral bioaccessibility at 40% incorporation level of the fortificant could be explained by the higher levels of antinutritional factors (phytates and polyphenols) in the fortified gruel. Kruger in 2020 stated that when selecting a plant-based fortificant, the relative levels of antinutrients versus micronutrients should be evaluated to estimate the potential effect on micronutrient bioavailability.

4. Conclusion

This study aimed to investigate the effect of food-to-food fortification of a local pearl millet gruel with a natural source of β-carotene (sweet potato) on the bioaccessibility of iron and zinc. The sensory evaluation revealed that the gruel fortified with mashed sweet potato at 20% incorporation level was the most favourably appreciated. The chemical analysis of mashed sweet potato revealed its richness in carotenoids. Although the addition of the fortificant increased the amount of phytates and polyphenols while reducing the amount of iron and zinc in fortified gruels, a significant increase in the bioaccessibility of these two trace elements was observed at the incorporation levels of 20 and 30%. Consumption of pearl millet gruels fortified with mashed sweet potato at moderate incorporation levels could provide a solution to the iron and zinc deficiencies prevalent in the Far North Region of Cameroon.

Data Availability

The data used to support the findings of this study are included within the article.

Additional Points

Practical Application. Consumption of pearl millet gruels fortified with mashed sweet potato could be an affordable and efficient solution to iron and zinc deficiencies in the Far North Region of Cameroon.

Disclosure

A previous version of this manuscript has been presented as a preprint in Research Square and is available at the following link: https://www.researchsquare.com/article/rs-1652018/v1 [50].

Conflicts of Interest

The authors declare that they have no conflicts of interest to disclose.

Authors' Contributions

Mawouma Saliou, Awoudamkine Emmanuel, and Ponka Roger conceptualized the study; Mawouma Saliou, Awoudamkine Emmanuel, Ndjidda Yaya Verlai, and Tedom Dzusuo William methodized the study; Mawouma Saliou, Awoudamkine Emmanuel, Ndjidda Yaya Verlai, and Tedom Dzusuo William investigated and analysed formally; Mawouma Saliou prepared and wrote the original draft; Mawouma Saliou and Ponka Roger reviewed and edited the manuscript; Mawouma Saliou, Awoudamkine Emmanuel, and Ponka Roger provided resources; Ponka Roger supervised the study.

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