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Nutritional Potentiality of Sorghum (*Sorghum bicolor*) *Tôh* Enriched with Flour of Shea Caterpillar (*Cirina butyrospermie*)

Mariam Tiékoungo Soro[1], Bernard Téhi Sea[1], Yves Djina[3, *], René Yadé Soro[1, 2], Natia Joseph Kouadio[1], Lucien Patrice Kouame[3]

[1]Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, UFR Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

[2]Laboratory of Biochemistry, Microbiology and Valorization of Agricultural Resources, Institute of Agropastoral, Management University of Péléforo Gon Coulibaly, Korhogo, Côte d'Ivoire

[3]Laboratory of Biocatalysis and Bioprocesses, Department of food science and technology, NANGUI ABROGOUA University, Abidjan, Côte d'Ivoire

Email address:

yvesdjina@yahoo.fr (Y. Djina)

[*]Corresponding author

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Abstract: In Côte d'Ivoire, malnutrition is still a public health problem. To remedy this, international institutions involved in this fight recommend the promotion of local resources rich in proteins such as insects and caterpillars. Thus, this study aims to enhance the enrichment of sorghum *tôh* with shea caterpillar flour (*Cirina butyrospermie*) in order to solve the problems of malnutrition. To do so, 25 young rats of Wistar strain, aged to 50±05 days with an average weight between 45 and 55±5 g were divided into 5 batches were fed respectively with *tôh* formulated with 5%, 10% and 15% of shea caterpillar flour during 15 days. At the end of this period, the nutritional parameters were calculated and biometric study was conducted to evaluate the impact of enriched food on well-being of young rats. The results obtained reveal interesting nutritional potentialities in most parameters. For example, the weight gains obtained varied from 4.86±0.45 g/d to 6.05±0.39 g/d in to rats fed with different formulations of *tôh* enriched with shea caterpillar flour and the diet STScF2 did not show any significant difference with control diet. In terms of biological value, values obtained vary from 84.12% to 90.64% while those of protein retention vary from 11.66±1.85 g to 12.99±0.89 g respectively for young rat fed with diets STScF3 and STScF1. Concerning biometric study, no abnormality was noted following the consumption of these foods. In view of the nutritional potential developed by young rats, the *tôh* enriched with 10% shea caterpillar four, could be retained to solve malnutrition problems.

Keywords: Shea Caterpillar, Sorghum *tôh*, Feed Formulation, Protein Efficiency, Net Protein Used, 6 Malnutrition

1. Introduction

Hunger and malnutrition are global public health problems. They affect nearly 800 million people worldwide and contribute to approximately 45% of under-five deaths in developing countries [25]. In Côte d'Ivoire, malnutrition is directly or indirectly responsible for 33% of child mortality [13]. According to Disseka and al. [7], this situation is explained by the fact that the diet of these children is poor and non-diversified. Indeed, the complementary foods given to children during the diversification period are for the most part, dishes based on local cereal such as sorghum [4]. Although available locally, sorghum-based preparations (porridges, *tôh* and flours) have insufficient protein and energy densities to cover the needs of the child [4]. As a result, their consumption by weaning children must be accompanied by protein-rich foods in order to meet their nutritional needs. A less expensive alternative for populations in developing countries is the use of new protein sources to substitute or complement existing ones [19]. Among these protein sources, snails, winged termites, insects and especially caterpillars are available and highly valued by local populations [10, 14, 20]. S*hea caterpillars* (*Cirina butyrospermie*) available in northern Côte d'Ivoire are a good source of protein (62.74%) of which 47.64% of essential amino acids are bioavailable [2, 9, 26]. In addition, these caterpillars contain a high content of linolenic acid (35.82%), a precursor of Omega 3. Thus, the combined use of sorghum flour and s*hea caterpillar* powder in local dishes such as sorghum *tôh* constitutes a nutritional and dietary asset in the fight against child malnutrition. However, for a better valorization of these composite flours, it is necessary to know their contribution to the well-being of the consumer. Thus, this study aims to evaluate the nutritional potential of sorghum *tôh* enriched with s*hea caterpillar* (*Cirina butyrospermie*) incorporated during the manufacturing process, using young growing rats.

2. Materials and Methods

2.1. Collection of Samples

Materials used in this study consists of sorghum (Sorghum bicolor) grain and shea caterpillar (*Cirina butyrospermie*) that were purchased from the bigger market of Korhogo city, Côte d'Ivoire. The material used for the formulation of the control diet consisted of corn starch flour (maizena, Côte d'Ivoire), palm oil ("Aya", Côte d'Ivoire), herring fish (*Clupea harengus*), cooking salt; all purchased from a supermarket in the city of Abidjan. The vitamins and minerals supplements were purchased in a pharmacy in Abidjan (Côte d'Ivoire).

2.2. Methodology

2.2.1. Production of Sorghum Flour

Sorghum (*Sorghum bicolor*) flour was obtained according to the method described by Lestienne *and al.* [18]. According to this method, sorghum grains were decanted three times by beating method, then sorted, and washed with a bleach solution prepared at 1% in order to limit further development of microorganisms. Then, they were steamed at 45°C for 24 h. Once dried, the seeds were ground using a blender (*Binatome*, China) and the resulting grind was sieved using a sieve of 250 µm of diameter. The resulting flour was stored in glass boxes and then kept at -6°C in the freezer for further use.

2.2.2. Preparation of Shea Caterpillar Flour

The collected shea caterpillars were sorted and then washed with water containing 1% (W/V or V/V) bleach. The washed shea caterpillars were dried in an oven (*Venticell*, Fisher Bioblock Scientific) at 45°C for 72 hours before being ground with a blender (Binatome, China) to obtain the flour. The resulting shea caterpillar flour was subsequently sieved using a 250 µm diameter sieve and then stored in a glass box in the refrigerator at -6°C.

2.3. Formulation and Preparation of Different Sorghum Tôh Enriched with Shea Caterpillar

For the formulation of 100 g of feed from sorghum flour, 5%, 10% and 15% of shea caterpillar flour were added respectively to 95%, 90% and 85% of sorghum flour and then the whole was mixed (table 1). For the preparation of the different sorghum *tôh*, 200 g of each mixture, previously dissolved in 600 mL of warm water (45-55°C), were introduced into 1000 mL of distilled water, and then the mixture was brought to a boil using an Electrolux brand hot plate (IKA Ret Basic, Germany). Then, the whole was homogenized with a wooden spatula until a slurry was obtained. A gradual dilution of 300 g of flour was applied to the slurry until a consistent and solid paste was obtained. Cooking for 15 min resulted in the different types of sorghum *tôh* enriched with shea caterpillar flour. Table 1 shows the different types of sorghum *tôh* enriched with different proportions of shea caterpillar flour. The formulation of the protein-based and protein-free control diets was carried out according to the indications of technical sheets of Pawlak and al. [22], which made it possible to formulate iso-caloric diets (400 Kcal/kg of dry matter). This protocol was modified for the protein-based diet in that the protein source used was fish (herring fish) instead of casein.

Table 1. *Presentation of the different sorghum tôh enriched with shea caterpillar flour.*

Types of sorghum *tôh* enriched with shea caterpillar flour	**STScF 1**	**STScF 2**	**STScF 3**
Quantity of shea caterpillar flour (%)	5	10	15
Quantity of sorghum flour (%)	95	90	85
Total quantity (g)	100	100	100

2.4. Nutritional Evaluation of Different Sorghum Tôh Enriched with Shea Caterpillar Flour

The *in vivo* nutritional study was performed according to the method described by Adrian and al. [1]. It was carried out using 40 young rats of Wistar strain from the animal house of the Biosciences UFR of the University Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire). The young rats, aged to 50±5 days, were divided into homogeneous batches of five (5) groups comprising five (5) rats each. The average weight of the young rats was between 45 and 55 g. The average temperature of the experimental room was 25°C and the percentage of humidity was 70%, with 12 hours of daylight and 12 hours of darkness. The young rats were divided as follows: one (1) group of five (5) young rats was fed with control diet (C D) based on herring fish protein, three (3) group of five (5) young rats were fed with different types of sorghum *tôh*, enriched with different proportions of shea caterpillar flour (STScF 1, STScF 2 and STScF 3) and one (1) group of five (5) young rats was fed with a diet without protein (DWP).

For the conducting of the experiment, the young rats were first arranged individually in metabolic cages designed to collect food scraps, feces and urine separately. Next, the young rats were acclimated for a period of five days during which they were all fed with the control diet. At the end of this time, each batch of young rats was fed *ad libitum* for a period of 15 days with the corresponding diet. Water was served *ad libitum* throughout the experimental period. During the experimental period, the food consumption of the rats was measured daily and the weight gain was assessed every 3 days between 8:30 and 9:30 am. Each food, before being offered, was weighed and the following day, the rejected food was also weighed, in order to determine the amount of food ingested. For the determination of dry matter, five (5) g of each feed was put in the oven for this purpose. The nitrogen (N) balance study consisted of collecting the feed and feces from each batch of rats and then drying them in the oven at 70°C for 12 h, weighing them, grinding them into a fine powder and storing them for the determination of nitrogen (N) content using the Kjeldahl method [3]. Urine was also collected in sample bottles, stored in 0.1N HCl to avoid ammonia formation and kept refrigerated until urinary N analysis. The calculated nutritional parameters are expressed in Table 2.

Table 2. Mathematical expression of nutritional parameters.

Nutritional parameters	Mathematical expression
Total dry matter intake (g/d)	DMI (g)=[Food served (g) - food refused (g)] × [dry matter rate]
Weight gain: WG (g/d)	WG (g/d)=Final weight - Initial weight / number of days
Coefficient of Food Efficiency: CFE	CFE=WG / DMI
Total Protein Intake: TPI (g/d)	TPI=DMI ×% Protein of diet
Coefficient of Protein Efficiency: CPE	CPE=WG/TPI
Apparent digestibility: Da (%)	Da=(I-Fe) × 100 / I
Digestibility: Dr (%)	Dr=[I - (Fe - Fpp)] × 100 / I
Protein Retention: PR (g)	PR=I - (Fe - Fpp) - (U- Upp)
Net Protein Utilization: NPU (%)	NPU=RP × 100 / I
Biological Value: VB (%)	BV=[I - (Fe - Fpp) - (U- Upp)] × 100 / [I - (Fe - Fpp)]

I: amount of dietary protein ingested; Fe: protein excreted in the feces of a subject other than the one on the protein-free diet; Fpp: protein excreted in the feces of a subject on the protein-free diet; U: protein excreted in the urine of a subject other than the one on the protein-free diet; Upp: protein excreted in the urine of a subject on the protein-free diet.

2.5. Biometric Study

At the end of the experimental phase (day 15), the young rats from each batch were sacrificed according to the method described by Bouafou and al. [5]. Organs such as the heart, liver, kidneys and lungs of each rat were removed, cleaned on adsorbent paper and weighed on a precision scale (the degree of precision was 0.5gr). The relative weight of the organs was evaluated according to the following mathematical expression:

Relative organ weight=[(organ weight/weight of animal) X 100]

2.6. Statistical Analysis

Statistical analyses of the data were performed using SPSS V20 software. Comparisons between dependent variables were determined using one-factor analysis of variance (ANOVA) and the DUNCAN test. Statistical significance was defined at the 5% level.

3. Result and Discussion

3.1. Results of Nutritional Evaluation of Sorghum Tôh Enriched with Shea Caterpillar (Cirina butyrospermie) Flour in to Young Rats Wistars

The results of nutritional evaluation of *tôh* prepared with sorghum and enriched with shea caterpillar (Carina butyrospermie) flour in to young rats Wistars shows in Table 3. There was significant ($p < 0.05$) difference between treatment. Analysis of zootechnical parameters and biometric study made it possible to evaluate the nutritional potential of the different types of sorghum *tôh* enriched with shea caterpillar flour in to young Wistar rats and to estimate the risks associated with their consumption.

Statistical analysis shows that the weight gain of young rats increased according to the rate of incorporation of caterpillar flour. However, the weight gain of young rats fed with *tôh* enriched of the formulation STScF2 and STScF3 did not differ significantly enough from those fed with control diet (CD). The positive impact of the high protein content of different formulation of sorghum *tôh* enriched resulted in daily weight gains ranging from 4.86±0.45 g/d to 6.05±0.39 g/d in the young rats fed. The lack of significant difference of the STScF 2 diet and STScF 3 diet with control diet (RTP) suggests that the consumption of these feeds could have a positive impact on the body of the consumers. Regarding the amount of dry matter ingested, statistical analysis reveals that the consumption of *tôh* enriched with shea caterpillar in formulations STScF1 (10.23±0.66 g / d) and STScF2 (10.27±1.20 g / d) did not show a significant difference with those fed with control diet (RTP) (10.41±1.03 g / d) while those fed with STScF3 (9.15±0.70 g / d) formulation showed a significant difference. The high quantities of dry matter ingested like the control diet insinuate that these diets had the same palatability towards the young rats. This result suggests that sorghum *tôh* enriched with shea caterpillar possess organoleptic and sensory characteristics that would favor its consumption [16]. The high amounts of dry matter intake are positively correlated with the amounts of total protein intake and protein retention in the different sorghum *tôh* enriched with shea caterpillar. This high protein content could be an asset for consumers of this food due to the beneficial effects of animal proteins due to their bioavailability and balance in all types of amino acids [23].

Concerning the feed efficiency coefficient, the values between 0.47±0.06 and 0.66±0.06 is also an indicator of positive impact of the consumption of these foods. Indeed, the feed efficiency coefficient reflects the assimilation of the nutrients in the feed. It is also the weight gain developed following the consumption of one gram of feed [16]. In terms of total protein intake, statistical analysis reveals a significant difference with higher intakes in to young rats fed with STScF1 diet, STScF2 diet and STScF3 diet compared to control diet (CD). Total protein intake are ranged from 1.87±0.23 g/d to 12.55±0.77 g/d respectively for juvenile rats fed with diet without protein (DWP) and the *tôh* of sorghum enriched with 10% of shea caterpillar flour. This performance is confirmed by the protein efficiency coefficient whose values are higher than the recommendations of FAO/WHO [12] and PAG [21] which is 2.7. This value shows that the proteins contained in the sorghum *tôh* enriched with shea caterpillar are very good nutritional quality.

Statistical analysis revealed a significant difference in the apparent digestibility of the young rats fed with different diet of enriched *tôh*. However, rats fed the STScF1 formulation of *tôh* appeared to have the highest digestibility rates compared to other types of *tôh* consumed by rats. Apparent digestibility ranges from 10.85±1.45% to 89.48±1.26% respectively for rats fed a protein-free diet (DWP) and a control diet (CD). As for the actual digestibility of the rats fed the control diet and the STScF1 diet, it had the highest digestibility rate compared to the STScF2 diet and the STScF3 diet. The actual digestibility values are between 88.96±0.76% and 97.04±0.35% respectively for the rats fed with the STScF1 diet and the control diet (CD). The high digestibility rates of the different formulations of sorghum *tôh* enriched with caterpillar show that the proteins contained in this food are more easily digested by the rats' organism. Indeed, according to FAO [11], an apparent digestibility of more than 70% of a food protein is preferably recommended in the diet of infants. Thus, the high values obtained with sorghum *tôh* enriched with shea caterpillar flour suggest that the consumption of this food could have a positive impact on the well-being of children and adults.

The difference was not significant between the rate of protein retention (PR) of the young rats fed with the formulations of *tôh* prepared with sorghum and enriched with shea caterpillar flour and the control diet. The amounts of net protein intake were 7.90±1.42; 12.99±0.89; 12.30±1.00 and 11.66±1.85 for young rats fed the control (CD), STScF 1, STScF 2 and STScF3 diets, respectively. This shows that the shea caterpillar was a source of growth protein for the rats fed the different formulations [12].

Table 3. *Nutritional potential of young rats fed controls diets and different formulations of tôh enriched with shea caterpillar flour.*

Nutritional parameters \ Diets	C D	STScF 1	STScF 2	STScF 3	DWP
Weight gain (g/d)	6.18±0.55[a]	4.86±0.45[b]	5.46±1.67[ab]	6.05±0.39[a]	-1.03±0.20[c]
Dry matter intake (g/d)	10.41±1.03[a]	10.23±0.66[a]	10.27±1.20[a]	9.15±0.70[b]	4.90±0.53[c]
CFE	0.59±0.02[ab]	0.47±0.06[c]	0.53±0.17[bc]	0.66±0.06[a]	-0.21±0.04[d]
TPI (g/j)	9.21±1.31[b]	11.77±.83[a]	12.55±.77[a]	12.15±1.62[a]	1.87±.23[c]
CPE	9.15±0.01[a]	5.18±0.65[c]	5.87±0.39[bc]	7.00±1.21[b]	-8.47±2.27[d]
Apparent digestibility (%)	89.48±1.26[b]	92.33±0.47[a]	84.44±1.08[c]	85.26±1.65[c]	10.85±1.45[d]
Digestibility (%)	97.04±0.35[a]	97.00±0.19[a]	88.96±0.76[b]	89.78±1.15[b]	-
Protein Retention (g)	7.90±1.42[b]	12.99±0.89[a]	12.30±1.00[a]	11.66±1.85[a]	-

Values are the mean±standard deviation of trials conducted in quintuplicate. Means with similar exponents on the same line are not significantly different at the 5% level ($p > 0.05$). STScF: Sorghum *Tôh* enriched with Shea caterpillar Flour; C D: control diet; DWP: Diet Without Protein.

Figure 1 shows the results of net protein utilization and biological value of young rats fed with sorghum *tôh* enriched with shea caterpillar flour. Statistical analysis revealed a significant difference in the rate of net protein utilization in to young rats fed with the different diets. Nevertheless, the highest rate was observed in to young rats fed with STScF 1 diet. The net protein utilization value are ranged from 75.54% to 87.92% respectively for rats fed with STScF 3 and STScF 1 diets. At the level of biological value, the results obtained reveal that there is no significant difference in the values of young rats fed with the formulations STScF1, STScF2 and control diet while those of the formulation STScF3 is statistically different to control diet. Thus, biological values varied from 84.12% to 90.64% respectively in to rats fed with the caterpillar-enriched sorghum *tôh* of the STScF3 diet and STScF1 diet. The high biological values confirm the positive impact of sorghum *tôh* enriched with shea caterpillar on young rats. Indeed, the biological value represents the proportion of protein actually used by the cells of the organism. These values are greater than 70%, standard recommended by FAO/WHO [12]. Thus the proteins contained in these different formulations are of very good nutritional quality and capable of promoting good growth. This nutritional efficiency was confirmed by the net protein values used which are greater than 80% in the STScF 1 and STScF 2 diets. In fact, the net protein used (NPU) reflects the absorption in quantity and in quality of the amino acids of diets by the body [15, 24]. Thus, the high values obtained in the results suggest that the proteins contained in the sorghum *tôh* of the different formulations have a good balance of amino acids and confirm the high values obtained in the weight gain of young rats. Thus, the STScF 2 diets, which use 10% of shea caterpillar flour and which enabled young rats to develop interesting zootechnical potential, which could be retained as the best formulation for sorghum *tôh* enriched with shea caterpillar.

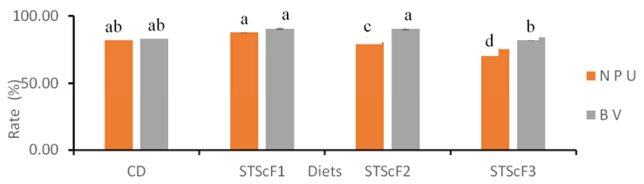

Figure 1. *Net Protein Utilization (NPU) and Biological Value (BV) of different formulation of sorghum tôh enriched with shea caterpillar flour submitted for feeding to young rats.*

Values are the mean±standard deviation of trials conducted in quintuplicate. Means with similar exponents on the same line are not significantly different at the 5% level ($p > 0.05$). STScF: Sorghum *Tôh* enriched with shea caterpillar Flour; CD: control diet; DWP: Diet Without Protein.

3.2. Biometrics of Organs of Young Rats Fed with Sorghum Tôh Enriched with Shea Butterfly (Cirina butyrospermie) Flour

Table 4 presents the results of biometric study performed on the organs of young rats fed with different diets enriched. For all organs, statistical analysis reveals a significant difference between the organ weights of young rats fed with control diet (CD) and those fed with other diets. However, the organ weights of the young rats fed the control diet (CD) did not differ significantly from those of the rats fed with different formulations of sorghum *tôh* enriched with shea caterpillar flour. Relative heart weights of young rats are ranged from 0.41±0.06% to 0.57±0.05% in young rats fed the STScF 3 diet and control diet, respectively. Relative weight of liver was ranged from 2.90±0.32 to 3.71±0.21 in to rats fed with STScF3 diet and the diet without protein (DWP), respectively. That of the kidneys varies from 0.66±0.11 to 1.02±0.07% respectively in to young rats fed with STScF3 diet and the diet without protein (DWP). Regarding the risks associated with the consumption of the different formulations of sorghum *tôh* enriched with shea caterpillar flour, the results obtained did not reveal any abnormality at the level of the organs studied compared to the control diet. The absence of abnormalities suggests that the consumption of these foods is safe for the heart, liver and kidneys of the young people and could rather contribute to the proper functioning of these organs. Therefore, the consumption of sorghum *tôh* enriched with shea caterpillar flour may not negatively impact cardiac activity [17], nor the disruption of physiological activity of liver through the production of bad cholesterol [8]; that of the kidneys through the production of urine and the elimination of waste [6].

Table 4. *Biometrics of organs of young rats fed with sorghum tôh enriched with shea butterfly (Cirina butyrospermie) flour.*

Diets / Nutritional parameters	C D	STScF 1	STScF 2	STScF 3	DWP
Heart	$0.49±0.05^{b}$	$0.49±0.06^{b}$	$0.45±0.06^{b}$	$0.47±0.03^{b}$	$0.57±0.05^{a}$
Liver	$3.24±0.48^{ab}$	$3.33±0.60^{ab}$	$3.02±0.34^{b}$	$3.14±0.47^{ab}$	$3.71±0.21^{a}$
Kidney	$0.82±0.07^{b}$	$0.91±0.08^{b}$	$0.85±0.05^{b}$	$0.90±0.07^{b}$	$1.02±0.07^{a}$

Values are the mean±standard deviation of trials conducted in quintuplicate. Means with similar exponents on the same line are not significantly different at the 5% level (p > 0. 0). STScF: Sorghum *Tôh* enriched with Shea caterpillar Flour; C D: control diet; DWP: Diet Without Protein.

4. Conclusion

The study of enrichment of sorghum *tôh* with shea caterpillar flour (*Cirina butyrospermie*) allowed the formulation of enriched food whose nutritional efficiency was demonstrated using young rats. The strong nutritional performances observed in terms of weight gain, protein efficiency ratio, digestibility, biological value and net protein used by young rats fed with sorghum *tôh* of the STScF 1 and STScF 2 diets show that the consumption of these enriched feeds has positively impacted their growth. In addition, the biometric study did not reveal any abnormalities in the organs studied. This suggests that there is no risk associated with the consumption of sorghum *tôh* enriched with shea caterpillar flour. In view of the absence of significant difference between the STScF1 and STScF2 diets, the STScF1 diet of sorghum *tôh* enriched with shea caterpillar flour can be retained for further studies in order to enhance its consumption by various segments of Ivorian society.

Declaration of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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