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Research Article

FOOD AND NUTRITIONAL SKILLS OF THE EDIBLE CATERPILLAR *CIRINA BUTYROSPERMI* VUILLET (LEPIDOPTERA: ATTACIDAE) ON NON-ORDINARY DIETS FOR INDUSTRIAL BREEDING

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ABSTRACT

The shea caterpillar *C. butyrospermi* has exceptional nutritional features. This insect is however univoltine, specific to shea and its larvae are mature only in July and August. Their food capacity apart from shea is poorly documented to suggest mass production and dietary diversification. This study aimed at evaluating the feeding and nutritional abilities of the caterpillar on non-ordinary natural diets. The approach consisted of feeding 21-day-old larvae with diets of choice or by imposition and then measuring some biological parameters of the edible insect. Nine diets including a control (shea leaves) were used in eight repetitions to the larvae in order to evaluate their preference index using the Chesson formula. Nutritional abilities were assessed for 12 days in a Fischer design with seven treatments (seven diets) randomized in four repetitions. Each treatment contained a single diet and one solitary or three gregarious larvae. The trace elements of the diets were measured by Inductively Coupled Spectrometry (ICP-AES). Caterpillars feed day and night and it does not matter whether they are gregarious or solitary. They preferentially feed on diets D1 (λ: 0.931) and D3 (λ: 0.791). Food rations were significantly higher in the control (989.86 mg/day), D3 diets (931.92 mg/day) and D1 (478.00 mg/day). Only the control and the D1 diet presented respective positive FCRs of 20.54% and 31.94% and respective positive AWGs of 53.63 mg/larva and 23.38 mg/larva. The pupation rate was 57.23% with the Control and 6.97% with the D1 diet. These two diets had similar levels of trace elements and macronutrients. The D1 diet could be used for mass breeding and contribute to the dietary diversification of the caterpillar.

Keywords: Edible insect, Diet, Mass breeding, Feeding behavior, Nutrition.

INTRODUCTION

In recent years, insect breeding has experienced real global expansion through various applications in the chemical industries, animal or human food (entomophagy) or biological control. Nowadays, this activity is developing on an industrial scale according to the recommendations of the Food and Agriculture Organization of the United Nations and foresees the production of more than tens of thousands of tons of insects per year (Van Huis *et al.,* 2013).

The caterpillar *Cirina butyrospermi* Vuillet, commonly called «chitoumu» in Burkina Faso has exceptional nutritional characteristics. It is very popular in the diet of

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many ethnic groups in Africa and contributes to the food security of cities and countryside (Thiombiano *et al.*, 2010)**.**The insect is rich in nutrients for human consumption with 63% protein, 15% fat, as well as vitamins and minerals (Anvo *et al.,* 2016b). The caterpillar can be powdered, fried or boiled and eaten with or without other accompanying meals. Insects have been found to be generally highly nutritious and good sources of proteins, fats, minerals, vitamins and energy compared to other animal foods (Van Huis *et al.,* 2015). Caterpillar meal is used in the feed of breed pullets and layers and thus contributes to the socio-economic development of populations through the financial resources generated (Sanon, 2005). It also has potential ingredients in fish feed (Anvo *et al.,* 2016a). Currently, insect fat has application in the food industry (Smetana *et al.,* 2016).

The environmental benefits of raising insects for food mainly lie in the better feed conversion efficiency of insects compared to that of chicken, pork and beef (Van Huis 2013; Jongema, 2014). Additionally, insects can be raised in organic waterways and emit fewer greenhouse gases and ammonia, requiring significantly less land and water (Van Huis *et al.*, 2013). Furthermore, a preliminary study carried out by Coulibaly *et al.*(2016) on caterpillar droppings revealed that, due to their carbon and nitrogen content, they constitute a means of ecological management of soil fertility.

Although *C. butyrospermi* is found in the southwest and west regions of Burkina Faso, its distribution is not homogeneous since it is completely absent in the provinces of Bougouriba and Poni, despite the presence of shea. The larvae have been absent from the Central Plateau since 1983, as well as from the provinces of Kossi and Mouhoun. Climatic factors and anthropogenic activities reduce the population of *V. paradoxa* and lead in certain localities to the disappearance of *C. butyrospermi*, the actions of which on shea appear to be detrimental (Ouédraogo, 1993; Payne *et al.,* 2020).

Notwithstanding the importance of shea caterpillars as human and animal food and even as a bioconverter (Coulibaly *et al.*, 2016), few studies have focused on dietary and nutritional behavior and mass breeding of shea caterpillars in Burkina Faso. Indeed, the insect is univoltine (only one generation per year) and specific to *Vitellaria paradoxa*shea;which makes its availability temporary during the year (July and August) andits difficult reproduction (Ande 2003, Adepoju & Daboh 2013). Wild *C. butryospermi* pupae were collected in Burkina Faso and sent to the UK for trials to break diapause (Dobermann *et al.,* 2017, Bama *et al*., 2018).The shea caterpillar sector requires more extensive actions for its preservation, its domestication through mass breeding and its transformation. However, the level of impact as well as the production and processing costs are not competitive compared to plant proteins (soybean for example) (Smetana *et al.,* 2016). This study aimed at evaluating the food and nutritional features of the edible caterpillar, *C. butyrospermi* on non-ordinary natural diets in semi-controlled conditions with a view to industrial production in Burkina Faso.

MATERIAL AND METHODS

C. butyrospermi larvae

The eggs of *C. butyrospermi* were collected in June 2022 from shea branches in Nasso (Bobo Dioulasso) and packaged in plastic trays until hatching. After hatching, the larvae of the same generation were fed with young fresh shea leaves and monitored in boxes in the laboratory (T° 30 ± 5°C, RH: 70 ± 10% PP: 12L/12D). The shea leaves were renewed morning and evening to allow the caterpillars to feed non-stop in conditions close to those observed in the wild in western Burkina Faso. The larvae were thus raised until the 21st ^{day} (stage 4) before being used for the tests.

Obtaining diets

During a preliminary survey carried out in May 2022 among 120 farmers in the Hauts- Bassins region, nine plant species were listed by farmers as secondary hosts of shea caterpillars. The young leaves of these plants served as the basis for the preparation of diets (500 mg) for the caterpillars.

Food Preference Test

The preference index considered the condition where the quantities of diets did not change significantly during the experiment due to their replacement as they were consumed. In this condition cylindrical plastic plates were arranged in eight repetitions. Each plate covered with a fine-mesh aeration muslin cloth contained nine diets of 500 mg each and two larvae previously weaned for 2 hours. This test was used to evaluate the appetite time, the frequency of choices and the preference index after 12 hours of observation. The preference index was estimated by the formula F1 adapted from Chesson (1978), where ri is the quantity of diet food i consumed by the caterpillar and ni the quantity of food presented at the start of foraging. A diet is said to be non-preferred if its preference index is zero (αi = 0).

$$\alpha i = \frac{ri/ni}{\sum_{j=1}^{9} rj/nj} \quad \text{i=1-9 (F1)}$$

Evaluation of the feeding and nutritional abilities of caterpillars

Fisher's design included four repetitions of each of seven randomized treatments consisting of fresh leaves of *V. paradoxa* (control) and dietsD1 to D6. These diets are offered as food substrates to the caterpillar larvae in plastic dishes covered with fine mesh muslin cloth. Each dish containing a single diet and one solitary or three gregarious larvae. The 5th instar larvae were transferred to jars containing sterilized sand to facilitate pupal emergence.

This test was used to evaluate after 12 days, the food ration, the weight of droppings, the average weight gain (AWG), the pupation rate and the feed conversion rate (FCR) or food ration' ratio on weight gain.

Evaluation of the nutritional composition of diets

The dosage of trace elements (Ca, K, Fe, Zn, Cu, Ni and Co) in the diets was carried out by the aqua regia method and analysis by Atomic Emission Spectrometry of Argon Plasma at Inductive Coupling (ICP-AES). In this process, 2 ml of aqua regia were added to 0.15 g of the mineral material (product calcined at 500 C° for 5 h). The whole is evaporated to dryness on a hot plate then dissolved in 25 ml of hydrochloric acid (2M) for analysis.

The protein content (TP) of the samples was determined by the Kjeldahl method according to NF V03050 (1970). The protein content is determined using the formula F2 where Ve is the burette drop for the distillate; Vb the fall of the cruet for the white; Pe the test portion; 0.2 the sulfuric acid titer; 0.014 the molar weight of nitrogen X 10-3; 6.25 the conversion factor; and %H the percentage by mass of water according to NF V03-707 (2000).

$$TP = \left(\frac{6{,}25 \times 0{,}014 \times 0{,}2 \times (Ve - Vb)}{Pe} \times 100\right) \times \frac{100}{100 - \%H} \quad \text{(F2)}$$

The fat content (TGM) of the diets was determined by the Soxhlet extraction method according to the international standard ISO-659 (1998). The extraction is carried out hot by soaking 5g per sample in 150 ml of hexane for 4 hours followed by rinsing the sample by rotavapor distillation. The lipid content is determined by weighing after cooling in a desiccator for 30 min. The percentage of fat to dry matter was calculated using the formula F3 where Pmg is the weight of the fat; Pe the test portion and %H the percentage by mass of water according to NF V03-707 (2000).

$$TMG = \frac{Pmg}{Pe} \times 100 \times \frac{100}{100 - \%H} \quad \text{(F3)}$$

The level of total sugars (TS) was determined by spectrophotometry according to the method of Montreuil and Spik (1969).The sugar content was determined using a calibration curve established with D-glucose (reference sugar). The percentage of total sugars relative to the dry matter was calculated and expressed by the relation (F4) where C is the concentration in g/l determined on the standard curve; FD the dilution factor (10); V the initial dilution volume (100 ml); Pe the test portion and %H the percentage by mass of water according to NF V03-707 (2000)

$$TS = \left(\frac{C \times FD \times V}{Pe} \times 100\right) \times \frac{100}{100 - \%H} \quad \text{(F4)}$$

Data analysis

Data were handwritten using a model in the EXCEL spreadsheet and the average values presented in the form of graphs or tables. Statistical analyzes were carried out using R 4.2.1 software. The homogeneity of variances was checked with the Bartlett test. The ration, the weight of the droppings and the macronutrient contents underwent an analysis of variance (fixed effect: treatment and feeding system) and the means were compared using the Tukey parametric test at the 5% threshold.

RESULTS AND DISCUSSION

The analysis of the preference index (ƛ), frequency (f) and appetency time (t) reveals (Figure 1) that the edible caterpillar feeds preferentially on D1 (ƛ: 0.931, t: 202.22 s; f= 19.10%) and D3 (ƛ: 0.791; t: 53.18 s; f: 32.00%). Diets like D2, D6, D5 and D4 were consumed with low respective preference indices of 0.182; 0.069; 0.039 and 0.023. Other diets such as D7, D8 and D9 were never grazed by the caterpillar. The food ration (Table I) varied significantly depending on the diets (F: 774.3; df: 21; P < 2.2e-16). The largest rations were obtained with the control (989.86 mg/day)and diets D3 (931.92 mg/day) and D1 (478.00 mg/day). The lowest rations are recorded with diets D2 (103.14 ± 17.81 mg/day), D4 (184.81 ± 39.83 mg/day), D6 (278.47 ± 43.89 mg/day) and D5 (478.00 ± 22.23 mg/day). In the same order, caterpillar droppings varied statistically (F: 4157; df: 21; P < 2e-16) as follows: the largest droppings were obtained with the control (484.58 ± 9.65 mg/day) and diets D1 (266.43 ± 9.00 mg/day) and D3 (166.02 ± 7.77 mg/day).

Table 1. Results of the analysis of variance of the food ration of *C. butyrospermi.*

Diet	Ration ± SE(mg/day)	Droppings ± SE(mg/day)
Control	989.86 ± 71.80 a	484.58 ± 9.65 a
D3	931.92 ± 47.32 b	166.02 ± 7.77c
D1	812.07 ± 31.99 cents	266.43 ± 9.00 b
D5	478.00 ± 22.23 d	100.69 ± 4.13 d
D6	278.47 ± 43.89 e	75.61 ± 4.51 e
D4	184.81 ± 39.83 f	40.17 ± 2.34 f
D2	103.14 ± 17.81 g	33.78 ± 3.83 f
F.	774.3	4157
Pr	< 2.2e-16	< 2nd-16
Meaning	***	***

Note: The means in the same column, assigned the same letter, are not significantly different at the 5% threshold (Tukey test); ***: Very highly significant.

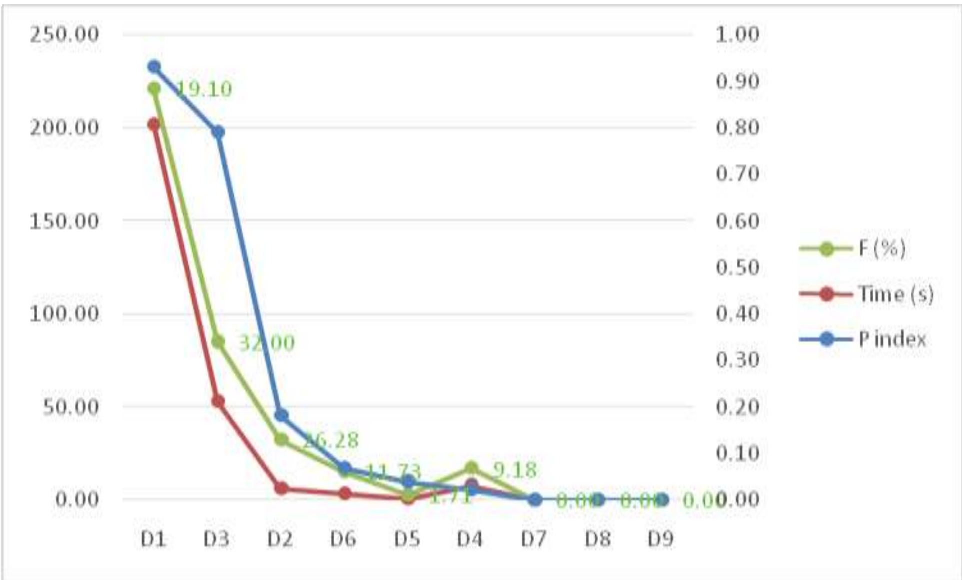

Figure 1. Analysis of food preference of *C. butyrospermi*.

Table 2. Analysis of the "group effect" on the caterpillar's food ration.

	Diff (δ)	lwr	upr	Prob
D1_lonely-D1_gregarious	-33.00	-111.33	45.33	0.95
D3_lonely-D3_gregarious	-33.00	-111.33	45.33	0.95
D4_lonely-D4_gregarious	-73.84	-141.67	-6.00	0.02
D5 _solitary- D5 _gregarious	-33.00	-111.33	45.33	0.95
D6_lonely-D6_gregarious	-33.00	-111.33	45.33	0.95
Solitary_Control-Ggregar_Control	-33.00	-111.33	45.33	0.95

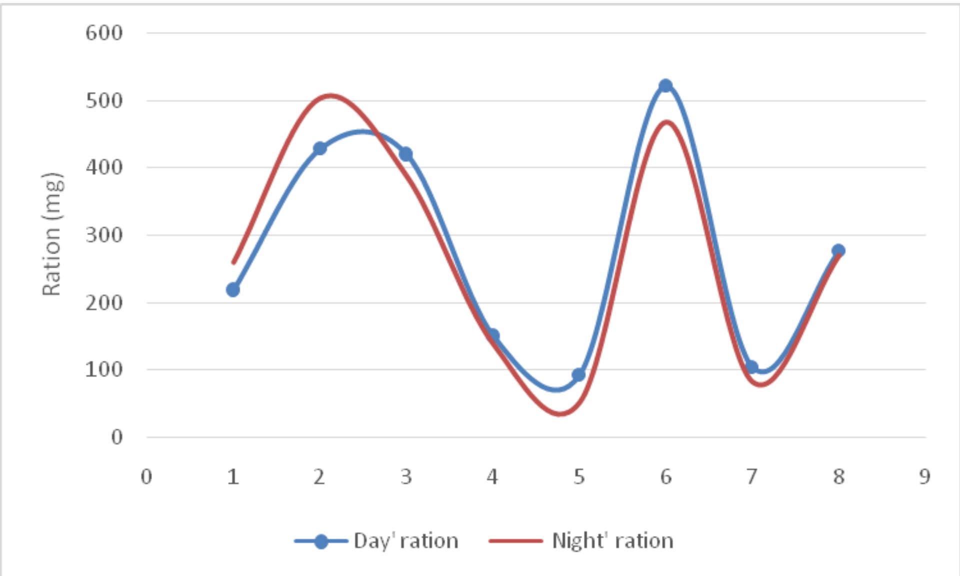

Figure 2. Analysis of the effect of photoperiod on the caterpillar's diet.

Table 3. ANOD of food conversion rates (FCR) by the caterpillar.

Interaction	Diff (δ)	lwr	upr	Pr
D3_lonely-D3_gregarious	-0.30	-2.36	1.76	1,000
D5_lonely-D5_gregarious	-0.30	-2.36	1.76	1,000
D1_lonely-D1_gregarious	-0.30	-2.36	1.76	1,000
D6_lonely-D6_gregarious	-0.30	-2.36	1.76	1,000
D4_lonely-D4_gregarious	0.16	-1.62	1.94	1,000
Solitary_Control-Ggregar_Control	-0.30	-2.36	1.76	1,000

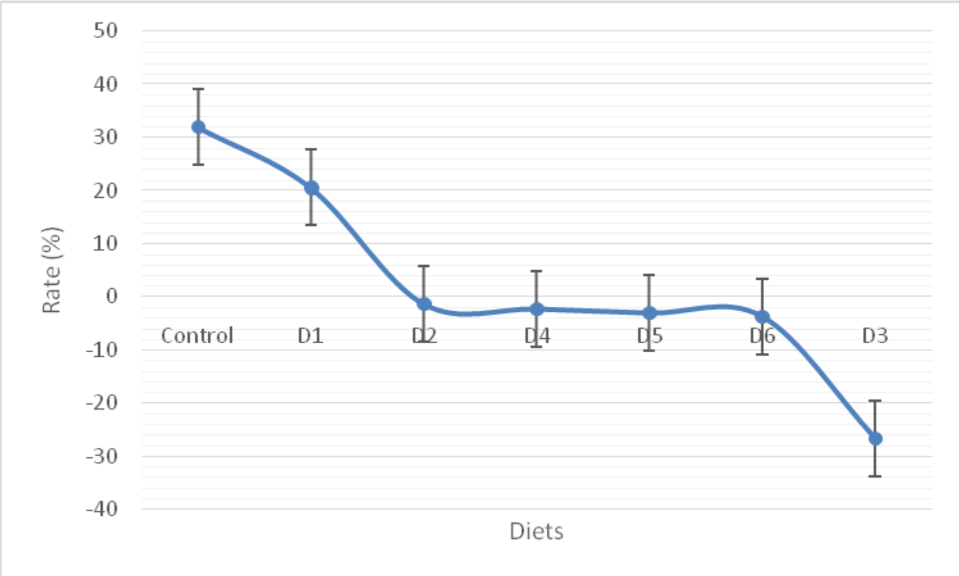

Figure 3. Evolution of food conversion rates depending on the substrates.

Table 4. Mineral element content of plants (mg/kg of raw product).

Diets	Ca_total	K_total	Fe_total	Zn_total	Cu_total	Ni_total	Co_total
Control	565.13	8514.39	153.00	12.00	2.00	3.33	0.00
D1	849.69	9786.65	273.00	76.00	5.00	6.00	15.00
D2	601.20	2348.79	383.00	13.00	9.00	7.00	0.00
D3	563.12	13799.18	298.00	10.00	3.00	3.00	0.00
D4	615.23	3816.79	177.00	16.00	15.00	4.00	0.00
D5	148.29	5774.12	176.00	9.00	4.00	6.00	0.00
D nd	176.35	9884.52	171.00	19.00	18.00	2.00	0.00

Figure 4. Average weight gain and pupation rate of caterpillars.

Table 5. Protein, fat and energy content of dietary diets (/100g DM).

Diets	Protein (g)	Fat (g)	Total sugars (g)	Energy (kcal)
Control	8.40 ± 1.49b	7.06 ± 0.56a	78.70 ± 0.75a	411.90 ± 23.22 a
D1	7.85 ± 0.95b	7.86 ± 1.66a	81.46 ± 0.30a	427.97 ± 21.41 a
D2	8.23 ± 1.25b	3.16 ± 1.32 a b	79.55 ± 1.60a	379.52 ± 13.86 ab
D3	12.17 ± 1.38 b	3.06 ± 1.67 ab	77.36 ± 1.90 a	385.60 ± 12.61 a b
D4	8.22 ± 1.25b	3.74 ± 1.00 a b	78.72 ± 0.98a	381.42 ± 24.13 ab
D5	19.65± 1.50a	1.86 ± 0.64b	71.04± 1.10b	379.48 ± 7.02 ab
D nd	7.86 ± 0.66 b	4.43 ± 0.81 ab	69.60 ± 0.94b	349.69 ± 16.40 b
F	15.86	4.03	28.81	3.65
Pr > F	0.0009	0.0451	0.0001	0.0473
Significant	***	*	***	*

Note: The means in the same column, assigned the same letter, are not significantly different at the 5% threshold (Tukey test); *: Significant; ***: Very highly significant.

Figure 2 shows that the caterpillar feeds day and night without distinction of photoperiod. The analysis of variance of the average rations (Table II) indicates no statistical difference between the gregarious and solitary modes (P; 0.597; F: 0.279). The average ration of the gregarious (273.196 mg/day) was, however, higher than that of the solitary (255.421 mg/day) (δ <0; P < 0.001). The feed conversion ratios (FCR) in Figure 3 were mostly negative, varying from -1.39 ± 0.38% for D2 to -26.68 ± 0.41% for D3.Only the FCRs of the control and the D1 diet were positive with 20.54 ± 0.68% and 31.94 ± 0.52%, respectively. The analysis of the differences (Table III) indicates that the food conversion capacity is higher in the gregarious than in the solitary (δ <0; P < 0.001).

Positive values (Figure.4) of average weight gain (AWG) were noted only in the control (53.63 mg/larva) and the D1 diet (23.38 mg/larva). Other weight gain values were negative ranging from -2.80 mg/larva (D3) to –138.22 mg/larva (D2). Furthermore, the pupation rate was 57.23% with the control and 6.97% with the D1 diet. Other diets did not allow the caterpillars to pupate. According to the results (Table IV) of the analysis of variance of the mineral composition of the diets, the highest contents of total calcium (849.69 mg/kg), total zinc (76.00 mg/kg) and total cobalt (15.00 mg/Kg) were recorded with the D1 diet while control provided respective contents of 565.13 mg/kg; 12.00 mg/kg and 0.00 mg/kg. The highest contents of total potassium (13799.18 mg/kg) were noted in the D3 diet, iron (383.00 mg/kg) and nickel (7.00 mg/kg) in D2 and copper (18.00 mg/kg) at Dnd. In Table V, the protein content (g/100g) of the diets varied very significantly (F: 15.86; Pr <0.001) from 19.65 ± 1.50 % for D5 diet to 7.85 ± 0.95% for D1; while the control was at 8.40 ± 1.49%. Regarding the fat content, it varied significantly (F: 4.03; P: 0.0451) from 1.86 ± 0.64 % for D5 diet to 7.86 ± 1.66 % for D1. D1 diet was close to the control which recorded 7.06 ± 0.56%. As for the sugar content, the means varied very significantly (F: 28.81; Pr: 0.0001) from 69.60 ± 0.94% for D diet to 81.46 ± 0.30 % for D1. The latter was very close to the control which recorded 78.70 ± 0.75 7%.

The same trend was observed for energy supply. This variable a significant variation (F: 3.65; Pr: 0.047) from 349.69 ± 16.40 kcal for D diet to 427.97 ± 21.41 kcal for D1. The control recorded 411.90 ± 23.22 kcal without significant difference with D1 diet.

The creation of protein additives for the African food market with significant turnover and the generation of alternative protein sources for animal and human food are the most promising areas for insect and entomo-related technologies. -industry. The shea caterpillar from Burkina Faso may be a real candidate. But can shea caterpillars be mass produced out of season using non-ordinary substrates?. This study revealed exceptional feeding abilities of this edible insect. It shows that the shea caterpillar preferentially feeds on diets D1 and D3. Diets D2, D6, D5 and D4 were only consumed with low decreasing indices from 0.182 to 0.023. Diets D7,D8 and D9 were not consumed. This result is similar to that of Dabiré *et al.* (2017) who showed that the leaves of certain shea plants are unsuitable for the growth and survival of the caterpillar. Another study have already shown that larval stages 3, 4 and 5 develop with difficulty with poor quality nutrition (older leaves) (Ouédraogo, M. (1993).

Furthermore, this study reveals that caterpillars feed day and night without distinction of photoperiod and whether they are gregarious or solitary. They were significantly higher in the control (989.86 mg/day) and diets D3 (931.92 mg/day) and D1 (478.00 mg/day). The other diets (D5, D6, D4 and D2) were lightly consumed at decreasing rations from 478.00 mg/day to 103.14 mg/day. Indeed, Schiavone*et al.,* (2017)'s complex proteomic study revealed that the quality of proteins produced by insects (hemolymph proteins typical of SWP, vitellogenin) depended on the rearing food substrate and sex. Shumo's results *et al.* (2019) also indicated variation in nutritional composition caused by differences in substrates. The FCR and AWG were mostly negative. Only the control and the D1 diet were positively measured with respective FCR of 20.54% and 31.94% and respective AWG of 53.63 mg/larva and 23.38 mg/larva. Overall, the FCR and AWG were higher in gregarious individuals than in solitary individuals. The quality of the substrate obviously has an impact on the development and survival of the larvae. According to Meneguz (2017) on the black soldier fly *Hermetia illucens* L, the feeding system considerably impacts the weight gain of the larvae. On the other hand, the pH of the diet does not directly impact the development of the larvae but only the growth duration. Indeed, the larvae can modify the pH and stabilize it during their growth. *Hermetiaillucens* larvae, for example, raise the pH of the solution (leachate) from 4.0 to 9.0 in 7 days (Popa and Green, 2012). In a similar context, Alattar (2012) determined a pH varying from 8 to 9 in 8 days of activity and the speed of pH change increased with larval density.

The low caterpillar pupation rate of 57.23% in the control and 6.97% in the D1 diet and the absence of a nymphal stage in the other diets would be linked to deficiencies which could be treated by adding nutritional supplements. According to Lehtovaara *et al.* (2017) lack of protein and fat in the diet prolongs development and leads to weight loss in *R. différens.* Under these conditions the omega-6/omega-3 ratio can be lowered below the recommended ratio which is 5:1. This study did not evaluate the effect of food substrates on the protein quality of the caterpillar which would have remained intact.All times, the diets were rich in trace elements (calcium, potassium, iron, zinc, copper, nickel and cobalt) and macronutrients (proteins, fats and sugars) at varying levels. Previous results (Smetana *et al.*, 2016) indicated that a mixture of protein concentrates (15–50% concentrated insects and soy) and water yielded analogs (texture and protein composition) of fibrous meats. The case of *C. butyrospermi* in this study bodes well for business prospects for the promotion of entomofood.

CONCLUSION

The objective was to evaluate the food and nutritional capabilities of the shea caterpillar on non-ordinary natural diets with a view to mass breeding. It appears that the caterpillars feed well day and night regardless of photoperiod, in a preferential manner and regardless of whether they are gregarious or solitary. Their rations are considerable on the D3 and D1 diets. They are capable of increasing their weight and developing to the nymphal stage by feeding on the D1 diet. It is possible to mass breed this insect for human consumption using the D1 diet as food substrates in mixed nutrition. Having not been able to deepen the analysis of the micro and macronutrients of the diets, additional studies should focus on the determination of the nutritional principles of the caterpillar with a view to developing well-balanced diets for mass breeding out of season on an industrial scale.

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