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The Protein Quality of *Cirina forda* Westwood (Lepidoptera: Saturniidae) caterpillar

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ABSTRACT

The quality of protein in *Cirina forda* Westwood caterpillar was elucidated by proximate and amino acid analyses of dried, processed and pulverized sample obtained from the market. A crude protein value of 64.49% on dry weight basis was recorded. Amino acid analysis indicated encouragingly high amounts of Lysine, Tyrosine, Leucine, Phenylalanine and valine. Methionine and Cysteine were however co-limiting. When compared with other insects, conventional and feed protein supplement sources, *C. forda* ranked equal or better. In the rat feeding trial, *C. forda* performed poorly as compared with skimmed milk powder despite the considerably high Digestibility Coefficient (72.31%) and Net Protein Utilization (95.02%) recorded. This was explained as mainly due to intolerance by the rats. The likelihood of *C. forda* protein profile meeting up the human protein requirement was discussed and its potential highlighted.

INTRODUCTION

Protein and energy malnutrition has continued to plague the developing world despite the benefits reaped from 'green revolution', which has transformed most of the developing world into exporters of grains (Defoliart, 1989). Most affected is animal protein, which is further limiting due to prohibitive prices and excessive dependence on conventional sources. Olaofe *et al,* (1998) confirmed an earlier observation (Umoh and Bassir, 1977) that conventional sources of animal protein are in limited supply and relatively expensive in Nigeria. A bridge of the gap in the human physiological protein requirement and supply, especially to peasants within the Nigerian society is desirable. One of the possible ways of achieving this is to identify or promote the utilization of lesser-known and cheaper sources of animal protein such as insects. One of such insects is *Cirina forda* (Lepidoptera: Saturniidae). The inventory of Nigerian edible insects (Ene, 1963; Akingbohungbe, 1992) did not include *C. forda* caterpillar, but Fasoranti and Ajiboye (1986) rated it as one of the most popular edible insect in Kwara state. Apart from *C. forda's* obscure status, a conspicuous dearth of information on its nutritional value have stood in the way of its full utilization as a protein source. This paper reports the protein composition and performance of *C. forda* in comparison with other sources, as well as, how well it meets human protein requirements.

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MATERIALS AND METHODS

Processed and dried sample of *C. forda* caterpillar was procured from Ipata market, Ilorin, Kwara state, Nigeria; pulverized and sifted through a sieve of 20 mesh size.

Biochemical analyses

Proximate analytical methods of A.O.A.C. (1975) were employed for nutrient analysis, while amino acid profile was obtained by acid hydrolysis and subsequent analysis on the PICO TAG amino acid analyzer (Waters). The quality of protein was rated using a Chemical Score (CS) calculated as a ratio of most limiting amino acid to the quantity of it in egg protein.

Rat Bioassay:

Food/Protein efficiency

Swaminathan (1986) method was employed in the evaluation of the nutritional value of *C. forda* protein on weanling CFT strains of albino rats obtained from the Central Food Technological Research Institute (CFTRI), Mysore, India. The rats were feed on corn-based diets compounded as shown in Table 1. This was compared with a control diet supplemented with skimmed milk powder as a protein source. Both diets were isonitrogenous and isocaloric reflecting the National Academy of Science- National Research council (NAS-NRC) recommendations (NRC, 1978).

Two groups of eight 2l-day old weanling rats were feed with each diet per group for 28 days. Food and water were provided ad lib. Weight of food consumed .were noted daily and initial/weekly weights of each rat were also noted. From these the following quality indices were determined using the formulae stated:

Protein Efficiency Ratio (PER) = Gain in body weight/Protein intake

Food Efficiency Ratio (FER) = Weight gained per unit diet consumed.

Table 1. Composition of the diets for the rats

Ingredients (Percentage of diet)	Control diet (g)	Experimental diet (g)
Skimmed milk powder (10)	921.05	Nil
Cirina forda powder (10)	Nil	542.72
Refined groundnut oil (9)	315.00	315.00
Vitaminized oil (1)*	35.00	35.00
Vitaminized starch (1)**	35.00	35.00
Salt mixture (4)***	140.00	140.00
Corn Starch (75)	2625.00	2625.00

* Vitamin A - 300mg
Vitamin D_2 - 2.3mg
Vitamin E - 10mg
(Dissolved in 1g of refined oil)

** Vitamin K - 0.5mg
Thiamine - 0.5mg
Riboflavine - 1.0mg
Pyridoxine - 0.4mg
Niacin - 4.0mg
Choline - 25.0mg
Inositol - 25.0mg
Vitamin B_{12} - 2.0mg
Biotin - 0.02mg
Folic acid - 0.2mg.
Calcium Pantothenate - 4.0mg.
Paraminobenzoic acid - 10.0mg.
(Dissolved in 1g of starch)

***Monobasic potassium hydrogen phosphate - 389g.
Anhydrous magnesium sulphate - 57.3g.
Calcium Carbonate - 381.4g.
Ferrous Sulphate - 27.0g.
Magnessium Sulphate - 4.91g.
Zinc Sulphate - 0.548g.
$CoCl_2.6H_2O$ - 0.2g.
Sodium Chloride - 139.2.
Potassium Iodide - 0.79g.

Metabolic Experiment

Three groups of five rats each were feed with each of the Skimmed milk, *C. forda* and no-protein diets. The weight of the rat, diet left over and faecal matters were noted daily for 10 days. Urine from each of the rats was collected daily in 1ml glycerol. Nitrogen (N) content of faecal sample for each rat was determined by Kjedahl's method while Biuret method was employed for urine sample. After 10 days the rats were sacrificed and oven dried to a constant weight, which was noted. The oily carcasses were deoiled individually with petroleum ether (60°C-80°C) and the ether evaporated in an oven at 80°C.Each deoiled carcass was crushed individually to fine powder and mixed properly. 2g of powdered sample was taken in triplicates and analyzed for protein by Kjedahl's method. Using the values obtained, the following indices were determined:

Digestibility Coefficient (DC) = N intake - (N in faeces - Endogenous faecal N)/N intake x 100.
Biological Value (BV) = N digested - N lost in metabolism/N digested x 100.
where: N digested = N intake - N in faeces on protein diet + N in faeces of non-protein diet.
N lost in metabolism = N in urine on protein diet - N in urine of non-protein diet.
Net Protein Utilization (NPU) = Body N in test group - Body N of non-protein group +N consumed by protein group N consumed by test group.
Net Protein Ratio (NPR) = Gain in weight of test group + loss in weight of non-protein group/protein intake.

RESULTS

The proximate composition of *C. forda* (Table 2) shows an encouragingly high crude protein value of 64.49%, ether extract of 21.45% and energy value of 479.61KJ/g on dry weight basis. These compare favourably with the proximate composition recorded for other food insects and conventional protein sources (Table 2). *C. forda* crude protein value is, however, lower than those of lesser-known protein sources except periwinkle (*Littorina littorea*).

The constituent amino acids in *C. forda* are as shown in Table 3. All amino acids except tryptophan, which was not determined, are represented. 44.53% of these are essential amino acids. *C. forda* had encouraging amounts of lysine, tyrosine, leucine, phenylalanine and valine, all of which were above 50mg/g of the protein. Histidine, methionine and cysteine were however considerably low in quantity, i.e. less than 30mg/g of the protein. The most limiting of these were methionine and cysteine and hence a low chemical score of 56.70 was recorder on *C. forda* as compared whole hen egg. A comparison of the amino acid quantities with those earlier reported from other lesser-known, as well as, conventional sources (Table 3) shows that *C. forda* rated higher in most cases except with leucine, lysine and methionine.

The rat feeding trial results showed that *C. forda* diet performance was considerably lower than with skimmed milk powder (control): with a protein efficiency and food efficacy ratios of 1.56 and 0.12 as compared with 3.49 and 0.28 respectively. The growth pattern recorded from the diets (Table 4) confirmed the poor efficiency of *C. forda*, as a rather low absolute growth rate value of 0.621g/day was recorded while 2.174g/day was recorded with skimmed milk powder. The weekly growth rate responses of the rats raised on *C. forda* diet were consistently below 1.00g/week during the 4 weeks of investigation.

Table 2. Proximate Composition of *C. forda* compared with other popular insect food and protein sources in Nigeria.

Scientific name (Common name)	**C forda* *(Manimani)*	[a]*Anaphe venata* (Eruku)	[b]*Macrotermes nigerienses* (Termite)	[b]*Rhyncophorus ferrigineus* (Palm weevil)	[b]*Orycetes boa* (Dung beetle)	[b]Cricket	[c]Smoked fish	[c]*Palamonetes varians* (Cray fish)	[c]*Vivipara quadrata* (Snail)	[c]*Littorea littorea* (Periwinkle)	[d]Beef	[d]Pork	[d]Fish
Moisture	9.76±1.53	6.61	48.84	84.72	75.10	52.94	1.45	2.02	76.42	79.98	(69.0)	(42.0)	(68.1)
Dry Matter	90.24±5.67	93.39	51.16	15.28	24.90	47.06	28.55	97.98	23.58	20.12	(31.0)	(58.0)	(31.9)
Organic matter	92.41±6.25	96.79	96.89	95.77	95.82	96.82	86.66	91.20	95.90	91.60	(99.0)	(99.4)	(98.8)
Ash	7.65±1.67 (6.90)	3.21	3.11	4.23	4.18	3.18	13.34	8.80	4.10	8.40	(1.0)	(0.6)	(1.2)
Crude Protein	64.49±3.12 (58.20)	60.00	61.80	65.63	62.45	64.44	75.31	74.84	65.29	60.93	(19.5)	(11.9)	(18.7)
Ether extract	21.45±3.07 (19.36)	23.22	25.60	22.40	21.26	24.80	11.01	5.53	3.10	2.34	(11.0)	(45.0)	(12.0)
NFE	7.15±1.51 (6.45)	NR	NR	NR	NR	NR	ND	10.83	27.50	28.33	(0.0)	(0.0)	(0.0)
Crude fibre	1.71±2.05 (10.56)	NR	9.49	7.74	12.11	7.58	NR	NR	NR	NR	NR	NR	NR
Energy value (KJ/g)	479.61±25.70	NR	NR	NR	NR	NR	26.39	19.14	20.13	19.52	NR	NR	NR

*Values are means of four replicates; Values in parentheses are on fresh weight basis; [a]Ashiru (1988); [b]Oyeleye (1988); [c]Umoh and Bassir (1977); [d]Jay (1978)
[a] & [b] - Other food insects; [c]Lesser known protein sources; [d] Conventional protein sources; NR- Not reported; NFE - Nitrogen Free Extract; ND - Not Determined.

Table 3. Amino acid profile of *C. forda* compared with other protein sources

Amino acid	Quantity: mg/g of protein		mg/g of N[b]					
	Manimani *C. forda*	Saturnid Moth *Hyalophora ceropia*[a]	Smoked Fish	Cray fish *Palamonetes* sp	Snail *Vivipara* sp	Periwinkle *Littorina* sp	Whole hen egg	Cow milk
Aspartic acid	121.6(756)	NR	NR	NR	NR	NR	NR	NR
Glutamic acid	169.2(1.056)	NR	NR	NR	NR	NR	NR	NR
Serine	61.7(386)	*203	NR	NR	NR	NR	NR	NR
Glycine	33.6(210)	-	NR	NR	NR	NR	NR	NR
Histidine'	23.2(145)	27	NR	NR	NR	NR	NR	NR
Arginine	44.6(279)	53	231	NR	NR	NR	NR	NR
Threonine'	48.6(304)	41	231	181	249	381	320	278
Alanine	53.4(334)	NR	NR	NR	NR	NR	NR	NR
Proline	47.4(296)	NR	NR	NR	NR	NR	NR	NR
Tyrosine'	64.5(403)	140	NR	NR	NR	NR	NR	NR
Valine'	56.1(351)	45	262	250	297	369	420	362
Methionine'	10.2(64)	14	119	119	65	129	133	NR
Cysteine'	3.5(22)	22	NR	NR	NR	NR	NR	NR
Isoleucine'	40.1(251)	33	244	238	238	331	390	295
Leucine'	60.2(376)	51	439	476	469	688	550	596
Phenylalanine'	54.2(339)	43	193	237	196	384	244	NR
Lysine'	66.7(417)	44	750	742	439	594	538	487
Tryptophan'	ND	ND	136	219	156	138	102	88

Total indispensable amino acid in *C. forda* = 427.3 (44.53%); Total dispensable amino acid in *C. forda* = 531.5 (55.47%); Total amino acid in *C. forda* = 958.8; First limiting amino acid - Methionine/cysteine; Chemical score = 56.70.; *Glycine-serine; ' = Essential amino acids; NR = Not reported; ND = Not determined; [a]Sourced from Landry *et al* (1986); [b]Sourced from Umoh and Bassir (1977); Values in parentheses are in mg/g of N

Table 4. Weekly growth pattern of rats fed with diets containing *C. forda* (experimental) and Skimmed milk (Control)

Number of days	Control diet Weight gained (g)	Growth rate	Experiment diet Weight gained (g)	Growth rate
7	12.63±2.12	1.803±0.286	3.08±0.57	0.531±0.143
14	21.13± 4.79	3.018±0.684	5.11±2.16	0.617±0.221
21	12.75±2.43	1.821±0.345	4.71±1.22	0.667±0.217
28	14.38±4.21	2.053±0.602	3.28±2.18	0.562±0.142
Absolute value	60.88 ± 7.32	2.174±0.260	16.65±5.36	0.621±0.142

All values are means of 8 replicates

The Net protein Utilization and Net Protein Ratio of *C. forda* were encouragingly high, i.e. 95.02% and 3.94 respectively. A high but lower Digestibility Coefficient of 72.31% as compared with 82.16% with skimmed milk powder was recorded. Biological Value showed a similar trend or lower value (47.57) with *C. forda* diet as compared with 58.11 from skimmed milk powder diet (Table 5).

Table 6 shows the result of the attempt to score *C. forda* with reference to human essential amino acid requirements using digestibility values obtained from the rat. *C. forda* proved to be a good and adequate source of isoleucine, phenyl-tyrosine, threonine and valine all with adjusted score of more than 1. Histidine, lysine and leucine were moderate, i.e.> 0.6. methionine/cysteine were confirmed as limiting with a score of 0.398.

Table 5. Protein Quality indicator values of *C. forda* composed diet as compared with skimmed milk composed diet

Indicator Value	*Cirina forda*	Skimmed milk
Protein Efficiency Ratio (PER)	1.56±0.31	3.49±0.29
Food Efficiency Ratio (FER)	0.12±0.02	0.28±0.08
Net Protein Utilization (NPU)	95.02%	ND
Net Protein Ratio (NPR)	3.94	ND
Digestibility coefficient (DC)	72.31%	82.16%
Biological Value (BV)	47.57	58.11

ND - Not determined; Values are means of 8 replicates

Table 6. Digestibility adjusted scores of essential amino acid content of *C. forda* as it applies to humans

Amino acid	Reference scoring pattern[a] (mg/g of protein)	*C. forda* Content (mg/g protein)[b]	*C. forda* Score[c]	Adjusted score (%)[d]
Histidine	19	23.2	1.22	0.882
Isoleucine	28	40.1	1.43	1.034
Leucine	66	60.2	0.91	0.658
Lysine	58	66.7	1.15	0.832
Methionine-Cysteine	25	13.7	0.55	0.398
Phenyl-Tyrosine	63	118.7	1.88	1.359
Threonine	34	48.6	1.43	1.034
Tryptophan	11	ND	ND	ND
Valine	35	56.1	1.60	1.157

[a]Suggested pattern of amino acid and requirement for 2-5year old children (FAO/WHO Joint Expert Consultation, 1990)
[b]Amino acid profile of *C. forda*
[c]Amino acid mg/g of protein (b)/Reference pattern(a)
[d]Adjusted score for digestibility i.e. amino acid score (c) x *C. forda* digestibility (72.31%)
ND - Not determined.

DISCUSSION

C. forda like all animal sources (Jay, 1978) showed a high crude protein value and its amino acid profile confirmed that it could be a reliable source of essential amino acids, such as lysine, tyrosine, leucine, phenylalanine and valine. The sulphur bearing amino acids, i.e. Methionine and cysteine are however very low and therefore limiting. This result is similar to that obtained on other insect species (Landry *et al*, 1986; Finke *et al*, 1985; 1989). The chemical score of *C. forda,* i.e. 56.70, underscores this limitation, as most of the other protein sources have limited amounts of Methionine and Cysteine.

The quality of protein is not determined solely by the quantity of essential amino acids, but also by the amounts of these amino acids available after digestion and how well it is able to meet the physiological requirements of the organism concerned (Swaminathan, 1986). As a protein source to rats, *C. forda* performed dismally when compared with skimmed milk powder. This was obviously not due to indigestion or non-availability, as a reasonably high digestibility value of 72.31% and Biological value of 47.11 were recorded. Non-utilization by the rat is also ruled out as 95.02% of the protein available is utilized. Thus the non-performance of C. *forda* could be hinged on the essential amino acid limitation occasioned by the sulphur group, a relatively lower digestibility rate, food intake and intolerance by the rats. Whereas the foremost is fundamentally a factor of the protein quality and can be resolved by supplementing the deficiency, all the other reasons are factors of the test organism. Test organisms are known to respond to insect protein differently. Whereas chicks did not show any deficiency when fed with insect supplemented diet (Landry *et al*, 1986), rats sowed a deficiency ((Finke *et al*, 1987; 1989), despite the fact that methionine and cysteine were limiting in both cases, the difference in food insect source not withstanding. Perhaps the natural food source of the test organism has a role to play in its tolerability to the insect diet, since rats are known to be herbivores. The slight diarrhoea noticed in some test rats during this study may be indicative of this intolerance. Finke *et al,* (1989) noticed a similar reaction in rats raised on diet containing a high level of tent caterpillar. Such a reaction has however not been documented on man.

Considering the most demanding human age group in terms of protein requirements, i.e. 2-5 years (FAO/WHO, 1990), *C. forda* caterpillar is adequate in respect of phenyl-tyrosine, valine, threonine and isoleucine. While histidine, leucine and lysine were slightly inadequate, but adequate for other less demanding age groups. *C. forda* caterpillar therefore offers a cheap, adequate and wide range of amino acids that could be readily tapped to improve protein deficiency amongst Nigerians. Its direct consumption by man may however require supplementation with the sulphur bearing amino acids.

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