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Effect of processing on the protein quality of four popular insects consumed in Southern Nigeria

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ABSTRACT

The effect of processing on the protein quality of four popular insects consumed in Southern Nigeria was carried out in the study. A crude protein content (% wet weight) of 35.18 ± 0.10, 8.38 ± 0.31, 11.76 ± 0.90 and 33.41 ± 0.20 were obt ained for IBL (Imbrasia belina), RP (Rhynchophorus pheonicis), OR (Oryctes rhinoceros) and MB (Macrotermes belicosus) respectively. These values on a dry weight basis were higher when compared with most conventional protein sources. Amino acid analysis revealed that the proteins contained all the essential amino acids, especially lysine, threonine and methionine which are the major limiting amino acids in the cereal and legume based diets. Biological assays, using weanling, male albino rats, suggested high nutritional and toxicological safety of the insects. The PER for OR, MB, and RP (% of casein) ranged from 94 -111 while IBL values were lower (51 – 71). The TD and BV for all the insects were not significantly different from the control (P ≥ 0.05), though boiling increased the BV of the proteins with the exception of value for raw IBL. The relative weights (g/kg live weight) of the liver, spleen, heart, kidney and lungs were significantly different for IBL only, but the rat serum enzyme activities for the boiled and fried insects showed no significant difference from the control (P≥ 0.05). Processing generally was observed to improve the biological parameters assayed for in the experiment. These results suggest these insects as good sources of essential nutrients which could go a long way in helping to solve most nutritional problems among the populations that consume them.

Key Words: *Imbrasia belina*, *Rhynchophorus pheonicis*, *Oryctes rhinoceros*, *Macrotermes belicosus*, Processing, Protein quality.

INTRODUCTION

The world has entered a period when the relationship between food availability in low-income countries (mainly in the tropics) and rising population is becoming increasingly perilous. In some

countries, this has already reached crises proportions [1, 2]. Malnutrition is responsible to a large extent for the high infant mortality rate in many parts of the world [3, 4, 5, 6, 7, 8, 9, 10]. A WHO report [11] states that nearly half of the world's populations are affected by one form or the other of nutrient deficiency. Due to inadequate diets, a third of the world's children fail to reach their physical and mental potential and many are made vulnerable to infectious diseases that account for half of all child deaths. For example severe malnutrition was the leading cause of low birth weight and death among children ranging from 1 – 5 years in a rural population in Palguar, India [12]. In many parts of the world, a single food stuff which could be a cereal or starchy food makes up 60 – 80 % of the diet. In these regions food of animal origin is in short supply. The amount may be as little as 11% of the diet in Africa, 9% in the near east and 5% in the Far East, compared to 20 – 30 % in Europe and 40% in the USA [6]. These developing regions have a Plant/Animal ratio of 9.3 as compared to 2.0 for the developed regions. It is common knowledge that plant proteins and their products, which thus form the major source of protein in these low – income countries are often lacking in quantity and quality [13]. It is estimated that proteins of vegetable origin constitute 81.4 % of overall available proteins, with cereals alone providing 57% while Oil seeds and nuts provide 16.8% [14].

The absence of suitable and acceptable protein supplements of high biological value that could be used for supplementary and mixed feeding of infants and young children and as part of the adult diet has been a major problem in Nigeria and other developing countries. Nutritional surveys [9, 10, 15, 16, 17, 18, 19] have highlighted the widespread incidence of protein energy malnutrition (PEM), due to inadequate protein intakes in developing countries. This was associated with low income group and inability to purchase animal protein. In Nigeria for example, the domestic demand for food and population change appear to double the capacity for food production [20, 21, 22]. It has been shown that in Nigeria, the daily average intake of protein *per capita*, does not meet the individuals gross requirement [21, 23]. The causes of the protein problem are multifarious. They are accentuated by such factors as the limited availability, seasonal variability and high cost of milk and animal protein sources which make them unavailable to the majority of people, especially in the low-income group [24].

The approaches to the protein problem among others should include the prevention of waste and making the best use of the supplies of protein that is available in different forms and from various sources. There is also a need to search for alternatives to the more conventionally accepted dairy and animal protein products.

In Africa, entomophagy is a traditional and culturally acceptable way by which low income persons supplement the meager protein content of their high carbohydrate diet [25, 26]. Human entomophagy is also widely practiced in Nigeria, but much remains to be done in the scientific appraisal of the nutritional attitude. Ordinarily, insects are not used as emergency food to ward off starvation in these indigenous groups, but are included as a planned part of the diet throughout the year or when seasonally available [27, 28]. The interest in the use of insect as food has been expressed in several reports [29, 30, 31]. The values reported showed that many of the insect studied had higher levels of protein and several other nutrients than beef, chicken and fish [32]. Despite these reports, there is a dearth of information on the proximate and biological evaluation of the nutrition quality of the protein in most insects consumed or the effect of various processing techniques on the protein quality of these rural delicacies. In addition, the association

of the consumption of the larvae of *Anaphe venata* with the incidence of seasonal ataxia in Western Nigeria [33] reinforces the need for a thorough study of these insects for nutrition quality and toxicological safety.

MATERIALS AND METHODS

Solvents and chemicals used in this study were mostly of the analytical reagent grade and were obtained from E. Merck (Darmstadt, West Germany), May and Baker (Dagenham, Essex, England), Sigma Chemicals Company (St. Louis, Missouri, U.S.A.). The chloroform and methanol were redistilled before being used in this study.

Rhynchophorus phoenicis (F) and *Oryctes rhinoceros* larvae were obtained live from Ilushi (on the bank of River Niger) in Edo State, Nigeria. *Imbrasia belina* larvae were obtained from Ogbomosho in Oyo State, Nigeria while *Macrotermes bellicosus* was obtained during their nuptial flight at Ekpoma, Edo State, Nigeria. The various species were identified in the Entomology Department, Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria. All live insects / insect larvae were used within twelve hours of collection. *Macrotermes bellicosus* (Termites) were dewinged before being used.

Lipid from the Insect/ larva was extracted with chloroform-methanol (1: 2, v / v) mixture as described by Bligh and Dyer [34]. Protein nitrogen was estimated according to the Kjeldahl William colorimetric method [35]. The amino acid profile of the insect/ larva samples was determined using the method described by Spackman *et al* [36]. The moisture content of the live insect/ larva was determined using the method of the Association of Official Analytical Chemists [37].

TABLE 1: Composition of Basal, Reference and Test Diets (g / kg)

Samples/ Treatment	Weight of samples	Vegetable Oil	Corn Starch	Sucrose	Glucose	Cellulose	Mineral Mix	Vitamin Mix	% Protein
RP(raw)	453.3	100	196.7	100	50	50	40	10	10
RP(boiled)	454.6	100	195.4	100	50	50	40	10	10
RP(fried)	453.3	100	196.7	100	50	50	40	10	10
MB(raw)	299.3	100	350.7	100	50	50	40	10	10
MB(boiled)	305.3	100	344.7	100	50	50	40	10	10
MB(fried)	299.3	100	350.7	100	50	50	40	10	10
OR(raw)	331.1	100	318.9	100	50	50	40	10	10
OR(boiled)	335.6	100	314.4	100	50	50	40	10	10
OR(fried)	331.1	100	318.9	100	50	50	40	10	10
IBL(raw)	284.3	100	365.7	100	50	50	40	10	10
IBL(boiled)	290.2	100	359.8	100	50	50	40	10	10
IBL(fried)	284.3	100	365.7	100	50	50	40	10	10
Casein(ref)	127.8	100	522.2	100	50	50	40	10	10
Corn starch(basal)	-	100	650	100	50	50	40	10	-

OR = *Oryctes rhinoceros*, IBL = *Imbrasia belina Lepidoptera*; MB = *Macrotermes belicosus*, RP = *Rhynchophorus phoenicis*

Formulation of Diets

Fourteen (14) experimental diets were formulated after the pattern of Jenkins and Mitchell [38] with slight modification. The diets were prepared following the proportions as shown in Table 1 below. The components were blended manually starting from the small components to ensure proper blending. Prepared diets were labeled in polythene bags and stored in the refrigerator at 4 ^{o} C until needed.

COMPOSITION OF MINERAL MIXTURE (J.T. Baker Co., Phillipsburg, NJ; g/kg)

Calcium phosphate ($CaHPO_4$)	500g
Magnesium oxide (MgO)	24g
Sodium chloride (NaCL)	74g
Potassium citrate ($K_3C_6H_5O$)	200g
Potassium sulphate (K_2SO_4)	52g
Manganous carbonate, $MnCO_3$(43-48%Mn)	3.5g
Ferric citrate (16-17%Fe)	6g
Cupric Carbonate, $CuCO_3$ (53-55% Cu)	0.3g
Potassium iodate (KIO_3)	0.01g
Sodium selenite (Na_2SeO_3,$5H_2O$)	0.01g
Chromium potassium sulphate [$CrK(SO_4)$, $12H_2O$]	0.55g
Zinc carbonate ($ZnCO_3$)	8.0g
Cellulose to make	1000g

Composition of Commercial Vitamin Premix (U.S. Biochem Corp, Cleveland, OH, mg/kg)

Thiamin-HCL	600mg
Riboflavin	600mg
Pyridoxine,-HCL	1600mg
Ca-pantothenate	0.6mg
Nicotinic acid	2.0mg
Inositol	4.0mg
Amino benzoic acid	6.0mg
Choline	1.3mg
Folic acid	200mg
Cyanocobalamin	1.0mg
Cholcalciferol	2500mg
Retinyl palmitate, vitamin A activity	320 UI
D-biotin	20mg
Menaquinine	50mg
Sucrose to make	1000g

Experimental animals

Eighty four (84) young weanling male albino wistar rats of about 23 days old (bred at the animal house of the College of Medicine, Ambrose Alli University, Ekpoma.) were used for the study. The animals were divided into fourteen (14) groups of six (6) animals per group. The animals were placed on a basal (nitrogen free) diet, a standard casein diet, a test diet of raw larvae/insect, a test diet of boiled insect/larvae (100 ^{0}C, 20min) and a test diet of fried larvae/insect (Deep fried

for 5 minutes). Each animal was caged separately for each of the groups to ensure proper metabolic study and monitor. Animals that showed symptoms of ill health were excluded from the experiment.

Feeding Regime

The rats were weighed to obtain their initial weights, after which they were placed on a 3- day adjustment period on the diets. Thereafter, the animals were placed on the diets and distilled water *ad libitum* for twenty eight days. All the diets except the protein free diet contained 10% protein (N x 6.25) by weight. Weighed foods were placed in small heavy porcelain mortars and about 5 ml of distilled water added and mixed to minimize spillage and scattering. Spilled food with the faecal contaminants were collected daily and dried. The dry spilt food was combined with the dry unconsumed food for the determination of total amount of food actually consumed by the rats in each of the groups. The proximate daily food consumption was determined by weight difference between the served food and the unconsumed food plus spilt food. The weights of the rats were monitored on a weekly basis.

Collection and Treatment of Urine and Faeces.

Urine was collected daily from each group of animals for the twenty eight day period they were on the diets. The urine for each cage was collected in small glass sample tubes containing 3 ml of (1% v/v) sulphuric acid. Additional precaution against urine losses were taken by washing the floor of the cage with 7 ml of (1% v/v) sulphuric acid, which is then added to that contained in the sample tube. The tube is then stoppered and stored in the refrigerator at 4^{o}C until analysed for nitrogen. Faecal collection for each group of animal was done daily. The faecal samples were dried to a constant weight in a Gallenkamp hot air oven size 2 at 85 ^{o}C, after which they were ground to a fine powder and then stored in a stoppered glass bottle container in the refrigerator at 4 ^{o}C until analysed for nitrogen.

Sacrifice of Animals

After the twenty eight day feeding period, the rats were weighed and their physical conditions such as fur, appearance and agility were noted and recorded prior to sacrificing them. The animals were put to sleep by placing them in a sealed container containing diethyl ether. Incision was made in the abdomen and extended to the thorax. Blood was collected directly from the heart with a syringe and needle and shared into 3 different containers containing K_3 EDTA, Lithium heparin while the third container had no anticoagulant in it. The kidneys, liver, lung, spleen and heart were dissected out quickly. These organs were visually inspected for possible abnormalities such as colour changes, lesions, and fatty liver. The organs were washed with normal saline and their weights determined gravimetrically.

Urine and faecal analysis

1 g of dried faeces and 10 ml aliquot of the urine were independently digested and analysed for nitrogen using the Kjeldahl William colorimetric method [35]. The values obtained for the protein free group were used to compute endogenous nitrogen losses.

Nutritional Evaluation of the Diets.

The protein qualities of the insect/insect larvae and casein diets were evaluated biologically, based on their ability to promote growth and nitrogen retention in the albino rats.

The protein efficiency ratio (PER) was determined using the method described by Osborne, Mendel and Ferry [39]. The biological value of the diets was determined using the nitrogen balance sheet method of Mitchell [40]. The net protein ratio (NPR) and protein retention efficiency (PRE) was determined using the method described Bender and Doell [41]. True digestibility was determined using the balance sheet method of Mitchel [40].

Haematological and Toxicological Parameters

Haematology is defined as the scientific study of the natural functions and disease of blood. Haematological parameter refers to those factors in the blood whose levels are usually determined in order to assess the degree of well being of an animal [42]. The relevant parameters measured included:- Red blood cell (RBC), White blood cell (WBC), Pack cell volume (PVC), Haemoglobin concentration (Hb), Mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin, (MCH), platelets count and differential leucocyte count which includes:- Neutrophils, oesinophils, basophils, monocytes and lymphocytes. The estimation of these parameters listed above was carried out using a blood cell counter (Beckman Counter Act Diff. Haematology Analyser).

Aspartate amino transferase (AST) activity was determined using the method described by Reitman and Frankel [43]. Alanine amino transferase (ALT) activity was determined using the method of Reitman and Frankel [43]. Alkaline phosphatase activity was determined using the method described by Deutsche [44]. GGT activity was determined by automatic method using the procedure specified for the ACE Clinical Chemistry Analyser (Schiapparelli Biosystems, Netherlands).

Urea is the main waste product of protein breakdown. It is formed in the liver, passes into the extracellular fluid and is excreted almost entirely by the kidneys. The measurement of urea is an important investigation in diagnosing kidney damage. The Urease –Berthelot method described by Weatherburn [45] was used for this estimation. The Biuret method described by Weichselbaum [46] was used for the estimation of total protein.

Calculation and Statistics

All results were expressed as Mean ± Standard error of the mean. The significance of the differences between the effects of control, basal and the test diets was measured ANOVA and the Student t-test (SPSS Inc. 1995) at 95% confidence limit.

RESULTS AND DISCUSSION

Table 2 shows the proximate composition of IBL, RP, OR and MB. RP had the highest moisture value while MB had the least moisture content. Lipid values revealed that MB had the highest lipid value of 31.46 % and when dehydrated the lipid value rose to 36.12%. IBL had the least lipid value of 15.16% (wet weight) but after dehydration the lipid value rose to 23.38%. The protein values observed in IBL where higher than for all the other insects studied with a value of 35.18%. This is closely followed by MB with 33.41%. Dehydration and eventually defatting is seen to increase relative concentration or proportion of the other nutrients encompassed in the proximate composition. The carbohydrate and ash values were highest in MB while RP larva had the least values.

TABLE 2: Proximate Composition (% wet weight) of some insects consumed in Southern Nigeria

INSECT	MOISTURE	LIPID	PROTEIN	CARBOHYDRATE	ASH
IBL	34.36 ± 0.25	15.16 ± 0.18 (23.38 ± 0.24)	35.18 ± 0.10 (54.26 ± 0.16) 70.81 ± 0.27^{+}	7.12 ± 0.55 (10.98 ± 0.46) 14.33 ± 0.20^{+}	7.38 ± 0.11 (11.38 ± 0.08) 14.86 ± 0.15^{+}
RP	61.85 ± 0.18	25.30 ± 0.20 (66.61 ± 0.35)	8.38 ± 0.31 (22.06 ± 0.26) 66.09 ± 0.28^{+}	2.10 ± 0.10 (5.53 ± 0.17) 16.56 ± 0.11^{+}	2.20 ± 0.08 (5.79 ± 0.13) 17.35 ± 0.08^{+}
OR	60.56 ± 0.41	14.87 ± 0.33 (38.12 ± 1.06)	11.76 ± 0.90 (30.15 ± 1.10) 48.72 ± 0.25^{+}	6.87 ± 0.24 (17.16 ± 0.41) 28.46 ± 0.18^{+}	5.51 ± 0.19 (14.13± 0.03) 22.83 ± 0.08^{+}
MB	12.60 ± 0.26	31.46 ± 0.57 (36.12 ± 0.28)	33.41 ± 0.20 (38.36 ± 0.70) 60.06 ± 0.28^{+}	12.41 ± 0.10 (14.25 ± 0.19) 22.31 ± 0.14^{+}	9.81 ± 0.04 (11.26 ± 0.11) 17.63 ± 0.08^{+}

Results represent the Mean ± SEM of three estimations. Values in brackets are % dry weight. $^{+}$ values are % lean weight of the larvae; OR = Oryctes rhinoceros, IBL = Imbrasia belina Lepidoptera; MB = Macrotermes belicosus, RP = Rhynchophorus phoenicis

Table 3 shows the amino acid composition of the protein in the insect/ larvae. The amino acids known to be essential to man were present in varying amounts in the protein portion of the insects, with leucine constituting the major essential amino acid for RP, OR and MB while for IBL, threonine was the major essential amino acid, with a value of 7.29 g/100g.

TABLE 3: Amino Acid Composition of Some Insects Consumed In Southern Nigeria (g / 100g Protein)

AMINO ACID	IBL	RP	OR	MB
LYSINE	4.15±0.21	3.99± 0.03	4.33±0.35	5.61±0.42
HISTIDINE	3.11±0.16	3.44± 0.14	3.02±0.07	2.91±0.10
ARGININE	6.85±0.45	5.06± 0.12	4.86±0.27	6.24±0.33
ASPARTIC ACID	6.35±0.20	7.02± 0.10	7.23±0.70	7.68±0.64
THREONINE	7.29±0.35	3.10± 0.13	3.22±0.10	3.15±0.12
SERINE	3.44±0.22	3.27± 0.04	3.01±0.06	4.16±0.32
GLUTAMIC ACID	14.49±0.81	12.91±0.70	12.80±0.32	16.47±0.44
PROLINE	2.49±0.04	2.11± 0.24	2.12±0.20	2.76±0.31
GLYCINE	3.12±0.18	2.95± 0.08	3.01±0.06	3.86±0.44
ALANINE	3.69±0.23	3.05± 0.11	3.79±0.17	3.99±0.28
CYSTEINE	2.30±0.15	2.20± 0.35	2.13±0.11	3.07±0.71
VALINE	3.17±0.20	2.80± 0.17	2.62±0.14	2.70±0.09
METHIONINE	2.21±0.10	2.05± 0.31	2.00±0.10	2.16±0.34
ISOLEUCINE	3.21±0.22	3.45± 0.03	2.89±0.21	3.01±0.25
LEUCINE	7.08±0.71	6.22± 0.54	6.13±0.45	7.42±0.23
TYROSINE	2.77±0.20	2.02± 0.09	2.21±0.32	3.15±0.14
PHENYL ALANINE	5.05±0.38	4.13± 0.67	4.01±0.18	4.28±0.07
TRYPTOPHAN	3.02±0.21	2.51± 0.15	2.04±0.41	2.13±0.17

Results represent the Mean ± SEM of three estimations.
OR = Oryctes bhinoceros, IBL = Imbrasia belina Lepidoptera, MB = Macrotermes belicosus, RP = Rhynchophorus phoenicis

Table 4 shows the protein quality of test samples (Insect/ larvae) after various treatments compared to casein. The rats kept on the protein free diet (Basal diet) showed signs of weakness at the end of the 4 week feeding period. Two of the rats eventually died before completion of the experiment. Some of the rats on this diet also appeared to have lost some of their furs. They also developed scaly tails, legs and hands. In contrast, rats kept on the other diets showed none of these symptoms. They looked normal, healthy and appeared agile. There were no obvious differences between rats kept on the reference feed and those kept on the test feeds.

TABLE 4 Protein Quality Evaluation

INSECTS / TREATMENT	Feed intake in 7 days (g)	Protein intake in 7 days	Total weight gain in 7 days (g)	PER	PER (% OF CASEIN)	NPR	PRE	BV	TD
OR (RAW)	59.50	5.95	6.81	1.15 ± 0.27	94.26	1.84 ± 0.18	29.44 ± 0.28	87.94 ± 4.14	89.30 ± 0.70
OR (BOIL)	59.90	5.99	8.14	1.36 ± 0.65	111.48	2.05 ± 0.09	32.80 ± 0.81	88.24 ± 3.96	90.08 ± 0.48
OR (FRIED)	60.40	6.04	7.77	1.29 ± 0.81	105.74	1.98 ± 0.68	31.68 ± 0.69	88.00 ± 6.01	89.69 ± 0.19
IBL (RAW)	49.40	4.94	4.30	0.87 ± 0.38	71.31	1.71 ± 0.41	27.36 ± 0.35	86.82 ± 3.89	86.03 ± 0.41
IBL (BOIL)	56.30	5.63	4.68	0.83 ± 0.23	68.03	1.57 ± 0.32	25.12 ± 0.41	87.67 ± 6.18	88.40 ± 0.36
IBL (FRIED)	55.20	5.52	4.00	0.73 ± 0.50	59.84	1.48 ± 0.15	23.68 ± 0.21	87.32 ± 5.14	87.79 ± 0.54
MB (RAW)	56.60	5.66	6.68	1.18 ± 0.28	96.72	1.92 ± 0.12	30.72 ± 0.51	88.55 ± 7.89	90.18 ± 0.09
MB (BOIL)	60.30	6.03	8.15	1.35 ± 0.19	110.66	2.04 ± 0.39	32.64 ± 0.88	88.64 ± 4.01	91.03 ± 0.87
MB (FRIED)	60.90	6.09	7.00	1.15 ± 0.09	94.26	1.83 ± 0.08	29.28 ± 0.23	88.67 ± 3.10	90.90 ± 0.28
RP (RAW)	65.40	6.54	8.59	1.31 ± 0.15	107.38	1.95 ± 0.61	31.20 ± 0.41	88.95 ± 8.14	91.79 ± 0.84
RP (BOIL)	70.80	7.08	8.73	1.23 ± 0.24	100.82	1.82 ± 0.46	29.12 ± 0.79	89.05 ± 7.41	92.58 ± 0.69
RP (FRIED)	68.40	6.84	8.27	1.21 ± 0.30	99.18	1.82 ± 0.38	29.12 ± 0.51	88.92 ± 6.36	92.12 ± 0.38
CASEIN	70.60	7.06	8.60	1.22 ± 0.61	100.00	1.81 ± 0.17	28.96 ± 0.45	89.20 ± 8.98	92.57 ± 0.42
BASAL	41.30	-	- 4.16	-	-	-	-	-	-

Results represent the Mean ± SEM of three estimations. OR = Oryctes rhinoceros, IBL = Imbrasia belina Lepidoptera, MB = Macrotermes belicosus, RP = Rhynchophorus phoenicis

There were differences in the overall feed intake among the different rat groups, compared to casein, which of course affected their protein intake. Animals on boiled RP larva consumed the highest amount of feed and they also recorded the highest weight gain. Boiled OR larva recorded the highest PER, closely followed by boiled MB and raw RP larva, while IBL (fried) recorded the lowest PER. The NPR followed the same pattern as observed for PER. Boiled OR larva recorded the highest PRE (32.80), followed by boiled MB and fried OR with PRE values of 32.64 and 31.68 respectively. The BV values for the test feeds and reference feeds were quite close, but boiled RP recorded the highest BV value of 99.05 and TD value of 92.58.

The relative organ weights of the rats are shown in Table 5. Rats fed raw IBL larva recorded the lowest liver weight, (3.01) even lower than those on the protein free (Basal) group while boiled RP larva recorded the highest liver weight of 4.21g while rats on casein diet recorded a liver weight of 4.11g. Rats fed fried OR larva recorded the highest spleen weight while rats fed raw IBL larva recorded the lowest spleen weights. The rats fed raw IBL recorded the lowest relative

heart weight while fried MB and boiled RP recorded the highest relative heart weights. The relative kidney weights showed raw IBL recording the highest value of 0.92 while boiled OR recorded 0.69.

The activities of some serum enzymes are shown in Table 6. There were differences observed in the activities of some of the enzymes studied. Some of these differences were significant when compared to casein values. The effects of boiling and frying on the haematological parameters are shown in Tables 7 and 8.

TABLE 5: Relative Organ Weights (g / 100g Live Weight)

ORGANS	OR			IBL			MB			RP			CASEIN	BASAL
	RAW	BOIL	FRY	RAW	BOIL	FRY	RAW	BOIL	FRY	RAW	BOIL	FRY		
LIVER	3.70 ±0.21	3.91 ±0.18	3.73 ±0.24	3.01 ±0.30	3.59 ±0.14	3.56 ±0.12	3.85 ±0.25	3.94 ±0.20	3.93 ±0.16	3.98 ±0.13	4.21 ±0.10	4.04 ±0.09	4.11 ±0.18	3.36+ ±0.05
SPLEEN	0.31 ±0.05	0.32 ±0.07	0.35 ±0.02	0.20 ±0.06	0.31 ±0.02	0.32 ±0.02	0.26 ±0.04	0.31 ±0.03	0.32 ±0.07	0.32 ±0.02	0.31 ±0.05	0.29 ±0.02	0.30 ±0.07	0.34+ ±0.01
HEART	0.40 ±0.05	0.45 ±0.02	0.43 ±0.04	0.35 ±0.07	0.51 ±0.05	0.49 ±0.02	0.49 ±0.02	0.51 ±0.02	0.54 ±0.02	0.48 ±0.04	0.54 ±0.02	0.51 ±0.03	047 ±0.05	0.33+ ±0.06
KIDNEY	0.70 ±0.03	0.69 ±0.05	0.72 ±0.04	0.92 ±0.06	0.82 ±0.06	0.83 ±0.05	0.74 ±0.03	0.70 ±0.04	0.75 ±0.04	0.73 ±0.05	0.71 ±0.02	0.72 ±0.03	0.72 ±0.05	0.98+ ±0.06
LUNGS	0.80 ±0.01	0.85 ±0.03	0.83 ±0.05	0.76 ±0.03	0.78 ±0.05	0.80 ±0.02	0.82 ±0.02	0.82 ±0.02	0.79 ±0.01	0.88 ±0.01	0.91 ±0.03	0.94 ±0.02	0.79 ±0.03	0.73+ ±0.01

Results represent the mean ± SEM of six estimations, + Results represent the mean ± SEM of four estimations.
OR = Oryctes rhinoceros, IBL = Imbrasia belina Lepidoptera, MB = Macrotermes belicosus, RP = Rhynchophorus phoenicis

TABLE 6: Activities (U/L) Of Some Serum Enzymes In Rats Fed Different Diets

ENZYME	OR			IBL			MB			OP			CASEIN	BASAL
	RAW	BOILED	FRIED	RAW	BOILED	FRIED	RAW	BOILED	FRIED	RAW	BOILED	FRIED		
ALP	374.25 ±37.69	375.75 ±24.62	397.00 ±51.88	586.00 ±26.20	549.75 ±61.15	554.28 ±38.22	466.25 ±16.76	452.00 ±34.71	459.00 ±27.43	360.25 ±53.11	358.50 ±60.35	380.25 ±52.96	312.50 ±57.33	582.00 ±10.44
ALT	20.75 ± 3.25	19.25 ±4.31	19.50 ±4.33	35.00 ±3.11	31.00 ±2.04	32.00 ±4.16	23.00 ±2.12	20.00 ±3.39	21.25 ±3.04	18.00 ±3.39	18.50 ±3.23	18.75 ±6.10	18.00 ±2.16	37.00 ±1.73
AST	40.00 ±4.30	41.00 ±2.68	41.50 ±4.33	55.00 ±5.18	40.00 ±3.58	41.00 ±2.04	42.00 ±4.45	40.00 ±3.94	40.50 ±4.09	38.00 ±5.21	37.00 ±2.04	37.75 ±4.50	37.25 ±3.33	48.33 ±7.62
GGT	10.50 ±2.50	13.25 ±1.49	11.50 ±0.96	7.58 ±0.85	8.75 ±1.80	8.00 ±0.91	9.75 ±1.31	10.25 ±1.49	10.25 ±1.08	11.25 ±1.32	14.25 ±1.44	13.00 ±1.29	14.00 ±1.41	-

Results represent the mean ± SEM of four estimations. ALP = Alkaline Phosphatase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, GGT = Gamma glutamyl transferase. . OR = Oryctes Rhinoceros, IBL = Imbrasia belina Lepidoptera, MB = Macrotermes belicosus, RP = Rhynchophorus phoenicis

The moisture content of the insects studied are observed to vary widely, but agrees with published data for insects of various species [47]. Table 2 shows the moisture values (%) for *Oryctes rhinoceros* (OR), *Rhynchophorus phoenicis* (RP), *Imbrasia belina* (IBL) *and Macrotermes bellicosus* (MB). Generally, insect larvae tend to contain more moisture than their adult counterparts. These insect/larvae are usually used as food supplements by those who feed on it. It is consumed as part of a meal or as a complete meal. When compared with conventional animal food supplements such as beef, chicken, pork, and fish which have a moisture content of 40-70% [48], the larva of RP and OR are seen as high moisture food supplement, while values for IBL are a little lower compared to these conventional foods. MB moisture value marks it as a low moisture food supplement. The high moisture content as observed for RP, OR and partially for IBL implies that most of the essential nutrients in the insect larvae will be in forms that are easily available to the body when the larvae are consumed as food. However, dehydration generally increases the relative concentrations of the food components (Table 2) and in addition improves the shelf-life/ preservation of the larvae.

TABLE 7: Effects of Boiling On The Haematological Data of Rats Fed Different Diets

PARAMETERS	OR		IBL		MB		RP		CASEIN	BASAL
	RAW	BOILED	RAW	BOILED	RAW	BOILED	RAW	BOILED		
PLASMA PROTEIN (g/dL)	5.30 ±0.25	5.75 ±0.22	4.20 ±0.18	5.02 ±0.15	5.10 ±0.12	5.80 ±0.14	5.60 ±0.35	5.90 ±0.20	5.85 ±0.31	2.55 ±0.20
BLOOD UREA (mg/dL)	33.75 ±4.13	32.75 ±4.82	38.25 ±2.95	35.00 ±3.67	34.25 ±6.42	33.00 ±2.97	32.75 ±3.01	30.25 ±2.59	32.00 ±3.29	46.33 ±5.24
PCV (%)	34.00 ±1.18	37.40 ±1.63	30.75 ±0.85	38.03 ±1.86	32.20 ±1.39	35.40 ±1.63	39.00 ±1.08	42.50 ±1.19	38.25 ±1.97	27.33 ±1.76
WBC (mm^3)	12320 ±1106.07	10340 ±746.73	13450 ±1155.06	14350 ±1201.73	13380 ±1044.70	13420 ±655.29	13275 ±793.07	12700 ±1585.88	14450 ±885.53	7133.33 ±920.75
RBC $X10^6$ UL	7.93 ±0.21	7.74 ±0.31	6.99 ±0.33	7.53 ±0.52	7.53 ±0.20	7.20 ±0.57	8.19 ±0.25	8.10 ±0.23	8.31 ±0.26	4.23 ±0.23
Hb (g/dL)	13.16 ±0.34	13.32 ±0.17	11.93 ±0.43	13.20 ±0.64	12.90 ±0.16	13.00 ±0.15	14.03 ±0.09	13.98 ±0.32	13.90 ±0.42	10.37 ±0.86
PLATELETS	646.25 ±23.57	650.00 ±24.15	645.25 ±45.61	625.75 ±49.29	615.25 ±15.80	630.5 ±16.43	689.25 ±18.86	648.75 ±25.69	635.00 ±27.23	460.00 ±25.17
MCV	58.15 ±2.39	55.35 ±2.26	60.43 ±3.08	53.63 ±1.78	56.00 ±2.71	54.75 ±2.30	60.30 ±4.30	53.53 ±1.59	57.02 ±0.93	44.97 ±4.32
MCH (pg)	19.98 ±0.82	18.73 ±0.46	20.93 ±1.65	19.83 ±0.72	18.45 ±0.55	18.15 ±0.72	18.45 ±0.31	18.18 ±0.30	19.40 ±1.11	15.23 ±0.50
MCHC (g/d)	32.30 ±0.49	31.80 ±0.47	32.40 ±0.21	31.88 ±0.19	32.23 ±0.38	31.38 ±0.54	32.83 ±0.47	32.13 ±0.37	32.33 ±0.33	27.98 ±0.23
% LYMPHOCYTES	81.20 1.39	80.20 2.46	84.25 ±4.39	80.50 ±2.5	82.20 ±2.69	80.60 ±1.29	81.75 ±0.85	82.25 ±1.32	81.00 ±0.41	65.67 ±2.96
% NUETROPHILS	16.20 0.80	17.60 0.93	13.25 ±1.38	17.00 ±0.19	16.00 ±0.84	16.40 ±1.29	15.50 ±0.96	15.75 ±0.75	16.25 ±1.44	34.33 ±2.98
% EOS	2.50 0.29	2.25 0.25	2.50 ±0.29	2.50 ±0.29	1.75 ±0.25	3.00 ±0.00	2.75 ±0.48	2.00 ±0.00	2.75 ±0.48	-

Results represent the mean ± SEM of four estimations. OR = Oryctes rhinoceros, IBL = Imbrasia belina Lepidoptera, MB = Macrotermes belicosus, RP = Rhyn chophorus phoenicis

TABLE 8: Effects of Frying On The Haematological Data Of Rats Fed Different Diets

	OR		IBL		MB		RP		CASEIN	BASAL
PARAMETERS	RAW	FRY	RAW	FRY	RAW	FRY	RAW	FRY		
PLASMA PROTEIN (g/dL)	5.30 ±0.25	5.70 ±0.16	4.20 ±0.18	4.90 ±0.16	5.10 ±0.12	5.70 ±0.35	5.60 ±0.35	6.01 ±0.14	5.85 ±0.31	2.55 ±0.20
BLOOD UREA (mg/dL)	33.75 ±4.13	33.00 ±2.89	38.25 ±2.95	36.25 ±2.95	34.25 ±6.42	33.75 ±3.33	32.75 ±3.01	31.00 ±1.08	32.00 ±3.29	46.33 ±5.24
PCV (%)	34.00 ±1.18	36.00 ±1.30	30.75 ±0.85	35.25 ±2.21	32.20 ±1.39	33.60 ±0.40	39.00 ±1.08	42.75 ±1.03	38.25 ±1.97	27.33 ±1.76
WBC (mm^3)	12320 ±1106.07	10000 ±692.10	13450 ±1155.06	14750 ±1286.14	13380 ±1044.70	14600 ±605.81	13275 ±793.07	14700 ±1640.63	14450 ±885.53	7133.33 ±920.75
RBC $X10^6$ UL	7.93 ±0.21	7.86 ±0.34	6.99 ±0.33	7.04 ±0.40	7.53 ±0.20	7.72 ±0.26	8.19 ±0.25	8.18 ±0.28	8.31 ±0.26	4.23 ±0.23
Hb (g/dL)	13.16 ±0.34	13.46 ±0.07	11.93 ±0.43	13.08 ±0.50	12.90 ±0.16	12.92 ±0.08	14.03 ±0.09	14.23 ±0.35	13.90 ±0.42	10.37 ±0.86
PLATELETS	646.25 ±23.57	670.00 ±32.49	645.25 ±45.61	636.50 ±28.13	615.25 ±15.80	645.50 ±22.19	689.25 ±18.86	668.25 ±51.55	635.00 ±27.23	460.00 ±25.17
MCV	58.15 ±2.39	56.23 ±1.16	60.43 ±3.08	57.03 ±1.56	56.00 ±2.71	55.83 ±1.41	60.30 ±4.30	58.13 ±3.35	57.02 ±0.93	44.97 ±4.32
MCH (pg)	19.98 ±0.82	18.25 ±0.63	20.93 ±1.65	19.73 ±0.63	18.45 ±0.55	18.00 ±0.15	18.45 ±0.31	18.27 ±0.40	19.40 ±1.11	15.23 ±0.50
MCHC (g/d)	32.30 ±0.49	32.20 ±0.34	32.40 ±0.21	32.03 ±0.22	32.23 ±0.38	31.68 ±0.30	32.83 ±0.47	32.50 ±0.45	32.33 ±0.33	27.98 ±0.23
% LYMPHOCYTES	81.20 1.39	80.40 ±1.21	84.25 ±4.39	81.25 ±4.48	82.20 ±2.69	80.90 ±1.16	81.75 ±0.85	80.75 ±0.75	81.00 ±0.41	65.67 ±2.96
% NUETROPHILS	16.20 0.80	16.80 ±0.58	13.25 ±1.38	16.00 ±1.08	16.00 ±0.84	16.60 ±1.08	15.50 ±0.96	17.00 ±2.38	16.25 ±1.44	34.33 ±2.98
% EOS	2.50 0.29	2.75 ±0.25	2.50 ±0.29	2.75 ±0.25	1.75 ±0.25	2.50 ±0.29	2.75 ±0.48	2.25 ±0.25	2.75 ±0.48	

Results represent the mean ± SEM of four estimations. OR = Oryctes rhinoceros, IBL = Imbrasia belina Lepidoptera, MB = Macrotermes belicosus, RP = Rhynchophorus phoenicis

Fat is the chief form in which energy is stored in insect larvae [49, 50, 51]. It is usually present in greatest amounts in the mature larvae before metamorphosis [52]. According to Fast [52] although fat content can reach as high as 41% wet weight, three-fourths of insect species studied contained less than 10% of wet weight as lipid. Those with fat content greater than 10% are primarily phytophagous. The lipid values of RP, OR, IBL and MB (Table 2) are in agreement with this statement [47]. Comparatively, the lipid values of these edible insects are higher than that found in most insects for which data are available [52]. The fat contents of these insects could have contributed to their highly acceptable flavour when fried or roasted. The lipid values of these insects when compared to lipids derived from conventional foods of animal origin [32] are found to be higher. Malnutrition in developing countries is as much, or more, a problem of calories deficiency as of protein deficiency [53]. The consumption of these insects could go a long way in taking care of the calorie needs in such communities. From the fat contents of these insects, a 100g sample will supply enough of the daily energy needs of very active people [54]. This is particularly relevant in the developing countries where much energy is expended in doing works that are usually done by machines in the industralised countries. Available data shows that of the insects analysed so far, 50% had a higher caloric value than soybeans, 87% were higher than corn, 63% were higher than beef, 70% were higher than fish, lentils and beans, while 95% were higher than wheat, rye or teosintle. [55, 56, 57, 58, 59]. Earlier studies [47, 53, 60, 61, 62, 63, 64, 65, 66] have shown that these insect/larval lipids are rich in the polyunsaturated fatty acids which are protective against atherosclerotic disorders. Another implication of the high fat content of these insects is that defatting them will markedly increase the relative proportions of the other nutrients encompassed in the proximate composition. This means that greatly increased protein contents can be achieved by defatting these insects/ larvae as can be seen in the protein values of the defatted samples (Table 2). From these studies, it was also shown that the levels of unsaturation in OR, IBL and RP were higher than for palm oil and coconut oil which are common household oils. The value for MB is same for palm oil but higher than coconut oil [67]. Insect fatty acids are similar to those of poultry and fish in their degree of unsaturation, with some groups being higher in linoleic and/or linolenic acids which are the essential fatty acids [60]. Nutritionally, a high level of saturated fatty acids in foods might be undesirable because of the linkage between saturated fatty acids and atherosclerotic disorders [68]. The presence of the essential fatty acids such as linoleic, linolenic and arachidonic acids further point to the nutritional values of the insect oils as edible oil. One implication of the high fat content in the insects studied is that it may increase susceptibility of the undefatted insect/larva to storage deterioration via lipid oxidation [69]. This may then be accompanied by increased browning reactions concurrent with reduced lysine availability [70]. Another implication of the high fat content is that defatting these insects will markedly increase the relative proportions of the other nutrients encompassed in the proximate composition. This means that greatly increased protein contents can be achieved by defatting these insects/ larvae as can be seen in the protein values of the defatted samples (Table 2).

The high protein contents of the insects (Table 2) are suggestive of the potential of the insect species in combating protein deficiency. The protein values observed justifies the cultural perception of the high nutritional value attached to entomophagy. The values show that the protein values for these insects are superior to that of beef and chicken as well as other conventional animal protein sources [32]. Proteins provide the chief structural elements of the muscle, glands and other tissues, but in larvae most of the proteins are found in the haemolymph

[51]. The fate and physiological role of these proteins are not fully defined, although it seems probable that they play a major role in insect metamorphosis [71]. All the amino acids commonly found in proteins have been identified in insects, which are known to have the same amino acid requirements as mammals [49, 72]. Table 3 shows the amino acid composition of the four insect/ larvae under study. All the amino acids known to be essential to man are found present in varying proportions in the protein portion of the insects. Of particular interest is the high level of leucine, lysine and threonine observed in the insects. Lysine and threonine are limiting amino acids in wheat, rice, cassava and maize based diets that are prevalent in the developing world [73, 74], while leucine and histidine have been reported to enhance the growth of infants and young children [75]. Fisher [76] revealed that among the rat, rabbit and chicken, the requirement pattern of the growing rat is most similar to that of a growing child, pointing out that neither requires arginine. He further showed that the requirement pattern of the child for sulphur amino acids and for lysine resembled that of the rat more than those of either rabbit or chick. The inclusion of these insects into the staple diets of these third world communities would boost their nutritional status. The values of the sulphur amino acids though not so high yet they meet the RDA values for these amino acids. Whole, matured insects as a source of protein, on one hand, are of somewhat lower quality than animal products because of the indigestibility of chitin [30, 55]. Despite this, the consumption of insects can to a substantial degree supplement the predominantly cereal diet with many of the protective nutrients [30]. Removal of chitin increases the quality of insect protein to a level comparable to that of products from vertebrate animals. It should be noted however that in insect larvae, most of these nutrients are in the haemolymph and could be easily absorbed in case of ingestion as food or feed. Comparison of the amino acids composition of these insects with conventional animal foods indicate that the supply of some of the essential amino acids were superior to those found in these conventional foods [77]. These insect proteins may constitute a cheaper source of protein supplement easily available and affordable to the natives within the localities where the insects are found.

The group of animals maintained on the protein-free control (Basal) diet consumed the least quantity of feed when compared with the other groups of animals kept on the experimental diets and standard casein diets (Table 4). All the diets with the exception of the protein free control group contained 10% crude protein (N x 6.25).

The lower feed intake of this group of animals correlated with their apparent growth failure, if weight gain is used as a measure of growth. The group of rats fed boiled RP larval diet consumed the highest quantity of feed and recorded the highest weight gain. This correlates weight gain to feed intake in the experimental diet. The feed intake and weight gain in this group was higher than that observed for the casein diet. The group of rats fed raw IBL larva consumed the least quantity of feed among the experimental diets, but recorded a weight gain higher than that observed for fried IBL larval diet, in spite of the fact that rats fed with fried IBL larval diet consumed a higher quantity of feed. Generally, the quantity of feed intake by rats fed Raw, Boiled and Fried Imbrasia belina larva were lower when compared with the other groups. This low feed intake (and indirectly low protein intake) also reflected in their low weight gain when compared to the other experimental diets. It is also observed that, all the groups of rats fed raw insect/ larval diets recorded lower feed intake compared to those fed with the boiled and fried versions, which also reflected in their lower weight gains, with the exceptions of raw IBL and raw RP diets where animals fed with the fried larvae recorded lower weight gains, compared to

those on the raw larval diets, in spite of their higher feed intake. The reduced feed intake by animals on the raw insect/ larval diets may have been due to either a deficiency and / or imbalance of amino acids and other nutrients; presence of adverse substances (antinutrients) in the protein source; or reduced palatability of the diets. Jacquot and Peret [78] reported that the nature of proteins included in diets influences the appetite of animals, and may cause significant reductions in food intakes during *ad libitum* feeding. This reduction in general food intake indicates a reduction in the intake of other essential dietary components such as calories. The reduction in food intake compounds the possible effects of limiting amino acids [38]. In addition, Passmore *et al* [79] reported that if the total energy intake is inadequate, some dietary protein is used for energy, and is therefore not available to satisfy protein needs. Thus, further increases in protein intake is of limited effectiveness and wasteful if energy needs are not being satisfied at the same time. The caloric (energy) values of the various experimental diets were not determined in this study. Rats fed with boiled insect/ larval diets recorded higher weight gains than those fed with raw or fried feed formulations, in spite of the higher feed intake by the rats fed especially with fried insect/ larvae as observed for OR, MB, raw IBL and RP. Oke [80] was of the opinion that the nutritional importance of a foodstuff in a diet depends on the nutrient composition of the raw foodstuff, the amount that is consumed and the extent to which the component nutrients are destroyed or lost during preparation of the diet. Similarly, Ensminger [81] observed that feed processing influences the nutritional value of feedstuffs by enhancing some nutrients and lowering others. The observed higher feed intake among the fried insect/ larva group could be due to the fat content of the insect/ larvae which contributes to its highly acceptable flavour when fried, thereby helping to improve the dietary intake of the diet [82]. On the other hand, these insect/ larvae all had a fat content higher than 10% (wet weight), which were also observed to be highly unsaturated. The implication of the high fat content is that it increases the susceptibility of the undefatted insect/ larvae to storage deterioration via lipid oxidation [69]. At the same time frying tends to increase browning reactions which is concurrent with reduced lysine availability [70, 83]. Available lysine values are generally lower in foods processed by techniques requiring heat input [84, 85, 86, 87, 88, 89] and drying treatments [83] and by non enzymatic browning [86, 90, 91]. Lysine (an essential amino acid) reduced availability may have resulted in the observed deceases in weight gain despite the higher feed intake in the fried insect/ larval diets. Of particular interest is the fact that weight gain of rats fed raw RP larva and IBL larva were higher than those fed fried versions of these larvae. These observations may be due to the explanations given above. With the exception of boiled RP larval diet, all the other diets recorded lower feed intake and lower weight gain compared to the casein diet. There was significant difference ($P \leq 0.05$) in feed intake between casein fed rats and rats fed with raw, boiled and fried OR, IBL and MB, while the difference in weight gain between the raw, boiled and fried IBL compared with casein was significant at $P \leq 0.05$. Looking at the amino acid credentials of these insect/ larvae, IBL tends to have the higher essential amino acid content than the other insect/ larvae. Despite this impressive array of essential amino acids, the feed intake and subsequently the weight gain of this group of rats fed the larval diet was lower than that observed for the other insect/ larvae groups. It is either that these essential amino acids are in a form unavailable to the animals or there is the presence of an antinutritional factor which makes these essential amino acids unavailable to the animals.

Osborne *et al* [39] and Henry [92] have reported maximum values of PER (Protein efficiency ratio) at lower protein concentrations for good quality proteins than for poor-quality proteins.

The PER values for OR (boil), OR (fried), MB (boil), RP (raw) and RP (boil) were higher than values obtained for the casein diets, while all the others were lower. This result is not too surprising as other researchers such as Calvert *et al* [93], Hale [94], Abdel Gawaad and Brune [95], Ocio *et al* [96], Finke *et al* [97] worked on various insects and discovered that many of the insect they studied had protein qualities that were superior to soy protein or casein as a source of protein. Interestingly, rats fed with boiled OR larva recorded the highest PER values, higher than those fed with boiled RP larval diet, though this group of rats recorded higher feed intake and weight gain. Rats fed with raw IBL larval diet recorded the lowest PER values which also corresponds with their low feed intake. Boiling tends to improve the PER values of the various insect/ larval diets, but for IBL larval diet, though the boiled sample recorded a higher weight gain, yet the PER value is lower than that for the raw sample. PER values for the fried samples are lower than the boiled samples despite the higher feed intake by the latter. This could be as a result of non availability of lysine due to increased browning reactions during the frying process [70]. With the exception of the IBL fed rats, all the others recorded higher Net protein ratio (NPR) values when compared to casein fed rats, with boiled OR larval diet fed rats recording the highest NPR value of 2.05 closely followed by boiled MB fed rats with an NPR value of 2.04 (Table 4). Though raw RP larva fed rats recorded a higher PER value compared to fried OR larva fed rats, yet the latter has a higher NPR value. Fried IBL larval diet recorded the lowest NPR values. The spread of protein retention efficiency (PRE) values were similar to the observed NPR values.

Very high biological values were observed for all the insect/ larval diets, though value obtained for the casein diet was higher. Comparison with results reported by Dreyer and Wehmeyer [30] for IBL shows that the results obtained in the present study are much higher. The high biological values of the insect/ larval diets support the general view that maximal utilization of good quality proteins occurs at lower levels of protein intake [92]. At higher levels, there is a tendency for increased metabolic wastage of dietary proteins.

The True Digestibility (TD) values strongly indicate the ease of bioavailability of the amino acid constituents of the insect/ larvae, an important factor in considering the nutritional quality of a protein [98]. Dreyer and Wehmeyer [30] reported a Digestibility value of 85.8% for IBL larva while the present study reports a slightly higher value of 86.03, 88.40 and 87.79 for the raw, boiled and fried IBL larval diets respectively. Boiling is observed to increase the True digestibility and Biological value of all the diets. Improved quality of food proteins have been ascribed to factors which range from denaturation of the protein and consequent enhancement of the digestion, to destruction of antinutritional factors which might be present in the food by heat treatment. While the unfolding of the protein for improved proteolysis may sound obvious, it is not possible to confirm the possibility of any anti-nutritional factor from these results. However looking at the proximate and chemical analysis credentials for IBL and comparing these with the observed low weight gain, Protein efficiency ratio, Biological value, True digestibility and other nutritional parameters, does not reflect the observed values.

Generally, the organs of the animals placed on the protein free diet were pale in appearance and diminished in size, as compared with those of the other animals placed on the standard casein diet and test diets. The liver and kidneys of the animals placed on the casein diet were brick red in colour with no lesions on them or any signs of fatty liver, while that of the animals placed on

the various test diets showed a fine brick red colouration, darker than that observed for the animals on the casein diet. There were no lesions on the various organs, or signs of fatty liver. The results of the organ weights clearly indicate that there was corresponding increase or decrease in the organ weights with increased protein intake and/ or quality. In particular the liver weight of the different group of rats fed the various diets correlated quite well with the level and quality of dietary protein intake. Rats fed boiled RP larval diet recorded the highest feed intake and also the highest relative liver weight of 4.21g closely followed by rats fed the casein diet. There is a direct correlation between feed intake and relative liver weights of the test animals. Expectedly animals fed raw IBL larva recorded the least feed intake and as well the least relative liver weight. This result is in agreement with the findings of Jenkins and Mitchell [38]. Raw insect/ larval diet for all the group of animals recorded lower relative liver weight while the boiled diets recorded higher values. These differences in relative liver weights may indicate stress and or possible changes in the relative composition of these tissues due to the test diets. The relative spleen weights were fairly constant with little variations among the various groups. Though animals fed the raw diets recorded slightly lower spleen weights compared to the boiled samples, the differences were not significant ($P>0.05$). The relative weights of the heart and lungs were similar to results observed for the spleen, and there were no significant differences ($P>0.05$) in the relative weights of the heart and lungs for the different group of rats. The relative kidney weight appears to be related to the PER of the protein source. Rats fed diets with the lowest PER values (index of protein quality) recorded the highest relative kidney weights. These observations were also reported by Tanaka *et al* [99], Kimiagar *et al* [100], and Jenkins and Mitchell [38]. Kimiagar *et al* [100] however demonstrated that the effects of the quality of dietary protein on the organ weights and liver enzymes could depend on the length of feeding.

Tanaka *et al* [99] ascribed the feeding of poor quality protein to significant increase in the serum activities of alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). A later report by Kimiagar *et al* [100] showed that these effects on liver enzymes depended on the length of feeding. AST is a mitochondrial enzyme and is also present in heart, muscle, kidney and brain, while ALT is a cytosol enzyme and is more specific to the liver than AST [101]. The normal range for these enzymes in rats could not be ascertained, but comparing observed results with the casein reference values shows increase in ALT and AST values for raw IBL fed rats, which was significant at $P \leq 0.05$. There were no significant differences in the casein values when compared to the other insect/ larval diets. Elevated levels of AST are usually observed in hepatic necrosis, myocardial infarction, muscle injury and congestive cardiac failure [101]. Increase in ALT activity has also been associated with vitamin D malnutrition and several diseases of the bone [102]. There were observed increases in the alkaline phosphatase activity in rats fed various levels of IBL larva ($P \leq 0.05$) compared to those fed casein diets. Rats fed casein diets showed lower levels of alkaline phosphatase activity compared to the other diets, but the differences were not significant ($P>0.05$) for these other insect/larvae. Alkaline phosphatase is present in the canalicular and sinusoidal membranes of the liver, but is also present in many other tissues e.g. bone, intestine and placenta. Serum alkaline phosphatase is raised in cholestasis from any cause, whether intra hepatic or extra hepatic [101, 103]. There were observed decreases in the activity of Gamma Glutamyl transferase (or transpeptidase) (A microsomal enzyme present in many tissues including the liver), in the animals fed the test Insect/ larval diets with the exception of boiled RP larva where the values were comparable to values observed in the casein fed animals. The differences were not significant compared with the casein values ($P \geq 0.05$) except for raw

IBL larval diet. These observed differences and apparent lack of any effects of the Insect/ larvae intake on these enzymes makes it difficult to ascertain any disease condition.

The observed haematological data results (Tables 7 and 8) show that the various protein sources had different effects on the total plasma protein concentrations. Rats fed the raw, boiled and fried OR, MB and RP had total plasma protein levels comparable to the casein group. Values for boiled IBL was comparable to casein, but values for the raw and fried IBL larva were significantly lower than casein values ($P < 0.05$). During protein depletion periods, there is usually a drop in plasma protein content and plasma volume, resulting in a steep reduction in the total amount of circulating plasma protein[104]. Blood urea levels for the various insect/ larval diets were similar to values observed for casein with the exception of values for raw and fried IBL fed rats. Fried IBL fed rats had blood urea values lower than those fed the raw samples. Miller and Payne [105] reported that, if the concentration of energy in the diet is too low, some of the protein is inevitably deaminated and used for energy. Since the feed intakes of rats fed raw, boiled and fried IBL larvae were low, the low energy level may have been one of the factors contributing to the higher levels of blood urea nitrogen due to deamination of amino acids in a bid to meet up with energy demands. Boiled RP fed rats recorded the highest feed intake and also the lowest blood urea nitrogen values. For the most part, plasma total protein and blood urea nitrogen levels reflected the quality of the protein sources. Blood urea nitrogen levels were lower in rats with the highest PER values. The higher concentration of blood urea nitrogen in the blood of rats with low PER values indicates the presence of large quantities of amino nitrogen resulting from the breakdown of tissue or dietary proteins or both. In this study PER values are positively correlated with plasma total protein and negatively correlated with blood urea nitrogen levels. The significant relationship between the biological value of the diets and blood urea nitrogen levels has been shown by several workers [105, 106]. There were no significant differences in the PCV, WBC, RBC, Platelet count, MCV, MCH, MCHC of the animals on the reference and Insect / larval diets ($P \geq 0.05$). Percentage lymphocyte, was only slightly elevated while percentage Neutrophils was slightly decreased in rats fed raw IBL, but this elevation or decrease was not significant at $P>0.05$.

The similarities in the values for both the raw, boiled and fried samples suggest that the animals were capable of utilizing these samples with almost equal efficiency, though values for the raw samples were usually lower than the others. This may however not be significant since most populations either boil or fry their insects before consumption.

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