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In vitro iron availability from insects and sirloin beef

Authors:

Latunde-Dada, G.O.[1]*, Yang, W.[2], Vera Aviles, M.[1]

Author affiliations:

[1]King's College London, Diabetes and Nutritional Sciences Division, Faculty of Life Sciences and Medicine, Franklin-Wilkins Building, London, SE1 9NH, United Kingdom.

[2]School of Marine Sciences, Ningbo University, Ningbo, Zhejiang 315211, China.

*Corresponding author: Dr G.O. Latunde-Dada, Diabetes and Nutritional Sciences, King's College London, Franklin Wilkins Building, 150 Stamford Street, London SE1 9NH. Tel: +44(0)2078484256; Fax: +44(0)2078484500; email: yemisi.latunde-dada@kcl.ac.uk.

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Abstract

Interest in the consumption of insects (entomophagy) as an alternative environmentally sustainable source of protein in the diet of humans has recently witnessed a surge. As knowledge of the nutrient composition and, in particular, the bioavailability of minerals from insects, is currently sparse. This study evaluated the availability of Fe, Ca, Cu, Mg, Mn, and Zn from four commonly eaten insects and compared these to sirloin beef. Soluble iron from the samples was measured by inductively coupled plasma optical emission spectrometry (ICP-OES). Iron bioavailability was determined using an *in vitro* simulated peptic-pancreatic digestion, followed by measurement of ferritin (a surrogate marker for iron absorption) in Caco-2 cells. Cricket and sirloin beef had comparably higher levels of Fe, Ca and Mn than grasshopper, meal and buffalo worms. However, iron solubility was significantly higher from the insect samples than beef. The complementation of whole-wheat flour with insect or beef protein resulted in overall increases in mineral content and iron solubility in the composite mixtures. Collectively, the data show that grasshopper, cricket, and mealworms contain significantly higher chemically available Ca, Cu, Mg, Mn and Zn than sirloin. However, buffalo worms and sirloin exhibited higher iron bioavailability that was comparable to $FeSO_4$. Commonly consumed insect species could be excellent sources of bioavailable iron and could provide the platform for an alternative strategy for increased mineral intake in the diets of humans.

Key words: solubility, bioavailability, insects, sirloin, whole-wheat

Introduction

The task of maintaining sustainable agricultural production and food security for the world's growing population is a challenge confronting the UN Food and Agricultural Organization and world leaders (1). Achieving this goal with alternative food sources and with agricultural practices that are environmentally safe and undisruptive to ecosystems is of prime importance. Food diversification through entomophagy (consumption of insects) by people in other countries apart populations in South and East Asia, Africa, South and Central America (2) has been highlighted (2) to contribute to food security.

The credibility of relying on insects as sources of protein in the diets of humans is substantiated by evidence of numerous nutrition, health, and environmental benefits. Insects can provide protein of comparable biological value to meat and fish (3). Insects also provide high-quality monounsaturated and polyunsaturated fatty acids and are rich sources of minerals and vitamins such as iron, zinc, copper, magnesium, selenium, biotin and pantothenic acid (4). Moreover, some insects have been reported to have significantly higher levels of iron than beef (5). For example, while the iron content of locusts (*Locusta migratoria*) is about three times more than beef, that of mopane caterpillar could be more than ten times higher (6).

Dietary haem from animal products is of significant importance in iron nutrition because it is much more bioavailable than non-haem iron (7) and therefore can provide a relatively larger amount of iron to the body. Consequently, haem iron from animal (i.e. meat) sources contributes about 10-25% of total food iron and has a higher bioavailability (about 15-38%) than non-haem iron *(8, 9)* in humans. Furthermore, the more highly bioavailable meat iron seems inert to inhibitory components of diets and the potential of meat to enhance non-haem iron absorption termed the 'meat factor' in a meal is phenomenal. Currently, populations subsisting predominantly on plant food sources of iron in the diets have a high incidence of iron-deficiency anaemia (IDA). In the UK, this deficiency afflicts about 5 million people and exerts debilitating consequences on cognition, physical performance, immunity, while also causing poor pregnancy outcomes, maternal deaths and other health problems (10). Edible insects are excellent sources of iron, and they could contribute to the prevention of IDA. However, there is little information on the relative absorption and bioavailability of the various insect species in human diets. Moreover, while the nature of iron compounds in edible insects is poorly understood, the forms of iron and bioavailability are important considerations if they are to replace meat in the diet. Consequently, substituting or replacing meat in the diet with insect products could have effects on iron nutrition and metabolism in the populace.

In light of the above, it is imperative to investigate the bioavailability and bioaccessibility of iron in the common insects that are being incorporated into human diets. Until now, insects have not been screened for chemical iron solubility and bioavailability. The aim of this study is to analyse mineral contents and availability from grasshopper (*Sphenarium purpurascens*) cricket (*Gryllus bimaculatus*), mealworm (*Tenebrio molitor*) and buffalo worm (*Alphitobius diaperinus)* samples and compare these with sirloin beef and whole-wheat flour. Furthermore, the potentials of animal proteins to enhance iron availability in whole wheat were also investigated.

Materials and Methods

Reagents and chemicals

Unless otherwise stated, all the reagents and chemicals used in this study were purchased from Sigma-Aldrich Company Ltd (Dorset, UK). Pepsin (EC232-629-3) and pancreatin (EC232-468-9) were stored at -20 °C. Solutions of enzymes were all prepared freshly just before use.

Insects, sirloin, and whole wheat samples

Grasshopper, crickets, mealworms, buffalo worms commercially farmed were purchased from Grub, UK; Durum wheat (*Triticum durum* L.; Svevo cv.), grains were provided by Millbo S.P.A., Trecate, Italy and sirloin steak was sourced from a local supermarket in the UK. Insect and whole wheat flour composite were prepared by weighing samples at a 1:1 ratio and mixed thoroughly before *in vitro* solubility studies.

Determination of iron content in insects and wheat samples

Samples were weighed in crucibles with lids. The samples were dried in an oven at 70°C overnight and cooled in a desiccator. Samples were charred over a Bunsen burner flame at a low heat to eliminate smoke before placing in a muffle furnace at 525°C for three hours during which all the organic matter was oxidized leaving remnants of clean white ash. Samples were oven-dried for 48 hours, cooled in a desiccator and reweighed. Mineral (Fe, Mg, Zn, Ca, Cu and Mn) concentrations in the samples were analysed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Thermo-Fisher). Plasma parameters and sample aspiration methods were performed according to the manufacturer’s recommendations. Mineral concentrations were extrapolated from the standard curve in the range of 0.1 –10 µg/mL. The internal standard, Yttrium (Merck Millipore), was added to each sample according to manufacturer’s specification to correct for sample losses due to volatility and evaporation.

In vitro mineral solubility and simulated peptic-pancreatic digestion

The solubility of minerals was done without digestive enzymes and by simulated peptic-pancreatic digestion (11) for iron uptake in Caco-2 cells. Enzymes and bile extract were demineralized with Chelex-100 (Bio-Rad Laboratories Ltd., Hercules, CA) before performing the experiments. One gram of samples (in quadruplicate) was added to 10 mL of isotonic saline solution (140 mM NaCl and 5 mM KCl) and was adjusted to pH 2.0 with HCl (1 M). During peptic digestion, 0.5 mL pepsin (16 mg/mL) was added and incubated at 37 °C for 75 min followed by pH was an adjustment to 5.5 with $NaHCO_3$ (1 M) to stop peptic digestion. Afterwards, 2.5 mL bile-pancreatin extract (8.5 mg/mL bile extract and 1.4 mg/mL pancreatin) was added and pH was adjusted to 7.0 with $NaHCO_3$ (1 M) to start pancreatin-bile digestion. The volume was brought to 15 mL by adding isotonic saline solution and incubated at 37 °C for 120 min. Following digestion, tubes were centrifuged at 3000 x g for 5 min and the supernatant of digests was retained for the experiment.

Mineral contents of Ca, Cu, Mg, Mn, Zn and Fe in the soluble fractions were determined using the MARS 6 Microwave reaction system. Samples (5 mL) and 5 mL of concentrated nitric acid (1 M) were added into reaction vessels and placed into the microwave digester. Digestion of the samples was carried out for an hour. The contents were then transferred into Falcon tubes containing 140 µL of 100 µg/mL Yttrium internal standards and the volume was made to 14 ml with deionized water. Ca, Fe, Cu, Mn, Mg, and Zn in the samples were read using the ICP-OES.

Cell culture

Caco-2 cells (ATCC; HTB-37) were utilized for the experiments. Cells at passage 25 were grown in Dulbecco’s Modified Eagle Medium (DMEM, Gibco, Life Technologies, UK), which contained 1% antibiotic solution, 25 mM HEPES and 10% fetal bovine serum. For the experiment, cells were trypsinised and seeded into 12-well plates in DMEM. Cells were incubated at 37 °C with 5% CO_2 and 95% air for 14 days while the medium was changed every two days.

The day before experiments, DMEM was replaced with minimum essential medium (MEM, Gibco Life Technologies, UK) and the cells were incubated at 37 °C for 24 h.. Sample digests were centrifuged and heated at 100ºC for 5 min to inactivate the digestive enzymes *(12)*. Afterwards, fresh MEM (0.5 mL) was added to the cells. Following this, each digest containing 20 µM iron was added to the cells. Cells were then incubated at 37 °C for 2 h for iron uptake. The control contained 20 µM $FeSO_4$ in MEM medium. Next, 0.5 mL of MEM was added to the cells, and these were incubated for a further 22 h. Following this incubation period, cells were washed with PBS and lysed with Mammalian Protein Extraction Reagent (MPER®, Thermo Fisher Scientific, Cramlington, UK). The cell lysate was centrifuged (5 min, 16,000 x g) to remove cell debris and the supernatant was used for ferritin and protein analysis. After that, cells were harvested in 100 mL PER protein lysate solution (Thermo Scientific) and analyzed for ferritin content using a commercially available ELISA (Ramco Laboratories, TX, USA). Experiments were carried out in triplicate and data expressed as ng ferritin per mg cell protein. Cellular protein concentration was determined according to Bio-Rad assay protocol (Bio-Rad Laboratories, UK).

Statistical analysis

Data were analysed with Microsoft Office Excel 2010 and Graph Pad software (USA). Data are shown as mean ± SEM. Comparison of means was analysed either by Student's unpaired t-test, or one-way analysis of variance (ANOVA) with Tukey's post-test for multiple comparisons. Significant differences were considered at $P<0.05$.

Results

Minerals content of samples

There were significant differences in the iron contents of the grasshopper, crickets, mealworms, buffalo worms, sirloin steak and whole-wheat flour analysed in the current study (Table 1). It is remarkable that only the cricket sample compared favourably in iron level to sirloin. Furthermore, Table 1 also shows high significant differences in the levels of Ca, Cu, Mg, Mn and Zn in the samples. While the cricket and sirloin share significantly higher levels of Ca, (155.82 and 126.13 mg/100 g respectively), the sirloin was distinctively higher in Mg and Mn (Table 1). Levels of Cu and Zn were however higher in cricket than sirloin (Table 1).

In vitro solubility of minerals from insects, sirloin and whole-wheat samples

. A classical *in vitro* technique was used to estimate chemical solubility of minerals from insects, sirloin, and whole-wheat samples. Iron solubility was significantly higher ($P>0.01$) from the insect samples than the sirloin (Figure 1). The solubility of iron from cricket was significantly (P>0.01) higher than grasshopper, mealworm and buffalo worms (Figure 1). Moreover, there were significant differences in the solubility of Ca, Cu, Mg, Mn and Zn from the insects, sirloin and whole-wheat samples (Table 2). Not surprisingly, mineral solubility, and in particular iron, from the whole-wheat samples was comparatively the lowest.

In vitro solubility of minerals from insects, sirloin and whole-wheat composite samples

Due to the potential dietary practice of incorporating insects into composite or mixed-meals, the solubility of insects and sirloin in the presence of whole-wheat sample was next investigated. The composite mixture of the insects and sirloin at a 1.1 ratio did not significantly affect iron solubility (Figure 2). Furthermore, Ca, and Zn levels were, in general, decreased in the insects/sirloin whole-wheat mixes as opposed to increased content of Cu in the mixes (Table 2 and 3).

In vitro bioavailability of iron from insects, sirloin and whole-wheat samples

To estimate the bioavailability of iron from the samples, an *in vitro* simulated peptic−pancreatic digestion was carried out followed by ferritin analysis (a surrogate marker for iron absorption) in Caco-2 cells. In contrast to iron solubility profile of the samples, buffalo worms and sirloin exhibited comparatively higher iron bioavailability than from grasshopper, cricket, and mealworms (Figure 3). Quite expectedly, iron bioavailability from the whole-wheat samples was relatively the lowest while that from $FeSO_4$ was significantly (P<0.01) higher than all the samples categories.

Discussion

In recent years, entomophagy has gained much attention worldwide and is being proposed by the Food and Agricultural Organization of the United Nations (FAO) as an initiative to enhance food security globally (1). This is attributable to the production practices that seem sustainable and less demanding on the ecosystems as well as the high nutritional quality of the insect products (2). In addition to being excellent sources of protein, and fatty acids, insects are also noted for their mineral profiles. However, to our knowledge, this is the first study to evaluate the bioaccessibility of some minerals, in particular iron, from four common edible insects. Grasshopper, cricket, mealworms and buffalo worms could provide excellent sources of Fe, Ca, Cu, Mg, Mn and Zn in human diets depending on the recipes and portion sizes. Variability in mineral levels across these insect samples is not surprising because they are heterogeneous species and the edible form could be at different stages of metamorphosis. Moreover, as they are now being farmed commercially, management practices and in particular their feeding and growth conditions could influence their mineral constituents. For example, Ca, Mg, Fe, Zn, Cu and Mn contents of cricket and mealworms were enhanced when they were fed on special diets *(13)*. The levels of these minerals in mopane caterpillar, a favourite edible species, are comparable to the insects analysed in the current study *(6)*. In contrast, however, the iron level (1562 mg/100 g dry matter) in crickets *(14)* is phenomenally higher than the value in this study. Quite remarkably, crickets share comparatively similar levels of Fe and Ca with sirloin (Table 1). There were no similarities in Cu, Mg Mn and Zn levels between the insects and sirloin (Table). The iron content of crickets was reported to be 180% greater than obtained from beef *(15)*. Finke *(15)* also reported comparable levels of copper, sodium, potassium, iron, zinc and selenium in both mealworms and beef.

In most cases, mineral solubility was higher from the four insect species than found in sirloin (Figure 1 and Table 2). In general, grasshopper, cricket, mealworms and buffalo worms would provide significant levels of soluble minerals than sirloin in the diet. Insects have traditionally been incorporated into food mixes and more recently included in processed foods such as biscuits, crackers, muffins and varied local snacks *(4)*. For example termite powder in muffins, crackers, and sausages amongst others are being considered for commercialization in Kenya *(14)*These initiatives attempt to ensure sensory and organoleptic acceptability as well as promote nutrient complementation for balanced diets. In light of this, mineral solubility was analysed in the current study in composites comprising 50% by weight replacement of the insect and sirloin samples with whole-wheat flour. The levels of Fe (Figure 2) and Ca, Mg, Zn, Cu and Mn (Table 3) decreased in the mixes and the protein-enhancing effect on non-haem iron absorption *(16)*was not evident in the current study. Iron bioavailability from buffalo worms compared favourably with sirloin and $FeSO_4$ in Caco-2 cells (Figure 3). Iron solubility and uptake in Caco-2 cells were significantly higher from the insect species than from whole-wheat plant product (Figure 1 and 3). Although the cricket sample had high iron content and solubility, it exhibited the lowest bioavailability in Caco-2 cells (Figure 1 and 3). The reasons for this observation are not clear. *In vitro* mineral solubility is a basic index of chemical availability, and it is a function of the cumulative actions of the pH, binding ligands, particle size, and synergistic interactions of enhancers and inhibitors in the food samples *(17)*. Moreover, it is useful for screening large samples and for predicting the trend of mineral availability. While iron solubility, in particular, has been reported to correlate positively with absorption, discrepancies of lack of correlation of *in vitro* with *in vivo* bioavailability studies are also evident in the literature *(18)*. Haemoproteins in meat are sources of highly bioavailable Fe in diets (7). However, the proportion of haemoglobin (Hb) and myoglobin vary according to meat type and part of an animal *(19)*. Quite notably, very few insects have haemoglobin and all are devoid of myoglobin *(20)*. It is reported that iron compounds in insects are mostly in form of ferritin, holoferritin and cytochromes *(21, 22)*. There is a dearth of information on the characterization and chemical composition of iron compounds in the diverse array of edible insects. Consistent with the literature on iron availability from animal sources compared to non-haem *(16, 23)*, iron solubility and bioavailability was lowest from whole-wheat flour. While insects as a composite meal with wheat flour was evaluated in the current study, future studies should evaluate bioavailability in the context of complete meals or snacks in human subjects. It is important to note that the Fe and Zn levels in the durum whole wheat sample were high when compared with values reported in *(24)*. The differences might be due to variation in the genotype, growing conditions of the wheat variety and exogenous sources of metals during processing *(25)*. Some studies have reported that wheat genotypes with high protein content tend to have higher micronutrient content, especially iron and zinc *(26)*. Similarly, residual high content of blood in the sirloin might also contribute to the high mineral levels. Another technical point worth noting also is particle size of the food analysed. Mineral solubility and bioavailability and in particular iron are influenced by dietary composition, interactions of enhancers and inhibitors of absorption as well as food particle size and the degree of physical encapsulation in cell components that may not be degraded by digestive enzymes *(18, 27, 28)*. Insects could provide significant proportions of daily recommendations of minerals and in particular, be excellent sources of bioavailable iron in the diets depending on the insect species. Further work is warranted on the nutrient composition of the vast array of insect types from both wild habitats and commercial insect farms. A compendium of these in food composition tables is also desirable. Moreover, human nutrition intervention studies on iron bioavailability from commonly consumed insects are overdue and are highly recommended.

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Author Contributions

GOL-D, designed the research; WY and MAV conducted the research; GOL-D, WY and MAV analysed data; GOL-D wrote the paper; GOL-D and WY had primary responsibility for final content. All authors read, contributed to and approved the final manuscript.

Table 1: Mineral concentrations in insects, sirloin and whole-wheat samples (mg/100 g) dry weight[a]

	Fe	Ca	Cu	Mg	Mn	Zn
Grasshopper	6.00±1.41	43.47±8.30	3.61±0.79	71.00±13.16	0.62±0.13	17.03±3.41
Cricket	12.91±0.12	155.82±2.37	3.18±0.45	91.74±0.92	4.79±0.06	32.11±0.48
Mealworms	7.04±0.15	97.59±1.83	1.80±0.17	224.19±3.11	1.09±0.01	17.86±0.08
Buffalo worms	6.58±0.32	51.64±2.44	2.61±0.16	138.01±7.52	0.94±0.04	19.10±0.95
Sirloin Beef	15.47±2.93	126.13±8.95	2.02±0.16	434.18±30.23	13.83±1.00	14.50±1.00
Whole-wheat flour	8.78±1.78	81.35±46.41	0.92±0.65	112.54±21.89	0.07±0.01	23.62±4.85

[a] Values are means ± SEM (n = 5).

Table 2: Mineral solubility from insects, sirloin and whole-wheat samples (mg/100 g)[a]

	Ca	Cu	Mg	Mn	Zn
Grasshopper	40.01±0.39	1.5±0.09	70.28±1.03	0.30±0.02	4.81±0.16
Cricket	75.62±13.55	1.03±0.07	54.84±8.97	0.53±0.23	4.24±0.37
Mealworms	72.61±5.89	1.10±0.11	196.04±21.16	0.41±0.04	3.47±0.73
Buffalo worms	20.36±2.25	0.69±0.12	53.95±14.49	0.02±0.01	0.87±0.21
Sirloin Beef	33.59±8.92	ND*	50.47±30.45	0.02±0.01	1.23±0.63
Whole-wheat flour	6.03±2.81	0.61±0.03	54.92±11.68	0.03±0.01	1.10±1.79

[a] Values are means ± SEM (n = 4). * ND: not detectable

Table 3: Mineral solubility from insects and sirloin in a 1:1 mixture with whole-wheat flour (mg/100 g)[a].

	Ca	Cu	Mg	Mn	Zn
Grasshopper	11.53±1.47	2.10±0.32	32.46±4.62	0.03±0	0.46±0.02
Cricket	10.62±1.17	1.10±0.14	54.49±5.21	0.05±0.01	0.37±0.03
Mealworms	19.85±1.34	1.31±0.11	71.16±7.24	0.06±0.01	0.45±0.02
Buffalo worms	14.18±3.39	0.67±0.02	95.29±5.73	0.11±0.02	0.20±0.01
Sirloin Beef	20.63±6.67	0.35±0.17	54.27±15.14	0.05±0.02	0.22±0.03

[a] Values are means ± SEM (n = 4).

Figure Legends

Figure 1. Iron solubility from grasshopper, cricket, mealworms, buffalo worms, sirloin beef and durum whole-wheat flour samples. Values are means ± SE (n = 4). Iron solubility from grasshoppers (P<0.001), cricket (P<00.1), mealworms (P<0.05) and whole wheat flour (P<0.05) are significantly different from the sirloin.

Figure 2. Iron solubility from grasshopper (A), cricket (B), mealworms (C), buffalo worms (D), sirloin beef (E) in a 1:1 mixture with durum whole-wheat flour. Values are means ± SE (n = 4). Significant differences are seen with the following comparisons AvB, (P<0.00), AvC, (P<0.05), AvD (P<0.001), BvC (P<0.01), BvE (P<0.001), CvE (P<0.05), D vE (P<0.05) only.

Figure 3. Iron bioavailability from grasshopper, cricket, mealworms, buffalo worms, sirloin beef, durum whole-wheat flour and $FeSO_4$ samples expressed as ferritin synthesis in Caco-2 cells. Data are means ± SE, (n=4). Iron bioaccessibility was significantly different between the sirloin and cricket (<0.05) and Whole wheat four (P<0.01) only.

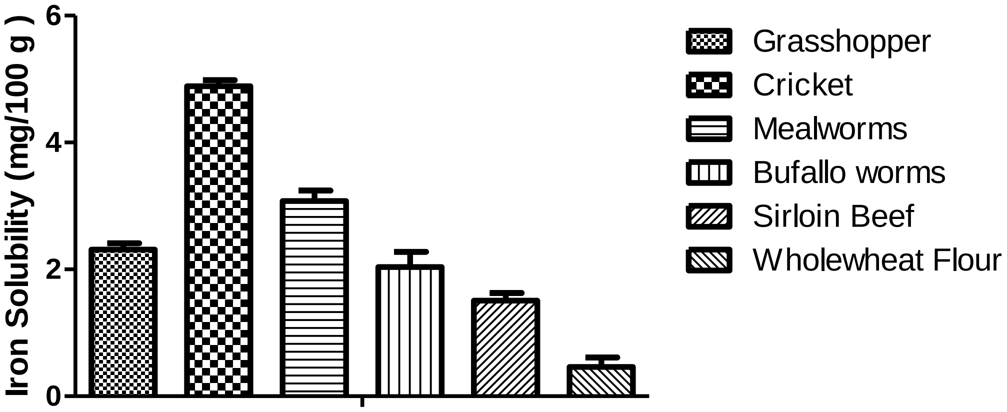
Iron Solubility (mg/100 g)
0
2
4
6
Grasshopper
Cricket
Mealworms
Bufallo worms
Sirloin Beef
Wholewheat Flour

Figure 1

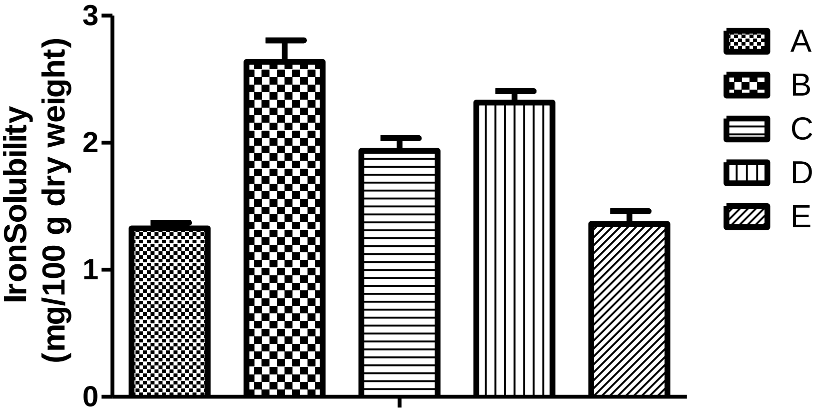

Figure 2

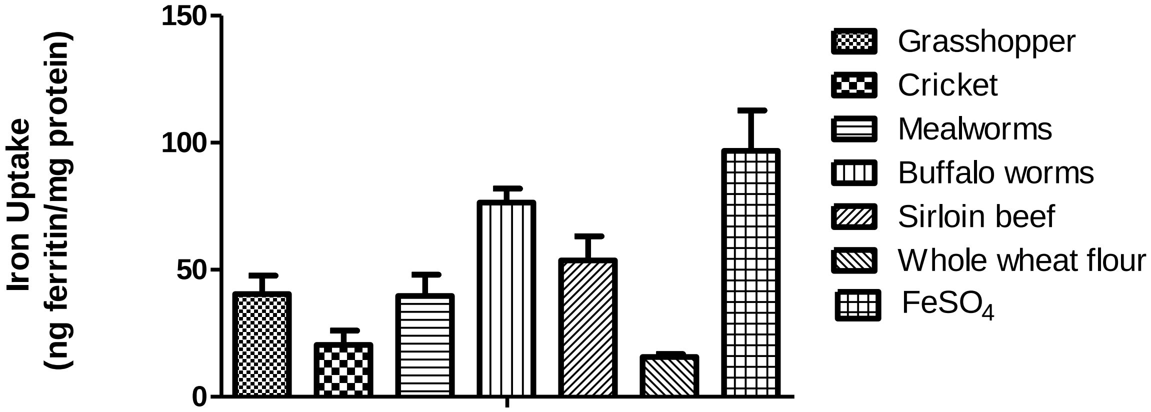
Iron Uptake
(ng ferritin/mg protein)
150
100
50
0
Grasshopper
Cricket
Mealworms
Buffalo worms
Sirloin beef
Whole wheat flour
FeSO4

Figure 3

TOC Graphic