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Research Article

Protein, Carbohydrate and Free Fatty Acid Profiles in *Ascaris suum* (Nematoda) Infecting Wild Pig *Potamochoerus africanus* (Suisdae) with Record of Heavy Metals Contents

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ABSTRACT

Ascaris suum is a widespread vertebrates' helminthes parasite, including humans. There is little study on *A. suum* in wild vertebrates, it has become necessary to evaluate the nutrients profile they accumulate from hosts and its ability to uptake heavy metals from their hosts feeds. The intestinal contents of matured *Potamochoerus africanus* were bought from various markets from 2023 to 2025. In 2023, nine, 2024 seven whereas in 2025 twelve intestines were bought and 2(15 parasite samples), 3(36 parasite samples) and 4(29 parasite samples) of the samples were infected with large matured *A. suum* species respectively. This recorded a high prevalence of 22.2%, 42.85 % and 32.10 % respectively. Fifteen of these samples were pooled into five sample sets for proximate composition, amino acid, carbohydrate and free fatty acid profiles using standard methods. The results show that *A. suum* is characterized by high moisture and carbohydrate content, low lipid reserves, and modest protein levels. The findings provide baseline biochemical data for *A. suum* in wild pigs. Also, histological assessment of the worm showed classical features of *A. suum* while thirteen were analyzed using (AAS) mg/kg. The results show varying levels of heavy metal accumulation with Zn (1.3877 ± 0.0792) as highest level whereas Ni (0.0000 ± 0.0000), and V (0.0000 ± 0.0000) were not detected. Also, the presence of Hg (0.0110 ± 0.0008) is rather worrisome, highlighting potential environmental contamination and risks to both wild and domesticated swine health and food safety.

Keywords: *Ascaris suum;* Heavy metals; Histological features; Nutrient profiles; *Potamochoerus africanus*

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INTRODUCTION

Phacochoerus africanus are hardy, and ubiquitous across savannah. In West Africa they can be found in Ivory Coast, Ghana, Benin, Nigeria, Cameroon, and Central Africa Republic. With their wide distribution and diurnal habits especially their unique vocals of an energetic representative, warthogs are the most famous of all of Africa's suids (AG, 2025). In Southeastern parts of Nigeria *P. africanus* are meat of choice during funerals and traditional masquerade rites. Although, *Ascaris suum* affects *P. africanus*, its transmission also affects other vertebrates such as *Rattus rattus* other than swine. It occurs through ingestion of infective eggs that remain viable in soil or feed for prolonged periods (Echi, *et al*., 2025). During larval migration through the host liver and lungs, pigs develop pathological lesions, including the formation of "milk spots" on the liver, which often leads to condemnation of organs at slaughter and increased economic losses (Stewart and Hale, 2017).

Parasites are having rising focus interests from ecological parasitology as bio-indicators for the assessment of environmental quality. This is because parasites respond in different ways to environmental hazards (Sures *et al.,* 1999).

Ascaris suum just like other visceral metazoan parasites obtain nutrients such as glucose, and amino acids by both facilitated diffusion and active transport from their hosts. These parasites do not depend on stored food of their hosts; the nutrients they can obtain from their hosts are sustainable as long as they stay in the lumen of their hosts.

Parasitic helminth nutrition is a heterotrophic nutrition as they obtain required nutrients from their hosts. A major part of energy source utilized by the parasite is from carbohydrates, the percentage and location of carbohydrates in the host, where the environment is rich for nourishment, normal development and reproduction of the parasite is accounted in the host diet, whereas amino acids and fatty acids are involved in the synthesis of macromolecules and egg production. Carbohydrates are very important food component due to its chief energy source in animal body. Essential nutrient component for all metabolic activities in animal tissues relies on glycogen. It is the most important agents for expression of the genetic material and this informs its abundance in all portions of cell structure and function of living cells (Sonune, 2014). Glycogen and trehalose appear to be the principal endogenous carbohydrates in acanthocephalans; however, their tissues are rich in glycogen. In acanthocephalans that affect rats the *Moniliformis dubius*, glycogen has been determined quantitatively and comparatively between worms recovered from male and female rats where male worms contained more glycogen per unit weight than the female worms (Laurie, 1959). The lumen-dwelling helminths, occupy environments where glucose availability is intermittent and oxygen tensions are low, rely on stored glycogen for energy and have modified their metabolic pathways to increase energy generation in the absence of oxygen e.g. adult *Hymenolepis diminuta, Haemonchus contortus* and *Ascaris suum.* Mitochondria from these organisms use unsaturated organic acids as well as oxygen as terminal electron acceptors, and cyanide-insensitive electron-transport-associated ATP synthesis is coupled to the excretion of reduced organic acids as end-products of carbohydrate metabolism (Monks and Richardson, 2011).

Generally, nematodesin vertebrates have not been known to show themselves as a good bio-indicator for heavy metals. For instance, the lack of appreciable heavy metal accumulation In *A. suum* is reliable with results for the nematode *Anguillicola crassus* in fish (Suresh *et al.,* 1998).

The disease of ascaris is as a result of the infection with the huge roundworm *Ascaris sp.* In pigs, *Ascaris suum* infects immeasurable pigs in the world, besides, poor hygiene and sanitation that may engender their infection in humans; the infection is common in organic and extensive farming systems (Leles *et al.,* 2012; Peng and Criscione, 2012). Infections in pigs are related to production losses due to poor growth and low feed conversion efficiency, with livers readily unfit for human consumption (Peng and Criscione, 2012). The need exists that Ascarid large worms of wild pigs be studied for heavy metals contents since the worms could affect both humans and pigs - domestic and wild.

MATERIALS AND METHODS

The intestinal contents of matured *Potamochoerus africanus* were bought from various markets in the Southeast from 2023 to 2025. The purchase localities of the samples had been listed in (Echi *et al.,* 2013). The samples were cleared of host's debris using glycerol and stored in 70 % ethanol prior to further analysis. Fifteen of these 29 samples were pooled into five sample sets (three worms per set) for proximate composition, amino acid, carbohydrate and free fatty acid profiles using standard methods.

Some were used for histological features of the parasite while thirteen of these 29 samples were analyzed using digestion and atomic absorption spectrometry (AAS) mg/kg for concentrations of cadmium (Cd), cobalt (Co), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), vanadium (V), copper (Cu), and zinc (Zn). The samples were burnt to ashes in a muffle furnace, after which 1g of it was placed in a beaker 10ml Aqua Regia: (Measure 75ml of Conc. HCl and 25ml conc. HNO3 into 100ml Volumetric Flask, 3:1 was added. Then, it was stirred to dissolve completely using a glass stirring rod. The solution was then cooled and filtered into a 100ml flask and further diluted to mark with distilled water (Radojevic and Bashkin, 2004), while Atomic Absorption

Spectrophotometer (AAS) mg/kg was used to read off the metal level at a particular wavelength (Lenntech, 2012).

The histological section analysis of some samples followed after fixation 10 % formalin – saline solution for days prior to paraffin processing: the fixed tissue was transferred through graded series of alcohol - 70 %, 80 %, 90 %, absolute 1 and 2 solutions for one and half hours in each case to dehydrate the tissues. The alcohol saturated tissue was then transferred to chloroform which cleared the tissue overnight. The chloroform was miscible with both the alcohol and the paraffin wax and also raised the refractive index of the tissue, imparting to it a transparent appearance. Then the tissue was embedded in molten paraffin wax, until the tissue was sufficiently impregnated with the wax, the tissue was embedded in fresh wax which solidified on cooling. The tissue was then sectioned at 5 – 6 microns thick, using microtome and stained using hematoxylin eosin stain. The sections were placed on glass slides and viewed and photographed under microscope at X 100 and X 400 objective lenses (Drury *et al.,* 1967; Echi *et al.,* 2014; Echi and Nnamdi, 2025).

Free fatty acids were extracted from each pooled sample and quantified by spectrophotometric assays. In one established procedure, unsaturated fatty acids can be chemically isomerized and then measured by UV. In practice, we used a commercial enzymatic/colorimetric assay kit (AOAC-approved) that detects free fatty acids (including linoleic, linolenic, palmitic, stearic, and oleic acids) via colorimetric reagent. The kit relies on enzymes to convert fatty acids to a colored product measured at a specific wavelength. This approach (analogous to the "FASafe" or similar test kits) provides rapid FFA quantification and is endorsed as an alternative to classical titration. All assays were performed in triplicate for each sample set, and concentrations were calculated using standard curves for each fatty acid.

Amino acid content of the worms was determined by chromatographic methods following acid hydrolysis. Briefly, parasite tissue was acid-hydrolyzed (e.g. 6 M HCl, 110 °C, 24 h) to release constituent amino acids. The hydrolysate was then analyzed by high-performance liquid chromatography (HPLC) and gas chromatography (GC) for amino acid separation and quantification. For example, Kar et al. used HPLC to profile the free amino acids of the trematode Fasciolopsis buski, identifying essential amino acids such as lysine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine and others. We followed similar protocols: amino acids were derivatized (e.g. with o-phthalaldehyde or PITC) and run on an HPLC column with UV/fluorescence detection, while GC (with appropriate derivatization) was used for volatile amino acids. These methods are in line with AOAC guidelines: HPLC (ion-exchange or reverse-phase) and GC–MS are established techniques for amino acid analysis, and AOAC has validated methods for many amino acids. Measured amino acids (including lysine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, etc.) were quantified against calibration standards and reported as µmol/g dry weight.

Total carbohydrate levels were measured by colorimetric (spectrophotometric) assay. In this study, we applied the anthrone–sulfuric acid method: samples were hydrolyzed with acid (e.g. HCl), reacted with anthrone reagent, and absorbance was read at 620 nm. This classical assay relies on the reaction of carbohydrate-derived furfural with anthrone, producing a blue-green complex that is quantifiable by spectrophotometry. A glucose standard curve was used to convert absorbance to carbohydrate concentration. This anthrone method is well-established for total sugar determination; for example, the anthrone test (AOAC 2000) has been widely used to estimate carbohydrates in biological samples. Carbohydrate content was expressed on a dry-weight basis (mg glucose equivalents per gram of sample).

Basic proximate analyses were carried out according to standard AOAC methods. Moisture content was determined by oven-drying a known weight of sample at 105 °C to constant weight, and dry matter was calculated as 100% minus moisture. Crude protein was measured by Kjeldahl nitrogen determination (digestion, distillation, and titration) with a conversion factor of 6.25. Crude fiber was determined by sequential acid and alkali digestion (Weende method). Ether extract (crude lipid) was measured by Soxhlet extraction with petroleum ether. Ash content was determined by incinerating a weighed sample in a muffle furnace at

550 °C. Nitrogen-free extract (available carbohydrate) was calculated by difference: 100% minus (moisture + crude protein + crude fat + crude fiber + ash). These proximate parameters (moisture, dry matter, crude protein, crude fiber, ether extract, ash, nitrogen-free extract) encompass the "Weende" analysis and are exactly the composition factors defined in AOAC methods. All proximate assays were performed in triplicate for each sample set, and results were reported on a dry-weight basis.

RESULTS

The intestinal contents of matured *Potamochoerus africanus* were examined from 2023 to 2025. In 2023, nine, 2024 seven whereas in 2025 twelve intestines resulting in 2(15 parasite samples), 3(36 parasite samples) and 4(29 parasite samples infections) respectively. This recorded a high prevalence of 22.2%, 42.85 % and 32.10 % respectively, which affirms the common occurrence of *A. suum* in the tropics.

The histological sections of the parasite, revealed the classical features of *A. suum*: a thick multilayered cuticle, hypodermis, radial muscle bands, and large reproductive structures, particularly in mid and posterior sections. The abundance of reproductive tissues explains the parasite's high fecundity and capacity for environmental contamination (Plate 1).

The concentrations of heavy metals detected in the *A. suum* make them the most hazardous to human health due to risks of carcinogenicity, neurotoxicity, and kidney damage. Lead occurs within the permissible range but close to the dangerous threshold. Copper, Zinc, Cobalt are within safe limits, while Nikel and Vanadium are not detected. Mercury is rarely detected in *Ascaris suum*, although this present detection is low it is still very dangerous and extremely toxic (Fig 1).

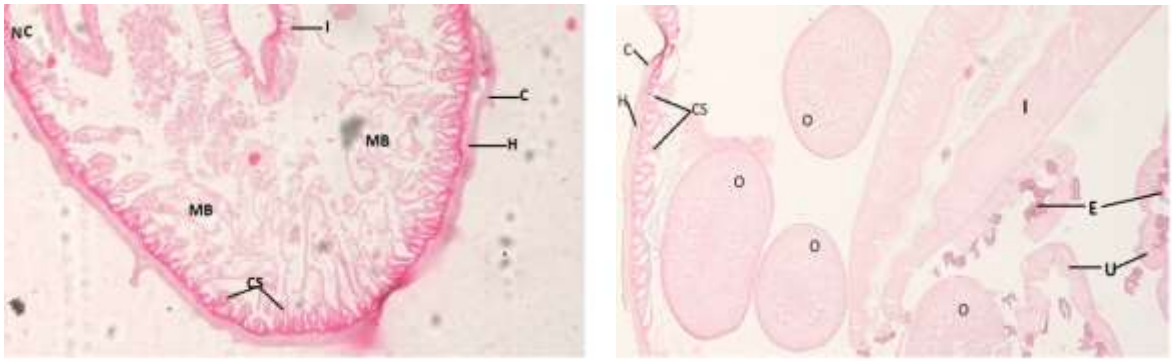

Plate 1: The histological sections of the anterior and posterior parts of *A. suum* , showing the classical features of *A. suum*

C – cuticle, H – hypodermis, O – ovary, E – eggs, U – uterus, CS – contractile spindle, N – nucleus
R – rachis, OW – ovarian wall, OO – oocyte, MB – muscle bags, I – intestine, NC – nerve cell.

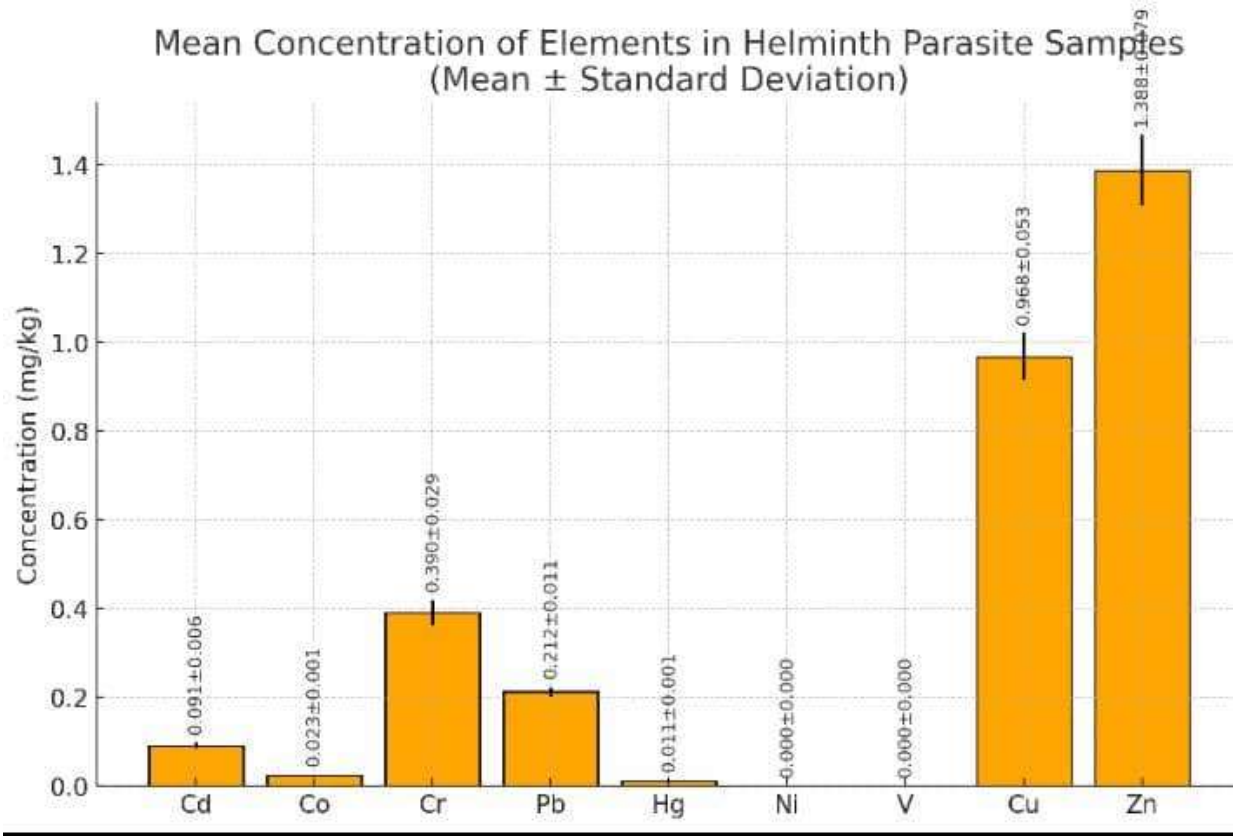

Figure 1: Heavy metals contents in helminthes – *Ascaris suum* recovered from the intestinal contests of matured *Potamochoerus africanus*

The carbohydrate levels measured by colorimetric (spectrophotometric) assay, show that the carbohydrate levels in the five sets of samples had very high contents ≥ 7.19. These high contents are indicative of over dependence of the parasites on the host for carbohydrate.

The amino acid content of the worms determined by chromatographic methods shows that Lysine, histidine, methionine and Isoleusine had a relatively ≤ 0.15 µmol/g dry weight. Whereas Arginine, Threonine, Valine, Phenylalanine and Leucine had values ≤ 0.05 µmol/g dry weight. However, after the values were subjected to ANOVA Analysis, the ANOVA F – Values indicate the following order of concentration: Lysine> histidine> Arginine> Threonine> Leucine> Phenylalanine> Isoleusine> methionine> Valine. The worms contain all essential residues; histidine, lysine, and methionine were among the most abundant (histidine ≈0.14–0.16 g/100 g; lysine ≈0.12–0.15 g/100 g; methionine ≈0.13–0.17 g/100 g), whereas other essential amino acids occurred at moderate concentrations (Fig. 2).

After the basic proximate analyses were carried out using standard AOAC methods, the moisture contents had highest percentage ≥ 83.00; the dry had values of ≥ 16.00, followed by the nitrogen and carbohydrate percentages of ≥ 7.00. The ash/mineral and crude protein had values ≥ 3.30 and ≥5.00 respectively. On a wet-weight basis, mean protein was approximately 7–8%, fat <0.2%, crude fiber ~2–3%, and ash ~1%, with the remaining fraction consisting primarily of carbohydrates and water. The differences in the parasites' proximate composition—including moisture, dry matter, crude protein, ether extract, crude fiber, and nitrogen-free extract—were all statistically significant ($p<0.05$) (Table 1).

After the Free fatty acids were extracted from each pooled sample and quantified by spectrophotometric assays and a commercial enzymatic/colorimetric assay kit (AOAC-approved) that detects via colorimetric reagent were analyzed. The free fatty acids - linoleic, linolenic, palmitic, stearic, and oleic acids values show that values of palmitic was highest ≥ 0.2, linoleic, and linolenic had values ≥ 0.13 while

stearic acid had the lowest values of ≤ 0.10. in other words, Palmitic acid (C16:0) was the predominant fatty acid (≈0.20–0.24 g per 100 g tissue), with oleic (C18:1) and linoleic (C18:2) acids present at moderate levels (~0.11–0.19 g/100 g). Stearic acid (C18:0) was the least abundant measured fatty acid (~0.07–0.09 g/100 g) (Table 2).

The adult *A. suum* rely primarily on host-derived carbohydrates, lipids and essential amino acids, consistent with a parasitic lifestyle of continual nutrient uptake.

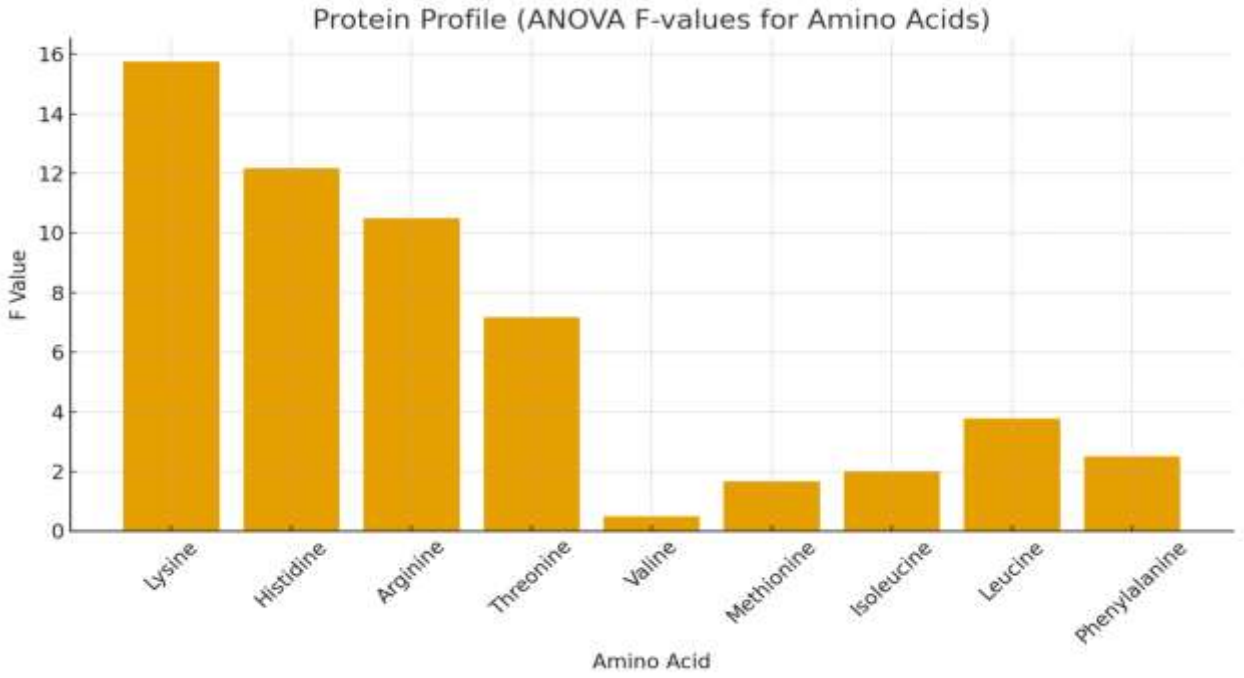

Figure 2: Protein Profile of *A. suum* result after ANOVA analysis

Table 1: Shows proximate analysis of *A. suum* recovered from *P. africanus*

Sample Code	Dry Matter (%)	Moisture (%)	Ash/Mineral (%)	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Nitrogen Free Extract (%)	Carbohydrate (%)
Sample 1 a	16.11	83.89	3.31	5.05	0.61	0.16	7.03	7.19
b	16.11	83.89	3.30	5.00	0.61	0.17	7.03	7.20
c	16.10	83.90	3.30	5.00	0.60	0.16	7.04	7.20
Sample 2 a	16.33	83.67	3.30	5.00	0.54	0.14	7.35	7.49
b	16.31	83.69	3.32	5.00	0.55	0.14	7.33	7.47
c	16.33	83.67	3.30	5.05	0.53	0.15	7.30	7.45
Sample 3 a	16.96	83.04	3.32	5.00	0.50	0.18	7.96	8.14
b	16.96	83.04	3.31	5.05	0.51	0.17	7.92	8.09
c	16.95	83.05	3.30	5.05	0.52	0.18	7.90	8.08
Sample 4 a	17.02	82.98	3.31	5.25	0.58	0.16	7.72	7.88
b	17.03	82.97	3.30	5.25	0.58	0.15	7.75	7.90
c	17.04	82.96	3.32	5.20	0.58	0.16	7.78	7.94
Sample 5 a	16.55	83.45	3.30	5.00	0.50	0.16	7.59	7.75
b	16.55	83.45	3.31	5.05	0.52	0.16	7.51	7.67
c	16.54	83.46	3.30	5.00	0.53	0.17	7.54	7.71

Table 2: Free fatty acid profile of *A. suum* recovered from *P. africanus*

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Linoleic Acid	0.19	0.15	0.18	0.19	0.15
(%)	0.19	0.15	0.17	0.18	0.16
	0.18	0.14	0.18	0.19	0.15
Linoleic Acid	0.14	0.14	0.14	0.15	0.14
(%)	0.13	0.13	0.14	0.15	0.13
	0.13	0.14	0.13	0.14	0.14
Palmitic Acid	0.23	0.20	0.23	0.23	0.21
(%)	0.22	0.20	0.23	0.24	0.20
	0.24	0.21	0.22	0.22	0.21
Stearic Acid	0.08	0.08	0.10	0.08	0.09
(%)	0.09	0.08	0.11	0.07	0.08
	0.08	0.09	0.10	0.08	0.09
Oleic Acid	0.14	0.14	0.13	0.11	0.10
(%)	0.13	0.13	0.13	0.10	0.10
	0.14	0.14	0.14	0.11	0.11

DISCUSSION

There are differences in the overall nutritional requirements among helminthes species. Similarly, the carbohydrate profiling also revealed a highly significant difference among the samples. The fatty acid analysis showed that while linolenic acid levels were consistent, the levels of linoleic, palmitic, stearic, and oleic acids varied significantly. This is particularly interesting as it points to potential differences in lipid metabolism or dietary intake of the worms. The amino acid profiling showed that lysine, histidine, arginine, threonine and leucine are required highly in A. suum for its metabolic needs. The hosts that harbor these worms are sapped of these amino acids at approximately higher levels than other amino acids whereas valine is required least.

The histological sections of the parasite itself revealed the classical features of *A. suum*: a thick multilayered cuticle, hypodermis, radial muscle bands, and large reproductive structures, particularly in mid and posterior sections. The abundance of reproductive tissues explains the parasite's high fecundity and capacity for environmental contamination (Crompton, 2001; Dold and Holland, 2011). High magnification fields also displayed vacuolations and possible degenerative changes, which may reflect stress induced by environmental contaminants. This observation supports earlier evidence that heavy metals can accumulate in helminths and modify their morphology or physiology (Stuart *et al*., 2016).

Heavy metal analysis using acid digestion and AAS detected measurable concentrations of cadmium, lead, zinc, and copper in parasite and tissue samples. Zinc and copper were the most abundant, which may reflect their routine supplementation in pig feed (EFSA, 2020). Conversely, cadmium and lead, though detected at lower concentrations, are of greater concern due to their toxicity and lack of biological role. Studies have shown that cadmium accumulates in the liver and kidney, impairing detoxification pathways, while lead exposure can interfere with neurological and hematological functions (FAO/WHO, 2019). The heavy metals found in these nematodes indicate that contamination occurred in perhaps other African environmental causes because *P. africanus* straddles across other African countries where such contamination could occur (AG, 2025). It is a documented fact that soils in Southeast, Nigeria contain safe levels of heavy metals. This is important because the rarity of non-migratory species such as *Hemisus guttatus* has not been linked to the presence of heavy metals and migratory animals such as *Milvus migrans parasitus* that visit the southeast areas seasonally perhaps accumulate such metals from environments other than Southeast environments (Echi *et al*., 2025).

The identification of *Ascaris suum* in *Rattus rattus* is noteworthy, highlighting the adaptability of certain

parasites to a range of hosts. *Ascaris suum*, a roundworm commonly found in pigs, is typically associated with domestic animals, but its presence in *Rattus rattus* emphasizes the potential for these parasites to exploit diverse hosts (Siddique *et al.,* 2015; Echi *et al.,* 2025). The presence of *Ascaris suum* in *Rattus rattus* suggests the possibility of these rodents participating in the transmission cycle of the parasite between wildlife, domestic animals, and potentially humans. This raises intriguing questions about the ecological dynamics of parasite transmission in environments where different host species coexist. The adaptability of *Ascaris suum* to infect both pigs and rodents challenges traditional host-parasite associations and prompts a reevaluation of the role of rodents in the epidemiology of this parasite (Siddique *et al.,* 2015). The ecological dynamics of *Ascaris suum* in *Rattus rattus* become particularly relevant in shared environments where wildlife, domestic animals, and humans interact. Understanding these dynamics contributes valuable insights into the complex interactions between different host species and the potential risks posed to human health. The ecological study of *Ascaris suum* in *Rattus rattus* becomes crucial for comprehending the intricate web of transmission routes and potential disease pathways in these shared ecosystems (South, 2012). In line with previous studies, the presence of parasites in *Rattus rattus* can have profound implications for both the health of the host and the ecosystems they inhabit. These parasites can compromise the overall well-being of the rats, leading to reduced reproductive success and survival, as observed with ectoparasites like fleas (Rae *et al.,* 2005). Additionally, endoparasites can lead to a range of health issues, including gastrointestinal problems and nutrient absorption difficulties, potentially affecting the growth and longevity of the host (Rae *et al.,* 2023).

Parasitic infection and heavy metals act synergistically to damage host tissue. For example, hepatic lesions from larval migration may worsen cadmium-induced hepatotoxicity, while renal inflammation may reduce the ability to excrete toxicants, leading to cumulative effects. This dual burden highlights that *A. suum* not only threatens pig health directly but also reflects environmental risks. Economically, parasitic liver lesions alone can lead to condemnation of up to 60% of pig livers at slaughter (Mateus *et al.,* 2015). When combined with the risks of heavy metal residues exceeding permissible limits, entire carcasses may be rejected, amplifying losses for farmers.

The zoonotic potential of *A. suum* and the toxicity of heavy metals represent a serious One Health concern, as humans may be exposed to both parasitic infections and chemical hazards through consumption of contaminated pork products (Nejsum *et al.,* 2012). Control of *A. suum* is challenging due to the extraordinary resistance of its eggs, which remain viable in soil for years (Katakam *et al*., 2016). Similarly, heavy metals are persistent environmental pollutants with no simple biological degradation. This persistence makes eradication impossible without integrated approaches. Measures such as strict hygiene, strategic deworming, feed quality control, and environmental monitoring are therefore essential for sustainable pig production both in the wild and domestication.

CONCLUSION

Ascaris suum remains an important helminthes parasite among vertebrates including humans other than the swine. This parasite affects the intestine of the affected vertebrates and saps the hosts of their metabolic needs. This makes the affected malnourished with other attendant pathologies. Also, their migration to various organs within the host induces mechanical damages, thereby paving the way for secondary infections. The migratory habit of the host - *Potamochoerus africanus* has shown that some African environments are replete with heavy metals contamination. This study affirms the fact that *A. suum* is a good environmental monitor as well as bio-indicator. Especially, as possible degenerative changes, which may reflect stress induced by environmental contaminants were detected. However, the detection of cadmium, Chromium and Mercury above permissible limits is a serious public health concern.

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