Journal of Chemistry and Allied Sciences
Volume 1, Issue 1 | 2025 | pp. 7~14
Original Research Article | ***Available online at*** **https://jcasjournal.org/**

COMPARATIVE ANALYSIS OF MACRO, MICRO AND TOXIC ELEMENTS IN COW BONES AND HORNS

Ita E. Uwidia[1*], Faith I. Akhidenor[1], Etinosa O. Oshodin[1]

[1]Department of Chemistry, Faculty of Physical Sciences, University of Benin, Edo State, Nigeria

Article History*: Received May 2025; Revised June 2025; Accepted June 2025; Published online July 2025*

Correspondent Author: *Dr. Ita E. Uwidia (ita.uwidia@uniben.edu; ORCID: https://orcid.org/0000-0002-7448-3549*
Tel: *+234 803 615 1318)*

Article Information

Citation: Uwidia, I. E., Akhidenor, F. I., & Oshodin, E. O. (2025). Comparative analysis of macro, micro and toxic elements in cow bones and horns. *Journal of Chemistry and Allied Sciences, 1*(1), 7~14.
DOI: https://doi.org/10.60787/jcas.vol1no1.38

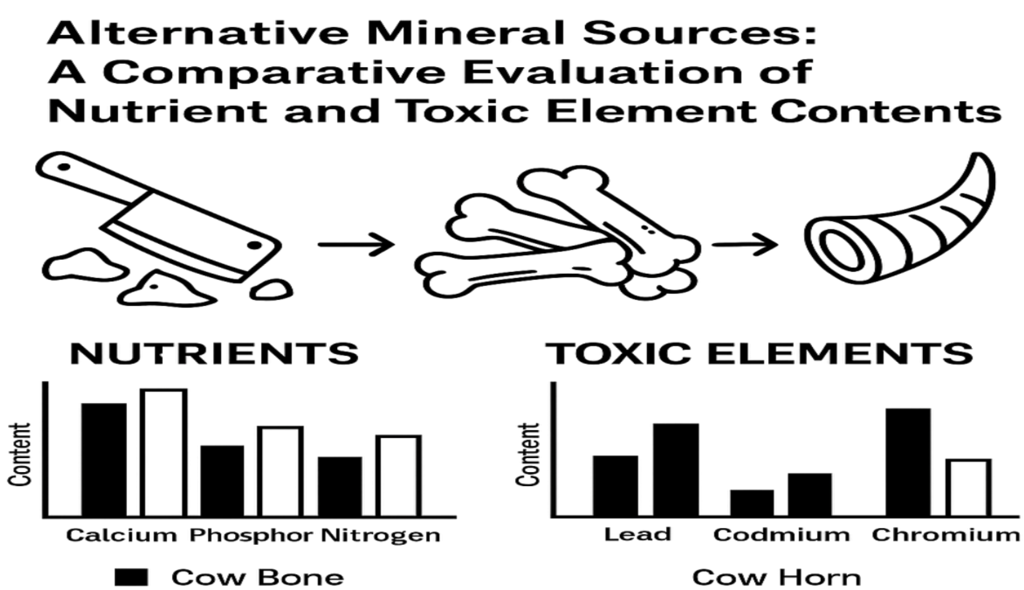
The Official Publication of the Tropical Research and Allied Network (TRANet), Department of Chemistry, *Federal University of Technology, Minna*

Abstract

Bones and horns from slaughtered cattle are often discarded as wastes, yet they contain vital minerals such as calcium and phosphorus. This study evaluates macro (Ca, P, N), micro (K, Mg, Zn, Cu), and toxic elements (Pb, Cd, Cr) in cow bones and horns to assess their potential for agricultural and nutraceutical use. One gram of each sample (except phosphorus, which was 0.5g) was digested. The digestion solutions were analysed using flame atomic absorption spectroscopy (Flame-AAS), flame photometry, and UV-visible spectroscopy. From the elemental analysis, the result revealed that cow bones had significantly higher Ca (5950 mg/kg) and P (3010 mg/kg) than horns ($p < 0.01$). Zinc was over 12 times higher in horns when compared to bones (1992 mg/kg vs. 154 mg/kg). Toxic elements (Pb, Cr, Cd) were detected in both samples and their concentration were above WHO/FAO limit, making these biomaterials suitable for most applications except human consumption. These findings support the sustainable reuse of cow bones and horns as nutrient-rich supplements, though heavy metal monitoring is critical.

Keywords*: Cow bones, Cow horns, Heavy metals, Nutrient recycling, Animal feed supplements, Slaughterhouse waste, Flame-AAS*

Graphical Abstract



1.0 INTRODUCTION

Bones and horns from slaughtered cattle, though traditionally discarded as wastes, contain vital minerals, particularly calcium and phosphorus which are essential for the development of bones, teeth, and eggshells. While these materials are usually disposed of as remains and wastes from slaughterhouses, they possess the potential they be used in the nutritional and agricultural processes. In areas such as West Sumatra, they perform everyday slaughtering which means that huge amounts of unusable bone substance will be disposed [1]. In Nigeria, more than 1870 tons of fresh cattle bones are produced every day, thus polluting the environment [2]. It is a hugely untapped source of macro and micro minerals that can be repurposed by recycling into livestock feed and biofertilizers, and it is a sustainable source of macro minerals.

The disorders that have been associated with elemental imbalances include osteoporosis and osteoarthritis in human beings and animals in the case of bones[3]. According to previous studies, the skeletal resiliency and strength are represented by the macro and trace elements such as magnesium and iron, respectively[4], [5].

The plant-based conventional poultry diets do not contain bioavailable phosphorus as it is present in the form of phytic acid, and this necessitates supplementation with inorganic phosphorus such as commercial dicalcium phosphate, which however is a costly and environmentally straining option because of the mining of non-renewable resources of phosphate rock [6]. Pollution can be reduced by substituting the use of these phosphate rocks with phosphorus from bone which helps in increasing the productivity of the animals.

Studies have shown that cow bone powder has high concentrations of iron, magnesium, carbohydrate and lipid content which could be used as a feed reinforcement in aquacultures despite its low moisture and protein content[7]. These characteristics are an indication of its potential to be a cost-effective, nutrient-rich additive. The existence of heavy metals like lead (Pb), mercury (Hg), and cadmium (Cd) may lead to population health hazards. Pb and Cd bioaccumulation in food chains necessitates stringent monitoring for feed and supplement applications and thus a strict toxicology evaluation is required before repurposing slaughterhouse wastes[3], [8]. Considering the increased pressure of minimizing environmental waste material, and maintaining the quality of feed throughout the livestock industry, the reuse of nutrient-rich byproducts of slaughter is a strategic solution.

Although most studies focus on bones alone; few compare horns or assess toxic elements for safe reuse, this study provides the first comparative analysis of macro, micro, and toxic elements in cow bones and horns from Benin City, Nigeria, with emphasis on nutraceutical and agricultural suitability. The study encompasses sampling and preparation of cow bone (ribcage and lower jaw) and matured cow horns samples in the determination of mineral qualification and quantification.

2.0 MATERIALS AND METHODS

2.1 Reagent and Instrumentation.

Reagents: The reagents used during the course of this study were of analytical grade and solutions were prepared using distilled water as a solvent. These are nitric acid, hydrochloric acid, potassium chloride, perchloric acid, Potassium dihydrogenphosphate, Phosphomolybdate Colorimetric Reagent, ascorbic acid, Kjeldahl catalyst, Sulphuric acid, alkaline sodium phenate solution, sodium potassium tartrate, and Sodium hypochlorite.

Standards: Certified reference materials (CRMs), such as NIST-traceable standards, were employed during instrument calibration.

Instrument Parameters: Flame Atomic Absorption Spectrophotometer (Model 210 VGP): Specific hollow cathode lamps and wavelengths: Ca (422.7 nm), Mg (285.2 nm), Zn (213.9 nm), Cu (324.8 nm), Pb (217.0 nm), Cr (357.9 nm) and Cd (228.8nm) were utilized. The system employed an air-acetylene flame, and the detection limits for each element were consistent with standard FAAS performance thresholds: Ca and Mg (0.02 mg/L), Zn and Cu (0.005 mg/L), and Pb (0.01 mg/L), Cr (0.2 mg/L), and Cd (0.005 mg/L). Flame photometer (Model 410 Sherwood) and UV/Vis Spectrophotometer, Model 721G UV-Vis Spec. were used.

2.2 Sample preparation.

The cow bones (ribcage bone and lower jaw) and matured horns were obtained from multiple waste sites from a local slaughterhouse located at Ikpoba slope, in Ikpoba Okha Local Government Area, Benin City, Edo state, Nigeria, between latitudes 6′2703 N and longitude 5°71’20′ E. The samples were cracked and washed several times in running water to remove traces of impurities and residual blood. Using a cutting knife, residual fats and tissues were removed. They were then chopped into smaller pieces, washed with water, and degrease water at 45°C. This was followed by rinsing with distilled water and sun drying for 3 days. The ambient humidity was not regulated throughout the drying period. The dried samples were ground to ≤150

  | DOI: https://doi.org/10.60787/jcas.vol1no1.38

µm using a mechanical grinder. The finely ground particles were used for the analysis.

2.2 Elemental analysis

2.2.1 Determination of Calcium, Magnesium and Heavy Metals

Using methods outlined by [9], [10] with some slight modifications, the concentration of calcium, magnesium, zinc, copper, chromium, lead and cadmium was determined and the digested sample was filtered using a whatman 1 filter paper into 100ml volumetric flask. Samples were digested in triplicate with 9 mL aqua regia (HNO_3:HCl, 1:3 v/v) at 120°C until fumes cleared. Calcium, magnesium and heavy metals were quantified via FAAS with calibration against NIST-certified standards (PerkinElmer 210 VGP; air-acetylene flame, λ = 422.7 nm for Ca, λ = 285.2 nm for Mg, λ = 213.9 nm for Zn, λ = 324.8 nm for Cu, λ = 217.0 nm for Pb, λ = 357.9 nm for Cr and λ = 228.8 nm for Cd and ther threshold for detection limit: Ca and Mg (0.02 mg/L), Zn and Cu (0.005 mg/L), and Pb (0.01 mg/L), Cr (0.2 mg/L), and Cd (0.005 mg/L). Recovery tests yielded 95% for all elements.

2.2.2 Total Potassium Concentration

According to methods highlighted by [9] with some slight modifications, the concentration of potassium in the bone and horn sample was determined.

Preparation of Potassium standards

0.477 g of potassium chloride was weighed and added to a 250 mL volumetric flask filled with 150 mL of distilled water. It was stirred until fully dissolved, after which the solution was diluted to the mark with more fresh water to get a standard stock solution of potassium. Using this stock, potassium concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm were made up. The standards were measured in flame photometer (Model 410 Sherwood) starting with the highest concentration, 100 ppm and a standard calibration curve was plotted.

Determination of Potassium concentration in Bone and Horn Sample

Samples were digested in triplicates with 9 mL aqua regia (HNO_3:HCl, 1:3 v/v) at 120°C until fumes cleared. The digested sample was filtered using a Whatman 1 filter paper into 100ml volumetric flask and made up to mark with distilled water. The absorbance of Potassium in the samples was determined using Flame photometer (Model 410 Sherwood) and the concentration of the sample was calculated in relation to the standard calibration curve.

2.2.3 Phosphorus Determination

According to methods highlighted by [11] with some slight modifications, the concentration of potassium in the bone and horn sample was determined.

Digestion Procedure for Phosphorus Determination

To 0.5g of the sample, 5ml of 65% nitric acid was added and then the mixture was boiled gently for 30minutes. After cooling, 2.5ml of 70% $HClO_4$ was added and the mixture was boiled gently until dense white fumes appeared. The mixture was then allowed to cool and 10ml of distilled water was added, followed by further boiling until the fumes were totally released and the solution became pale yellow. The solution was then made up to 100ml with distilled water.

Determination of Phosphorus concentration in Bone and Horn Sample

200ml of Phosphomolybdate Colorimetric Reagent was measured into a beaker and 1.056g of ascorbic acid was added and stirred to dissolve. The mixture was than label Reagent B. 1ml of the digested sample filtrate was pipetted into a test-tube and diluted with 9ml of distilled water and 4ml of reagent B was added, well shaken and allowed to rest for the colour to develop. The colored solution was well shaken and the absorbance value was determined at 660nm after 15minutes using UV/Vis Spectrophotometer, Model 721G UV-Vis Spec. The working standard was treated the same way as the digested sample filtrate and standard calibration curve was plotted.

2.2.4 Nitrogen Determination

The concentration of nitrogen in the matured bone and horn sample was determined using methods highlighted by [12] with some slight modifications.

Digestion Procedure for Nitrogen Determination

1.0g of the sample and 1gm of Kjeldahl catalyst was weighed into a digestion flask, and 10ml of concentrated H_2SO_4 was added and heated on a sand bath heating system till copious fumes were given off completely and the liquid became pale yellow. It was then cooled, diluted, filtered into 100ml volumetric flask, made up to mark with distilled water.

Determination of Nitrogen concentration in Bone and Horn Sample

5ml of the sample filtrate was pipetted from the digest into a test-tube. 9ml of distilled water was added and well shaken. 2.5ml of alkaline sodium phenate solution was added and well shaken. 1ml of sodium potassium tartrate was then added and well shaken. 2.5ml of sodium hypochlorite was added and well shaken by hand and the mixture was allowed to rest for 15minutes for color development then read using a UV/Vis Spectrophotometer, Model 721G UV-Vis Spec at 630nm. The blank were treated the same way as the sample filtrate and standard calibration curve was plotted by reading absorbance value against concentration (ppm).

2.3 Statistical Analysis

The values obtained from the analysis were of triplicate determinations. Data analysis was performed using IBM SPSS Statistics version 31.0 for Windows. The results was expressed as the mean ± standard deviation (SD). A one-way ANOVA was conducted followed by either the Student-Newman-Keuls (SNK) post hoc test. Statistical significance was set at ($p < 0.05$). Student Newman Keuls test was used instead of others poc hoc test because the SNK test is more sensitive in detecting differences between the mean values of different groups of data, particularly in datasets where differences are expected to be gradual. Albeit being a little less conservative, SNK provides higher resolution that will facilitate exploratory analysis of natural variation.

3.0 RESULTS AND DISCUSSION

3.1 Macro-Elements

The result for analysis of macro-element, such calcium, phosphorus and Nitrogen present in the cow bone and matured horn are presented in Table 1 The analysis revealed that Cow bones had 2.1 times the concentration of Calcium (5950 vs. 2840 mg/kg; $p< 0.01$) and 1.5 times the concentration of Phosphorus (3010 vs. 2050 mg/kg; $p<0.001$) than horn. Ca/P ratio in bones of the ratio of 5950/3010 mg/kg corresponds well to the fundamental functions of the body organs and tissues of body parts as bones are principally mineral warehouses, mainly calcium and phosphorus. Bones consist mostly of crystals of hydroxyapatite deposited in collagen matrices which makes them a rich source of calcium and phosphorus [13], [14]. Horns have minimal mineral content but a high level of sulfur-containing amino acids like cysteine and methionine, which accounts for the nitrogen value comparable to that of bones[15]. These macro-element results indicate there is great potential of bone-derived mineral supplementation to replacement of mined phosphate minerals used as nutraceuticals for human consumption and animal feed and fertilizers, which are costly and environmentally challenging.

Studies have shown that although phosphorus mining which is a process linked to strip mining and chemical leaching, has major ecological costs, valorization of these bone and horn samples neutralizes these issues and allows the final closure of the nutrient loop [16], [17]. Likewise, nitrogen-rich horn materials could be integrated into organic fertilizers or bioplastics, reducing synthetic polymer dependence while enhancing soil nitrogen availability[18]. [1] reported that bone meal (ribs) contained 27920mg/kg of Ca and 360mg/kg of P whereas [19] reported cow horn contains 80100mg/kg of Ca. The lower Ca/P concentration in cow bones and horn observed may be due to the geographical location of the sample sorted. Local soil mineral profile directly affect the plants animal consume and in Benin City, Nigeria, tropical soil suffers from leeching due to heavy rainfall reducing available-minerals.

Table 1: Results of macro-elements obtained from the analysis

Macro-Element	Bone Sample (mg/kg)	Horn Sample (mg/kg)
Calcium (Ca)	5950 ± 59.03	2840 ± 54.51
Phosphorus (P)	3010 ± 36.06	2050 ± 41.07
Nitrogen (N)	1930 ± 61.80	1990 ± 39.05

Value: Mean of triplicate determination ± Standard Deviation

3.2 Micro-Element

The findings of the micro-element including potassium, magnesium, zinc and copper were reported on Table 2. As Table 2 shows, the potassium and magnesium levels in bones (691 mg/kg) and horn (721 mg/kg) were very comparable but also the level of trace elements such as zinc and copper were significantly higher in the horns than in the bones. Zinc was also enriched in horns (1992 mg/kg) compared to 154 mg/kg in bones which is a 12 times difference. The result revealed that, copper was concentrated almost twice as much in horns sample compared to the bones sample (1.998 mg/kg compared to 0.998 mg/kg). Zinc is also utilized in enzymatic processes, immune regulation, and sustainable skin. Horns' keratin matrix explains their high zinc and copper concentration [15], [20] [16], [21]. Although not as pronounced, potassium and magnesium are vital in osmotic adjustment and enzyme reaction in the plant and animal body [21]. Their existence is conducive to the diversified mineral content of both the anatomical constituents. [19] reported cow horn contains 8000mg/kg K, 20000mg/kg Zn, and 2100mg/kg Cu, whereas [1] reported bone meal (rib) contains 6.41mg/kg Zn and 0.05mg/kg Cu. The discrepancies in micro-element concentration may be a result of the environmental contamination of the sampling site. These data indicate the possible utilization of cow horns as powerful sources of trace elements, especially zinc and copper which could be utilized in supplementing livestock and human with micronutrients at a strict and regulated portions. The lack of these elements may negatively affect the condition of the hooves, growth dynamics, and local immunity, which causes economic losses in livestock agriculture [22].

Table 2: Result of micro-elements obtained from analysis

Macro-Element	Bone Sample (mg/kg)	Horn Sample (mg/kg)
Potassium (K)	691 ± 29.51	721 ± 34.70
Magnesium (Mg)	103 ± 9.53	92 ± 10.14
Zinc (Zn)	154 ± 21.17	1992 ± 69.21
Copper (Cu)	0.998 ± 0.15	1.998 ± 0.14

Value: Mean of triplicate determination ± Standard Deviation

3.3 Toxic-Element

The results identified toxic heavy metals Lead (Pb), Cadmium (Cd), and Chromium (Cr) in both bone and horn samples with practically equal values in structures. The finding of the toxic component in Table 3 indicates an excess amount of lead and cadmium (1.996 and 0.998 mg/kg, respectively) while horns also contained twice the amount of chromium as the bones (1.998 vs. 0.998 mg/kg). Bioaccumulation of lead and cadmium can be very unhealthy for both humans and animals because, according to studies by Fatima et al., chronic exposure to lead causes nephropathy, neurotoxicity, and developmental disorders, whereas cadmium interferes with bone metabolism and renal activity[23]. The toxicity of chromium therefore largely relies on its chemical specification since although Cr (III) is relatively non-toxic and required in low doses, Cr(VI) is a carcinogen and cytotoxic [24]. Cadmium levels (0.998 mg/kg Bone and 0.996mg/kg Horn) vastly exceeded WHO limits (0.000003 mg/kg) which suggests potential bioaccumulation risks in food chains, requires rigorous risk assessment protocols, including speciation analysis, decontamination, and environmental sourcing traceability.

Table 3: Results of Toxic-elements obtained from the analysis

Toxic-Element	Bone Sample (mg/kg)	Horn Sample (mg/kg)
Lead (Pb)	1.996 ± 0.98	1.998 ± 0.11
Cadmium (Cd)	0.998 ± 0.28	0.996 ± 0.06
Chromium (Cr)	0.998 ± 0.02	1.998 ± 0.17

Value: Mean of triplicate determination ± Standard Deviation

3.4 Comparative Study

Table 4 shows a comparative study of the results obtained from the analysis in relation to previous studies by[7], [25] as well as the guideline limit for daily nutritional consumption state by a joint consultation of World Health Organization and Food and Agricultural Organization of the United Nation[26], [27]. Compared to previous studies and WHO/FAO standards, the current results consistently show cow bones and horns to possess mineral concentrations exceeding daily consumption recommendations. Notably, horn zinc content was 44 times greater than global daily intake recommendations (1992 mg/kg vs. 45 mg/kg), and calcium and phosphorus also surpassed limits.

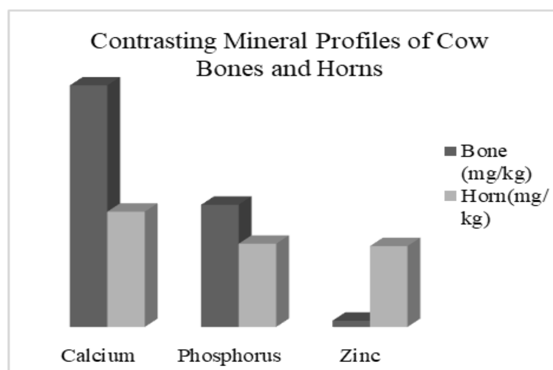


Table 4: Comparative Analysis of Cow Bone and Horn from Previous Studies in Relation to WHO/FAO Nutritional Guidelines

The bone and horn Samples from Benin City may not represent other regions. Pooled samples could mask individual animal variability. Existing research by [7], [25] substantiated the same elemental patterns in the bones and horns of other geographic areas, and this fact evidences reproducibility and accuracy of the data. Discrepancies in Zn (0.144 vs. 154 mg/kg) may reflect regional soil contamination. Excess mineral concentrations, particularly zinc, lead, and cadmium which demands cautious application in nutraceutical consumption and supplements. Assuming an average adult body weighing 60kg ingested 10g of cow horn powder daily, the estimated daily intake (EDI) for both lead and cadmium would be 0.000333mg/kg/day and the hazard quotient (HQ) would be 0.033 for lead, suggesting a low immediate risk and 111.0 for cadmium which is extremely toxicologically unsafe under assumed intake conditions. The elemental concentration in both cow and horns greatly exceeds the limit per kilogram of material; thus, unregulated or chronic consumption may lead to potential bioaccumulation in the food chain, posing long-term health risk. Based on these observations, restrictive dosage measures is required, particularly when used as either feed or nutraceutical products. Exploiting such nutrient-rich properties would be possible with precision dosing without any undesirable health outcomes.

Table 4: Comparative Analysis of Cow Bone and Horn from Previous Studies in Relation to WHO/FAO Nutritional Guidelines

Parameters	Result Obtained		Previous Studies				Nutritional Guidelines for Daily Consumption
	Bones(mg/kg)	Horn(mg/kg)	Bones(mg/kg)	Reference	Horn(mg/kg)	Reference	WHO/FAO (mg/kg)
Calcium (Ca)	5950 ± 59.03	2840 ± 54.51	7231.60 ± 0.04	[25]	300.00	[7]	3000
Phosphorus (P)	3010 ± 36.06	2050 ± 41.07	2168.70 ± 0.03	[25]	70.00	[7]	70
Nitrogen (N)	1930 ± 61.80	1990 ± 39.05	NA	-	NA	-	830 (Protein value)
Potassium (K)	691 ± 29.51	721 ± 34.70	31.2 ± 0.02	[25]	710.00	[7]	3150
Magnesium (Mg)	103 ± 9.53	92 ± 10.14	84.2 ± 0.02	[25]	30.00	[7]	350
Zinc (Zn)	154 ± 21.17	1992 ± 69.21	0.144 ± 0.00	[25]	NA	-	45
Copper (Cu)	0.998 ± 0.15	1.998 ± 0.14	0.001 ± 0.00	[25]	NA	-	2.00
Lead (Pb)	1.996 ± 0.98	1.998 ± 0.11	0.003 ± 0.00	[25]	NA	-	0.01
Cadmium (Cd)	0.998 ± 0.28	0.996 ± 0.06	0.024 ± 0.00	[25]	NA	-	0.000003
Chromium (Cr)	0.998 ± 0.02	1.998 ± 0.17	NA	-	NA	-	0.05

Value: Mean of triplicate determination ± Standard Deviation
NA: Not analyzed in cited studies.

4.0 CONCLUSION

The comparative mineral analysis of cow bones and matured horns from this study shows potential for sustainable exploitation in the agricultural and nutraceutical supplementation. Compared to cow bones, superior concentrations of macro-elements, notably calcium (5950 mg/kg) and phosphorus (3010 mg/kg), proved the applicability of cow bones as a natural mineral reservoir and justification to employ cow bones in the place of mined phosphate in fertilizers and supplements. On the contrary, the horns rich in keratin showed significantly higher micro-elements such as zinc (1992 mg/kg) and copper (1.998 mg/kg) content, which makes them highly relevant in enhancing livestock feed on a micro-element/micronutrient basis used by livestock in immunity boosting and strong hooves/horns.

The anatomical samples showed a similar level of nitrogen indicating that their composition is rich in proteins and that they can be used in organic fertilizer and biopolymer preparation. Nonetheless, its incorporation into diet or feed related applications requires strict decontamination procedures as well as environmental observation due to the presence of toxic heavy metals, including lead, chromium, and cadmium because when compared with WHO/FAO daily intake recommended guidelines, it is very evident that some of the concentrations of minerals particularly zinc and heavy metals surpass the recommended levels. Cow bones and horns, therefore, provide a very good means of nutrient loop closure, less dependency on synthetic mineral supplementation and also use of more resources as it would be a reliable source of nutrients.

Conflict of Interest
The authors declare that they have no conflict of interest.

Data Availability Statement:
All data supporting this study are available upon request from the corresponding author.

Authors' Contribution
Faith I. Akhidenor performed formal analysis, investigation, data curation and original draft writing while, Ita E. Uwidia and Etinosa O. Oshodin contributed to conceptualization, supervision, methodology and writing review and editing.

Authors' Declaration
The authors certify that this research is original, has not been published previously, and is not under consideration by any other journal. We assume full responsibility for the integrity of the data and the accuracy of the reported findings and will accept all liability for any claims about the content

Ethical Declarations Human/Animal Studies
The authors declare that no human/animal was used for the studies

Acknowledgments
The authors gratefully acknowledge the Department of Chemistry, University of Benin (UNIBEN) for providing the research facilities and enabling environment that supported this work. Special appreciation goes to the dedicated laboratory staff for their technical assistance and valuable contributions during experimental procedures.

REFERENCES

[1] K. Khalil, R. Reswati, F. Ferawati, Y. F. Kurnia, and F. Agustin, "Studies on Physical Characteristics, Mineral Composition and Nutritive Value of Bone Meal and Bone Char Produced from Inedible Cow Bones," 2017, Accessed: Aug. 02, 2025.

[2] B. V. Iriso, P. A. John, S. S. Bello, and C. E. Ajinwo, "Frequency of Abattoir Waste Generation and Management in Port Harcourt Metropolis," *Niger. J. Anim. Prod.*, pp. 1064–1067, 2018, doi: 10.51791/njap.vi.6199.

[3] K. Buddhachat *et al.*, "Elemental Analysis of Bone, Teeth, Horn and Antler in Different Animal Species Using Non-Invasive Handheld X-Ray Fluorescence," *PLOS ONE*, vol. 11, no. 5, p. e0155458, May 2016, doi: 10.1371/journal.pone.0155458.

[4] Ż. Ciosek, K. Kot, D. Kosik-Bogacka, N. Łanocha-Arendarczyk, and I. Rotter, "The Effects of Calcium, Magnesium, Phosphorus, Fluoride, and Lead on Bone Tissue," *Biomolecules*, vol. 11, no. 4, Art. no. 4, Apr. 2021, doi: 10.3390/biom11040506.

[5] M. Li, F. Deng, L. Qiao, X. Wen, and J. Han, "The Critical Role of Trace Elements in Bone Health," *Nutrients*, vol. 16, no. 22, Art. no. 22, Jan. 2024, doi: 10.3390/nu16223867.

[6] P. Sannamani, J. Venkata Reddy K., and K. Rengarajan, "Role of Phytic Acid and Phytase in Poultry Nutrition." Accessed: Aug. 02, 2025. [Online]. Available: https://thepoultrypunch.com/2019/05/role-of-phytic-acid-and-phytase-in-poultry-nutrition/

[7] I. E. Uwidia, O. K. Chisom, and O. E. Uwidia, "Comparative Characterization and Assay of Cow Horn Waste and Fish Feed as Biomaterials for Reinforcement in Aquaculture Feeds," in *TMS 2023 152nd Annual Meeting & Exhibition Supplemental Proceedings*, Cham: Springer Nature Switzerland, 2023, pp. 686–694. doi: 10.1007/978-3-031-22524-6_61.

[8] M. Balali-Mood, K. Naseri, Z. Tahergorabi, M. R. Khazdair, and M. Sadeghi, "Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic," *Front. Pharmacol.*, vol. 12, Apr. 2021, doi: 10.3389/fphar.2021.643972.

[9] R. A. Bisergaeva and Y. N. Sirieva, "Determination of calcium and magnesium by atomic absorption spectroscopy and flame photometry," *J. Phys. Conf. Ser.*, vol. 1691, no. 1, p. 012055, Nov. 2020, doi: 10.1088/1742-6596/1691/1/012055.

[10] A. H. Uddin, R. S. Khalid, M. Alaama, A. M. Abdualkader, A. Kasmuri, and S. A. Abbas, "Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry," *J. Anal. Sci. Technol.*, vol. 7, no. 1, p. 6, Jan. 2016, doi: 10.1186/s40543-016-0085-6.

[11] S. Pradhan and M. R. Pokhrel, "Spectrophotometric Determination of Phosphate in Sugarcane Juice, Fertilizer, Detergent and Water

Samples by Molybdenum Blue Method," Scilit. Accessed: Aug. 02, 2025. [Online]. Available: https://www.scilit.com/publications/ae7c7648810d1ce0d3fa411f6637e9d4

[12] Y. B. Cho, S. H. Jeong, H. Chun, and Y. S. Kim, "Selective colorimetric detection of dissolved ammonia in water via modified Berthelot's reaction on porous paper," *Sens. Actuators B Chem.*, vol. 256, pp. 167–175, Mar. 2018, doi: 10.1016/j.snb.2017.10.069.

[13] D. Churchill, M. D. Sikiric, B. Čolović, and H. F. Milhofer, *Contemporary Topics about Phosphorus in Biology and Materials*. BoD – Books on Demand, 2020.

[14] M. Peacock, "Phosphate Metabolism in Health and Disease," *Calcif. Tissue Int.*, vol. 108, no. 1, pp. 3–15, Jan. 2021, doi: 10.1007/s00223-020-00686-3.

[15] S. Banasaz and V. Ferraro, "Keratin from Animal By-Products: Structure, Characterization, Extraction and Application—A Review," *Polymers*, vol. 16, no. 14, Art. no. 14, Jan. 2024, doi: 10.3390/polym16141999.

[16] M. C. Ogwu, M. E. Patterson, and P. A. Senchak, "Phosphorus mining and bioavailability for plant acquisition: environmental sustainability perspectives," *Environ. Monit. Assess.*, vol. 197, no. 5, p. 572, Apr. 2025, doi: 10.1007/s10661-025-14012-7.

[17] A. R. Jupp, S. Beijer, G. C. Narain, W. Schipper, and J. Chris Slootweg, "Phosphorus recovery and recycling – closing the loop," *Chem. Soc. Rev.*, vol. 50, no. 1, pp. 87–101, 2021, doi: 10.1039/D0CS01150A.

[18] S. Žibutis, J. Pekarskas, and L. Česonienė, "Effect of horn shaving and horn core powder fertilizers on the dynamics of mineral nitrogen in the soil of organic farm," *Ekologija*, vol. 58, no. 3, Jan. 2013, doi: 10.6001/ekologija.v58i3.2534.

[19] I. O. Ambali *et al.*, "Suitability of cow horn as filler in an epoxy composite," *J. Appl. Sci. Environ. Manag.*, vol. 23, no. 3, Art. no. 3, Apr. 2019, doi: 10.4314/jasem.v23i3.17.

[20] P.-H. Lin, M. Sermersheim, H. Li, P. H. U. Lee, S. M. Steinberg, and J. Ma, "Zinc in Wound Healing Modulation," *Nutrients*, vol. 10, no. 1, Art. no. 1, Jan. 2018, doi: 10.3390/nu10010016.

[21] M. Ameen *et al.*, "Potassium in Plants: Possible Functions, Mechanisms and Proteomics Under Abiotic Environmental Stress," in *Metals and Metalloids in Plant Signaling*, T. Aftab, Ed., Cham: Springer Nature Switzerland, 2024, pp. 73–110. doi: 10.1007/978-3-031-59024-5_5.

[22] G. M. Hill and M. C. Shannon, "Copper and Zinc Nutritional Issues for Agricultural Animal Production," *Biol. Trace Elem. Res.*, vol. 188, no. 1, pp. 148–159, Mar. 2019, doi: 10.1007/s12011-018-1578-5.

[23] G. Fatima, A. M. Raza, and P. Dhole, "Heavy Metal Exposure and its Health Implications: A Comprehensive Review," *Indian J. Clin. Biochem.*, Apr. 2025, doi: 10.1007/s12291-025-01322-3.

[24] J. B. Vincent, "Chromium: Sources, Speciation, Toxicity, and Chemistry," in *Chromium in Plants and Environment*, N. Kumar, C. Walther, and D. K. Gupta, Eds., Cham: Springer Nature Switzerland, 2023, pp. 43–62. doi: 10.1007/978-3-031-44029-8_3.

[25] O. Nwazulu Innocent, O. Malachy, L.-C. Adindu, and O. Uchechukwu Felicia, "Comparative Mineral Profiles and Yields of Food Grade Ash from Cow, Goat and Pig Femur Bones," *Int. J. Food Sci. Biotechnol.*, vol. 6, no. 4, p. 107, 2021, doi: 10.11648/j.ijfsb.20210604.13.

[26] K. Yoshida *et al.*, "Daily Intake of a Commercially Available Vegetable Juice Has Beneficial Effects on Metabolic Syndrome-Related Biomarkers in Young, Healthy Japanese Adults," *Food Nutr. Sci.*, vol. 7, no. 10, Art. no. 10, Aug. 2016, doi: 10.4236/fns.2016.710084.

[27] K. S. Patel *et al.*, "Exposure of Fluoride in Coal Basin," *Int. J. Clean Coal Energy*, vol. 05, no. 01, Art. no. 01, 2016, doi: 10.4236/ijcce.2016.51001.

