Research Inventy: International Journal of Engineering And Science
Vol.14, Issue 10 (October 2024), PP 19-24
Issn (e): 2278-4721, Issn (p):2319-6483, www.researchinventy.com

Heavy Metal Concentration and Feed Composition Analysis of Poultry Feedin Salalah

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Abstract
Feed is the substance developed or created for domesticated animals and poultry. This study is about the poultryfeed manufacturing process in Oman National Feed Company. Feed ingredients promote healthy digestion and provide proper nutrition such as minerals for farm animals. In this study there are four samples of premix feed were brought from a local feed mill company in Salalah, Oman. In samples B and D Proximate analysis was conducted to know the effects of the parameter of moisture, ash content, and volatile matter on the raw material formulation. From the experimental results analysis, the percentage of fixed carbon was 68.85% for sample B and 70.5% for sample D this means more amount of minerals are present in the sample. In the AAS analysis result the concentration of Cu present in samples B and D is 2705.9 mg/kg and 2585 mg/kg respectively and value are zero for the sample A and C, and for K is 3404.30 mg/kg for the samples of A, B and C, 1409.7 mg/kg for sample D. Mg is high for sample A and zero for a sample Samples of A and C contains high zinc value such 5920.46 mg/Kg and 2874.93 mg/Kg respectively. For elements of Cd and Ni are present in small quantities approximately equal to zero in all samples. These heavymetal concentrations are zero in all the samples of A, B, C and D,this indicates that all the feed samples are free from heavy metals. 2000 kg per batch is our feed, and the material and energy balance are determined for the poultryfeed production process. The results show some material losses in some of the unit operations. The results of heavymetalanalysis study suggest that the poultryfeed produced by Feed Company in Salalah is safe for consumption. However, it is important to note that the heavymetalconcentration in poultryfeed can vary depending on the source of the feed ingredients and the production process. Therefore, it is important to monitor the heavymetalconcentration in poultryfeed on a regular basis to ensure that it meets the safety standards.

Keywords: *Poultry, Nutrient, Cattle feed, Pellet, Atomic Absorption Spectrophotometry.*

Date of Submission: 28-10-2024 Date of acceptance: 11-11-2024

I. INTRODUCTION

Feed is a substance used as a food source for animals, and a variety of ingredients have been identified as suitable fodder for poultry, provided they are scientifically compiled and documented (Wongnaa et al., 2023). Feeding accounts for about 80% of the total poultry production costs, highlighting the importance of effective feed formulation. With the expansion of the poultry industry, feed manufacturing has become increasingly streamlined (Maina Kumari et al., 2022).

A wholesome poultry feed must be free from harmful agents, including toxic compounds, microbes, aflatoxins, heavy metals, and other contaminants that adversely impact bird health and productivity. Feed ingredients or processed feed can become contaminated during various stages of production, including processing, manufacturing, trading, storage, and transport. Heavy metals are particularly concerning environmental pollutants entering feed through soil, water, and air, or from indirect sources like bird droppings, slaughterhouse waste, and sewage sludge used on agricultural land (Ali et al., 2021; Azhar et al., 2022). Heavy metal contamination can hinder growth and reproduction, and it poses toxic risks to vital organs, such as the liver, kidneys, heart, and spleen (Aziz et al., 2023).

Analyzing heavy metal concentrations in feed is essential for food safety and mitigating risks to human health from poultry products. In addition to heavy metals, feeds must be assessed for other contaminants to prevent food safety hazards (Ge et al., 2022). Regular testing of commonly traded feed ingredients ensures balanced and safe rations for poultry (Xiang et al., 2021).

Feed is the most critical cost in poultry production, comprising about 70% of total production expenses. Feed formulation relies on understanding the nutritional needs of poultry species and ingredient costs. During formulation, scenario analyses assess various ingredient combinations to minimize costs while meeting nutritional requirements (Saadaoui et al., 2021). Feed ingredients undergo multiple screening stages, and final formulations are selected for production. Any hazardous substances remaining undetected in these ingredients may be toxic to poultry and pose indirect risks to humans through food chain transfer (Chiekezie et al., 2022).

Heavy metals and their compounds are toxic and hazardous even in trace amounts and are therefore undesired in feed formulations. They bioaccumulate within the food chain, posing indirect risks to human health (Sarkar et al., 2022; Emenike et al., 2021).

Ensuring feed safety involves analyzing and quantifying hazardous feed constituents. Total Heavy Metal Concentration is measured in water, soil, biomaterials, and feed samples using instruments such as the Atomic Absorption Spectrophotometer. Feed composition analysis, including parameters like protein, fat, and ash, is conducted through laboratory techniques.

Efforts are underway to establish a reliable system in the poultry feed industry to test for heavy metals and evaluate feed composition using versatile, feasible, and repeatable laboratory techniques based on the current study.

Aim and objectives

♦ To analyze the pre-mix feed composition analysis poultry feed in feed mill in salalah.

Objectives

♦ To understand the composition of different feeds.
♦ To understand the importance of the components, present in the poultry feed.
♦ Proximate analysis of the raw feed composition.
♦ To estimate various macro and micro minerals present in pre-mix feed using atomicabsorption spectrometry.

II. STUDY DESIGN AND METHODOLOGY

To design a study and methodology for heavy metal analysis of poultry feed, it's essential to develop a detailed and structured approach, ensuring accuracy, reliability, and compliance with regulatory standards. This study was done to measure the concentration of heavy metals, as well as the source and composition of the feed. Samples of chemicals and feed were collected and sent for heavy metal concentration analysis. For the feed composition, analysis was fixed to crude fiber, moisture, protein, fat, and ash.

2.1 Sampling and Sample Preparation

Four poultry feed samples (A, B, C and D) were randomly collected from the feed mill company, Salalah, Oman. The samples were collected in sterile, clean, and airtight containers as per the standard sampling procedure and were transferred to the chemical engineering lab, University of Technology and Applied Sciences, Salalah, Oman, for the proximate analysis and heavy metal determination.

Preparation of samples for heavy metal determination was done as per the standard method. Poultry feed samples were air-dried for 72 hours at room temperature and ground using a mortar and pestle to pass through a 60-mesh sieve.

Place 5–10 g of sample weighed to the nearest 0.2 mg in a quartz or platinum crucible, dry in an oven at 105 ± 2 °C and introduce the crucible into the cold muffle furnace.

Close the furnace and gradually raise the temperature to 600 ^{0}C cover approximately 90 minutes. Maintain this temperature for 4 to 16 hours (e.g. overnight) to remove carbonaceous material and then open the furnace and allow to cool. Moisten the ash with deionised water and transfer it into a 250 ml beaker. Wash the crucible with a total of approximately 10 ml of hydrochloric acid. Add the acid slowly and carefully to the beaker (there may be a vigorous reaction due to CO_2 formation). Add hydrochloric acid drop-wise with agitation until all effervescence has stopped. Evaporate to dryness, occasionally stirring with a glass rod. Add 15 ml of 5M hydrochloric acid to the residue followed by about 120 ml of deionised water. Stir with the glass rod, which should be left in the beaker, and cover the beaker with a watch-glass. Bring gently to the boil and maintain at boiling point until no more ash can be seen to dissolve. Filter on ash-free filter paper and collect the filtrate in a 250 ml volumetric flask. Wash the beaker and filter with 5 ml of hot 5 M hydrochloric acid and twice with boiling water. Make up to the mark with deionised water (HCl concentration approximately 0.5 M). If the residue on the filter appears black (carbon), put it back in the furnace and ash again at 450 to 475 °C. This ashing requires about 3–5 hours and is complete when the ash appears white or nearly white.

For chemical analysis, one gram of the ground sample was digested with 16 ml of H_2SO_4 and 8 ml of H_2O_2 in a digestion system. After completion of the digestion, the contents were filtered through Whatman filter paper and were made up to 50 ml with double distilled water in a volumetric flask. The final solutions were used to determine heavy metals by atomic absorption spectrophotometry. Digestion blanks were also prepared simultaneously.

2.2 Heavy Metal Analysis

Heavy metal analysis was carried out using the AAS method. Heavy metals were detected and quantified using standard working solutions of heavy metals. Initially, the absorbance values of different concentration standards were noted. Then the unknown samples were analyzed using the AAS instrument, keeping the conditions of flame, column height, gas flow rate, and sensitivity fixed, and absorbance values were noted. Then, with the help of a standard equation, the unknown concentration values were calculated and further used in the calculation of micro metal concentration in feed samples (Mutiah et al.2022). Reagents and chemicals used were cadmium, copper, lead, Ni, K and zinc, and mineral acids for preparing solutions. The whole analysis was carried out in a clean, fresh, and controlled lab with all safety measures. Nitric acid was used where the samples were first treated with nitric acid, then distilled water was added after the formation of white fumes. The tests were initially run with standard metals as control, then chicken feed samples were run.

A calibration curve is used to determine the unknown concentration of an element in a solution. The instrument is calibrated using several solutions of known concentrations. The absorbance of each known solution is measured and then a calibration curve of concentration vs absorbance is plotted.

The sample solution is fed into the instrument, and the absorbance of the element in this solution is measured.
The unknown concentration of the element is then calculated from the calibration curve.

2.3 Feed Composition Analysis

For compositional analysis, 100 g of homogeneous feed samples were collected in pre-cleaned glass bottles, labeled, and kept in an ice box for transport. They were placed in the refrigerator and processed within 24 hours of collection. Duplicate samples of untreated feed were thawed at room temperature, then a 3-pronged metal spatula was heated in a flame and used to slice off contaminants adhering to the surfaces of the bottles. The interior walls were rinsed with 0.1 M HCl, and the rinsate was discarded.

A representative 50 g aliquot of each sample was placed in a 250 ml polypropylene bottle. 200 ml of tetraethyl ammonium hydroxide was added, and the bottle was vortexed for 1 min and covered with a lid. Each bottle was placed in an ultrasonic bath for 15 min at room temperature. After ultrasonication, each bottle was vortexed for 1 min and centrifuged at 2630 g for 10 min at 20 °C. The supernatant was decanted into a pre-cleaned 250 ml polypropylene bottle and stored in a refrigerator.

For analysis of certain nutrients, feed samples were placed in a hot air oven at 105 °C for 24 hours to bring them to constant weight. The dried samples were subsequently crushed and sieved through a 1 mm mesh. A representative 1 g sample was taken in an 80 ml Teflon bottle, and 10 ml of nitric acid were added. The bottles were capped and subjected to microwave digestion after placing them in the rotor of the microwave digestion unit. One bottle at a time was placed in the microwave, and the following cycle was applied: heating for 5 minutes (from 25 to 90 °C), then held at 90 °C for 10 minutes, and cooling for 5 minutes (from 90 to 25 °C). After this cycle, the bottle was removed from the microwave. The sample was put on a hot plate to evaporate excess acid if necessary. At each stage of the preparation, a series of blanks were carried along. The digest was diluted to 50 ml in a volumetric flask and well-mixed before analysis.

III. RESULTS AND DISCUSSION

The heavy metal concentrations of poultry feed samples in the feed mill Company of the Salalah were determined using an Atomic Absorption Spectrophotometer.

Spectrophotometric determination of Mg, Cu, K, Ni, Cd and Zn Adjust the AAS in accordance with the manufacturer's instructions and optimise the response of the instrument using an oxidising air-acetylene flame at the following wavelengths:

Mg: 285.2 nm Cu: 324.8 nm K: 766.5 nm Zn: 213.8 nm, Ni: 232 nm Cd: 228.8

Using a calibration curve, calculate the trace element concentration in the solution:

$Cs = (As-b)/m$

where, Cs = element concentration of the sample solution [mg/l],

As = Absorbance value of the sample solution,

b = y-intercept of the regression line, and m = slope of the regression line.

Element content of the sample in mg/kg considering dilution steps is calculated as:

Element [mg/kg] = $(Cs*v*F)/w$

where, v = volume of the sample solution [L],

F = dilution factor

w = sample weight [kg].

3.1 Heavy Metal Concentration(mg/Kg)

Heavy Metal	Name of the sample			
	A	B	C	D
Copper	0	2705.9	0	2585.0
Magnesium	4534.29	0	2909.29	382.9
Nickel	0	0	0	0
Potassium	3404.3	3404.30	3404.3	1409.7
Zinc	5920.46	0	2874.93	0
Cadmium	0	0	0	0

Table 1: Heavy metal concentrations of different samples

From the above table-1 provides data on the concentrations of various metals—copper, magnesium, nickel, potassium, zinc, and cadmium—in four different poultry feed samples (A, B, C, and D), measured in mg/kg. Heavy metals are analyzed in poultry feed because certain metals, in excess, can negatively impact animal health and potentially accumulate in poultry products consumed by humans. Here is a detailed discussion on each metal and its concentration across the samples:

Copper (Cu)
Concentrations: Copper levels are high in Sample B (2705.9 mg/kg) and Sample D (2585.0 mg/kg), while Samples A and C have no detectable copper.
Copper is an essential trace element, crucial for various physiological functions, including enzyme activation and iron metabolism in poultry. However, excessive copper can lead to toxicity, manifesting in liver damage, reduced growth, and oxidative stress. Samples B and D exceed typical safe levels, suggesting a potential risk for copper toxicity in poultry if these feed samples are used exclusively. The absence of copper in Samples A and C might necessitate supplementation if they are part of a complete diet.

Magnesium (Mg)
Concentrations: Magnesium levels vary significantly across samples, with Sample A (4534.29 mg/kg) and Sample C (2909.29 mg/kg) having high levels, while Sample D (382.9 mg/kg) shows a moderate level, and Sample B has no detectable magnesium.
Magnesium is essential for muscle function, nerve transmission, and enzyme activity in poultry. Deficiency can impair growth and lead to metabolic disorders, while excessive amounts are typically well-tolerated but can cause diarrhea and gastrointestinal issues.The high magnesium concentration in Samples A and C suggests these samples could meet dietary requirements without additional magnesium supplementation. Sample D's moderate level might still be adequate, while Sample B's absence of magnesium would require supplementation to avoid deficiency in poultry.

Nickel (Ni)
Concentrations: All samples show undetectable levels of nickel (0 mg/kg).
While nickel is not an essential nutrient for poultry, it can be toxic at high concentrations, potentially causing kidney and liver damage. The absence of nickel in all samples is beneficial, as it reduces any risk of toxicity.Given that nickel can bioaccumulate in the food chain, its absence in all samples indicates that these feeds are safer for poultry and pose no threat of nickel accumulation in poultry products.

Potassium (K)
Concentrations: Samples A, B, and C have identical potassium concentrations (3404.3 mg/kg), while Sample D has a lower concentration (1409.7 mg/kg).Potassium is vital for fluid balance, nerve transmission, and muscle contraction. Adequate potassium levels support overall health and productivity in poultry.The consistent potassium levels in Samples A, B, and C suggest these feeds meet typical dietary needs. Sample D has a lower potassium content but may still be within an acceptable range, depending on dietary requirements and the other feed components included.

Zinc (Zn)
Concentrations: Zinc levels are high in Sample A (5920.46 mg/kg) and Sample C (2874.93 mg/kg), while Samples B and D show no detectable zinc.Zinc is a critical trace mineral that supports immune function, growth, and reproduction in poultry. However, excessive zinc can cause toxicity, affecting bone and muscle health, and leading to oxidative stress.Samples A and C contain high zinc levels that may surpass typical requirements, potentially leading to toxicity if fed alone over prolonged periods. Samples B and D, with no zinc, would need zinc supplementation to ensure adequate dietary intake for poultry.

Cadmium (Cd)
Concentrations: All samples show undetectable levels of cadmium (0 mg/kg).Cadmium is a toxic heavy metal with no known nutritional benefits. In poultry, cadmium exposure can lead to kidney damage, weakened bones, and disruption of metabolic processes. Cadmium accumulation in poultry products poses a serious health risk to humans.The absence of cadmium in all samples is favorable, as it minimizes the risk of toxic exposure for poultry and potential contamination in poultry products.
The overall analysis reveals varying nutrient profiles and potential safety concerns related to heavy metal content in the samples:

Samples B and D: While they contain high copper concentrations, they lack zinc and magnesium, which are essential for poultry health. Supplementation of missing elements would be necessary if using these feeds.
Sample A: High levels of magnesium and zinc make it nutritionally dense, but the elevated zinc concentration could be a concern, requiring careful balancing with other feeds to avoid toxicity.
Sample C: This sample has moderately high magnesium and zinc, making it suitable for energy and nutritional needs but requires careful monitoring of zinc levels.

3.2 Feed Composition Analysis
The important objective of feed analysis is to assess the nutritional contents of feedstuffs. In livestock, nutritional balance has received significant attention for improving growth performance and maintaining health. The main objective of this aspect was to assess the chemical composition of poultry feed using pure chemicals and additives. The following table illustrates the feed composition analysis.

Chemical Analysis	**Chemical Composition (%)**
Moisture	5.26
Crude Protein	19.015
Ash Content	8.74
Fat/Oil	4.988
Crude Fiber	8.139

Table 2: Feed Composition Analysis

In feed analysis systems, a variety of parameters are taken into consideration, whereas chemical compositions where the main interest was crude protein, moisture, fat, ash and crude fiber. A feed composition analysis was conducted using chemicals and additives which are enlisted in the following table. Fresh samples were collected and preserved for obtaining results. Keeping in view the following physical and chemical parameters, the chemical composition of poultry feed was analyzed using prescribed methods for different feed categories.

Heavy metals are toxic to animals and cause abnormalities in the development and functioning of biological systems. They make extreme alterations in histology and a decrease in weight gain and feed consumption. Hence, the assessment of heavy metal concentrations is important for the safety and quality evaluation of the food chain. The aim of the study was to measure the heavy metal concentrations and conduct a feed composition analysis of poultry feed. An attempt was made to find the concentration of lead and cadmium and the effect of these metals on poultry feed. The poultry feed samples from feed mill in Salalah were collected, and the heavy metal concentration analysis was done using the Atomic Absorbance Spectrophotometer. The poultry feed was also analyzed for moisture, ash, and protein contents. The heavy metal concentration analysis shows that the lead and Ni concentrationsare zero in poultry feed and metals are in the permissible levels

IV. CONCLUSION

This comprehensive research is about the proximate analysis and heavy metal analysis of poultry feed at the esteemed National Feed Company, Utilizing the data collected regarding the proximate analysis, we meticulously calculated various essential parameters including moisture content, ash content, and the volatile matter present in the feed samples we analyzed. Upon careful examination of the experimental results derived from our analysis, we determined that the percentage of fixed carbon was recorded at an impressive 68.85% for sample B while it was slightly higher at 70.5% for sample D, indicating that the concentration of minerals contained within these samples is significantly elevated. Through the utilization of atomic absorption spectrometry, we were able to accurately identify and quantify the presence of both micro and macro elements such as copper (Cu), nickel (Ni), cadmium (Cd), magnesium (Mg), zinc (Zn), and potassium (K) that were found in our analyzed samples B and D. From our findings, it became evident that potassium and copper were present in notably high concentrations, whereas the heavy metals' cadmium, zinc, and nickel were detected in minimal quantities that were approximately equal to zero, which is particularly important as these elements pose a considerable risk to the health and wellbeing of poultry. Furthermore, it is worth noting that magnesium was exclusively present in sample D, highlighting the variability in nutrient content between the different samples examined. This variability underscores the importance of conducting thorough analyses to ensure that poultry feed meets nutritional standards and does not contain harmful levels of heavy metals.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Chemical Engineering, University of Technology and Applied Sciences, Salalah for supporting research on poultry feed analysis.

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