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Monitoring of Organochlorine Pesticides Residue Contamination Levels in Poultry Feeds in North Shewa Zone, Amhara Region, Ethiopia

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Abstract

Background and Objectives: The consumption of food products from animal origin and environmental exposure remain the main sources of organochlorine pesticides residues (OCPs) in human diets. This study assessed levels of OCPs in poultry feed obtained from three feed mills in north shewa Zone, Ethiopia. Materials and Methods: The proximate analysis was carried out using method described by AOAC and analysis of organochlorine pesticides with GC-ECD. Results: The proximate analysis mean levels are as follows: Moisture (broiler feed (6.33±0.8%), layer mash (7.00±1.8%)), ash (broilers (8.33±2.6%), layers (9.17±4.9%)), fibre (broilers (0.33±0.2%), layers (0.20±0.2%)), dry matter (broilers (93.7±0.8%), layers (93.0±1.7%)). The mean OCPs values ranged from 0.34-14.8 ng kg⁻¹ in broilers and 0.20-5.22 ng kg⁻¹ in layers mash. In broilers feed, aldrin, endosulfan I and endrin, exceeded the Maximum Residue Limits (MRL) for animal feed for OCPs, while in layers mash, aldrin and endrin aldehyde exceeded the MRL limits. Conclusion: These results indicated the presence of organochlorine pesticides in poultry feeds which could bioaccumulate and eventually lead to the contamination of table eggs and table meats as a result of these endocrine disruptors. The study observed that from the detected analytes seven of them above the international MRL values and suggested that stringent monitoring of use of pesticides in poultry feed and food of animal origin is required.

Key words: Persistent organic pollutant, organochlorine pesticides, monitoring, animal feed, GC-ECD


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Data Availability: All relevant data are within the paper and its supporting information files.
INTRODUCTION

Pollution by pesticides is a predicament of escalating importance for ecological, evolutionary, nutritional and human health reasons. Organochlorine (OC) pesticides, a group found within the persistence organochlorine pesticide (POPs) has become a potential toxic pollutant of the environment in the last few decades. They can be highly accumulated in the environment due to their properties which include; chemical inertness, persistence, lipophilic nature and very less biodegradability. In the developed countries, the use of products containing OC are banned and/or restricted, developing countries still derived application from the use of some of these OC pesticides. Their low cost relative to application in control of crop pest and plant vectors increased usage. Although most of them leave the products or degrade in soil, water and atmosphere, residues of these compounds are still found to some extent in various substances and can be transferred to human via consumption of agricultural products. The consumption of OC by human through food items is potentially harmful to human health, hence, there is a need for further monitoring, to suggest reduction of OC in agricultural food products.

The consumption of food products from animal origin and environmental exposure remain the main sources of OCs in human diets. Researches confirmed that human suffered from toxic accumulation of OCs mostly via diet as compared to inhalation and dermal exposure. Their bioaccumulation and biomagnification abilities when found in living tissues are responsible in part to their high toxicity. Therefore, efficient monitoring, identification and extraction of OCs in food of animal origin (i.e., meat, milk, egg and other dairy products) is an important safety mechanism for human nutrition. Organochlorine pesticides entered into the animal food chain through contaminated feed and/or water ingested by animals. Application of pesticides in livestock area through treatment of building, equipment/tools and other forms of disinfection and/or quarantine is another pathway for the OCs contamination in animal food. According to Salar-Amoli and Ali-Esfahani, OCs are carcinogenic in humans and animals. Immunology studies confirmed toxicity of selected OCs and their metabolites in human foetus and neonatal via in vitro and in vivo.

Poultry farming is one of the most important aspects of agriculture, contributing immensely to meet animal protein demand of increasing global population through egg, dairy products and meat production. The main mean by which pesticides entered into animal body is through feed and fodder. The pesticides accumulate in animal body tissues and in their associated products such as egg, milk and meat. According to Suleiman et al. and Aycicek et al., most of the organised OC pesticide residue-monitoring programmes are centred on food crops, vegetables and fruits with very few reports on status in feed and fodder consumed by livestock animals. Feed constituted 70% of the total cost of production in poultry farming. The composition and quality of feedstuffs used in feed manufacturing is an important part in the food chain and has implication on the associated poultry products consumed by man. Therefore, determination of OCs residue levels in animals (poultry) feed is highly essential. Poultry birds may be fed with feeds compounded with contaminated plant origin. The use of contaminated fish powder as a protein source in feed manufacturing or from the remnants of vehicle exhausts, which are hit by air to the source of fodder and water to drink used in poultry. Chicken meat constitute an important part of the Ethiopian diet and the content of OCs influences the quality of the final products.

In this study, north shewa zone, Amhara region, basically Debre birhan and Ataya town was chosen, being an agrarian community, where many subsistent and large scale farming as well as large scale poultry farming was taking place. The use of herbicides and pesticides to control weed and preserve agricultural products was prevalent among the farmers. This study aimed at assessing the concentration of organochlorine pesticides residue in poultry feed using GC-ECD. The outcome of this study will be an eye opener to the people to understand the environmental position of poultry and poultry feed and respectively standardize effectual counter measure to control their pollution.

MATERIALS AND METHODS

Description of the study area: The study was conducted in Debre Berhan and Ataya towns in North Shoa Zone of Amhara National State located between 1059559-1077353 North latitude and 548800-567000 East longitudes at an average elevation range of 2765-2850 m above sea level (masl) with a total area of 146.27 km² along the main road from Addis Ababa to Dessie at 115 km North East of the capital. According to Central Statistics Agency (CSA) National Population and Housing Census, 65,231 people of whom 31,668 were males and 33,563 females lived in the town.

Sampling methods: For this study, purposive sampling and stratified random sampling in selected three towns. Stratified sampling technique was used considering the variability of the nature of the population. Sample size of households that
participate was determined by using a sample technique\textsuperscript{16}. Sampling was conducted during 15 February-09 April, 2018 G.C. from three different feed mills (three each at the LGAs). The poultry feeds (broiler and layers mash) were collected from three feed mills in Ataya and Debre berhan, north shewa zone, Ethiopia, namely: Tateke Farm is situated in Debre berhan, a capital city in north shewa zone while Hidasa Farm and Royal are situated in Ataya, a semi-urban city in north shewa zone. These two towns are predominantly farmer and perhaps, teaching and other civil service.

Pesticide standards with the corresponding purity were obtained from Adamitulu Pesticide Preparation Company, Zuway, Ethiopia. The entire reagents used were: Dichloromethane (GFS Chemicals, Inc. 867 Mckinley Avenue, Columbus, OH 43223) and n-hexane (GFS Chemicals, Inc. 867 Mckinley Avenue, Columbus, OH 43223), Acetone, Silica (MerkKGAa, 64271 Darmstadt, Germany) and Sodium Sulphate (VMR International Ltd, Poole, England) Nitric acid- HNO3 (BDH laboratory Supplies Poole, 1TD England) Tetraoxosulphate (IV) acid-H2SO4 (BDH Chemicals Ltd Poole, England), Sodium Hydroxide-NaOH, Methanol (GuangdongGuanghuaSci-Tech Co. Ltd, Shantou, Guangdong, China) Hydrogen Fluoride-HF and Perchloric acid (Kermel) and Agilent 78970A GC equipped with electron capture detector was used for the chromatographic separation.

**Proximate analysis:** The experimental samples were subjected to some proximate analysis which includes: moisture, ash and crude fibre. In carrying out the analysis practically, methods used vary according to the food material being studied and also in details of evaluation, basically in accordance with standard methods described by the Association of Official Analytical Chemist\textsuperscript{17}.

**Ash content:** Ash content of the samples were determined by subjecting the sample with known weight (2 g) to ignition in a muffle furnace for 4 h at 300°C for 45 min to pre-ash the sample and at 550°C for 3 h 15 min to complete ashing, until a light grey ash was obtained, after which the samples was cooled in a desiccator and weighed\textsuperscript{17}. The ash (%) was calculated from the equation:

\[
\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Original weight of sample}} \times 100
\]

Dry matter content (%) = 100-Ash content (%)

**Moisture content:** Moisture content of the samples was according to the standards of AOAC\textsuperscript{17}. A known weight of the feed sample is subjected to drying in an oven at 105°C for 2 h. The loss in weight is reported as moisture content\textsuperscript{17}. This is calculated thus:

\[
\text{Moisture content (\%)} = \frac{\text{Difference in weight}}{\text{Original weight of sample}} \times 100
\]

\[
\text{Dry matter content (\%)} = 100-\text{Moisture content (\%)}
\]

**Crude fibre:** The crude fibre (%) was determined as loss of ignition of dried lipid-free residue after digestion with 1.25% H2SO4 and 1.25% NaOH. About 10 mL of acetone was added to dissolve any organic matter\textsuperscript{17}. The fibre (%) was obtained with the equation:

\[
\text{Crude fibre (\%)} = \frac{\text{Weight of residue} - \text{Weight of ash}}{\text{Original weight of sample}} \times 100
\]

**Sample preparation for OCPs:** Then, the samples were packed with polyethylene plastic bag and were kept in a refrigerator for a week until sample preparation started for the analysis. Before sample preparation the samples were further is stratified by the size (weight) into three portions as large, medium and small to make homogeneous sample. Then about 300-350 g was taken from each portion to make a sub-sample\textsuperscript{18} of 1 kg.

**Extraction procedure for OCPs:** The poultry feed samples were extracted using soxhlet extraction method (USEPA method 3540). A dried, sieved feed sample (20 g) was weighed into extraction thimble and placed in a soxhlet extractor. Extraction was done for 10 h using distilled dichloromethane. The extract was concentrated by distilling off part of the solvent. The concentrated extract was cooled to room temperature and was concentrated further to about 1 mL under a stream of nitrogen gas of 99.99% purity. The reduced extract was preserved for chromatographic clean-up prior to GC-ECD analysis\textsuperscript{18,19}.

**Clean-up procedure for OCPs:** Clean-up method (USEPA Method 3630C) was used in this project work. A column of about 15 × 1 cm (internal diameter) was packed with about 5 g of activated silica gel prepared in a slurry form in n-hexane. About 0.5 cm\textsuperscript{3} of anhydrous sodium sulfate was placed at the top of the column and allowed to sink below the sodium sulphate layer. Elution was done with 2 × 10 mL portion of the extracting solvent. The eluate was collected, dried with anhydrous sodium sulphate and then evaporated to dryness.
Gas chromatography-electron capture detector analysis:

Gas chromatography from grace agri food laboratory service PLC, Addis abeba, Ethiopia was used to determine the presence and levels of organochlorine in both broiler's and layers mash. The dried eluate above was reconstituted with 1 mL n-hexane and 0.5 mL of 20 ppm of the internal standard. Qualitative and quantitative analysis of the OCPs was carried out with the aid agilent 78790A GC-ECD. The levels of OCPs were calculated from the relationship given:

\[
\text{Concentration of analyte (ng g\textsuperscript{-1})} = \frac{\text{Concentration of analyte (ng mL\textsuperscript{-1})} \times \text{dilution factor}}{\text{Mass of sample (g)}}
\]

The system was fitted with DB 17(30 m×250 μm × 0.25 μm) column. A 1 μL aliquot of prepared sample extract was injected into the column in splitless mode at an injector and interface temperature of 250 °C. There was a split flow rate of 2 mL min\textsuperscript{-1} during an oven temperature program of 150 °C which was increased to 280 °C at 6 °C min\textsuperscript{-1}, the total run time was 21.67 min.

Statistical analysis: The data were processed with Microsoft Excel software 2007 version. The data were subjected to descriptive analysis using Statistical Package for Social Sciences (SPSS) software 20 version was used to check the presence of significant difference at 95% confidence and the significance difference were determined at the p<0.05 level. Two-way Pearson correlation were used to assess the strength of association between the OCPs compounds.

RESULTS AND DISCUSSION

Proximate composition: The proximate composition of broiler and layers mash obtained from three different feed mill is presented in Table 1. The ash content for broiler feed ranged from 5-10% with mean value of 8.33%. These values was in agreement with the ash content (%) of broiler feed produced having a range of 5-15%, while layers mash ranged from 5-12.5% with mean value\textsuperscript{14} of 9.17%. There was no significant difference between the two feed types in the three locations.

Crude fibre is a measure of the quantity of indigestible cellulose, lignin and other components of this type in present foods. It is the residue of plant materials remaining after solvent extraction followed by digestion with dilute acid and alkali. These components have little food value but provide the bulk necessary for proper functioning in the intestinal tract. The result in Table 1 showed that the crude fibre content for broilers feed ranged between 0.20 and 0.58%, with mean value of 0.33% while layers mash ranged from 0.10-0.40% with mean value of 0.20%. The values obtained is lower than the recommended nutrient values which ranged from 5-7% for both broilers and layers mash, respectively\textsuperscript{20}. The highest crude fibre content was obtained in the broilers feed from Hidasa Farm (0.58%) and the lowest in the layers mash from Tekke and Hidasa farms (0.10%).

Moisture content which is the amount of water in a material or substance ranged from 6.0-7.0% in broilers feed with a mean of 6.33%, these values are in agreement with 4-10% ranged for broiler feed and 6-8% for layers mash obtained in Kano. Bukar and Saeed\textsuperscript{14} obtained as the moisture content (%) of broilers feed in Kano that ranged from 4-10%, while for layers mash it ranged from 6.0-8.0% with a mean value of 7.0%, the values obtained are not too far from the results which\textsuperscript{14} obtained as the moisture (%) content of layers mash in Kano that ranged from 7-14%. The highest moisture content (%) was obtained in layers mash from Royal farm 8.00±2.8%, while the lowest was obtained in Broilers feed from Tateke farm 6.0±0.0%. The dry matter content which is the amount of material remaining after removal of water, ranged from 93.0-94.0% in broilers feed with a mean of 93.7%, while for layers mash, it ranged from 92.0-94.0% with a mean value of 93.0%.

Concentrations of organochlorine pesticides residue: The distribution of OCPs in poultry feed for all three feed mills were presented in Table 2 and 3. The results of the OCPs studied fell into three categories: Dichlorodiphenylethanes (pp-DDT), Cyclodienes (aldrin, dieldrin, endrin, endrin aldehyde, endosulfan I) and chlorinated benzenes/ cyclohexane (Delta-BHC). The concentration (ng kg\textsuperscript{-1}) of detected OCPs in broilers feed obtained from three feed mills

<table>
<thead>
<tr>
<th>Feed mill</th>
<th>Ash (%)</th>
<th>Fibre (%)</th>
<th>Moisture (%)</th>
<th>Dry M (%)</th>
<th>Ash (%)</th>
<th>Fibre (%)</th>
<th>Moisture (%)</th>
<th>Dry M (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hidasa</td>
<td>5.00±0.0</td>
<td>0.58±0.3</td>
<td>7.00±0.0</td>
<td>93.0±1.4</td>
<td>12.3±3.5</td>
<td>0.10±0.0</td>
<td>7.00±0.0</td>
<td>93.0±1.1</td>
</tr>
<tr>
<td>Royal</td>
<td>10.0±0.0</td>
<td>0.20±0.1</td>
<td>6.00±0.0</td>
<td>94.0±0.0</td>
<td>10.0±0.0</td>
<td>0.40±0.1</td>
<td>8.00±2.8</td>
<td>92.0±2.8</td>
</tr>
<tr>
<td>Tateke</td>
<td>10.0±0.0</td>
<td>0.30±0.1</td>
<td>6.00±0.0</td>
<td>94.0±0.0</td>
<td>5.00±7.1</td>
<td>0.10±0.0</td>
<td>6.00±1.4</td>
<td>94.0±0.0</td>
</tr>
<tr>
<td>Mean</td>
<td>8.33±2.6</td>
<td>0.33±0.2</td>
<td>6.33±0.8</td>
<td>93.7±0.8</td>
<td>9.17±4.9</td>
<td>0.20±0.2</td>
<td>7.00±1.8</td>
<td>93.0±1.7</td>
</tr>
</tbody>
</table>

Dry M: Dry matter

Table 1: Proximate composition of broilers feed and layers mash obtained from three feed mills.
is shown in Table 2. Aldrin and endosulfan I was detected in all the feed mills while endrin aldehyde was not detected in all the feed mills. All the values of OCPs in broiler feed recorded in these farms were below the maximum residue limits.

The mean value of OCPs in layers mash obtained from three feed mills is presented in Table 3. Hidas Farm recorded the mean values (ng kg⁻¹) as follows: aldrin (3.44), Delta-BHC (0.20), pp-DDT (0.72) while dieldrin, endosulfan I, endrin and endrin aldehyde were below detection limit. These values were below the maximum residue limits. Royal Farm had mean values (ng kg⁻¹) of aldrin (3.97), endosulfan I (3.63), endrin (1.95) while delta-BHC, dieldrin, endrin aldehyde and pp-DDT were below detection limit. Tateke Farm had the mean levels (ng kg⁻¹) of Aldrin (1.77), endrin aldehyde (5.22), pp-DDT (0.89), delta-BHC, dieldrin while endosulfan I and endrin were below detection limit. Similarly, the levels of the OCPs in layers mash in these feed mills were below the maximum residue limits.

The total mean values (ng kg⁻¹) of OCPs in broilers and layers mash of all the feed mills presented in Table 4. Despite the evidence of presence of the three categories of OCPs in all the feed mills, Endrin aldehyde and dieldrin were not detected in broilers and layers mash. All the detected pesticides did not exceed th MRLs established by European Union for each compound. However, aldrin has the highest level of OCPs of 14.8 ng kg⁻¹ for broilers and 3.06 ng kg⁻¹ for layers mash while delta-BHC has the lowest value was of 0.34 for broilers and 0.07 ng kg⁻¹ for layers mash. The presence of aldrin in this feed indicates the need for concern for public health point of view because it’s much higher toxicity than other OCPs21,22. Statistically there was a significant difference in broilers and layers mash, with exception of endrin aldehyde. In feed composition, the percentage of protein source (soya beans, groundnuts etc) in broilers is always higher than that of layers mash, hence greater fat content in broilers. The deposition of fat in broilers gives rise to an increase in the bioaccumulation of OCPs in broilers. Similarly, despite the fact the protein content in layers mash is low compared to broiler mash, the accumulation over a period of time could lead to higher concentration of OCPs in layers bird.

The mean values of aldrin in broilers and layers mash obtained in this study were above the value in poultry eggs and chicken in Jordan which was found to be below the detection limit23. Rabinder et al.9 monitored the level of OCPs residues in poultry feed, chicken muscle and eggs in a selected farm in Punjab India and reported the value of 0.65 mg kg⁻¹ for δ-BHC in poultry feed. This value is higher than the level of δ-BHC in broilers mash (0.34 ng kg⁻¹) and layers mash (0.07 ng kg⁻¹) obtained in this study (Table 3). In this study, dieldrin concentration in broilers is 1.66±2.6 ng kg⁻¹ but not detected in layers mash. Dieldrin was not also detected in the OCPs study in poultry feed in Jordan23. The presence of dieldrin in broiler field and endrin in both broilers and layers mash is a strong indication of epoxidation and conversion of aldrin to dieldrin and endrin24. Dieldrin is the most prevalent and environmentally persistent among the cyclodienes, its low concentration in broilers and below detection limit in layers mash suggest fresh input of aldrin since dieldrin has a low biotransformation and evaporation ratio that suggest their persistent in the environment than aldrin25,26.
Table 5: Correlation of OCPs in layers and broiler mash

<table>
<thead>
<tr>
<th>Feed type</th>
<th>Aldrin</th>
<th>δ-BHC</th>
<th>Dieldrin</th>
<th>Endosulfan</th>
<th>Endrin</th>
<th>Endrin CHO</th>
<th>pp-DDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ-BHC</td>
<td>-0.344</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dieldrin</td>
<td>0.938**</td>
<td>-0.245</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endosulfan</td>
<td>0.951**</td>
<td>-0.243</td>
<td>0.958**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endrin</td>
<td>-0.047</td>
<td>-0.356</td>
<td>-0.316</td>
<td>-0.044</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endrin CHO</td>
<td>-0.324</td>
<td>-0.245</td>
<td>-0.2400</td>
<td>-0.366</td>
<td>-0.316</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>pp-DDT</td>
<td>0.628</td>
<td>-0.560</td>
<td>0.632</td>
<td>0.435</td>
<td>-0.391</td>
<td>0.326</td>
<td>1</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2 tailed)

concentration of pp-DDT in broilers (0.60 ng kg⁻¹) and in layers (0.53 ng g⁻¹) in this study are much lower compare to 0.95 ng g⁻¹ in poultry feed in Beijing, China⁹. Evidence of OCPs have been found in poultry feed in Bangladesh²³, food basket eggs in Tehran⁸, cheese in Turkey⁹ and rabbit feed¹⁰, all these have been traced to animal feed.

**Correlation analysis of OCPs in all the feeds:** The general approach for studying OCPs interaction was achieved by computing the Pearson’s correlation coefficient. The data in Table 5 showed that aldrin was strongly correlated with dieldrin and endosulfan while dieldrin was strongly correlated with endosulfan at p<0.01 level. All other OCPs were either weakly correlated or negatively correlated. A strong correlation indicates that the feeds are from the same source.

**CONCLUSION AND FUTURE RECOMMENDATIONS**

This study investigated proximate composition, level of OCPs in broilers and layers mash, from selected farm mills in Amhara region, North shewa zone, Ethiopia. The data obtained in the proximate analysis represent great variations among the quality of the poultry feeds from selected feed mills. Each feed mill has its own formulation which doesn’t remain constant throughout the whole year but changes according to the feasibility of the constituents of the poultry feed. The existing information about the composition and nutritive value of the poultry feed permit the poultry farmers to select the better choice of feed and it’s ration for the better growth and health of the poultry on the basis of cost, palatability and energy. Of all 16 OCPs compound analysed, only 7 were detected in the feed. The source of this could be as a result of use of herbicides, pesticides and other chemicals. Despite the ban of some OCP compounds for agricultural purposes, its continue use is still evident as revealed in the results obtained.

This may be as a result of geochemical soil composition, the use of fertilizer and other environmental contaminants. However, there was either not detected or very low in both broilers and layers mash obtained from the feed mill. Moreover, the analysis have indicated that these fresh products are contaminated with 7 pesticides so it is clear that consumption of foods containing unsafe amount of pesticide residues are of public health concern, consequently entailing additional health cost. By the same token, this may affect economic development.

Thus, all concerned bodies of the country need to play their crucial role of ensuring that foods consumed by the general public are not of health concern. Even though, the concentrations of the detected analytes are lower than the reference MRL. Therefore, in order to have safe fresh produce, a variety of measures such as laws, regulations, standards and a system of effective inspection and laboratory analysis are urgently needed.

**SIGNIFICANCE STATEMENTS**

The study of the levels of pesticide residue in food item is very limited in the country. Therefore, the contamination status of animal feed by pesticide residues at all is unknown. This calls for an extensive study of the residue status of agricultural products. Thus the study will significant to show preliminary data regarding the pollution status of the poultry feed by pesticides. In addition, this research work can have the following outcome; It serves as baseline information for the development of standards regarding maximum pesticide residue limit in poultry feed and to protect the society from acute and chronic health hazard the status of contamination must be known. Also it contributes for the economic development of the country. That is pesticide residues in agricultural products may also have a detrimental effect on export agricultural crops.

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