International Journal of Veterinary Sciences and Animal Husbandry 2023; 8(4): 317-318

ISSN: 2456-2912
VET 2023; 8(4): 317-318

www.veterinarypaper.com
Received: 13-03-2023
Accepted: 19-04-2023

Deepak Soni
M.V.Sc, Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Vijay J Jadhav
Professor, Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Pallavi Moudgil
Scientist, Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Khushbu
M.V.Sc, Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Corresponding Author:
Deepak Soni
M.V.Sc, Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Determination of cyromazine and Melamine residues in poultry eggs

Deepak Soni, Vijay J Jadhav, Pallavi Moudgil and Khushbu

DOI: https://doi.org/10.22271/veterinary.2023.v8.i4e.622

Abstract
Cyromazine (CYR) and Melamine residues (MEL) in 40 samples of eggs were detected from layer farms located in selected districts of Haryana (Ambala, Jind and Panipat) using. High Pressure Liquid Chromatography (HPLC-UV). The separation was achieved using C18 column using acetonitrile and acidified TFA as a mobile phase (0.05%) with a flow rate of 1 ml/minute with UV detection at wavelength 214 and 230 nm at column temperature of 28 °C. Out of these, CYR was detected in 03 samples above the LOD (5.3 µg/kg). MEL was not detected in any of the sample analyzed. Cyromazine in the eggs can appear if the layer is subjected to fed cyromazine contaminated feed deliberately.

Keywords: Eggs, cyromazine, melamine, LOD, HPLC, Haryana

Introduction
Insect growth regulator cyromazine, also known as Trigard or Larvadex and having the chemical name N-cyclopropyl-1,3,5-triazine-2,4,6-triamine, is used to control flies in cow dung, field crops, vegetables, and animal foodstuffs (Chou *et al.*, 2003) [4]. Maximum residual levels (MRLs) of cyromazine in chicken feed must not be higher than 5.0 ppm, and feeding of feed treated with cyromazine must cease at least 72 hours prior to slaughter (EPA, 1999) [5]. Due to its larvicidal activity in the manure, layer farmers augment the meal of layer birds with CYR (Berry, 2003) [2]. Cyromazine is highly successful at controlling pests, however it is harmful to both people and the environment, like other chemicals used in the agriculture. Melamine (MEL), which can bind with its analogs like cyanuric acid to form crystals, is a metabolite product of CYR that undergoes metabolism by dealkylation or environmental degradation in both plants and animals. These crystals are a major public health concern because they may cause significant renal toxicity and carcinogenic effects in humans (Sancho *et al.*, 2005; Baynes *et al.*, 2008) [7, 3]. When consumed, CYR and MEL residues may cause major health effects in consumers. These residues can also be found in the eggs of layer birds fed CYR-treated feed (Bao *et al.*, 2011) [1].
Cyromazine and melamine can be detected using a variety of techniques, including isotope dilution method, High Pressure Liquid Chromatography (HPLC) with ultra-voilet detector, Gas Chromatography, and GC-MS. Consumers should be concerned owing to cyromazine residue and its metabolite as there is an evidence of its residues appear in the eggs by Bao *et al.*, 2011 [1]. As a result, the present study was conducted to determine the residue levels of cyromazine and melamine in varous poultry farms in Haryana.

Materials and Methods
Samples
From June to September 2022, a total of 40 egg samples were directly obtained from layer farms located in particular areas for this investigation. A total of 40 Layer farms located in the Jind, Ambala and Panipat district were selected. 05-10 egg samples were collected randomly from 20 farms of Jind district, 15 of Ambala district and 05 of Panipat district. After collection, eggs were homogenized and stored in a refrigerator at 4 °C upto analysis.

Reagents
Analyical standard of cyromazine and melamine were procured from Sigma-Aldrich Chemicals Private Limited, Bengaluru, India with purity more than 98%. Acetonitrile, n-hexane (HPLC grade) were procured from Fisher scientific. Whereas, 25% ammonia solution, trifluoroacetic acid (TFA), were procured from Central Drug House (P) Ltd. (CDH), New Delhi.

Instrumentation
A Shimadzu Prominence™ UHPLC system equipped with DGU-20A5R degasser, SIL- 20A HT autosampler and LC-20AD pump which was connected to C18 column (Chromasol® 4.0 mm x 250 mm having porosity 5 µm procured from Intek Chromasol Pvt Ltd, Bangalore-560099, India) housed in CTO-10AS column oven with SPD-20A detector was used for analysis of cyromazine and melamine. Lab solutions software was used for data processing.

Chromatographic conditions
The separation was achieved using C18 column using acetonitrile and acidified TFA as a mobile phase (0.05%) with a flow rate of 1 ml/minute with UV detection at wavelength 214 and 230 nm at column temperature of 28 °C.

Standard preparation
For prepartion of standard reagent, 100 mg of cyromazine and melamine was weighed and diluted in HPLC grade water. Further, the stock solutions was prepared by diluting the mobile phase. The solution were stored at 4 °C.

Sample preparation
5 g of egg sample was weighed and homogenized. Weak HCL solution (0.01M) was used for denaturation of protein. After that, the content was transferred to 25 mL centrifuge tube and vortexed for 1 min. For extraction and clean up, liquid liquid extraction technique was used using n-hexane. The pesticide is fat soluble and comes upwards when kept standing for several minutes. The fat soluble phase was collected and subjected to rotary evaporation. The residue diluted with mobile phase and was filtered using 0.2 µm syringe filter. Use 20 µL for HPLC analysis.

Results and Discussion
Out of the 40 samples, 03 samples that were procured from Ambala district were above the limit of detection (LOD 5.3 µg/kg) of cyromazine. Whereas, None of the sample was found positive for melamine residues. The concentration of analytes determined in each of the egg sample collected from various layer farms was compared with the MRL set forth by the international regulatory authority i.e. Codex Alimentarious Commission. It was found that all the samples were below the maximum regualtary limit as set by CAC (300 µg/kg) for cyromazine. The results are corroborate with the findings of Bao *et al.*, (2011) [1] in which melamine was not detected in any of the sample. In another study from Taiwan, Chou *et al.* (2003) [4] analyzed 10 egg samples collected from markets and found the concentration of both the analytes below the LOD. Miao *et al.* (2010) [6] showed absence of MEL and its metabolites *viz*. cyanuric acid (CA), Ammelide (AMD), ammeline (AMN) in market eggs of different brands.
Cyromazine in the eggs can appear if the layer is subjected to feed cyromazine contaminated feed deliberately. Melamine present in very acute amount leads to not present in the eggs as only 7% of cyromazine is degraded into melamine.

Conclusion
None of the Sample was found above the maximum regulatory limit. Cyromazine appears in eggs only if fed cyromazine contaminated feed in excess level. Melamine presence is negligible in the eggs. Monitoring levels need to be strengthened for usage of cyromazine in the commercial layer farms.

References

1. Bao H, Wei R, Ming C, Wang R. Residues of cyromazine and its metabolite melamine in eggs of laying hens following consumption of cyromazine contaminated feed. Journal of Livestock Science. 2011;2:1-10.
2. Berry JG. Fly control in the poultry house. Oklahoma Cooperative Extension Service, 2003. https://www.thepoultrysite.com/articles/fly-control-in-the-poultry-house. Accessed on 19.01.2023.
3. Baynes RE, Smith G, Mason SE, Barrett E, Barlow BM, Riviere JE. Pharmacokinetics of melamine in pigs following intravenous administration. Food and Chemical Toxicology. 2008;46:1196-1200.
4. Chou SS, Hwang DF, Lee HF. High Performance Liquid Chromatographic Determination of cyromazine and Its Derivative Melamine in Poultry Meats and Eggs. Journal of Food and Drug Analysis. 2003;11(4):290-295.
5. EPA US. Cyromazine; Pesticide Tolerance, 1999. 64, 50043- 50050. (http://www.epa.gov/EPA-PEST/1999/September/Day-15/p24047.htm). Accessed on 05.05.2023.
6. Miao H, Fan S, Zhou PP, Zhang L, Zhao YF, Wu YN. Determination of melamine and its analogues in egg by gas chromatography-tandem mass spectrometry using an isotope dilution technique. Food Additives and Contaminants. 2010;27(11):1497-1506.
7. Sancho JV, Ibanez M, Grimalt S, Pozo OJ, Hernandez F. Residue determination of cyromazine and its metabolite melamine in chard samples by ion-pair liquid chromatography coupled to electrospray tandem mass spectrometry. Analytica Chemica Acta. 2005;530:237-243.