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Fecal Metabolites Analysis In Cattle: An Important Clue For Estimation of Bio Magnification.

Yellepeddi K Karthik[1], Krishnasatya A[1*]

[1] Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

[*]**Corresponding Author:** Dr. Krishna Satya A, Associate Professor, Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Abstract:

Accumulation of toxins such as pesticides or other harmful material into food chain is called bio magnification and it is a major problem in food chain. . During the cultivation of crops, pesticides are widely used to control pests such as insects, bacteria, fungi, molds, rodents and weeds. Due to climate changes, pesticide usage is increasing and they are also toxic to other organisms, including beneficial insects, non-target plants, birds, water, air, soil, and crops. These pesticide residues are detected in trace amounts on a fruit or vegetable or crop produced. These chemical residues are impacting human health through food and environmental contamination. Majority of feed supplied to cattle is rice grass and other grass, there is a need to assess the safety of feed with respect to bio magnification. So it is very important to feed the animals with proper feed. Large number of plants and chemical agents will become poisonous to animals. Most of the dairy farms use hotel and food wastes as one of their feed source along with their nutritional diet. But most of the human diet crops are cultivated with Pesticides and they were using as feed to cattle which is leading to bio magnification, so it is important to monitor those agents to maintain animal health. Our study demonstrated a primary data for the evaluation of bio magnification in cattle by analysing dung samples with GC-MS. Further progression of this research will help us to understand the level of bio magnification in cattle.

Keywords: Biomaginification, pesticides, food chain, fecal metabolites and GC-MS.

Introduction:

Accumulation of toxins such as pesticides or other harmful material into food chain is called biological magnification which is also known as bio amplification or bio magnification. During the cultivation of crops, pesticides are widely used to control pests such as insects, bacteria, fungi, molds, rodents and weeds. Pesticides are used to kill pests are also toxic to other organisms, including beneficial insects, non-target plants, birds, water, air, soil, and crops. It is important to note that the used pesticide residue is detected in trace amounts on a fruit or vegetable or crop produced. When these pesticides enter into food chain and they are consumed by other animals, these toxic substances then move up the food chain progressively greater concentrations. These chemical residues are impacting human health through food and environmental contamination. Due to climate changes, pesticide usage is increasing as a result pesticide pollution increasing [1]. Analysis of pesticides require accurate methods to detect pesticides in food products, GC-MS/MS allow us to detect pesticides [2]. Almost 404 articles are published regarding pesticide presence and review report considered only 15 studies and review report concluded that mass spectroscopy is the most widely used technique

to determine the presence of pesticides and pesticide residues are present in banana [3]. In a study 15% of food items are detected with pesticide residues [4] and raw vegetables and fruits are tested with Ultrahigh performance liquid chromatography-tandem Q/Orbitrap high-resolution mass spectrometer (UPLC-Q/Orbitrap-HRMS) using established data base of 392 pesticides, 95% samples are detected with one or more pesticides within limits and co-occurrence of multiple residues are severe in muskmelon and peach [5]. As majority of dairy farms uses rice grass and other grass as feed, there is a need to assess the safety of feed with respect to bio magnification. So it is very important to feed the animals with proper feed. Large number of plants and chemical agents will become poisonous to animals. Most of the dairy farms use hotel and food wastes as one of their feed source along with their nutritional diet. But most of the human diet crops are cultivated with Pesticides and they were using as feed to cattle which is leading to bio magnification, so it is important to monitor those agents to maintain animal health.

Materials and Methods

Sample collection:

Cows and buffalo dung samples are collected from local dairy farms located at Hyderabad. Samples are collected carefully in 4 × 6 inches zip lock bags from healthy animals and placed them in Thermocol box with ice packs. Samples are stored at -20°C in laboratory till the extraction of metabolites [6].

Extraction of Metabolites:

All samples are allowed to reach room temperature prior to the extraction. In 15ml falcon tubes, 1±0.05g of dung sample is weighed. 5 ml of 90% methanol (Methanol: Qualigens, Milli Q water) is added to sample. Methanol added samples are vortexed to mix well for 30 seconds by using vortexer. After vortexing, samples are centrifuged at 1500g for 5 minutes. Pellets are discarded and supernatants are collected in 5ml cryogenic vials (CORNING®). Vilas are sealed with para film and stored at -20°C till further processing [6].

HPLC analysis:

HPLC analysis was performed with Waters alliance e2695 HPLC system equipped with 2998 PDA detector and Empower 3 Software. Extracted metabolite solution was centrifuged to settle the particles and diluted to 2fold with Milli Q water. Diluted sample was filtered through 0.2µ syringe filters (Millipore) and analysed with the following method conditions [7].

HPLC Method	
Run time	20 minutes
Mobile phase	Acetonitrile and Milli Q water
Gradient programme	Programme starts with 30% acetonitrile, reaches to 50% by 3rd minute and reaches to 100% by 8th minute. 100% acetonitrile continue till 14th minute, reaches to 30% by 18th minute and continue till 20th minute.
Flow rate	1mL per Minute
Sample injection volume	100µL
Sampler temperature	15±5°C
Column	Phenomenex C18 Kinetex 2.6µm 100A° (150*4.6mm)
Column temperature	35±5°C
Detector wavelength	194nm, 214nm, 240nm, 254nm, 280nm and 310nm.

Table 1: HPLC Programme parameters to analyse fecal hormones.

Fractions collection:

HPLC eluted components were collected at detector outlet as manual fractions in glass vials. Eluted fractions were completely evaporated in an oven at 100°C. Fractions were dissolved in methanol and stored at -20°C till further analysis.

Analysis of components by GC-MS:

Silylation Procedure: During sylilation procedure, methanol is evaporated to dryness using nitrogen gas. To the residue, 100µL Acetonitrile and 100µL BSTFA (silylating reagent) are added and heated at 60°C for 1 hour. Samples are submitted to CSIR- IICT (Hyderabad, India) for further analysis.

GC-MS-MS analysis procedure at CSIR- IICT: Sample is evaporated and dissolved in 0.5 mL acetonitrile at the time of GC-MS analysis. Analysis is performed in Agilent 6890 Gas chromatography installed with chemstation software (Agilent Technologies, Palo Alto, CA, USA) equipped with HP-5MS capillary column (30m length, 250µm internal diameter and 0.25µm film thickness) and 5973N mass selective detector. 1µL sample is injected and analysed with the following method parameters and results are compared in NIST library to identify compounds [6].

Run time	30 minutes
Column initial temperature	50°C with 2 minutes hold-up time
Column final temperature	280°C with 5 minutes hold-up time
Temperature ramp	10°C/minute
Carrier gas flow	Helium gas at 1.2mL flow rate/minute constant flow
Inlet temperature	250°C
GC-MS interface temperature	280°C
Sample injection mode	Split mode with 10:1 split ratio
EI source temperature	230°C
Quadrapole analyser temperature	150°C
MS scan range	m/z 29 to 600

Table 2: GC-MS Programme parameters to analyse sample fractions.

Results and discussion:

Extracted metabolites are analysed and monitored in HPLC with specific wavelengths but components that are eluting out with different absorption capacity will not be appeared in chromatogram. Hence when the eluted fractions analysed in GC for identification of hormone metabolites showed trace amounts of chemicals. Generally fecal sample investigation provide some information about feed. After investigating the traced chemicals they are observed as polymer industry related chemicals, pesticides, veterinary preservatives of medicines and antibiotics. During the treatment of animals veterinary medicines will be injected into body which contains preservatives such as glycerol and benzoic acids. Synthetic hormones are detected in GCMS analysis. Generally synthetic hormones administered will be utilised by the animal and will be excreted in feces but at the same time those synthetic hormones may permeate to milk also, in that case they enter into food chain and lead to bio magnification. During treatment of animals, injected antibiotics traces are found in feces. Major concern in GCMS analysis output is presence of polymers related chemicals and pesticides related chemicals. Cyclo hexanol, Piperlene, Trsiloxane, Tetrazole and Triazole 4 amine are the few traces observed in GCMS analysis. Cyclo hexane is an organic compound which is the major feed stock of polymer industry. Cyclo hexose is moderately toxic and few studies reported that it is carcinogenic. 1, 3-pentadiene is also known as Piperylene is an organic compound that is used as monomer in plastic manufacturing. Most of the food is transporting through plastic bags, the chemicals that are used in their manufacture entering into food chains of cattle. Dairy farms collect food wastage from hotels and houses where these plastic bags are disposing in that waste might entering into food chain of cattle which is becoming gate way for food chain of humans. These chemicals are dangerous and carcinogenic. So care must be taken in cattle feed to avoid bio magnification impact on humans. Piperylene is classified as hazardous chemical to our health as it is a potential

carcinogen and mutagen. Trisiloxane is a surfactant often referred to as superwetters or superspreaders. They are added to pesticides for the enhanced activity of pesticides. It promotes the rapid spreading of the active substance over the hydrophobic surfaces of leaves. Trisiloxane is reported as toxic material. Tetrazole works effectively as antibacterial, anticancer, antifungal and antihypertensive potency agent. 4-Amino-1, 2, 4-triazole is used as an intermediate for the preparation of fluconazole which is antifungal agent. Presence of these agents in feces represents their intake through physical administration or feed. During cultivation of rice and grass, used pesticides are entering into food chain of cattle which are detected in feces and there might be a chance for entering of those pesticides in human food chain through beef and milk.

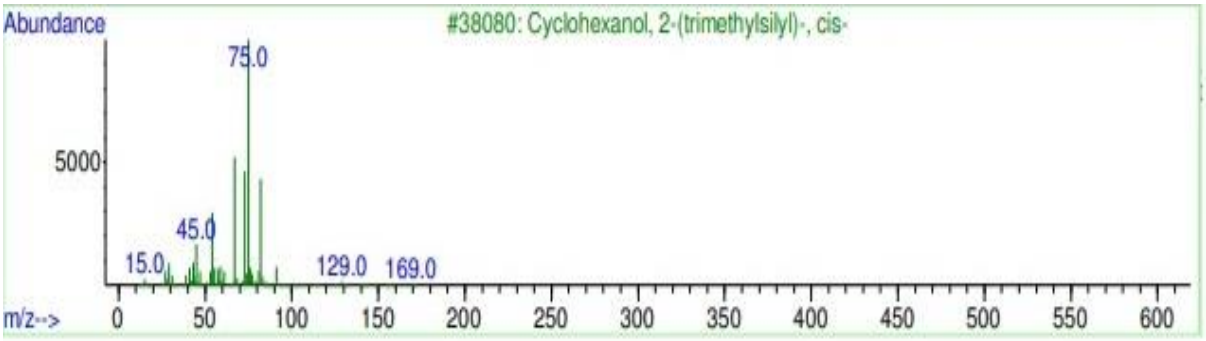

Figure 1: Cyclo hexanol identification in method development sample by GCMS.

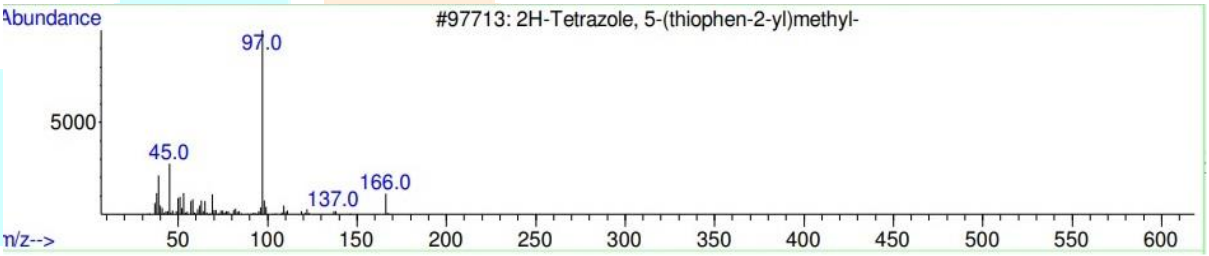

Figure 2: Tetrazole identification in method development sample by GCMS.

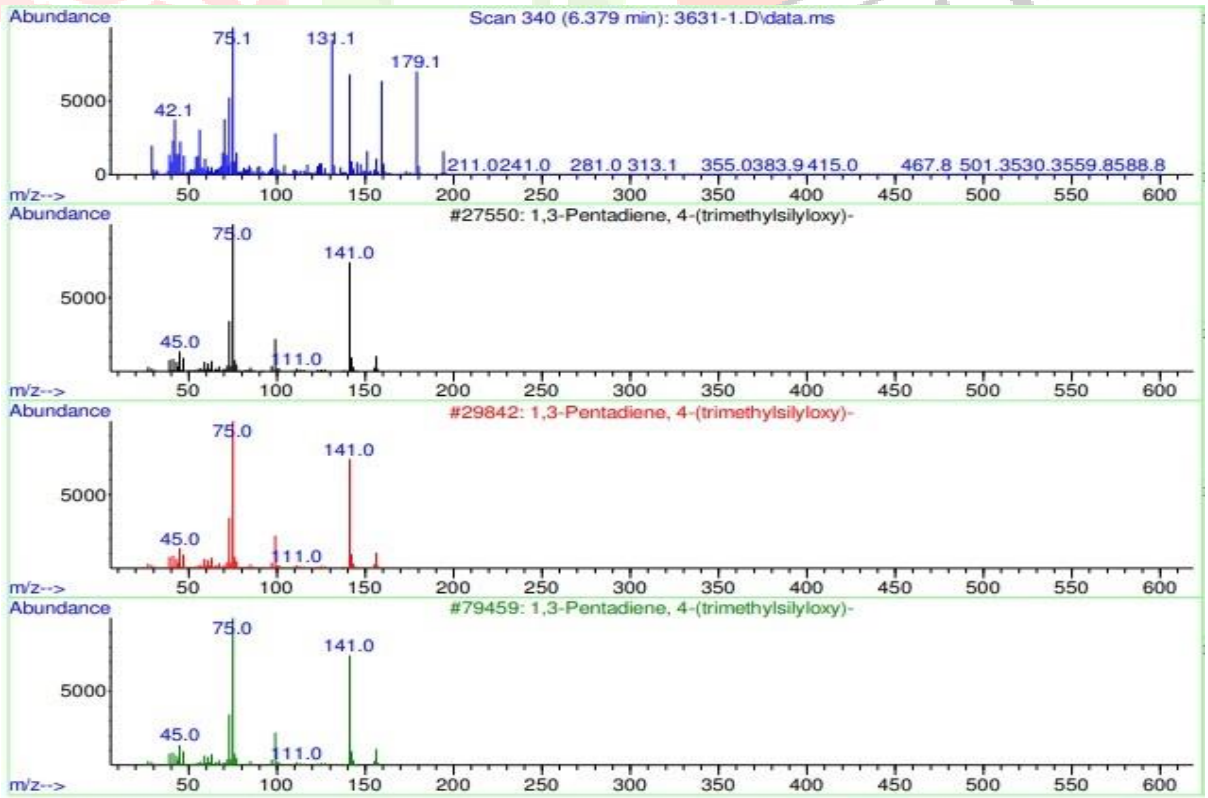

Figure 3: Pentadiene identification in method development sample by GCMS.

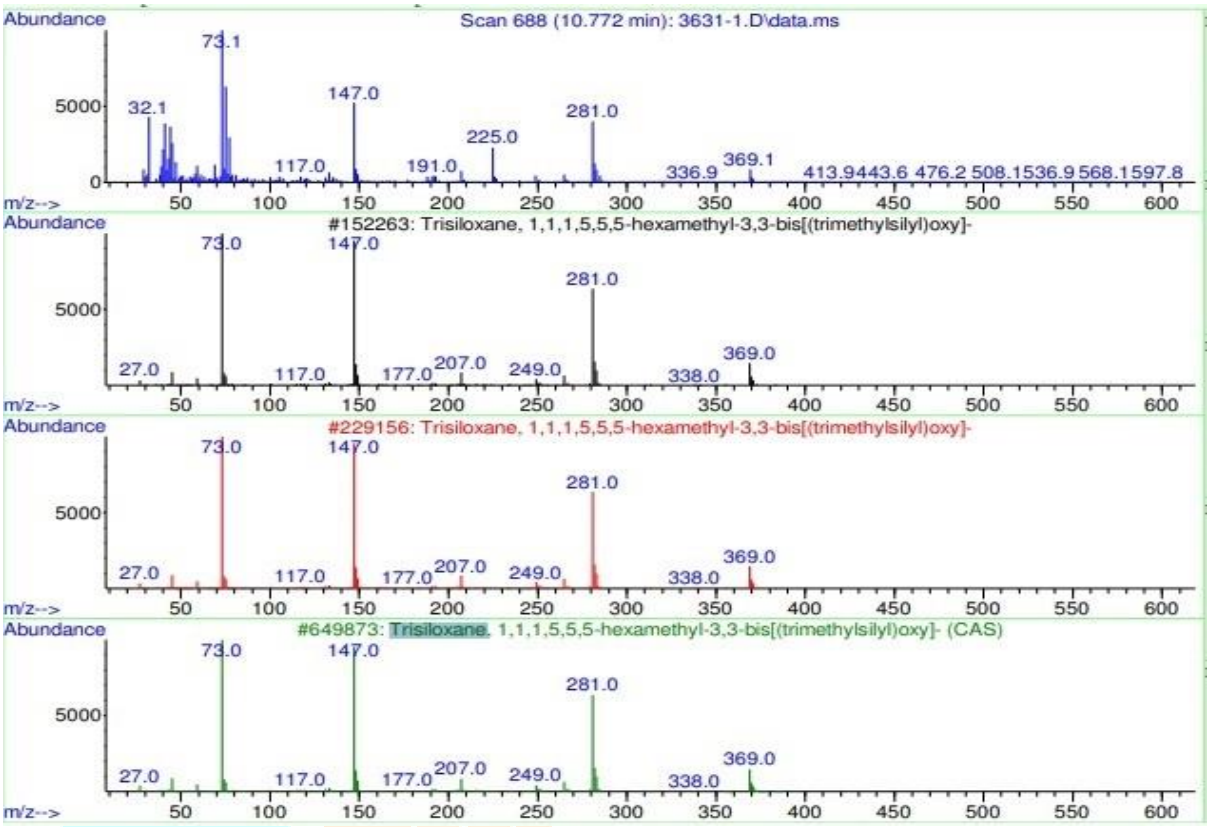

Figure 4: Trisiolxane identification in method development sample by GCMS.

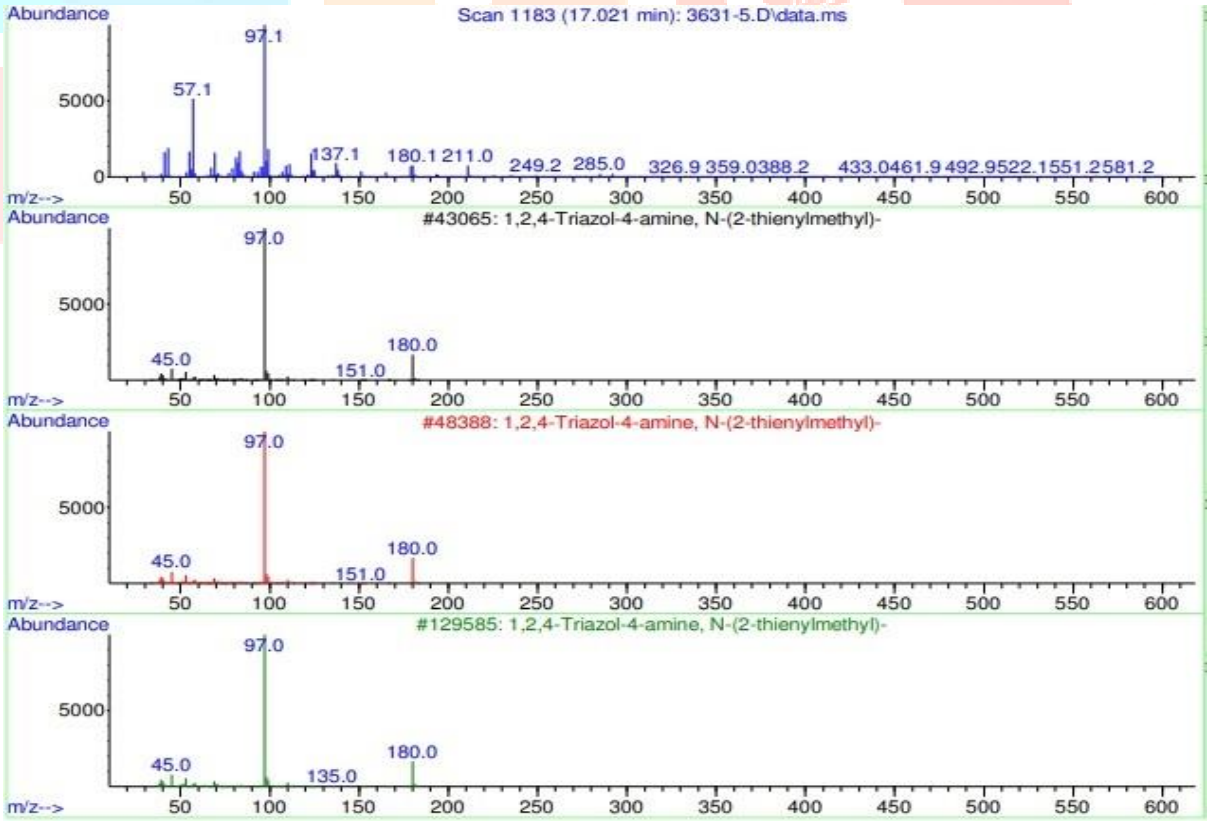

Figure 5: Triazole 4--amine identification in method development sample by GCMS.

Conclusion:

Food play a crucial role in human health and cattle are the animals that are producing essential diet to humans. Biomagnification is the severe problem that is facing by mankind and research need to be progressed to assess the biomagnfication and its impact on human health. As of now very limited information is available on biomagnification , in our study, we assessed few dung samples to understand the feasibility of biomagnification assessment by non-invasive methods. Our study provided a clue to investigate biomagnification by using dung and it can be further investigated to evaluate the level of biomaginification in cattle and its impact on human health.

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Declaration of interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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