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Detection of Urea Adulteration in Oilseed Cakes using 4-Dimethylaminobenzaldehyde Reagent and Foldscope

T. Anitha[1], S. Bindu Madhuri[1], G. Alexander[2], A. Sarat Chandra[1], M. Venkateswarlu[2]

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ABSTRACT

Background: Costly livestock feed ingredients like oilseed cakes are more frequently adulterated with urea by feed manufacturers and suppliers to derive economic benefit. However, a simple method to detect adulteration of urea in oilseed cakes under field conditions is not available. In this context, the present study was carried out to evaluate the usefulness of 4-dimethylaminobenzaldehyde reagent for the detection of urea adulteration in oilseed cakes using foldscope under field conditions.

Methods: Six commonly used oilseed cakes for livestock feeding were procured from three different sources and processed in duplicate. A minute quantity of dry powdered oilseed cake or oilseed cake adulterated with urea (10 g/kg) was taken on a glass slide, treated with 4-dimethylaminobenzaldehyde reagent and air dried. The air dried sample was spread and covered with transparent cello tape and examined under foldscope with a standard light source. The foldscope images were captured using smart phone camera. Approximate area covered with yellow colour in foldscope images of oilseed cakes adulterated with urea at three different concentrations also evaluated.

Result: Oilseed cakes adulterated with urea on treatment with 4-dimethylaminobenzaldehyde reagent showed yellow colour covering almost entire area of many fields in foldscope images, while the colour of oilseed cakes devoid of urea was mostly pink to violet. The oilseed cakes adulterated with urea at different concentrations varied in the approximate area covered with yellow colour in foldscope images. The 4-dimethylaminobenzaldehyde reagent can be used to detect urea adulteration in oilseed cakes using foldscope under field conditions.

Key words: 4-Dimethylaminobenzaldehyde, Adulteration, Foldscope, Oilseed cakes, Urea.

INTRODUCTION

Oilseed cakes are commonly used as protein sources for feeding of livestock and poultry. Because of high protein content, oilseed cakes are generally costly. Oilseed cakes like groundnut cake, mustard cake and soyabean meal are frequently adulterated with cheaper non-protein nitrogen substances like urea in order to increase their protein content and thereby to obtain financial gain (Uppal *et al*., 2004). Urea can be incorporated in adult ruminant rations through concentrate mixture, crop residues, silage and complete feed as a source of non-protein nitrogen for microbial protein synthesis in limited amounts without any adverse effect. However, higher level of inclusion of urea in ruminant rations may induce ammonia toxicity (McDonald *et al*., 2010). Further, urea is not recommended in the rations of non-ruminants and pre-ruminants as it may produce adverse effects even at lower level of inclusion.

Protein content of feedstuffs including oilseed cakes are mostly determined through kjeldahl method. In kjeldahl method, nitrogen content is determined first and the protein content is derived by multiplying nitrogen content with a factor. Therefore, it is not possible to detect adulteration of urea in oilseed cakes while estimating protein content through kjeldahl method.

Several methods such as spectro-colorimetric, enzymatic, high performance liquid chromatography coupled with fluorescence detection and tandem mass spectrometry are available for quantification of urea in feedstuffs (Kramer *et al*., 2021; Pibarot and Pilard, 2012). Among the available methods, spectro-colorimetric methods and enzymatic (urease) methods are commonly used for urea analysis in feedstuffs due to their simple protocol (Pibarot and Pilard, 2012). An analytical method that is applicable to detect urea in feedstuffs on the spot is highly useful for avoiding of purchase of urea adulterated costly feed ingredients like oilseed cakes. However, above mentioned methods are not suitable for detection of urea on the spot under field conditions.

Foldscope is a portable, cheap paper microscope with the magnification power of 140 X developed at Stanford University, United States (Cybulski *et al*., 2014). It can be

[1]Department of Livestock Production Management, College of Veterinary Science, P.V. Narsimha Rao Telangana Veterinary University, Hyderabad-500 030, Telangana, India.
[2]Department of Animal Nutrition, College of Veterinary Science, P.V. Narsimha Rao Telangana Veterinary University, Hyderabad-500 030, Telangana, India.

Corresponding Author: G. Alexander, Department of Animal Nutrition, College of Veterinary Science, Mamnoor, Warangal-506 166, Telangana, India. Email: dralexvet@rediffmail.com

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used routinely for microscopic examination of feed under field conditions. Spectro-colorimetric method of urea analysis is based on the reaction of urea with 4-dimethy laminobenzaldehyde (4-DMAB) reagent (FAO, 2011; Pibarot and Pilard, 2012). Yellow colour was observed under foldscope when urea was examined after addition of a drop of 4-DMAB reagent. Similarly, yellow colour spots were observed in the foldscope images of urea adulterated soyabean meal treated with 4-DMAB reagent. Hence, using 4-DMAB and foldscope, urea adulteration in soyabean meal can be detected under field conditions (Alexander, 2018). In this context, the present study was conducted to assess the usefulness of 4-DMAB reagent to detect adulteration of urea in various oilseed cakes using foldscope.

MATERIALS AND METHODS

This study was carried out during the year 2019 at Department of Animal Nutrition, College of Veterinary Science, P.V. Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad.

Foldscope

Foldscopes manufactured by Foldscope Instruments, Inc., California, United States were supplied by Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi. Foldscopes were provided as a sheet of paper along with accessories. Foldscopes were assembled as per the guidelines of the manufacturer and used for the experiments. The magnification power of foldscope was 140 X (Cybulski *et al.*, 2014). Chargeable LED light was used as light source. The LED light was attached to foldscope using magnetic couplers while viewing and capturing foldscope images with smart phone camera.

Collection and processing of oilseed cakes

The oilseed cakes that are commonly included in the rations of livestock such as soyabean meal, groundnut cake, un-decorticated cottonseed cake, decorticated cottonseed cake, sunflower cake and rapeseed meal were collected from three different sources. Two hundred and fifty grams of sample was collected for each oilseed cake as per the standard sampling procedure and stored in the polythene zip lock covers. Further, sub sample of 25 g was taken from 250 g of sample collected for each oilseed cake from three different sources. The samples were crushed with mortar and pestle to reduce particle size. The crushed samples were passed through 0.3 mm screen and preserved in plastic containers for examination under foldscope.

Preparation of urea adulterated oilseed cakes

Urea was obtained from Merck Life Sciences Private Limited, Mumbai. The different powdered oilseed cakes were mixed with urea powder to achieve the urea concentration of 10 g/kg.

Preparation of 4-dimethylaminobenzaldehyde reagent

The 4-DMAB reagent was prepared by dissolving 1.6 g of 4-DMAB in 100 ml of aqueous methanol (960 ml/l) and mixing the resultant solution with 10 ml of concentrated hydrochloric acid (FAO, 2011). Aqueous methanol was used to dissolve 4-DMAB instead of aqueous ethanol which was used in FAO (2011) procedure. This reagent was kept for a maximum period of two weeks at 4°C in a refrigerator.

Slide preparation, foldscope examination and image capturing

A minute quantity of powdered oil seed cake or urea adulterated oil seed cake was taken on a glass slide and treated with 2 drops of 4-DMAB reagent. After evaporation of reagent, the air dried sample on the slide was spread uniformly and covered with transparent cello tape. The processed slide was inserted into foldscope in such a way that the sample side of slide was close to lens. The oilseed cakes samples were evaluated in duplicate. A standard light source (Chargeable LED light) was used throughout the study period. The foldscope images were photographed using a smart phone camera (Redmi Note 7 Pro) by adjusting the zoom function of camera. The images were captured as indicated earlier to obtain 6 images for each oilseed cake of adulterated and unadulterated samples.

Determination of approximate area covered with yellow colour in foldscope images of oilseed cakes adulterated with urea at three different concentrations

Soyabean meal, groundnut cake and un-decorticated cottonseed cake collected from three different sources were adulterated with urea at 1, 5 and 10 g/kg concentration as described earlier. The samples were processed in duplicate for each concentration and for each oilseed cake to obtain six slides for each concentration. These urea adulterated samples were examined under foldscope after treating with 4-DMAB reagent. Thirty fields in foldscope were observed for each slide for presence of yellow spots. Accordingly, total of 180 fields were observed for each oilseed cake at specific concentration. Based on the approximate area covered with yellow colour, one point was given when 25 per cent or less of the foldscope field covered with yellow colour. Accordingly, two, three and four points were provided when 25 to 50, 50 to 75 and 75 to 100 per cent of the fields covered with yellow colour, respectively. The percentage of area covered with yellow colour in foldscope images was calculated by dividing the score obtained with the maximum possible score (120) for each slide and the mean and standard error were calculated based on the values obtained from six slides.

Detection of adulteration of urea in oilseed cakes from different dairy farms and poultry farms

While conducting field demonstration programme on the use of foldscope for quality control of feed ingredients at different dairy and poultry farms of Telangana, oilseed cakes used for feeding in the farms were collected. The oilseed cakes were evaluated for the presence of urea using 4-DMAB reagent and foldscope on the spot and also later at the laboratory. The un-decorticated cottonseed cake and groundnut cake were collected and evaluated for presence of urea from 5 and 4 dairy farms, respectively. Rapeseed meal was collected and evaluated from 2 dairy farms while

soyabean meal was collected and evaluated from 2 dairy farms and 2 poultry farms.

RESULTS AND DISCUSSION

Detection of adulteration of urea in oilseed cakes using 4-dimethylaminobenzaldehyde reagent and foldscope

This method is based on the concept that 4-DMAB reacts with urea in the presence of hydrochloric acid to produce yellow-green colour which can be used for the detection of urea (Basova *et al*., 2011). Further, urea treated with 4-DMAB reagent showed yellow colour under foldscope (Alexander, 2018).

Foldscope images of 4-DMAB treated soyabean meal, groundnut cake, un-decorticated cottonseed cake, decorticated cottonseed cake and sunflower cake showed pink to violet colour (Fig 1). The colour of powdered rapeseed meal on treatment with 4-DMAB appeared light pink and orange (Fig 1) due to original characteristic orange colour of seed coat of rapeseed meal. However, urea adulterated (10 g/kg) soyabean meal, groundnut cake, un-decorticated cottonseed cake, decorticated cottonseed cake, sunflower cake and rapeseed meal in foldscope images were almost completely covered with golden yellow colour in many fields on reaction with 4-DMAB reagent (Fig 2) suggesting that urea adulteration in oilseed cakes can readily be detected by treating the oilseed cakes samples with 4-DMAB reagent and then examination with foldscope. This is in accordance with observations of earlier study on detection of urea in soyabean meal using 4-DMAB reagent and foldscope (Alexander, 2018). However, yellow colour was not observed in foldscope images on the characteristic orange colour structure of seed coat of urea adulterated rapeseed meal (Fig 2). Therefore, caution may be required while evaluating rapeseed meal containing low level of urea through this method.

In the original procedure (FAO, 2011), 4-DMAB was dissolved in aqueous ethanol. However, in the present study, aqueous methanol was used as solvent to dissolve 4-DMAB due to restrictions associated with procurement of ethanol. Aqueous methanol had been used to dissolve 4-DMAB in previous studies (Mahipalsinh *et al*., 2017).

Determination of approximate concentration of urea in oilseed cakes using 4-dimethylaminobenzaldehyde reagent

The oilseed cakes adulterated with urea at different concentration differed in the approximate area covered with yellow spots (Table 1). Preliminary study confirmed that urea adulteration in oilseed cakes can be detected using 4-DMAB reagent and foldscope. However, the usefulness of this method to describe the extent of adulteration of urea in the oilseed cakes samples was not known. Therefore, three selected oilseed cakes were adulterated with urea at three different concentrations and the area covered with yellow colour in foldscope images on treatment with 4-DMAB was measured by assigning a value as described in materials and methods.

The area (%) under yellow colour in foldscope images for different oilseed cakes ranged from 6.66 to 8.19, 29.99 to 34 and 59.58 to 67.35 for the urea concentration of 1, 5 and 10g/kg, respectively. Therefore, it may be possible to roughly specify the extent of adulteration of urea in the oilseed cakes using 4-DMAB reagent and foldscope. It is also evident from the results of the present study that the method is sensitive enough to detect urea concentration of less than 1 g/kg in oilseed cakes.

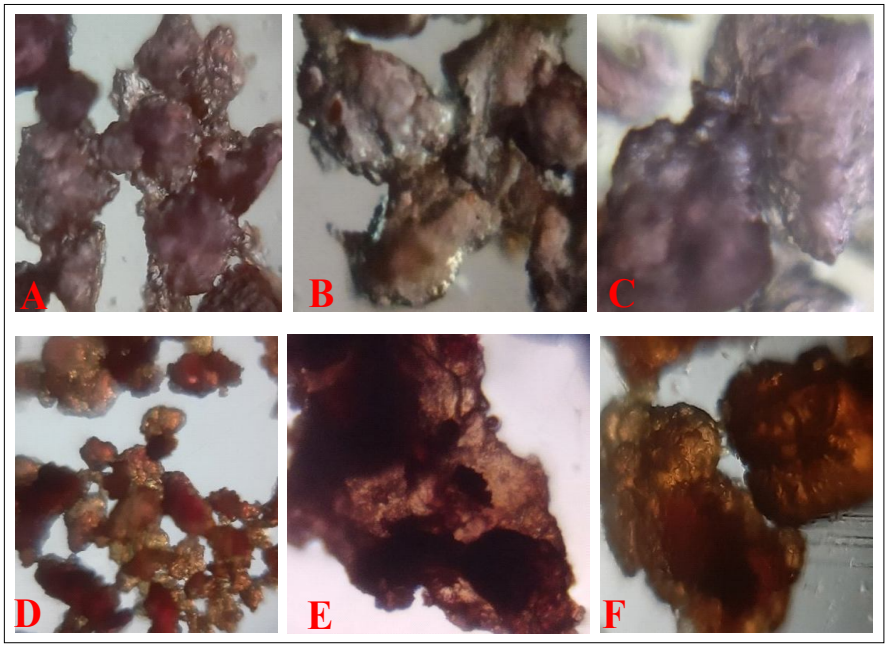

Fig 1: Foldscope images of oilseed cakes treated with 4-DMAB reagent: (A) Soybean meal; (B) Groundnut cake; (C) Un-decorticated cottonseed cake; (D) Decorticated cottonseed cake; (E) Sunflower cake; (F) Rapeseed meal.

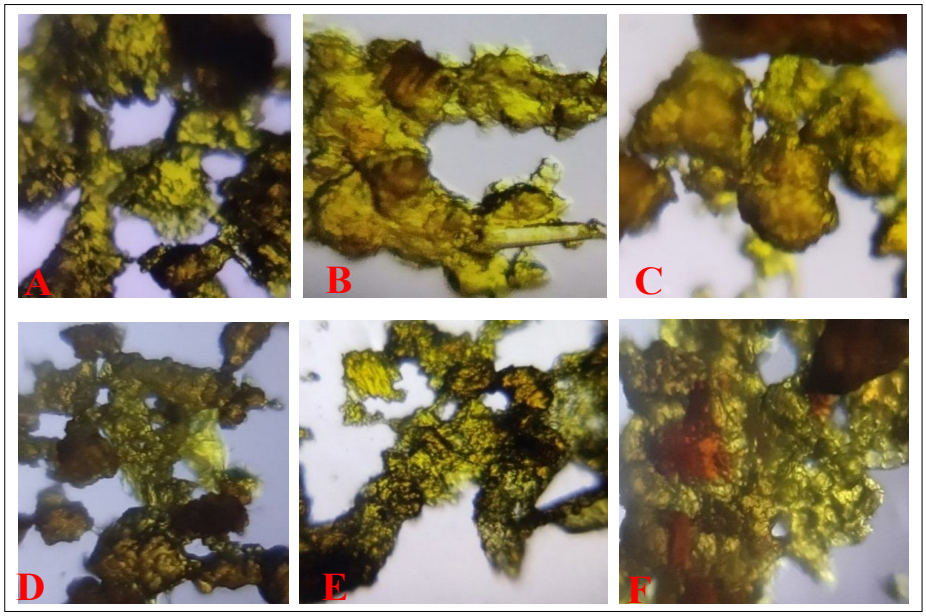

Fig 2: Foldscope images of urea adulterated oilseed cakes treated with 4-DMAB reagent: (A) Soyabean meal; (B) Groundnut cake; (C) Un-decorticated cottonseed cake; (D) Decorticated cottonseed cake; (E) Sunflower cake; (F) Rapeseed meal.

Table 1: Approximate area covered with yellow colour in foldscope images of oilseed cakes adulterated with urea on treatment with 4-dimethylaminobenzaldehyde reagent.

	Urea concentration (g/kg)		
	1	5	10
Soyabean meal	6.66±0.48	29.99±0.94	59.58±0.64
Groundnut cake	7.35±0.50	32.35±0.95	63.60±0.98
Un-decorticated cottonseed cake	8.19± 0.84	34.0± 0.79	67.35±0.89

Since free amino acids react with 4-DMAB and absorb at 435 nm, urea content may be overestimated when urea is determined through spectro-colorimetric method in food samples containing significant levels of free amino acids. Methionine was detected as urea when methionine containing samples were analysed for urea by spectro-colorimetric method (Pibarot and Pilard, 2012).

Soyabean meal was found to contain various free amino acids and the total free amino acid content of soyabean meal was 4.25 g/kg (Li and Wu, 2020). In the present study, soyabean meal treated with 4-DMAB reagent did not show any yellow colour under foldscope suggesting that the level of free amino acids in soyabean meal may not be sufficient to produce yellow colour on reaction with 4-DMAB reagent. Therefore, free amino acids in oilseed cakes may not interfere with detection of urea in oilseed cakes using 4-DMAB reagent and foldscope.

Detection of urea adulteration in oilseed cakes from different dairy farms and poultry farms

Un-decorticated cottonseed cake and groundnut cake are commonly used oilseed cakes as protein sources in the rations of dairy animals of small holder as well as organized dairy farms of Telangana and other states of India. Soyabean meal is the most common protein source in the broiler and layer chicken diets throughout the world. Urea was detected in 4 un-decorticated cottonseed cake and 2 groundnut cake samples out of 5 un-decorticated cottonseed cake and 4 groundnut cake samples evaluated using 4-DMAB reagent and foldscope. However, urea was not detected in any of the soyabean meal and rapeseed meal samples. Even though, the number of samples evaluated in the present investigation was less, the results obtained indicated that un-decorticated cottonseed cake and groundnut cake may be adulterated with urea to a considerable extent under field conditions.

The current method can be readily used to detect urea adulteration in oilseed cakes under field conditions without a smartphone and artificial light source if sufficient sunlight is available at the place of testing and image documentation is not necessary.

CONCLUSION

The 4-DMAB reagent on reaction with urea in oilseed cakes showed yellow colour under foldscope. This colour change can be used to detect adulteration of urea in various oilseed cakes using foldscope under field conditions. Approximate concentration of urea in oilseed cakes can also be determined using this method.

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Conflict of interest: None.

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