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Correlative Studies between Quantitative and Biological Estimations of Aflatoxins Extracted from Cattle Feeds and their Ingredients of Gujarat State*

A. B. Fulsoundar and P. C. Shukla**

Animal Nutrition Department, Gujarat Agricultural University, Anand Campus, Anand.

ABSTRACT

The study was carried out to estimate the biologically positive samples quantitatively for their crude aflatoxins content on Beckman DU-2 spectrophotometer by using standard mixture of aflatoxins (B_1, B_2, G_1 and G_2) procured from V. P. Chest Institute, New Delhi. It was found that deoiled Sheria (Hibiscus cannabinus, Linn.) cake had the minimum aflatoxin content (0·05 ppm.) while banti (Echinochloa stagmina, Beaur.) a cereal grain had the maximum of the toxin content (5·44 ppm.).

An attempt was made to calculate the amount of the crude aflatoxins as per the suggestion of Robertson, Pons and Goldblutt (1967). It was observed that the calculated quantitative values obtained by this method of each of the samples were comparatively higher than the spectrophotometric method. The general range (calculated) by this method was found to be 0·07 to 7·31 ppm.

The correlations were worked out between the concentrations of the aflatoxins (ppm) of both the methods with the zone of inhibition of bacterial growth (diameter in cm). It was observed that both the correlations (i) spectro-photometric concentration of aflatoxin and zone of inhibition (0·967) and (ii) Robertson et al (1967) method concentration and the zone of inhibition (0·798**) were statistically significant ($P < 0.01$). These results thereby suggests that zone of inhibition can be used for approximate quantitative estimations, when the costlier equipment like Beckman DU-2 spectrophotometer is not available.**

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INTRODUCTION

CONSIDERING the increased severity of unexplained diseases and their effects on a subsequent deterioration of animal health, an attempt was made to assess commonly used concentrates and compounded cattle feeds for their extent of aflatoxin content and to correlate the quantity of aflatoxin (ppm) with zone of inhibition (diameter in cm) of bacterial growth.

METHODS AND MATERIALS

The feed samples were quantitatively analysed for their presence of aflatoxin as per A.O.A.C. (1970) and biologically analysed to confirm the presence of aflatoxins by using the micro-organism as per the suggestions of Sheth (1973) and Subrahmanyam and Rao (1973).

Biologically positive samples for aflatoxins were quantitatively analysed on Beckman DU-2 spectrophotometer by using the standard aflatoxin mixture (B_1,B_2,G_1 and G_2) procured from V. P. Chest Institute, New Delhi as standard and methanol as organic solvent used to dissolve aflatoxins. The standard curve

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of above mentioned standard was prepared by plotting its different concentrations against their optical density at 363 mcg wave length and it was observed that aflatoxin content as per the curve was 43·5 mcg/optical density and accordingly optical density readings of test samples were taken and quantity was calculated.

The quantity of the crude aflatoxins (ppm) obtained by both the methods were correlated with the zone of inhibition (diameter in cm) of bacterial growth.

RESULT AND DISCUSSION

The results of quantitative analysis on Beckman DU-2 spectrophotometer and calculated quantity of aflatoxins as per Robertson *et al* (1967) method along with their biological effects on bacterial growth are given in Table 1.

TABLE 1

Quantitative results of biologically positive feed samples by spectrophotometric method and Robertson et al calculation method and their correlation with zone of inhibition.

Sr. No.	*Class and Code No. of feed sample*	*Zone of inhibition (cm.)*	*Method A (ppm)*	*Method B (ppm)*
1.	2.	3.	4.	5.
I	Deoiled groundnut cake			
	A_{17}	0·5	0·326	0·440
	A_{20}	0·7	0·734	0·990
	P_1	1·1	0·863	1·160
	R_4	1·1	0·691	0·930
	B_4	0·5	0·245	0·330
	W_1	0·8	0·614	0·830
II	Expeller groundnut cake			
	A_1	1·2	0·965	1·300
	A_2	1·5	1·604	2·160
	A_3	1·8	2·855	3·840
	A_{18}	0·6	0·133	0·180
	A_{19}	1·0	0·653	0·880
	M_9	0·5	0·473	0·640
	W_2	0·3	0·188	0·250
III	Deoiled Cottonseed cake			
	C_1	0·3	0·582	0·780
	W_6	1·0	0·734	0·990
IV	Deoiled *kardi* cake			
	A_{23}	1·1	1·251	1·680
	R_{18}	1·5	1·590	2·150
	S_{19}	1·7	2·813	3·780
V	Deoiled *Salseed* cake			
	A_9	1·4	1·223	1·640
	A_{24}	0·6	0·533	0·720
	M_7	1·2	2·719	3·650
	P_9	1·0	0·674	0·910
	R_8	1·0	1·319	1·770
	S_{14}	0·9	0·631	0·850
	W_7	1·6	2·202	2·960
	W_8	1·7	2·800	3·760

TABLE 1 (Contd.)

1.	2.	3.	4.	5.
VI	Deoiled Soyabean cake S_7	1·3	1·631	2·190
VII	Deoiled Sunflower cake S_{17}	1·1	1·448	1·940
VIII	Deoiled *sheria* cake A_{21}	0·9	0·136	0·180
IX	Expeller *sheria* cake A_{22}	0·2	0·054	0·070
X	Deoiled *ambadi* cake S_{18}	0·9	0·632	0·850
XI	Deoiled rubber seed cake W_{16}	2·7	5·166	6·940
XII	Cereal and damaged grains			
	A_{30} Barley	1·6	1·830	2·460
	M_3 *Banti*	1·9	5·438	7·310
	A_{33} Maize	1·0	1·332	1·790
	M_{15} Maize	1·5	2·202	2·960
	S_{16} Maize	1·4	2·189	2·940
	P_{11} Damaged *jowar*	0·5	0·315	0·420
	P_4 Damaged Wheat	0·4	0·277	0·370
	R_5 Damaged Wheat	1·4	1·550	2·080
	R_6 Damaged Wheat	0·6	0·370	0·500
XIII	Cereal byproducts and other feeds			
	A_{37} Maize grit	1·3	1·618	2·170
	W_{10} Maize gluten	1·9	3·262	4·380
	M_{11} Rice bran	1·7	2·882	3·870
	S_{15} Wheat bran	1·8	4·840	6·500
	A_8 Mango kernels	1·3	1·142	1·530
	A_{10} Tapioca	0·7	0·707	0·950
	W_{18} Tapioca	1·0	0·767	1·030
XIV	Compounded cattle feeds			
	A_{12}	1·7	2·460	3·310
	M_{12}	0·8	0·663	0·890
	S_{13}	1·7	1·870	2·510
	B_2	1·3	1·577	2·120
	B_3	1·6	2·433	3·270
	Correlation(r) =		0·967**	0·798**

Method A = Spectrophotometric method
Method B = Robertson *et al* calcualtion method
** Significant at 1% level

It is seen that ranges of aflatoxins content among the samples examined as per spectrophotometric method and Robertson *et al* (1967) calculation method are 0·05 to 5·44 ppm and 0·07 to 7·31 ppm respectively. Deoiled *sheria* cake has shown the minimum aflatoxin (0·05 ppm) while *banti* a cereal grain has shown the maximum of the toxin (5·44 ppm). The aflatoxins content ranges show that even the same type of ingredients would have a large variation. This may be as a result of different factors, either favouring the growth of fungi and production of toxins or inhibiting the growth or absence of infection.

TABLE 2

The quantitative ranges of aflatoxins of each class of the cattle feeds.

Sr. No.	*Class of feed*	*Ranges of aflatoxins*	
		Method A (ppm)	*Method B (ppm)*
I	Decoiled groundnut cake	0·25 to 0·86	0·33 to 1·16
II	Expeller groundnut cake	0·13 to 2·86	0·18 to 3·84
III	Other decoiled cakes	0·05 to 5·17	0·07 to 6·94
IV	Cereal and damaged grains	0·28 to 5·44	0·37 to 7·31
V	Cereal byproducts and other feeds	0·70 to 4·84	0·95 to 6·50
VI	Compounded cattle feeds	0·66 to 2·46	0·89 to 3·31

Method A = Spectrophotometric method
Method B = Roberston *et al* calculation method

The quantitative ranges of aflatoxins content of each class are given in Table 2.

Normally groundnut cake and other cakes are considered to be the sources of aflatoxin problems. However, the results here show that different classes of cattle feed ingredients are positive and are having larger amount of aflatoxins as compared to groundnut cake.

It is also observed from the table that aflatoxin content of each individual sample was comparatively higher in Robertson *et al* (1967) calculation method than spectro-photometric method of the respective samples. The quantitative results (0·05 to 5·44 ppm) and (0·07 to 7·31 ppm) of the respective methods are in agreement with the results (0·5 to 5 ppm) reported by Gopal (1970).

The correlations between the concentration of aflatoxins (ppm) and the zone of inhibition of bacterial growth (diameter in cm) were carried out. It was observed that the 'r' values under spectrophotometric method and Robertson *et al* (1970) calculation method were 0·967** and 0·798*** respectively. It is apparent from the above '*r*' values that the zone of inhibition of bacterial growth is significantly ($P < 0·01$) correlated with the concentrations of both the methods. It is also seen that the correlation between concentration of aflatoxins by spectrophotometric method and zone of inhibition is better than Robertson *et al* (1967) calculation method. It is therefore concluded that biological test by using *Bacillus megaterium* can be used for quantitative determinations when equipment like Beckman DU-2 spectrophotometric is not available.

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