## Ubiquitous contamination with multiple mycotoxins and other fungal metabolites in dietary rations of dairy cattle in Punjab, Pakistan

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### Introduction

Pakistan is third in worldwide dairy production ranking, behind India and USA. The dairy cows are usually fed total mixed rations (TMR) containing various ingredients, including roughages, cereal grains, and agro-industrial by-products (1). Due to favorable climatic conditions in South Asia, feedstuffs are vulnerable to mould infection and colonization with successive mycotoxin contamination leading to animals and public health problems. This is indicated by far higher levels of aflatoxin (AF) M1 observed than those of recommended level by European Union (2). As feed is the central source of AFM1precusrsor (AFB1), contamination of in dairy cattle diets should also be monitored to kept the risk at minimum. This exploratory study aimed to determine via a LC-MS/MS method the presence, cooccurrence, and concentrations of mycotoxins and other secondary metabolites in 30 dairy farms of Punjab-Pakistan.





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The samples were collected during June - July 2020 from 30 dairy farms (each with minimum 200 animals herd size) located in Punjab-Pakistan (n = 30) in such a way that each administrative division contributed equal farms. A total of 30 incremental samples from feed bunk right after serving were pooled and 1 - 1.5 kg sample was vacuum-packed, and stored at -20 °C. The frozen TMR were thawed at room temperature for 24 hours and air-dried at 65 °C for 48 hours. Then, the dried TMR were milled to a final particle size of  $\leq$  0.5 mm, using a cutting mill following the previously reported procedures (3). Finally, the samples were analyzed via LC-MS/MS (Spectrum 380®) for mycotoxins analysis. The concentration values are presented on a dry basis in parts per billion (ppb).

### Results



# Alternariol 40 37 40 40 7 27 40 27 20 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40 40

		Alternariol	ternariolmethylether	Infectopyrone	Tentoxin	Tenuazonic acid	Aflatoxin B1	Averufin	Kojic acid	Sterigmatocystin	Versicolorin C	Ergometrinine	Beauvericin	Bikaverin	Epiequisetin	Equisetin	Fumonisin B1	Fumonisin B2	Fumonisin B3	Moniliformin	Monocerin	Nivalenol	Siccanol	Zearalenone	Flavoglaucin	Griseofulvin	Oxaline	Phenopyrrozin	estiomycin Derivate	
l	Questiomycin Derivate	17	40	43	43	40	13	27	43	23	27	33	43	43	43	43	40	43	33	37	43	17	43	17	43	33	40	40	43	10
	Phenopyrrozin -	40	87	87	97	97	40	67	97	33	60	73	97	97	97	97	93	97	77	87	90	40	93	43	97	70	80	97	40	
Penicillium-	Oxaline –	37	77	77	83	80	30	63	83	33	57	63	83	83	83	83	77	83	63	73	80	40	80	40	83	73	83	80	40	20
	Griseofulvin-	37	67	70	73	70	23	53	73	30	50	53	73	73	73	73	67	73	57	63	73	37	70	37	73	73	73	70	33	
	Flavoglaucin-	40	90	90	100	97	40	70	100	37	63	73	100	100	100	100	93	100	77	87	93	40	97	43	100	73	83	97	43	
ſ	Cycloaspeptide A-	23	30	33	33	30	10	20	33	20	23	17	33	33	33	33	27	33	17	27	33	20	33	17	33	30	30	30	20	- 30
	Zearalenone -	27	40	40	43	43	17	33	43	20	33	30	43	43	43	43	40	43	40	40	43	23	40	43	43	37	40	43	17	
	Siccanol -	40	87	87	97	93	37	67	97	37	60	70	97	97	97	97	90	97	73	83	90	40	97	40	97	70	80	93	43	
	Nivalenol -	27	33	37	40	40	7	30	40	27	27	27	40	40	40	40	37	40	30	33	40	40	40	23	40	37	40	40	17	- 40
	Monocerin -	37	83	87	93	90	37	63	93	33	57	70	93	93	93	93	87	93	77	80	93	40	90	43	93	73	80	90	43	
	Moniliformin	37	77	80	87	87	37	60	87	30	57	63	87	87	87	87	83	87	70	87	80	33	83	40	87	63	73	87	37	
Fusarium _	Fumonisin B2	10 27	67	70	77	77	37	53	77	27	47	67	77	77	77	77	77	77	77	70	77	30	73	40	77	57	63	77	43	- 50
	Fumonisin B1 -	37	83	83	93	93	40	70	93	33	57	73	93	93	93	93	93	93	77	83	87	31	90	40	93	72	11	93	40	
	Equisetin-	40	90	90	100	97	40	70	100	37	63	73	100	100	100	100	93	100	77	87	93	40	97	43	100	73	83	97	43	
	Epiequisetin -	40	90	90	100	97	40	70	100	37	63	73	100	100	100	100	93	100	77	87	93	40	97	43	100	73	83	97	43	60

	Proportion of ingredient (% DM) <sup>1</sup>													
Mycotoxin / Metabolite	Rhodes grass hay	Wheat straw	Corn grain	Soybean meal	Canola meal	Molasses	Commercial concentrate							
Aflatoxin B1	0.26	0.03	0.06	0.00	0.07	0.41*	-0.26							
Kojic acid	-0.12	-0.07	0.10	0.06	0.07	0.38*	-0.23							
Ergometrinine	-0.23	0.26	0.39*	0.31	0.38*	0.54**	-0.36							
Ergot alkaloids	-0.23	0.27	0.40*	0.31	0.38*	0.53**	-0.35							
Bikaverin	0.08	0.13	0.47*	0.47*	0.49**	0.31	-0.58**							
Fumonisin B1	-0.16	0.22	0.54**	0.54**	0.52**	0.36	-0.56**							
Fumonisin B2	-0.09	0.21	0.55**	0.56**	0.57**	0.32	-0.47**							
Fumonisin B3	0.02	0.18	0.47	0.42*	0.35	0.27	-0.50**							
Moniliformin	0.16	-0.36	0.14	0.13	0.17	0.36	-0.56**							
Neoechinulin A	0.38*	-0.26	-0.12	-0.03	-0.06	0.03	-0.40*							
Questiomycin Derivate	-0.06	0.41*	-0.29	-0.23	-0.19	-0.12	-0.12							
from Penicillium	0.23	0.10	-0.19	-0.18	-0.11	0.32	-0.05							

#### 5 10 15 20 25 30 35 40 45 50 55 60

Number of detected metabolites / sample

### Conclusions



Our results indicated ubiquitous contamination of TMRs with multiple mycotoxins. The most prevalent were *Fusarium* toxins like fumonisin B1 (FB1) (93%), B2 (FB2) (100%), B3 (FB3) (77%). Aflatoxin B1 (AFB1) was evidenced in 40% of the samples, and 7% exceeded the EU maximum limit for feeding dairy cattle (5 µg/kg). The dietary ingredients like corn grain, soybean meal, and canola meal were related to increased contamination of some mycotoxins (like FB1, FB2, and FB3) in TMRs from the Province of Punjab, Pakistan.

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